THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

VOLUME 157

August TO DECEMBER, 1979

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Communications relative to manuscripts should be sent to Dr. Charles B. Metz, Marine Biological Laboratory, Woods Hole, Massachusetts 02543 between June 1 and September 1, and to Dr. Charles B. Metz, Institute For Molecular and Cellular Evolution, University of Miami, 521 Anastasia, Coral Gables, Florida 33134 during the remainder of the year.

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CONTENTS

No. 1, August, 1979

No. 2, October, 1979

No. 3, December, 1979

Volume 157 Number 1

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INSTRUCTIONS TO AUTHORS

THE BIOLOGICAL BULLETIN accepts original research reports of intermediate length on a variety of subjects of biological interest. In general, these papers are either of particular interest to workers at the Marine Biological Laboratory, or of outstanding general significance to a large number of biologists throughout the world. Normally, review papers (except those written at the specific invitation of the Editorial Board), very short papers (less than five printed pages), preliminary notes, and papers which describe only a new technique or method without presenting substantial quantities of data resulting from the use of the new method cannot be accepted for publication. A paper will usually appear within four months of the date of its acceptance.

The Editorial Board requests that manuscripts conform to the requirements set below; those manuscripts which do not conform will be returned to authors for correction before review by the board.

1. ***Manuscripts.*** Manuscripts must be typed in double spacing (*including* figure legends, foot-notes, bibliography, etc.) on one side of 16- or 20-lb. bond paper, 8½ by 11 inches. They should be carefully proof-read before being submitted and all typographical errors corrected legibly in black ink. Pages should be numbered. A left-hand margin of at least 1½ inches should be allowed.

2. ***Tables, Foot-Notes, Figure Legends, etc.*** Tables should be typed on separate sheets and placed after the Literature Cited. Because of the high cost of setting such material in type authors are earnestly requested to limit tabular material as much as possible. Similarly, foot-notes to tables should be avoided wherever possible. If they are essential, they should be indicated by asterisks, daggers, etc., rather than by numbers. Foot-notes are not normally permitted in the body of the text. Such material should be incorporated into the text where appropriate. Explanations of figures should be typed double-spaced and placed on separate sheets at the end of the paper.

3. ***A condensed title*** or running head of no more than 35 letters and spaces should be included.

4. ***Literature Cited.*** The list of references should be headed LITERATURE CITED, should conform in punctuation and arrangement to the style of recent issues of THE BIOLOGICAL BULLETIN, and must be typed *double-spaced* on separate pages. Note that citations should include complete titles and inclusive pagination. Journal abbreviations should normally follow those of the U. S. A. Standards Institute (USASI), as adopted by BIOLOGICAL ABSTRACTS and CHEMICAL ABSTRACTS, with the minor differences set out below. The most generally useful list

Continued on Cover Three

NOTICE TO SUBSCRIBERS

Effective with the first issue of Volume 158 (February, 1980), the subscription price for THE BIOLOGICAL BULLETIN will be raised. Subscription per volume (three issues) will be $27.00 (this is $54.00 per year for six issues); single numbers will be $10.00 each. Continuing 1980 subscriptions for which payment has actually been received before November 1, 1979 will be honored at the old rate.

Vol. 157, No. 1 Auguust, 1979

THE BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE MARINE BIOLOGICAL LABORATORY

EIGHTY-FIRST REPORT, FOR THE YEAR 1978 NINETY-FIRST YEAR

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II. CERTIFICATE OF ORGANIZATION

(On File in the Office of the Secretary of the Commonwealth)

No. 3170

We, Alpheus Hyatt, President, William Stanford Stevens, Treasurer, and William T. Sedgwick, Edward G. Gardiner, Susan Mims and Charles Sedgwick Minot being a majority of the Trustees of the Marine Biological Laboratory in compliance with the requirements of the fourth section of chapter one hundred and fifteen of the Public Statutes do hereby certify that the following is a true copy of the agreement of association to constitute said Corporation, with the names of the subscribers thereto:-

We, whose names are hereto subscribed, do, by this agreement, associate ourselves with the intention to constitute a Corporation according to the provisions of the one hundred and fifteenth chapter of the Public Statutes of the Commonwealth of Massachusetts, and the Acts in amendment thereof and in addition thereto.

The name by which the Corporation shall be known is THE MARINE BIOLOGICAL LABORATORY

The purpose for which the Corporation is constituted is to establish and maintain a laboratory or station for scientific study and investigations, and a school for instruction in biology and natural history.

The place within which the Corporation is established or located is the city of Boston within said Commonwealth.

The amount of its capital stock is none.

In Witness Whereof, we have hereunto set our hands, this twenty seventh day of February in the year eighteen hundred and eighty-eight, Alpheus Hyatt, Samuel Mills, William T. Sedgwick, Edward G. Gardiner, Charles Sedgwick Minot, William G. Farlow, William Stanford Stevens, Anna D. Phillips, Susan Mims, B. H. Van Vleck.

That the first meeting of the subscribers to said agreement was held on the thirteenth day of March in the year eighteen hundred and eighty-eight.

In Witness Whereof, we have hereunto signed our names, this thirteenth day of March in the year eighteen hundred and eighty-eight, Alpheus Hyatt, President, William Stanford Stevens, Treasurer, Edward G. Gardiner, William T. Sedgwick, Susan Mims, Charles Sedgwick Minot.

(Approved on March 20, 1888 as follows:

I hereby certify that it appears upon an examination of the within written certificate and the records of the corporation duly submitted to my inspection, that the requirements of sections one, two and three of chapter one hundred and fifteen, and sections eighteen, twenty and twenty-one of chapter one hundred and six, of the Public Statutes, have been complied with and I hereby approve said certificate this twentieth day of March A.D. eighteen hundred and eighty-eight.

CHARLES ENDICOTT
Commissioner of Corporations)

III. ARTICLES OF AMENDMENT

(On file in the office of the Secretary of the Commonwealth)

We, James D. Ebert, President, and David Shepro, Clerk of the Marine Biological Laboratory, located at Woods Hole, Massachusetts 02543, do hereby certify that the following amendment to the Articles of Organization of the Corporation was duly adopted at a meeting held on August 15, 1975, as adjourned to August 29, 1975, by vote of 444 members, being at least two-thirds of its members legally qualified to vote in the meetings of the corporation:

VOTED: That the Certificate of Organization of this corporation be and it hereby is amended by the addition of the following provisions:

"No Officer, Trustee or Corporate Member of the corporation shall be personally liable for the payment or satisfaction of any obligation or liabilities incurred as a result of, or otherwise in connection with, any commitments, agreements, activities or affairs of the corporation.

"Except as otherwise specifically provided by the Bylaws of the corporation, meetings of the Corporate Members of the corporation may be held anywhere in the United States.

"The Trustees of the corporation may make, amend or repeal the Bylaws of the corporation in whole or in part, except with respect to any provisions thereof which shall by law, this Certificate or the Bylaws of the corporation, require action by the Corporate Members."

The foregoing amendment will become effective when these articles of amendment are filed in accordance with Chapter 180, Section 7 of the General Laws unless these articles specify, in accordance with the vote adopting the amendment, a later effective date not more than thirty days after such filing, in which event the amendment will become effective on such later date.

In Witness whereof and Under the Penalties of Perjury, we have hereto signed our names this 2nd day of September, in the year 1975, James D. Ebert, President; David Shepro, Clerk.

(Approved on October 24, 1975, as follows:

I hereby approve the within articles of amendment and, the filing fee in the amount of $10 having been paid, said articles are deemed to have been filed with me this 24th day of October, 1975.

PAUL GUZZI
Secretary of the Commonwealth)

IV. BYLAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

(Revised August 11, 1978)

I. (A) The name of the Corporation shall be The Marine Biological Laboratory. The Corporation's purpose shall be to establish and maintain a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history.

(B) Marine Biological Laboratory admits students without regard to race, color, sex, national and ethnic origin to all the rights, privileges, programs and activities generally accorded or made available to students in its courses. It does not discriminate on the basis of race, color, sex, national and ethnic origin in employment, administration of its educational policies, admissions policies, scholarship and other programs.

II. (A) The members of the Corporation ("Members") shall consist of persons elected by the Board of Trustees, upon such terms and conditions and in accordance with such procedures, not inconsistent with law or these Bylaws, as may be determined by said Board of Trustees. Except as provided below, any Member may vote at any meeting, either in person or by proxy executed no more than six months prior to the date of such meeting. Members shall serve until their death or resignation unless earlier removed, with or without cause, by the affirmative vote of two-thirds of the Trustees then in office. Any Member who has attained the age of seventy years or has retired from his home institution shall automatically be designated a Life Member provided he signifies his wish to retain his membership. Life Members shall not have the right to vote and shall not be assessed for dues.

(B) The Associates of the Marine Biological Laboratory shall be an unincorporated group of persons (including associations and corporations) interested in the Laboratory and shall be organized and operated under the general supervision and authority of the Trustees.

III. The officers of the Corporation shall consist of a Chairman of the Board of Trustees, President, Director, Treasurer and Clerk, elected or appointed by the Trustees as set forth in Article IX.

IV. The Annual Meeting of the Members shall be held on the Friday following the Second Tuesday in August in each year at the Laboratory in Woods Hole, Massachusetts, at 9:30 a.m. Subject to the provisions of Article VIII(2), at such meeting the Members shall choose by ballot six Trustees to serve four years, and shall transact such other business as may properly come before the meeting. Special meetings of the Members may be called by the Chairman or Trustees to be held at such time and place as may be designated.

V. Twenty five Members shall constitute a quorum at any meeting. Except as otherwise required by law or these Bylaws, the affirmative vote of a majority of the Members voting in person or by proxy at a meeting attended by a quorum (present in person or by proxy) shall constitute action on behalf of the Members.

VI. (A) Inasmuch as the time and place of the Annual Meeting of Members are fixed by these Bylaws, no notice of the Annual Meeting need be given. Notice of any special meeting of Members, however, shall be given by the Clerk by mailing notice of the time and place and purpose of such meeting, at least 15 days before such meeting, to each Member at his or her address as shown on the records of the Corporation.

(B) Any meeting of the Members may be adjourned to any other time and place by the vote of a majority of those Members present or represented at the meeting, whether or not such Members constitute a quorum. It shall not be necessary to notify any Member of any adjournment.

VII. The Annual Meeting of the Trustees shall be held promptly after the Annual Meeting of the Corporation at the Laboratory in Woods Hole, Massachusetts. Special meetings of the Trustees shall be called by the Chairman, the President, or by any seven Trustees, to be held at such time and place as may be designated. Notice of Trustees' meetings may be given orally, by telephone, telegraph or in writing; and notice given in time to enable the Trustees to attend, or in any case notice sent by mail or telegraph to a Trustee's usual or last known place of residence, at least one week before the meeting shall be sufficient. Notice of a meeting need not be given to any Trustee if a written waiver of notice, executed by him before or after the meeting is filed with the records of the meeting, or if he shall attend the meeting without protesting prior thereto or at its commencement the lack of notice to him.

VIII. (A) There shall be four groups of Trustees:

(1) Trustees (the "Corporate Trustees") elected by the Members according to such procedures, not inconsistent with these Bylaws, as the Trustees shall have determined. Except as provided below, such Trustees shall be divided into four classes of six, one class to be elected each year to serve for a term of four years. Such classes shall be designated by the year of expiration of their respective terms.

(2) Trustees ("Board Trustees") elected by the Trustees then in office according to such procedures, not inconsistent with these Bylaws, as the Trustees shall have determined. Except as provided below, such Board Trustees shall be divided into four classes of three, one class to be elected each year to serve for a term of four years. Such classes shall be designated by the year of expiration of their respective terms. It is contemplated that, unless otherwise determined by the Trustees for good reason, Board Trustees shall be individuals who have not been considered for election as Corporate Trustees.

(3) Trustees ex officio, who shall be the Chairman, the President, the Director, the Treasurer, and the Clerk.

(4) Trustees emeriti who shall include any Member who has attained the age of seventy years (or the age of sixty five and has retired from his home institution) and who has served a full elected term as a regular Trustee, provided he signifies his wish to serve the Laboratory in that capacity. Any Trustee who qualifies for emeritus status shall continue to serve as a regular Trustee until the next Annual Meeting whereupon his office as regular Trustee shall become vacant and be filled by election by the Members or by the Board, as the case may be. The Trustees ex officio and emeriti shall have all the rights of the Trustees, except that Trustees emeriti shall not have the right to vote.

(B) The aggregate number of Corporate Trustees and Board Trustees elected in any year (excluding Trustees elected to fill vacancies which do not result from expiration of a term) shall not exceed nine. The number of Board Trustees so elected shall not exceed three and unless otherwise determined by vote of the Trustees, the number of Corporate Trustees so elected shall not exceed six.

(C) The Trustees and Officers shall hold their respective offices until their successors are chosen in their stead.

(D) Any Trustee may be removed from office at any time with or without cause, by vote of a majority of the Members entitled to vote in the election of Trustees; or for cause, by vote of two-thirds of the Trustees then in office. A Trustee may be removed for cause only if notice of such action shall have been given to all of the Trustees or Members entitled to vote, as the case may be, prior to the meeting at which such action is to be taken and if the Trustee so to be removed shall have been given reasonable notice and opportunity to be heard before the body proposing to remove him.

(E) Any vacancy in the number of Corporate Trustees, however arising, may be filled by the Trustees then in office unless and until filled by the Members at the next Annual Meeting. Any vacancy in the number of Board Trustees may be filled by the Trustees.

(F) A Corporate Trustee or a Board Trustee who has served an initial term of at least 2 years duration shall be eligible for re-election to a second term, but shall be ineligible for re-election to any subsequent term until two years have elapsed after he last served as a Trustee.

IX. (A) The Trustees shall have the control and management of the affairs of the Corporation. They shall elect a Chairman of the Board of Trustees who shall be elected annually and shall serve until his successor is selected and qualified and who shall also preside at meetings of the Corporation. They shall elect a President of the Corporation who shall also be the Vice Chairman of the Board of Trustees and Vice Chairman of meetings of the Corporation, and who shall be elected annually and shall serve until his successor is selected and qualified. They shall elect a Treasurer and Clerk to serve one year, and Board Trustees as described in Article VIII (B). They shall appoint a Director of the Laboratory for a term not to exceed five years, provided the term shall not exceed one year if the candidate has attained the age of 65 years prior to the date of the appointment. They may choose such other officers and agents as they may think best. They may fix the compensation and define the duties of all the officers

and agents of the Corporation and may remove them at any time. They may fill vacancies occurring in any of the offices. The Board of Trustees shall have the power to choose an Executive Committee from their own number as provided in Article X, and to delegate to such Committee such of their own powers as they may deem expedient in addition to those powers conferred by Article X. They shall from time to time elect Members to the Corporation upon such terms and conditions as they shall have determined, not inconsistent with law or these Bylaws.

(B) The Board of Trustees shall also have the power, by vote of a majority of the Trustees then in Office, to elect an Investment Committee and any other committee and, by like vote, to delegate thereto some or all of their powers except those which by law, the Articles of Organization or these Bylaws they are prohibited from delegating. The members of any such committee shall have such tenure and duties as the Trustees shall determine; provided that the Investment Committee, which shall oversee the management of the Corporation's endowment funds and marketable securities, shall include the Chairman of the Board of Trustees, the Treasurer of the Corporation, and the Chairman of the Corporation's Budget Committee, as *ex officio* members, together with such Trustees as may be required for not less than two-thirds of the Investment Committee to consist of Trustees. Except as otherwise provided by these Bylaws or determined by the Trustees, any such committee may make rules for the conduct of its business; but, unless otherwise provided by the Trustees or in such rules, its business shall be conducted as nearly as possible in the same manner as is provided by these Bylaws for the Trustees.

X. (A) The Executive Committee is hereby designated to consist of not more than ten members, including the ex officio Members (Chairman of the Board of Trustees, President, Director and Treasurer); and six additional Trustees, two of whom shall be elected by the Board of Trustees each year, to serve for a three-year term.

(B) The Chairman of the Board of Trustees shall act as Chairman of the Executive Committee, and the President as Vice Chairman. A majority of the members of the Executive Committee shall constitute a quorum and the affirmative vote of a majority of those voting at any meeting at which a quorum is present shall constitute action on behalf of the Executive Committee. The Executive Committee shall meet at such times and places and upon such notice and appoint such sub-committees as the Committee shall determine.

(C) The Executive Committee shall have and may exercise all the powers of the Board during the intervals between meetings of the Board of Trustees except those powers specifically withheld from time to time by vote of the Board or by law. The Executive Committee may also appoint such committees, including persons who are not Trustees, as it may from time to time approve to make recommendations with respect to matters to be acted upon by the Executive Committee or the Board of Trustees.

(D) The Executive Committee shall keep appropriate minutes of its meetings and its action shall be reported to the Board of Trustees.

(E) The elected Members of the Executive Committee shall constitute as a standing "Committee for the Nomination of Officers," responsible for making nominations, at each Annual Meeting of the Corporation, and of the Board of Trustees, for candidates to fill each office as the respective terms of office expire (Chairman of the Board, President, Director, Treasurer, and Clerk).

XI. A majority of the Trustees, the Executive Committee, or any other committee elected by the Trustees shall constitute a quorum; and a lesser number than a quorum may adjourn any meeting from time to time without further notice. At any meeting of the Trustees, the Executive Committee, or any other committee elected by the Trustees,

the vote of a majority of those present, or such different vote as may be specified by law, the Articles of Organization or these Bylaws, shall be sufficient to take any action.

XII. Any action required or permitted to be taken at any meeting of the Trustees, the Executive Committee or any other committee elected by the Trustees as referred to under Article IX may be taken without a meeting if all of the Trustees or members of such committee, as the case may be, consent to the action in writing and such written consents are filed with the records of meetings. The Trustees or members of the Executive Committee or any other committee appointed by the Trustees may also participate in meeting by means of conference telephone, or otherwise take action in such a manner as may from time to time be permitted by law.

XIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees then in office.

XIV. These Bylaws may be amended by the affirmative vote of the Members at any meeting, provided that notice of the substance of the proposed amendment is stated in the notice of such meeting. As authorized by the Articles of Organization, the Trustees, by a majority of their number then in office, may also make, amend, or repeal these Bylaws, in whole or in part, except with respect to (a) the provisions of these Bylaws governing (i) the removal of Trustees and (ii) the amendment of these Bylaws and (b) any provisions of these Bylaws which by law, the Articles of Organization or these Bylaws, requires action by the Members.

No later than the time of giving notice of the meeting of Members next following the making, amending or repealing by the Trustees of any Bylaw, notice thereof stating the substance of such change shall be given to all Corporation Members entitled to vote on amending the Bylaws.

Any Bylaw adopted by the Trustees may be amended or repealed by the Members entitled to vote on amending the Bylaws.

XV. The account of the Treasurer shall be audited annually by a certified public accountant.

XVI. The Corporation will indemnify every person who is or was a trustee, officer or employee of the Corporation or a person who provides services without compensation to an Employee Benefit Plan maintained by the Corporation, for any liability (including reasonable costs of defense and settlement) arising by reason of any act or omission affecting an Employee Benefit Plan maintained by the Corporation or affecting the participants or beneficiaries of such Plan, including without limitation any damages, civil penalty or excise tax imposed pursuant to the Employee Retirement Income Security Act of 1974; provided, (1) that the Act or omission shall have occurred in the course of the person's service as trustee or officer of the Corporation or within the scope of the employment of an employee of the Corporation or in connection with a service provided without compensation to an Employee Benefit Plan maintained by the Corporation, (2) that the Act or omission be in good faith as determined by the Corporation (whose determination made in good faith and not arbitrarily or capriciously shall be conclusive), and (3) that the Corporation's obligation hereunder shall be offset to the extent of any otherwise applicable insurance coverage, under a policy maintained by the Corporation or any other person, or other source of indemnification.

V. REPORT OF THE DIRECTOR

To: The Trustees of the Marine Biological Laboratory

"We should be careful to get out of an experience only the wisdom that is in it—and stop there; lest we be like the cat that sits down on a hot stove-lid. She will never sit down on a hot stove-lid again—and that is well; but also she will never sit down on a cold one anymore."

—Mark Twain

The calendar year to which this report refers saw many changes. Not the least of these was the departure of James Ebert for the Presidency of the Carnegie Institution of Washington. Ebert's tenure extended through about half of the year, although he was present in person, and by way of generous advice and guidance from Washington in spirit, for a far larger part of it. Other changes have taken place; some in management, some in programs and courses, and some in the Laboratory's rules of operation. A most significant event of the latter kind was adoption by the Trustees, in February of 1979, of a comprehensive seven-year plan for programs, space utilization, and campus improvement.

Many of the transitional changes, like the Trustees' plan and its related actions, came in 1979, and will therefore not be covered in this report. Their occurrence is felt, however, at the time of writing, and their impact upon the style of life and work at the Laboratory will doubtless increase in the year to come.

James D. Ebert

Although a *State of the Institution* report is not the appropriate place for a tribute to its retired chief executive officer, there being better occasions and places, I cannot let pass the opportunity to mark the debt we owe to Dr. Ebert. His long association with the MBL, which we trust will continue, reached a peak of intensity during an influential tenure as the Laboratory's first full-time Director and President. Always forceful and direct in his views, more analytical and better-prepared than most who disagreed with him, Ebert brought about a series of major alterations in the way the MBL functions, meeting successfully, thereby, a number of crises in finance and management. He was able to accomplish this without any infringement of program quality nor of the traditional rights and responsibilities of the Corporation.

I have a more detailed view of his efforts than is generally available, owing to the records of his work, upon which we are daily dependent, and to my now frequent contacts with friends of the Laboratory to whom Ebert first took the MBL's case. There can be no denial of the fact that his efforts were as fruitful as they were tireless and self-sacrificing. I believe that his contribution to the Laboratory will stand permanently as having been central to its survival in strength.

Research

To separate research from education is a process even more artificial for the MBL than it is for universities. The intertwining of the two is here less an accident and more a design than in any other scientific institution. It has been so ever since Director Whitman wrote in the first Annual Report:

"Other things being equal, the investigator is always the best instructor. The highest grade of instruction in any science can only be furnished by one who is thoroughly imbued with the scientific spirit, and who is actually engaged in original work. Whence the propriety—and, I may say, the necessity—of linking the function of instruction with that of investigation."

It is nevertheless useful to report separately upon research activity at the MBL, to the extent that is characterized by numbers of persons so engaged, by the sums awarded and spent for the purpose, and by the facilities given up to it, as opposed, for example, to those employed solely for classroom activities.

We can be proud that in a time of declining real support for research nationwide, and of exponentially-growing costs and demands upon physical facilities, the MBL has been able, upon a comparatively uncertain financial base, to enlarge the scope and impact of its research, and to do so without denying access to its facilities by any properly qualified person or group.

There are four broad areas of research that together encompass most, although not all, investigative activities at the Laboratory. These are (1) Ecology, (2) Cellular, Developmental, and Reproductive Biology, (3) Neuroscience, Physiology, and Biophysics, and (4) Marine Biology and Biomedicine. In each of these areas the Laboratory provides for permanent (or year-round) as well as transient (mainly, but not exclusively summer) programs.

In 1978 there was growth in all four, as measured by people, funds, and—since no decline of standards was, or will ever be permitted—by impact upon biology as a whole, *i.e.*, by quality. The simple argument underlying this last conclusion is from information theory, in which one obtains first the information content per individual in an assemblage (the units being "bits" or "nats," or some other, depending upon the base of logarithms chosen), and then the information content of the entire assemblage by multiplying the result by the number of individuals.

Lest this be interpreted as an argument for unlimited growth, I make haste to demur. It is not. It is, rather, a way of saying that, within limits, growth accompanied by maintained standards is *ipso facto* an increase in total quality.

The Ecosystems Center, the Boston University Marine Program, the Laboratory of Sensory Physiology, and Laboratory of Biophysics, and a group of some twenty individual research programs operated at the MBL have all performed as well or better in 1978 than they did in 1977. By performance I mean the sum of those measures employed in our system of program evaluations: important publications; grants awarded; honors and awards to staff; presentations of important lectures; quality of students and other participants attracted; and the like.

These activities, which comprise the currently permanent part of the MBL's research effort, have already reached a size and level of achievement such that to single out one or two for special mention here would be impolitic. It may well be that we shall soon need a separate Annual Report on Research, in order to do justice to the accomplishments of our permanent programs. Suffice it to say that the health and quality of year-round research at MBL are no longer at issue. The issue will instead be, for the future, to control growth while still retaining a decent flexibility of operation, so that life at MBL does *not* decay to the level of that lived in many universities, where changes in space assignment are accomplished only at the expense of internecine warfare.

In the summer of 1978 research space at the Laboratory was fully occupied, except for a few rooms that fell vacant at a late hour, owing to changes of plans. For the summer of 1979 there were far more applications even than in the prior year. We have been able, at least at the time of writing, to accommodate all those whose proposed research was judged appropriate to our standards by the Research Space Committee. This was accomplished by several devices, including the use for research of rooms not formerly rented for that purpose, and a vigorous effort to encourage the sharing of space, when that would not interfere with research quality. I may say that applicants so approached by us, including Corporation members who have enjoyed a certain luxury in past summers, responded magnificently. No such suggestion for sharing failed to be received courteously and to be considered seriously.

The above is a quantitative statement, of course, about the summer research program, but the argument made earlier about population and information content applies: there is a significant and continuing increase in the demand for research space and facilities here assignable for such use, and the overall quality is rising. All indicators suggest that the trend will continue.

It is evident from the foregoing that we shall have a great deal of decision-making to do in the future as regards deployment of space and facilities. This is a challenge that we ought not to fear: far better to be in a position of making fine distinctions—however fallible peer judgments may sometimes be, and however painful rejection is—than to be unable to assign all the available space for want of good contenders.

A few sample numbers: overhead recovery from non-MBL research grants (via space charges) was $424,631 in 1977, $503,576 in 1978, and we have budgeted $562,699 for 1979. The corresponding figures for MBL-administered grants (government and private) are $251,916; $297,136; $391,766. These number do, of course, have an inflation component, and hence do not imply a *proportionate* increase in the absolute size of the research effort, but they do reflect a real and impressive size increment nevertheless. Were it not that the inflationary rise of operating costs easily matches them, I might be moved to call such figures "healthy."

Some losses and gains of research persons should be mentioned, although of course space limitation prohibits anything like a comprehensive listing. There was the very tragic loss of Dr. Fred Lang, of the B.U.M.P., in an automobile accident, and that must be mentioned. Two other honored and well-known Corporation members died in the course of the year: Jean Clark Dan and Lester G. Barth.

It is a personal pleasure for me and splendid news for the Laboratory that Professor Shinya Inoué, of Pennsylvania, a distinguished cell biologist and long-time Corporation member, will henceforth be in permanent residence at the MBL, and will play an important role in the planning and growth of our cell biology programs. Several additional appointments of importance have been made or are in the making. They will be announced in due course.

Education

As indicated earlier, every research activity at the MBL has a closely-related educational component; hence a mere catalogue of the formal courses does not convey the pervasiveness of learning and instruction that characterizes work here. As is true for research, however, a simple listing can indicate something of the *scope* of the program, and of changes taking place within it, for Corporation members already familiar with it.

Each of the five January courses offered in 1978 was continued in 1979: Behavior (J. Atema); Comparative Pathology of Marine Invertebrates (F. Bang); Developmental Biology (W. Vincent); Ecology (G. Woodwell); and Neurobiology (Alan Fein, together with E. F. MacNichol, Jr. and others, who carried on following the death of Fred Lang). One hundred fourteen students completed courses in the now well-established January semester, to which contributions were made by a group of distinguished teachers from around the nation, as well as from the Woods Hole resident community. The January semester is an obvious success, but its growth in future years will be small, limited by the space and facilities available for it.

Since it is clear that the January semester is to be a recurrent MBL activity, we shall be engaged during months to come in a number of management and quality control initiatives. These will bring the entire program under the same sort of continuous review, eventually, as is applied to the summer courses, possibly by the same committee of the Corporation. We will also attempt to regularize the financial arrangements, i.e.,

those governing tuition charges, reimbursements and compensation for faculty and guest speakers, and the assignment of operating costs among various cost centers of the budget. It is even now clear, however, that the January semester places no great financial burden upon the Laboratory, while yielding large benefits to the community intellectual life during what was formerly a slow month. Of course it provides invaluable educational benefits to the students, whose many letters attesting thereto are a gratifying chapter of our files.

All seven of the distinguished summer courses continued under the same directorates as in 1977, with one hundred forty-eight participants. During the year a number of searches for directorships due to become vacant in 1980 were initiated. Thomas Reese and John Hildebrand have been appointed co-directors of the Neurobiology Course to succeed Edward Kravitz. Outcomes of other searches will be announced in the summer of 1979. The excellent Microbial Ecology program has been elevated, under stimulus of the recommendations of an *ad hoc* committee and with approval of the Standing Committee on Instruction, to the status of a regular MBL summer course. Ably directed in 1978 and in prior years by Holger Jannasch, it will for 1979 and 1980 have Jannasch as co-director with Harlyn Halvorson, who will then continue as Instructor-in-Chief for an additional three years. A major effort is underway to provide this new course with suitable quarters in the Loeb Building by the summer of 1980.

Because of the tragic death of Mrs. Gelperin in May, 1979, Alan Gelperin will be unable to serve, at least for 1979, as director of the course on Neural Systems and Behavior. Faculty member Ronald Hoy agreed promptly and generously to step in as interim director. For this reason we anticipate a smooth operation of the relatively new offering despite the absence, hopefully a temporary one, of its energetic first director.

Active planning was begun in April, 1979, for a 1980 summer course in the Biology of Parasitism. The planning group includes, in addition to a number of MBL administrators and our own Frederik Bang, director of the January course on comparative pathology and a scholar of international reputation in the field, several accomplished members of the Harvard and Rockefeller University faculties, together with scientific officers of the Rockefeller and Edna McConnell Clark Foundations. The MBL Committee on Instruction, also represented among the planners (by Chairman Kaminer), will be concerned with detailed proposals during the summer of 1979.

The spring course in Aquatic Veterinary Medicine, a combined offering of the University of Pennsylvania, Cornell University, and three of the four Woods Hole institutions, directed energetically by Donald Abt, was offered for the second time, and with pronounced success. Interest in the program is sufficiently high to justify not only its continuation, but also expansion of the effort to include post-course research and, possibly, a number of more permanent activities centered at the MBL.

Morton Maser's program of continuing education, in the form of Short Courses, continues to merit the praise accorded it in Dr. Ebert's 1977 report. In 1978 there were eleven such courses, most of them concerned with advanced instrumentation and research technology, and featuring faculties of an exceptional expertise. Among the Instructors-in-Chief were Robert Allen, Blair Bowers, Lee Peachey, Bruce Wetzel, Russell Steere, Eduardo Macagno, Yukata Kobayashi, Patrick O'Farrell, Robert Ivarie, and Maser. A total of 157 participants came to Woods Hole in the months of March, April, May, October, November and December for these high-intensity courses. Their quality has been the subject of uniformly favorable comment, from students and faculty alike. A most enthusiastic article about them has been published in the *Norelco Reporter*.

Funding of all the formal educational offerings is at a high level, although as university science administrators well know, it is impossible to recover the current *real*

costs of advanced laboratory teaching. Nearly every summer course receives direct support from government or private grants, or from a combination of the two. Those few courses that were temporarily without directed support in 1978 have it or are very likely to have it in 1979. With reorganization of the financing and accounting for the January Semester, it will become practical to initiate an energetic program of fund-raising for that venture. Short Courses come very close to paying for themselves; indeed, they are upon a simple cash basis truly self-supporting.

This section cannot close without mention of the Boston University Marine Program, which continues as a leading opportunity, nationally, for graduate students seeking to specialize in marine biology. The MBL's pride in the Program and pleasure in its fine students and faculty is matched by opinion of the faculty and central administration at Boston University. An outstanding new appointment to the B.U.M.P. faculty has been made this year (Dr. Sidney Tamm, a cell biologist who will work with the already prominent MBL motility group). Dr. Christopher H. Price, an active investigator in the field of neurobiology, has been appointed to replace Dr. Lang.

Scientific Meetings and Conferences

Among the blessings that some of us take for granted at the MBL is the skill and efficiency with which housing and other maintenance needs of transient participants in our programs are handled. It is a task of the greatest complexity, involving not only the work of a specialized support staff, but also attention to detail, patience, and tact, coupled with a willingness to deal under pressure with many demands for exceptions to rules. All this Homer Smith does, as he has done for many years, quietly and effectively.

The more remarkable, therefore, is the rapidly increasing exploitation of our Swope Center, comprising as it does a significant additional burden of work. This is done, again, with minimum fuss, and by a support staff that would be viewed as skeletal by the management of a small motel.

In 1978, there were only two months (June and December)—and those with good reason—during which there were *not* significant numbers of working visitors to the MBL, residing in the Swope Center and making use of the Laboratory's facilities for one or another scientific or educational purpose. The total number of such persons reached a remarkable 2,300. Activities and institutions represented included: Massachusetts Marine Educators, St. Mary's College, Purdue University, National Marine Fisheries Service, Yale University, East Coast Nerve Net, International Symposium on the Spermatozoon, M.I.T., the University of Pennsylvania, Harvard University, Drew University, Upsala College, the Society of General Physiologists, the Marine Science Librarians, University of Maryland, Maine Audubon Society, a conference on Biomedical Applications of *Limulus*, Brown University, the New England Estuarine Society, Simmons College, and many others.

The value of such utilization of our facilities, quite aside from the implied financial benefits, is incalculable. All the Woods Hole institutions are beneficiaries (as is the village as a whole), and those perceived values attest to the wisdom of MBL people whose labors and gifts made the Swope Center a reality.

Once again, our problem is now one of choice among worthy competing alternatives, rather than of recruitment of users,—one of growth control, rather than of its simple encouragement. Again, I should be inclined to describe the whole phenomenon as unqualifiedly healthy, were there not the ghost of concern about inexorable inflation of operating costs, which, however carefully *they* are held down by means of economies, tend to alter what should be a surplus-producing function to one that may just pay for itself. The matter of costs and charges in this sphere will receive serious review in the year to come.

Management

Significant changes in management structure and technique will certainly take place in the two or three years ahead, but we have been very cautious about changes during this year of transition. Caution is demanded for more than the usual reasons of morale: most changes in management that *ought* to be made will cost more than current systems, but we are determined that administration shall set an example of cost-consciousness for the institution as a whole.

Francis Bowles, who has served effectively as head of the Department of Research Services, has relinquished that position in favor of an association with the Ecosystems Center, which provides him a deserved increase of opportunity to pursue his own research and technical interests. He has been replaced by Morton Maser, who adds this responsibility to his already major commitment in continuing education and in the operations of service laboratories. In recognition thereof, Maser has been named Assistant Director for Educational and Research Services, with a broad range of defined duties and responsibilities concerned with research support services, equipment space deployment, admissions, and the day-to-day operations of our formal courses. He will work closely with the Director, with the Instructors-in-Chief, and with the Committee on Instruction in an effort to bring an increase of order and predictability into what is an unnecessarily variable system of management.

All activities enumerated in earlier sections need and deserve published notice at a number of levels of specificity—from in-house posting of events schedules to journal articles and press releases. These are the purview of the Public Relations Department which, with minimal staff under the direction of Anne Camille Maher, has met the demand with honor. Among particularly useful ventures of the past year (quite aside from such public events as MBL Day) have been the appearance of a regular and timely calendar of scientific activities, and a new and greatly improved directory of personnel and programs.

Our able Controller, Edward Casey, and his competent staff continue to get things done accurately and on time, and to accomplish that miracle with a staff, space, and machinery that would be more appropriate to an institution half the size of the MBL. Some relief from the burden of work and responsibility will have to be provided for that department soon. In the meantime it is to be commended for its management of accounting and financial control functions. Among those are the duties of Joan Howard, MBL Grants and Contracts Administrator, who deals competently with a volume of government support for research and teaching that would, in better-endowed circumstances, call for an office full of people.

The Library, too, deserves commendation, for the self-effacing but highly professional style with which its services are managed and rendered to the entire community, again under circumstances of fiscal restraint. We will be announcing during the summer of 1979 a number of encouraging initiatives and gifts which, all together, will make easier the work of our Library staff and improve significantly the facilities and services offered to users.

Support

Details of Foundation, Corporate, and other support received during 1978 appear below in a separate listing. For the present it is sufficient to note that Foundation support, specifically, came to $719,142 in 1978, as compared with $763,699 in 1977. This is a good showing, considering that it was a year of management transition, requiring a certain temporary slowing of fund-raising activity. In fact gifts received early in calendar year 1979, and others in prospect, make it certain that the two-year totals for 1978 and 1979 will exceed by a large factor those obtained in any prior period of similar

length. This will, it is hoped, be the subject for considerable discussion and action beginning in the late summer of 1979. It is to be noted that the Annual Campaign yielded twice as much support in 1978 as in 1977.

Atlantic Richfield Foundation
Charles Ulrick & Josephine Bay Foundation
Charles E. Culpeper Foundation
Fred Harris Daniels Foundation, Inc.
Arthur Vining Davis Foundations
Henry L. and Grace Doherty Charitable Foundation
Eastern Associated Foundation
Exxon Corporation
Foundation for Microbiology
Walter Henry Freygang Foundation
Friendship Fund
General Electric Foundation
Gillette Charitable & Educational Foundation
Grace Foundation, Inc.
Grass Foundation
Lillia Babbitt Hyde Foundation
IBM Corporation
Edward Bangs Kelley & Elza Kelley Foundation
Henry P. Kendall Foundation
Charles A. King Trust
Josiah Macy, Jr. Foundation
NL Industries
Jessie Smith Noyes Foundation
Pfizer, Inc.
Rockefeller Foundation
Rowland Foundation, Inc.
Sandoz Foundation
Alfred P. Sloan Foundation
Seth Sprague Educational & Charitable Foundation
Surdna Foundation
UPS Foundation

Conclusion

I take the liberty, in closing, of making a personal comment. I hope to be forgiven for it, because the comment is in effect a message to Trustees, but one that ought to be proffered in print.

I have now had a sufficient opportunity to study the MBL and its operations to have some confidence in a judgment of its health. I find the Laboratory in a state of flourishing health; this by comparison with other academic and research institutions with which I have been, and in some cases remain, associated. By health I refer to the *entire* organism; its history, its personality, its prospects, and its physical body. Parts of the body need attention, to be sure, and some growth of the parts is urgent; but that can and will be done. There is every reason to believe that the unique and influential history of the Laboratory is predecessor to a future just as unique and of just as great an influence.

To play a role in fashioning that future, and in solving the important problems that must be solved, is a privilege. To be sure the privilege has to be paid for; the work is as taxing, physically and psychologically, as any I have ever done, and despite every

effort to the contrary I have had to slow somewhat, for a year or so, my efforts in research that I care about a great deal. I am certain now, however, of what I could not have been certain in July of 1978, when I arrived for full-time residence in Woods Hole: the job can be done. It is as important as any job it has ever been my good fortune to work at.

1. The Staff

MARINE ECOLOGY

I. Instructors

John M. Teal, Woods Hole Oceanographic Institution, co-director of course
Ivan Valiela, Boston University Marine Program, co-director of course
Charlene Van Raalte, Hampshire College

II. Assistants

Mike Petrovski, Boston University Marine Program
Christopher Van Raalte, Dalhousie University

III. Special Lecturers

Arne Jensen, University of Aarhus
Thomas Fenchel, University of Aarhus
Bruce J. Peterson, Ecosystems Center
John E. Hobbie, Ecosystems Center
James W. Porter, University of Georgia
Karen G. Porter, University of Georgia
Lauren Haury, Woods Hole Oceanographic Institution
Robert Paine, University of Washington
Joy Geiselman, Woods Hole Oceanographic Institution
Wendy Wiltse, Woods Hole Oceanographic Institution
Donald Rhoads, Yale University
Thomas Jordan, Boston University Marine Program
Richard Haedrich, Woods Hole Oceanographic Institution
John Steele, Woods Hole Oceanographic Institution
Susan Peterson, Woods Hole Oceanographic Institution
John Mason, Woods Hole Oceanographic Institution
William Odum, University of Virginia
Howard Sanders, Woods Hole Oceanographic Institution
C. A. S. Hall, Cornell University

IV. Lectures

I. Valiela	Introduction to course
J. Teal	Introduction to the oceans
C. Van Raalte	Marine ecology and Woods Hole
A. Jensen	Salt marsh vegetation in Denmark
I. Valiela	Structure and function of salt marsh ecosystems
A. Jensen	Phenology and growth of *Halimione*
B. Peterson	Techniques and results of phytoplankton production studies
B. Peterson	The role of plankton in the global carbon cycle
I. Valiela	Nutrient budget of a salt marsh

T. Fenchel	Microbial processes in estuarine sediments I
J. Hobbie	Bacteria in aquatic systems I
J. Hobbie	Bacteria in aquatic systems II
J. Porter	Life in three dimensions: open ocean *vs.* terrestrial ecosystems
J. Porter	Life in two dimensions: substrate-bounded marine ecosystems
J. Porter	The ecology of marine invertebrate-algal symbioses
K. Porter	Grazing and planktonic community structure
L. Haury	Patchiness in Cape Cod Bay
R. Paine	Plant-herbivore relationship in marine systems
R. Paine	Disturbance and diversity in the intertidal
J. Geiselman	Chemical defenses in marine algae
R. Paine	Higher order carnivores
W. Wiltse	Effects of the predatory snail, *Polinices*, on community structure
T. Fenchel	Microbial processes in estuarine sediments II
C. Van Raalte	Nitrogen fixation in salt marshes
D. Rhoads	Infaunal deposit-feeding: climax mud communities
D. Rhoads	Bioturbation and sediment binding
T. Jordan	Nitrogen budget of mussels
R. Haedrich	The nature of deep sea fishes
J. Steele	Problems with the management of marine resources
S. Peterson	Changing values in uses of salt marshes
I. Valiela	Experimental studies of salt marsh pollution
J. Mason	Distribution and behavior of Atlantic blue fin tuna
W. Odum	The ecology of mangroves
W. Odum	Detritus feeding in coastal ecosystems
W. Odum	Venice: a case study
H. Sanders	The West Falmouth oil spill
C. A. S. Hall	Environmental impact of coastal zone energy facilities
C. A. S. Hall	Models and decision making: Hudson River

EMBRYOLOGY

I. Instructors

Joan V. Ruderman, Harvard University, co-director
Tom Humphreys, University of Hawaii, co-director
Susan V. Bryant, University of California, Irvine
Gary L. Freeman, University of Texas
Cyrus Levinthal, Columbia University
Eduardo R. Macagno, Columbia University

II. Consultants

R. K. Hunt, Johns Hopkins University
J. P. Trinkaus, Yale University
J. R. Whittaker, Wistar Institute

III. Assistants

Clarissa Cheney, University of Pennsylvania
Nigel Holder, University of California, Irvine
Marc Laufer, University of Pennsylvania

IV. Lectures

G. L. Freeman	Localization and the specification of form in development
G. L. Freeman	Localization of embryonic determinates in *Cerebratulus* eggs and early embryos
N. H. Verdouk	The origin of spatial organization in Molluscan development
M. R. Dohmen	The relationship between local surface differentiation and cytoplasmic localization
J. R. Whittaker	Localization and development in Ascidians eggs and embryos: I and II
L. Wolpert	Pattern formation in chick limb development
J. P. Trinkaus	Meroblastic development and gastrulation in *Fundulus* embryos
J. P. Trinkaus	Mechanisms of cell movement *in vivo* during gastrulation
J. V. Ruderman	Maternal mRNA and localization
T. Humphreys	The marine sponge, *Microciona prolifera* and other aggregating cell systems
T. Humphreys	The molecular organization of *M. prolifera* aggregation factor complex and its active fragments
Lan Bo Chen	Studies on intercellular fibronectin matrices
R. K. Hunt	Specification of positional information in amphibian eye development
R. K. Hunt	Positional information in neural development
C. Levinthal	Three dimensional computer reconstruction of neural anatomy
E. R. Macagno	Development of synaptic connections in invertebrate visual systems
C. Levinthal	Development and characterization of identified cells in isogenic fish
S. Bryant	Pattern regulation in amphibian limb regeneration: I and II
L. Jaffee	Activation and patterning of animal eggs and oocytes by steady ion currents
S. Inoué	Mitosis: Cell structural organization and mechanisms
N. deTerra	Control of cell division and morphogenesis in *Stentor*
N. B. Gilula	Gap junctions and communication between cells
N. B. Gilula	Communication in development and differentiation
P. Bryant	Pattern formation in the imaginal disks of *Drosophila*
J. Saunders	Positional signaling in chick limb bud development
N. Holder	Programmed development of the chick wing
L. Iten	Pattern regulation in chick limbs
S. Kaufman	Control of sequential commitment in *Drosophila* development
K. Kalthoff	Analysis of an anterior morphogenetic determinant in the egg of an insect, *Smitta sp.*, Chironimidae
N. LeDouarin	Migration and differentiation of neural crest cells studied in avian embryos with interspecific chimeras
J. Rosenbaum	Control of flagellar protein synthesis in Chlamydomonas
R. Sager	Somatic cell genetics: Methodology
R. Sager	Somatic cell genetics: Application to some fundamental problems in biology
G. Stent	Neuroembryology of the leech

Y. Peng Loh	Corticotropin and endorphin peptides. Biosynthesis and their role as intercellular messengers
N. Marcus	Normal and aberrant phenotypes in the developing sea urchin
P. Grant, S. Sharma, C. Levinthal, and R. K. Hunt	New looks at retinal-tectal connections, a microsymposium

NEURAL SYSTEMS AND BEHAVIOR

I. Instructors

Alan Gelperin, Princeton University, director of course
James L. Gould, Princeton University
Adrianus Kalmijn, Woods Hole Oceanographic Institution
Ronald Hoy, Cornell University
David J. Prior, University of Kentucky
William Kristan, Jr., University of California, San Diego
Randolf Menzel, Free University, Berlin
Fernando Nottebohm, Rockefeller University

II. Assistants

Christina Myles, Manchester Community College
S. Reingold, Princeton University

III. Visiting Lecturer

J. Nicholls, Stanford University

IV. Special Lecturers

George Gerstein, University of Pennsylvania
Robert Capranica, Cornell University
William G. Quinn, Princeton University
Eric R. Kandel, Columbia University
Larry Cohen, Yale University
Tom Eisner, Cornell University
Donald Griffin, Rockefeller University
Michael V. L. Bennett, Albert Einstein University
Eduardo Macagno, Columbia University
Gunther Stent, University of California, Berkeley

V. Lectures

J. Gould	Introduction to classical ethology
J. Gould	Neuroethology of *E. coli*
J. Gould	Communication in honey bees
J. Gould	Orientation and navigation in honey bees
A. Kalmijn	Electroreception in fish
A. Kalmijn	Active and passive electro-orientation
A. Kalmijn	Physics and physiology of electroreception
A. Kalmijn	Magnetic orientation
R. Hoy	Genetics and neurobiology of cricket song

R. Hoy	Acoustic interneurons
R. Hoy	Specificity and mechanisms of neural regeneration
D. Prior	Temperature acclimation in neural systems
D. Prior	Behavioral switching
D. Prior	Environmental modulation of reflex pathways
J. Nicholls	The leech: Sensory and motor neurons
J. Nicholls	The leech: After-effects of activity
J. Nicholls	The leech: Chemical and electrical transmission and quantal analysis
B. Kristan	Pattern generation
B. Kristan	Leech heartbeat control system
B. Kristan	Leech swimming I
B. Kristan	Leech swimming II
A. Gelperin	Neuroethology of molluscan learning
A. Gelperin	Serotonin and synaptic modulation
A. Gelperin	Comparative physiology of feeding
R. Menzel	Color vision in invertebrates
R. Menzel	Wavelength selective behavior
R. Menzel	Physiology of insect learning
T. Eisner	Herbivore-plant interactions II
F. Nottebohm	Selective learning
F. Nottebohm	Evolution of vocal learning
F. Nottebohm	Origins and mechanisms of hemispheric dominance
F. Nottebohm	Birdsong, a neuroethological model

EXPERIMENTAL MARINE BOTANY

(Comparative Biology and Biochemistry of Algae)

I. Instructors

Jerome A. Schiff, Brandeis University, director of course
James Fiore, Suffolk University, laboratory instructor
Harvard Lyman, State University of New York at Stony Brook, laboratory instructor
David Mauzerall, Rockefeller University
Robert Troxler, Boston University

II. Consultants

Robert L. Guillard, Woods Hole Oceanographic Institution
Frank A. Loewus, Washington State University
Ralph S. Quatrano, Oregon State University

III. Staff Associates

Seymour Cohen, State University of New York at Stony Brook
Catherine Fussel, Pennsylvania State University
Hans Gaffron, Woods Hole, Massachusetts and Sanibel, Florida
Andrew Holowinsky, Brown University

IV. Assistants

Joanne Luebbert, State University of New York at Stony Brook
Scott Schatz, University of Rhode Island
Sharon Subyak, Suffolk University

V. Special Lecturers

Vernon Ahmadjian, Clark University
Mary M. Allen, Wellesley College
Martha Berliner, Simmons College
Annette Coleman, Brown University
Franklin Fong, Texas A & M University
Jane Gibson, Cornell University
Robert L. Guillard, Woods Hole Oceanographic Institution
Peter Hepler, University of Massachusetts
Peter Heywood, Brown University
Lionel Jaffe, Purdue University
Alfred Loeblich, Harvard University
Lynn Margulis, Boston University
Eytana Padan, Hadassah Medical School
Carl Price, Rutgers University
Joel Rosenbaum, Yale University
Wolfhardt Rüdiger, University of Munich
Gregory Schmidt, Rockefeller University
James R. Sears, Southeastern Massachusetts University
Raymond E. Stephens, Marine Biological Laboratory
Arthur I. Stern, University of Massachusetts
Stan Watson, Woods Hole Oceanographic Institution
Robert Wilce, University of Massachusetts
C. L. F. Woodcock, University of Massachusetts

VI. Lectures

J. A. Schiff	Chemical Phase of Evolution; biogeochemistry
J. A. Schiff	Appearance of Oxygen
J. A. Schiff	Evolution of Procaryotes
J. A. Schiff	Evolution of Eucaryotes & Organelles
J. A. Schiff	Evolution of Life Cycles
J. A. Schiff	Nutritional Cycles
J. A. Schiff	Metabolism of Nitrogen and Sulfur
L. Margulis	Microbial Evolution
C. L. F. Woodcock	Biology of *Acetabularia*
R. Troxler	Biosynthesis of Heme and Chlorophyll, Early Stages
R. Troxler	Biosynthesis of Heme and Chlorophyll, Later Stages
R. Troxler	Open Chain Tetrapyrroles
D. Mauzerall	Photochemical Principles
D. Mauzerall	Photochemistry of Photosynthesis
J. Gibson	Photosynthetic Electron Transport
A. Stern	Photophosphorylation
L. Jaffe	Development of Polarity
J. Rosenbaum	Control of Flagellar Development in *Chlamydomonas*
R. E. Stephens	Microtubules in Plant Development
A. Holowinsky	Wall Synthesis, Deformation and Morphogenesis in Algae
F. Fong	Phototaxis in *Euglena*
J. Sears	Vertical Distribution of Algae
R. Wilce	Phytogeographic Studies in the North Atlantic
J. Fiore	Life Histories of the Brown Algae
P. Hepler	Stomatal Mechanisms
M. M. Allen	Biology of Blue Green Algae (Cyanobacteria) I

S. Cohen	Polyamines
J. A. Schiff	Chloroplast Development I
H. Lyman	Chloroplast Development II and III
A. Holowinsky	Circadian Rhythms
H. Lyman	Quantitative Measurement of Cell Constituents
H. Lyman	Pigment Methodology
H. Lyman	Xanthophyta, Chrysophyta, Bacillariophyta
A. Loeblich	Unique Cytology and Molecular Biology of Dinoflagellates
J. A. Schiff H. Lyman	Euglenophyta and Cryptophyta
P. Heywood	Chloromonads
H. Lyman	Chlorophyta I: Unicells
J. A. Schiff	Development of Single Cells from *Prasiola*
A. Coleman	Chlorophyta II: Biology of the Colonial Green Flagellates
J. Fiore	Field and Preservation Methods
R. Guillard	Phytoplankton Ecology I and Ecology II
J. Fiore	Chlorophyta III, Chlorophyta IV and Chlorophyta V
C. Price	Principles Affecting the Isolation of Cells and Subcellular Structures
J. Fiore	Pheophyta I, II, and III
J. Fiore	Rhodophyta I, II and III
J. Fiore	Distribution of Eastern Macroalgae
A. Holowinsky	Phytochrome-Controlled Responses
Stan Watson	Procaryotic Membranes
M. M. Allen	Biology of Blue Green Algae (Cyanobacteria) II
S. Cohen	Cyanophages
G. Schmidt	*In Vitro* Synthesis and Assembly of Chloroplast Proteins
V. Ahmadjian	Experimental Studies of the Lichen Symbiosis
M. Berliner	Protoplasts of Unicellular Green Algae
H. Lyman	Algal Symbioses
E. Padan	Facultative anoxygenic photosynthesis in blue-green algae
W. Rüdiger	Studies of phytochrome and chlorophyll synthesis

NEUROBIOLOGY

I. Instructors

Edward A. Kravitz, Harvard Medical School, director of course
Jonathan B. Cohen, Harvard Medical School
Edward J. Furshpan, Harvard Medical School
David D. Potter, Harvard Medical School
John G. Hildebrand, Harvard Medical School
Gerald D. Fischbach, Harvard Medical School
Peter R. MacLeish, Harvard Medical School
Story C. Landis, Harvard Medical School
John Heuser, University of California, San Francisco Medical School
Paul O'Lague, University of California, Los Angeles
Thomas S. Reese, National Institutes of Health

II. Staff Associates

S. Matsumoto, Harvard Medical School
B. Battelle, National Institutes of Health
R. Harris-Warrick, Harvard Medical School
M. Nelson, Harvard Medical School

S. Glusman, Harvard Medical School
D. Landis, Harvard Medical School
M. Rheuben, Penn State University

III. Assistants

Susan Huttner, University of California, Los Angeles
J. LaFratta, Harvard Medical School
Ruth Siegel, Harvard Medical School
R. Neubig, Harvard Medical School
B. Reese, National Institutes of Health

IV. Symposia and Seminars

Symposium I Slow Physiological Events

S. W. Kuffler, Harvard Medical School
R. W. Tsien, Yale University
E. R. Kandel, Columbia University

Symposium II Selectivity

D. Purves, Washington University
B. G. Wallace, Stanford University
U. J. McMahan, Stanford University
J. Diamond, McMaster University

Special Saturday Seminars

C. Armstrong, University of Pennsylvania
T. N. Wiesel, Harvard Medical School
R. Rahamimoff, Hebrew University, Israel

Regular Seminar Series

D. D. Potter, Harvard Medical School
J. Brown, State University of New York at Stony Brook
A. E. Stuart, Harvard Medical School
T. S. Reese, National Institutes of Health
R. Llinas, New York University
D. Goodenough, Harvard Medical School
L. Jan, Y. N. Jan, Harvard Medical School
E. Macagno, Columbia University
M. Guthrie, Commission to Combat Huntington's Disease
R. Murphy, State University of New York Albany
J. Cartaud, Paris
H. Pollard, National Institutes of Health
R. Hoy, Cornell University
T. Seeley, Harvard University
J. Kirz, State University of New York at Stony Brook

V. Lectures

J. Heuser	Structural features of nervous systems
S. Landis	Presynaptic structure

S. Landis	Postsynaptic structure
T. Reese	Growth cones and synaptic development
G. Fischbach	Myogenesis and development of chemosensitivity
G. Fischbach	Neurogenesis (neural tube) and development of electrical excitability
N. LeDourain	The neural crest
P. MacLeish	Synapse formation: I. Early stages of transmitter release
G. Fischbach	Synapse formation: II. Organization of the postsynaptic membrane
J. Cohen	Subcellular fractionation of Torpedo electric organ
R. Neubig	Identification of neurotransmitter receptors by ligand binding
J. Cohen	Biochemical characterization of the nicotinic cholinergic receptor
J. Cartaud	Morphological studies of nicotinic cholinergic receptors and acetylcholinesterase
J. Cohen	Neurotransmitter receptors and their responses, an overview
J. Hildebrand	Introduction. Acetylcholine
J. Hildebrand	Catecholamines
E. Kravitz	GABA
M. Nelson	Neuroethology
E. Kravitz	Amines and Modulation
B. Battelle	Cyclic AMP
E. Kravitz	Peptides

PHYSIOLOGY

I. Instructors

K. E. Van Holde, Oregon State University, director of course
Mark Mooseker, Yale University
Ray Stephens, Marine Biological Laboratory
Jay Brown, University of Virginia
John Wooley, Princeton University
Tom Baldwin, University of Illinois
F. Dahlquist, University of Oregon
Susan Astrin, Institute of Cancer Research

II. Consultants

Ruth Sager, Sidney Farber Institute for Cancer Research
Alex Rich, Massachusetts Institute of Technology

III. Staff Associates

Al Christopher, University of Chicago
Miriam Baldwin, University of Illinois
W. Dentler, University of Kansas
Jerre Summers, Cancer Research Institute
K. Tatchell, Oregon State University

IV. Assistants

Pat Galvin, University of Denver
David VonHippel, University of Oregon

V. Special Lecturers

Phil Bassford, Harvard Medical School
Su Chung, Harvard University
Noel deTerra, Haneman Hospital
H. Eisenberg, Weizmann Institute, Israel
Sarah Elgin, Harvard University
R. Gennis, University of Illinois
N. B. Gilula, The Rockefeller University
Alfred Goldberg, Harvard Medical School
Steve Harrison, Harvard University
Rhu Chi Huang, John Hopkins University
S. Inoué, University of Pennsylvania
I. Isenberg, Oregon State University
Robert Jackson, The Rockefeller University
Nicole Le Douarin, Marine Biological Laboratory
Allan Maxam, Harvard University
T. Pollard, Johns Hopkins University
Hans Ris, University of Wisconsin
Joel Rosenbaum, Yale University
Peter Setlow, University of Connecticut Health Center
Albert Szent-Györgi, Marine Biological Laboratory
Andrew Szent-Györgi, Brandeis University
L. Tilney, University of Pennsylvania
Anna Marie Weber, University of Pennsylvania

VI. Lectures

K. E. Van Holde	Introduction to cromatin structure: Evidence from nuclease digestion and hydrodynamic studies
J. Wooley	Introduction to chromatin structure: evidence from electron microscopy and scattering studies
T. Baldwin	Bioluminescence and bacterial luciferase
Andrew Szent-Györgi	Introduction to contractile proteins
A. M. Weber	Properties of the actin filament
M. Mooseker	The control of actomyosin-mediated motility in non-muscle cells
L. Tilney	The role of actin in non-muscle motility
M. Mooseker	Actin-membrane association
R. Stephens	Microtubules, I and II
A. Maxam	Nucleic acid chemistry and DNA sequences
W. Dentler	Microtubule membrane interaction in cilia and flagella
K. E. Van Holde	DNA-protein interactions in the nucleosome
S. Chung	Characterization of the histone core complex
J. Wooley	Molecular architecture of the nucleosone
S. Elgin	Chromosomal structure and function in *Drosophila*
I. Isenberg	Histone-histone interaction
H. Ris	Higher order structure in chromosomes
J. Brown	The fluid mosaic model of membrane structures
R. Gennis	*E. coli* pyruvate oxidase—studies on lipid-protein interactions
T. Wegmann	Structure and function of histocompatability antigen on a membrane protein

P. Bassford	Transport and secretion in gram negative bacteria
R. Dahlquist	Lipid-protein interactions
R. Dahlquist	Bacterial chemotaxis, a model sensing system
S. Inoué	Mitosis cell structural organization and mechanisms
N. deTerra	Control of cell division and morphogenesis in *Stentor*
N. B. Gilula	Gap junctions and communication between cells
N. B. Gilula	Communication in development and differentiation
H. Eisenberg	Protein-DNA interactions
S. Astrin	Control of transcription in eukaryots
S. Astrin	Control of endogenous viral gene expression in chick embryos
J. Brown	Protein components of the L-cell plasma membrane
A. Goldberg	Studies on the mechanism of protein breakdown in animal and bacterial cells
T. Baldwin	Role of proteases in controlling enzyme levels
R. Jackson	Signal peptidase and its role in the transfer of polypeptides across the RER membranes
P. Setlow	Protein regulation during bacterial spore germination
R. C. Huang	Initiation of RNA synthesis *in vitro*
N. LeDouarin	Migration and differentiation of neural crest cells studied in avian embryos with interspecific chimeras
J. Rosenbaum	Control of flagellar protein synthesis in chimeras
R. Sager	Somatic cell genetics I. methodology
R. Sager	Somatic cell genetics II. Application to some fundamental problems in biology
S. Harrison	Protein flexibility and macromolecular assembly: virus structures at high resolution
A. Rich	Molecular structure and biological function of t-RNA
Albert Szent-Györgi	The living state

RESEARCH PROGRAM IN MICROBIAL ECOLOGY

I. Instructors

Jane A. Gibson, Cornell University
Robert E. Hungate, University of California, Davis
Holger W. Jannasch, Woods Hole Oceanographic Institution
Alex Keynan, The Hebrew University, Jerusalem
Edward R. Leadbetter, University of Connecticut, Storrs
Jeanne S. Poindexter, Public Health Research Institute, New York

II. Consultants

Harlyn O. Halvorson, Brandeis University
J. Woodland Hastings, Harvard University
Roger Y. Stanier, Institute Pasteur, Paris
Edward O. Wilson, Harvard University

III. Staff Associates

Russel L. Cuhel, Woods Hole Oceanographic Institution
Mark A. Schneider, Amherst College
Craig D. Taylor, Woods Hole Oceanographic Institution

IV. Assistant

Stephen J. Giovannoni, Boston University

V. Special Lecturers

Richard P. Blakemore, University of New Hampshire, Durham
Alasdair Cook, Cornell University
Bernard D. Davis, Harvard University
Arnold L. Demain, Massachusetts Institute of Technology
Ian Dundas, University of Bergen, Norway
John W. Farrington, Woods Hole Oceanographic Institution
Tom Fenchel, University of Aarhus, Denmark
James G. Ferry, Virginia Polytechnical Institute, Blacksburg
Joel C. Goldman, Woods Hole Oceanographic Institution
Everett P. Greenberg, Harvard University
Harlyn O. Halvorson, Brandeis University
J. Woodland Hastings, Harvard University
Etana Padan, The Hebrew University, Jerusalem
Antonio H. Romano, University of Connecticut, Storrs
E. G. Ruby, Harvard University
Ivan Valiela, Marine Biological Laboratory
John B. Waterbury, Woods Hole Oceanographic Institution

VI. Lectures

H. W. Jannasch	Introduction to microbial ecology I and II
H. W. Jannasch	Continuous culture of microorganisms
H. W. Jannasch	Continuous culture in microbial ecology
H. W. Jannasch	Practice of the chemostat
H. W. Jannasch	Experiments in deep sea microbiology
J. A. Gibson	The photosynthetic bacteria I and II
J. A. Gibson	Uptake measurements in microbial ecology
J. A. Gibson	Nutrient uptake in cyanobacteria
J. A. Gibson	Survival of prokaryotes: envelope changes
E. R. Leadbetter	Ways of making a living: anaerobes
E. R. Leadbetter	Ways of making a living: aerobes
E. R. Leadbetter	Microbial attack on hydrocarbons
E. R. Leadbetter	Survival of procaryotes: ecological aspects
E. R. Leadbetter	Microbiology of the tooth surface
R. E. Hungate	Analysis of a microbial ecosystem
R. E. Hungate	Cultivation of anaerobes
R. E. Hungate	The rumen as an ecosystem I and II
J. S. Poindexter	The bacterial prostheca: occurrence, structure and possible function
J. S. Poindexter	The bacterial prostheca: developmental studies
J. S. Poindexter	Survival of prokaryotes: the vegetative cell
A. Keynan	Introduction to the light emitting bacteria II
A. Keynan	Experimentation with bacterial bioluminescence
A. Keynan	Survival of prokaryotes: the endospore
C. D. Taylor	The biology of methane formation
C. D. Taylor	The ecology of methane formation
C. D. Taylor	The effect of pressure on bacterial growth
R. L. Cuhel	Psychrophilic bacteria

R. P. Blakemore	Magnetotactic bacteria
B. D. Davis	Mechanism of protein secretion across membranes
A. Cook	Microbial degradation of pesticides
A. L. Demain	Do antibiotics have a function in natural populations of microorganisms?
I. Dundas	Halophilic bacteria
J. W. Farrington	Biochemistry of methane in oceanic environments
T. Fenchel	Microbial transformations in sediment I and II
J. G. Ferry	The biology of sulfate reduction
J. G. Ferry	Sulfate reduction in marshes and estuaries
J. C. Goldman	Continuous culture of algae
E. P. Greenberg	The Spirochaetes
H. O. Halvorson	Survival of prokaryotes: molecular aspects
J. W. Hastings	Introduction to the light emitting bacteria I
E. Padan	Anoxic photosynthesis in cyanobacteria
A. H. Romano	The biology of Sphaerotilus
E. G. Ruby	Taxonomy and distribution of luminous bacteria
I. Valiela	The Sippewisset marsh
J. B. Waterbury	Marine cyanobacteria

YEAR-ROUND PROGRAMS

1978

BOSTON UNIVERSITY MARINE PROGRAM

The Staff

Arthur Humes, Boston University Marine Program, director
Jelle Atema, Boston University Marine Program
Björn Ganning, University of Stockholm, Sweden
Stjepko Golubic, Boston University
C. K. Govind, University of Toronto
Frederick Lang, Boston University Marine Program
Charles Lent, Brown University
Ivan Valiela, Boston University

THE ECOSYSTEMS CENTER

George M. Woodwell, director

John E. Hobbie, senior scientist; Daniel B. Botkin, associate scientist; Francis P. Bowles, Jerry M. Melillo, Bruce J. Peterson, assistant scientists; Richard H. Burroughs, senior fellow; John T. Finn, postdoctoral fellow; Erene V. Pecan, assistant to the director and research associate; Mary Louise Montgomery, assistant to the director; Richard A. Houghton, Paul A. Steudler, research associates; William J. Behrens, Joanne Clark, Colleen M. Cavanaugh, Keith N. Eshleman, John V. Helfrich, Voytek Kijowski, Frederic Lipschultz, Katherine C. Parsons, David S. Schimel, Jeffrey B. Shelkey, Hedy Sladovich, research assistants; Paul Detwiler, graduate research fellow; James T. Morris, William B. Bowden, James P. Reed, graduate research assistants; Michelle Dionne, graduate assistant; Gregg Dionne, Lawrence Hobbie, Ann Lewandowski, Andrea R. Turner, laboratory assistants; Mark White, technician; Joan M. Upton, secretary; Nancy L. Campbell, Jeanne Ferrari, Frances A. Seymore, typists.

JANUARY COURSES 1978

BEHAVIOR

(Offered Jointly by Boston University Marine Program and the Marine Biological Laboratory)

I. Instructors

Jelle Atema, Boston University, director of course
Adrianus Kalmijn, Woods Hole Oceanographic Institute
Terry Crow, Marine Biological Laboratory
Tony Swain, Boston University
Vincent Dethier, University of Massachusetts
Robert Barlow, Syracuse University
Margaret Nelson, Harvard University
Behrus Jahan-Parwar, The Worcester Institute
Izja Lederhendler, Marine Biological Laboratory
Daniel Stenzler, Boston University
Lehr Brisbin, University of Georgia
John Palmer, University of Massachusetts
Mel Kreithen, Cornell University
Elizabeth Russell, The Jackson Laboratory
Timothy Williams, Swarthmore College
Douglas S. Riggs, Hampshire College
William Watkins, Woods Hole Oceanographic Institute
Kathy Payne, The Rockefeller University
Bernt Würsig, State University of New York at Stony Brook
Jane Frick, Woods Hole, Massachusetts
Dietland Müller-Schwarze, State University of New York College of Environmental Science and Forestry
Arthur Silverstein, Johns Hopkins School of Medicine
Emil Menzel, State University of New York at Stony Brook
Bori Olla, National Marine Fisheries Service
Alastair Stuart, University of Massachusetts at Amherst
George Michel, Boston University
Francis Bowles, Marine Biological Laboratory
Stuart MacKay, Boston University
Daniel Alkon, Marine Biological Laboratory

II. Lectures

J. Atema	Introduction
J. Atema	Sensory physiology and behavior
J. Atema	Chemo- and mechanoreceptors
A. Kalmijn	Electroreception with and without electric organs
J. Atema	Evolution of chemical senses
A. Kalmijn	Electroreception: the detection of inanimate electric fields
A. Kalmijn	The physics and physiology of electroreception
T. Crow	Statistical considerations in the design of behavioral experiments
A. Kalmijn	Electroreception: more questions with or without answers
V. Dethier	Insect taste: Food discrimination in plant-feeding insects
T. Swain	Chemical compounds in plants affecting insect herbivores
V. Dethier	What chemosensory neurons tell the brain

R. Barlow	The eye and the brain
R. Barlow	Vision and *Limulus*: a multidisciplinary analysis
M. Nelson	Introduction to neural control of behavior
M. Nelson	Hissing and social behavior in cockroaches
B. Jahan-Parwar	Chemosensory behavior in the seahare, *Aplysia*
I. Lederhendler	Social behavior in *Aplysia*
D. Stenzler	The burial alarm response and olfaction in the mud snail
L. Brisbin	Ecology, domestication and the behavior of dogs: some principles and their application to present-day problems of men, wolves, and dogs
L. Brisbin	The domestic dog today: Training and tracking
J. Atema	Catfish social behavior data analysis
J. Palmer	Introduction to biological rhythms
J. Palmer	Biological rhythms in shore dwelling animals
J. Palmer	Human rhythms
M. Kreithen	Introduction to bird migration
M. Kreithen	Orientation and infra-sound
T. Williams	Pleiotropism and the nature of the W-Locus in the mouse
D. Riggs	Selected topics in biomathematics
W. Watkins	Sperm whale acoustic behavior
K. Payne	Annual change in songs of Humpback whales
B. Würsig	Group composition and stability of coastal bottlenose porpoises
D. Smith	Introduction to social behavior in birds
D. Smith	Acoustic and visual signals in redwing blackbirds
J. Frick	Migration of green turtles
D. Müller-Schwarze	Introduction to pheromones
D. Müller-Schwarze	Mammalian pheromones
D. Müller-Schwarze	Predator-prey relations in Antarctic bird communities
D. Müller-Schwarze	Ecology of the reindeer culture
A. Silverstein	The generation of immunologic diversity: Phylogenists *vs.* Ontogenists
E. Menzel	Social organization in Chimpanzees
E. Menzel	Social organization in Macaques
E. Menzel	Primate intelligence
B. Olla	Social behavior and rhythms in marine fishes as related to environmental factors
A. Stuart	Social behavior of insects
A. Stuart	Termite communication and social behavior
J. Atema	Social behavior and pheromones in lobsters
G. Michel	The European Ethologists as seen by an American Psychologist
J. Atema	Behavior assays in pollution research
F. Bowles	Territories and social behavior of lobster fishermen
S. MacKay	Bio-medical telemetry in behavioral studies
D. Alkon	Associative training in a nudibranch mollusc *Hermissenda*
D. Alkon	Neural substrates of associative training in *Hermissenda*

DEVELOPMENTAL BIOLOGY

I. Instructors

Walter S. Vincent, University of Delaware, director of course
Nancy H. Marcus, Woods Hole Oceanographic Institution

James D. Ebert, Marine Biological Laboratory
Kenneth T. Edds, Marine Biological Laboratory
Susan Gerbi, Brown University
Shinya Inoué, University of Pennsylvania
William Massover, Brown University
Richard Miller, Temple University
Raymond E. Stephens, Marine Biological Laboratory

II. Assistant

David Moss, University of Delaware

III. Special Lecturers

Eugene Bell, Massachusetts Institute of Technology
David Francis, University of Delaware
Harlyn Halvorson, Brandeis University
Arthur Humes, Boston University Marine Program
Hans Lauffer, University of Connecticut
Dolores Schendel, Sloan-Kettering Institute
Richard Tasca, University of Delaware

IV. Lectures

W. S. Vincent	Introduction to course
W. S. Vincent	Gametogenesis I, II, and III
N. H. Marcus	Differentiation and development of sea urchins in laboratory culture I and II
W. Massover	Comparative oogenesis I
W. Massover	Maturation and ovulation I and II
W. Massover	Vitellogenesis
S. Inoué	Spicule development in sea urchins I and II
S. Inoué	Techniques of phase and polarizing microscopy
S. Inoué	Experimental analysis of spindle structure I
S. Inoué	Experimental analysis of mitosis
J. D. Ebert	Ionic regulation of embryonic induction, differentiation in growth
R. Miller	Gamete activation I and II
W. S. Vincent	Principles and practices of cell fractionation and gradient analysis I
R. Miller	Fertilization I and II
M. D. Maser	Techniques and demonstration of electron microscopy
K. T. Edds	Cleavage and control of cell divisions I and II
W. S. Vincent	Principals and practice of gradient analysis II
E. Russell	Plieotropism and the nature of the W-locus in mice
K. T. Edds	The role of microfilaments in cell division
W. S. Vincent	Processing of eucaryotic transcripts
W. S. Vincent	Sequence conservation in ribosomal RNA
S. Gerbi	DNA sequence organization on eucaryotic genomes I and II
H. Lauffer	Analysis of gene activity in *Chironomus* development
H. Lauffer	Specific message isolation and identification in *Chironomus*
J. Collier	Normal development of Spiralia

J. Collier	Experimental analysis of the development of spiralians
J. Collier	Molecular biology of spiralian development I and II
E. Anderson	Early embryogenesis in mammals
A. Silverstein	The generation of immunologic diversity: phylogenists *vs.* ontogenists
R. E. Stephens	Chemistry and structure of microtubules
R. E. Stephens	Morphogenesis of cilia
H. O. Halvorson	New techniques in approaching developmental problems
J. D. Ebert	Birth defects: Biological and ethical considerations
W. S. Vincent	The politics of the recombinant DNA controversy
N. H. Marcus	Phenotypic plasticity in marine invertebrates
Albert Szent-Györgyi	Protein, ascorbic acid and cancer

ECOLOGY

I. Instructors

George M. Woodwell, The Ecosystems Center, director of course
Daniel B. Botkin, The Ecosystems Center
John E. Hobbie, The Ecosystems Center
Jerry M. Melillo, The Ecosystems Center

II. Special Lecturers

S. H. Berwick, Yale
Kenneth O. Emery, Woods Hole Oceanographic Institution
T. Fenchel, University of Aarhus, Denmark
C. A. S. Hall, Cornell University
Holger W. Jannasch, Woods Hole Oceanographic Institution
Marilyn J. Jordan, The Ecosystems Center
Thomas E. Lovejoy, World Wildlife Fund
Bruce J. Peterson, The Ecosystems Center
G. Rowe, Woods Hole Oceanographic Institution
J. H. Ryther, Woods Hole Oceanographic Institution
Howard L. Sanders, Woods Hole Oceanographic Institution
Lawrence B. Slobodkin, State University of New York at Stony Brook
Frederick E. Smith, Harvard University
J. Steele, Woods Hole Oceanographic Institution
Ivan Valiela, Boston University

III. Lectures

G. M. Woodwell	Structure of the biosphere I and II
H. Sanders	Natural communities and evolutionary strategies I and II
L. Slobodkin	Evolution: The development of species
L. Slobodkin	Group selection: The development of communities
K. O. Emery	Geological factors: Continental drift
G. M. Woodwell	Climatic factors: The vegetation of the earth
G. Rowe	The oceans: Distribution of primary production
G. Rowe	The oceans: Secondary production
G. M. Woodwell	Primary production and the metabolism of the earth
G. M. Woodwell	The world carbon budget: The predominance of forests
J. E. Hobbie	The world carbon budget: The role of oceans
J. E. Hobbie	Primary productivity in aquatic systems I and II

C. A. S. Hall	Secondary productivity I and II
S. H. Berwick	Biogeography I and II
D. B. Botkin	Succession and stability I and II
G. M. Woodwell	Nutrients and communities I and II
H. W. Jannasch	The microbiology of S-transformations I and II
B. J. Peterson	Nutrient limitation in aquatic ecosystems
B. J. Peterson	Phosphorus cycle of the ocean
J. M. Melillo	Nutrient cycling in forested basins I and II
I. Valiela	Nutrient cycling in a salt marsh
G. M. Woodwell	Ecosystems, energy and world politics
T. E. Lovejoy	Endangered species
J. H. Ryther	Oceanic productivity
J. Steele	The North Sea fishery I and II
S. B. Peterson	Northwest Atlantic fishery I and II
K. O. Emery	Mineral resources of the deep sea and international politics
F. E. Smith	Urban ecology I and II
M. J. Jordan	Ecological effects of toxins
G. M. Woodwell	Biotic impoverishment and the threshold dilemma: A major challenge for science and government

NEUROBIOLOGY

(Offered jointly by Boston University Marine Program and the Marine Biological Laboratory)

I. Instructors

Alan Fein, Marine Biological Laboratory, director of course
Frederick Lang, Boston University Marine Program, director of course
Edward F. MacNichol, Jr., Marine Biological Laboratory, laboratory director

II. Special Lecturers

William J. Adelman, nih-nincds, Woods Hole
Daniel L. Alkon, nih-nincds, Woods Hole
Jelle Atema, Boston University Marine Program
Robert Barlow, Syracuse University
Thomas Carew, Columbia University
Melvin Cohen, Yale University
Vincent G. Dethier, University of Massachusetts
Frederick Dodge, Rockefeller University and IBM Watson Labs
Eric Frank, Harvard Medical School
Donald Frazier, University of Kentucky
Alfredo Gorio, The Rockefeller University
Rami Grossman, nih-nincds
Adrianus Kalmijn, Woods Hole Oceanographic Institution
Ehud Kaplan, Rockefeller University
Charles Lent, State University of New York at Stony Brook
Simon LeVay, Harvard Medical School
Douglas S. Riggs, Hampshire College
Elizabeth S. Russell, The Jackson Laboratory
Arthur Silverstein, Johns Hopkins University

III. Lectures

A. Fein	Cell permeability and the plasma membrane
S. LeVay	Microscopic anatomy of the nervous system
J. Atema	Evolution of chemical senses
M. Cohen	Evolution of neuron structure and function
A. Kalmijn	Physics and physiology of electroreception
A. Fein	The resting membrane potential
A. Fein	Cable properties of neurons
A. Fein	Action potentials
A. Fein	Saltatory conduction in myelinated axons
W. Adelman	Neuronal currents, channels, spaces and clefts
V. Dethier	What chemosensory neurons tell the brain
R. Barlow	The eye and the brain
R. Barlow	Vision in *Limulus*: a multidisciplinary analysis
F. Dodge	A horseshoe crab-eye view of Great Harbor
E. Kaplan	Receptor properties of *Limulus* lateral eye *in situ*
F. Lang	Synaptic transmission—The soups *vs.* the sparks
F. Lang	Mechanisms of transmitter release
F. Lang	Ionic basis of synaptic potentials
A. Fein	Role of intracellular Ca^{++} and Na^{++} in adaptation of *Limulus* photoreceptors
F. Lang	Quantal release
F. Lang	Excitation-secretion coupling
E. Russell	Pleiotropism and the nature of the W-locus in the mouse
F. Lang	Origin and fate of synaptic vesicles
F. Lang	Neurotrophic influences
A. Gorio	Intracellular recording of synaptic potentials
D. Riggs	Pleasures and pitfalls of biological modeling
A. Gorio	Acetylcholine compartments in the mouse diaphragm
A. Gorio	The mode of action of black widow spider venom
L. Marshall	Factors influencing reinnervation of skeletal muscle
D. Riggs	The steady-state behavior of biological feedback systems
E. Frank	Formation of nerve-muscle synapses in tissue culture
D. Riggs	Fitting straight lines when both X and Y are subject to error
F. Lang	Developmental neuroethology: Physiological basis for changes in fight and flight behavior during growth of the lobster
D. Frazier	Putative transmitters in vertebrate respiratory neurons
A. Silverstein	The generation of immunologic diversity: Phylogenists *vs.* ontogenists
E. Kravitz	Three neurohormones in the lobster: Studies on the cellular localization, release and physiological actions of octopamine, serotonin, and dopamine
C. Lent	Organizational and neural properties of leech CNS
R. Grossman	Conduction of action potentials along nonhomogenous axons
T. Carew	The utility of the marine mollusc *Aplysia* for the cellular analysis of behavior
C. Lent	Cellular studies of amine neurons in leech
D. Alkon	Associative training in a nudibranch mollusc, *Hermissenda*
D. Alkon	Neural substrates of associative training in *Hermissenda*

COMPARATIVE PATHOLOGY OF MARINE INVERTEBRATES

I. Instructors

Frederick B. Bang, Johns Hopkins University
Betsy Bang, Johns Hopkins University
Jack Levin, Johns Hopkins University
Robert Prendergast, Johns Hopkins University
C. Austin Farley, National Marine Fisheries Service at Oxford, Maryland
Kenneth Edds, Marine Biological Laboratory
Jack Marchalonis, Frederick Cancer Research Center
Carol Reinisch, Sydney Farber Cancer Research Institute in Boston

II. Lectures

F. B. Bang	Cellular clumping in seastars, hermit crabs, and clotting factors in *Carcinus*
J. Levin	Extracellular clotting
J. Levin	*Limulus* and endotoxin
J. Levin	Comparison of invertebrates and vertebrates
B. G. Bang	Evolution of mucociliary system
B. G. Bang	Sipunculus and urn cells
F. B. Bang	Comparative aspects of mucociliary systems
K. Edds	Inflammations of Invertebrates
C. A. Farley	Normal Anatomy of the Oyster
C. A. Farley	Pathology of the Oyster
C. A. Farley	Infectious Diseases in the Oyster
C. A. Farley	Tumors in Oysters
C. A. Farley	Virus Diseases in Oysters
F. B. Bang	Specific Bacteriological and Virus Diseases of crabs and lobsters
J. Pearce	Pathological pollution aspects of ecology
C. Reinisch	Tumors and regeneration
C. Reinisch	Recognition in invertebrates
R. Prendergast	Starfish factor—effects in vertebrates
F. B. Bang	Elements of immune response—Sipunculus, stars
R. Prendergast	Introduction to the vertebrate immune response
J. Marchalonis	Agglutinins and antibodies
J. Marchalonis	The evolution of the immune response

LABORATORY OF BIOPHYSICS, NINCDS–NIH

William J. Adelman, Jr., chief of the laboratory and head, section on neural membranes
Daniel L. Alkon, head, section on neural systems
Alan J. Hodge senior scientist; Jay Wells, research physiologist; David E. Goldman, guest worker; Terry Crow, staff fellow; Izia Lederhendler, Robert J. French, Mitsuo Tabata, visiting fellows; Jonathan Shoukimas, Thomas Jerussi, Leon Shiman, IPA fellows; Joseph Neary, biochemist; Richard Waltz, mathematician programmer; Clyde Tyndale, electronics specialist; Ruthanne Mueller, research technician; June Harrigan, mariculturist

LABORATORY OF SENSORY PHYSIOLOGY

Edward F. MacNichol, Jr., director
Alan Fein, associate scientist and deputy director; Ferenc I. Hárosi, associate

scientist; Ete Z. Szuts, assistant scientist; Barbara Ann Collins, senior research associate; D. Wesley Corson, postdoctoral fellow; Joseph Levine, graduate student, Harvard University; Kathleen French, graduate student, Boston University Marine Program; Steven L. Goodman, research assistant; Menachem Hanani, National Institutes of Health; Theodore P. Williams, Florida State University; Yvette Kunz Ramsay, University of Dublin; Leo E. Lipetz, Ohio State University; Paul Witkovsky, Guido Hassin, State University of New York at Stony Brook, visiting scientists

NATIONAL FOUNDATION FOR CANCER RESEARCH

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INDEPENDENT YEAR-ROUND PROGRAMS

Raymond Stephens, principal investigator
Melanie Pratt; Mary E. Porter

Shinya Inoué, principal investigator
Andrew Eisen; Mark B. Fienberg

Kenneth Edds, principal investigator
Norman R. Jarvis

Eric Ball, principal investigator

D. Eugene Copeland, principal investigator

Judith Grassle, principal investigator
Fern Birtwistle; Cynthia Lanyon-Duncan; Linda Philbin-Munson

Ruth D. Turner, principal investigator
Carl J. Berg; Gregory A. Tracey

Robert Rice, principal investigator
Priscilla Roslansky; Susan M. Houghton; Rebecca Lash

Eric Kandel, principal investigator
Carl J. Berg; Thomas Capo; Susan Perritt

Keith R. Porter, principal investigator
Randolph H. Byers; Mark McNiven

Lewis Tilney, principal investigator
Laurinda A. Jaffe

YEAR-IN-SCIENCE

1977–1978

The Year-in-Science program at the MBL is for advanced undergraduates and beginning graduate students. For undergraduates the program is equivalent to a university honors program; for beginning graduate students it is designed to accommodate those who require the staff and special facilities of the laboratory in support of their research. Students join staff of the MBL in a variety of studies, participate in courses at

the Woods Hole Oceanographic Institution, the Boston University Marine Program, and in regularly scheduled seminars with staff of The Ecosystems Center and others at the MBL.

THE STAFF

GEORGE M. WOODWELL, director, The Ecosystems Center
DANIEL B. BOTKIN, associate scientist
FRANCIS P. BOWLES, assistant scientist
JOHN E. HOBBIE, senior scientist
JERRY M. MELILLO, assistant scientist
BRUCE J. PETERSON, assistant scientist

THE LABORATORY STAFF

(Including all whose services began or ended during the year)

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FRANCIS P. BOWLES, *Coordinator of Research Services*
EDWARD G. CASEY, *Controller*
JANE FESSENDEN, *Librarian*
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LEWIS M. LAWDAY, *Assistant Manager, Department of Marine Resources*
KARLENE LUKOVITZ, *Assistant Editor, The Biological Bulletin*
ANNE C. MAHER, *Public Relations Officer*
MORTON D. MASER, *Coordinator of Continuing Education*
LAURIE A. MORSE, *Assistant Editor, The Biological Bulletin*
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Susan Nickerson
Simone St. Jean
Clayton Sears
Glenn I. Shear
Gilbert F. Silvia
Merilyn A. Smart
Christopher Stone
Jane E. Sylvia
Frederick Thrasher
Frederick Ward
Ralph Whitman
William Whittaker

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Linda M. Golder
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Andrew Hodgdon
David Juers
Lowell V. Martin
Joan Peters-Gilmartin
Frank E. Sylvia

2. Investigators; Research Fellowships; Students

Independent Investigators, 1978

Adejuwon, Christopher A., Research Fellow, The Population Council, The Rockefeller University
Armstrong, Clay M., Professor of Physiology, University of Pennsylvania

Armstrong, Peter B., Associate Professor of Zoology, University of California
Arnold, John M., Professor, University of Hawaii, Kewalo Marine Laboratory
Astrin, Susan M., Assistant Member, The Institute for Cancer Research
Babb, Richard S., Research Fellow, Albert Einstein College of Medicine
Baldwin, Thomas O., Assistant Professor of Biochemistry, University of Illinois, Urbana
Bang, Frederik B., Professor of Pathobiology, The Johns Hopkins School of Hygiene and Public Health
Bang, Betsy G.
Barlow, Robert B., Professor, Syracuse University
Bauer, G. Eric, University of Minnesota
Baumgold, Jesse, Staff Fellow, NIMH, National Institutes of Health
Beauge, Luis A., Associate Professor of Biophysics, University of Maryland, School of Medicine
Begenisich, Ted, Assistant Professor of Physiology, University of Rochester
Bell, Eugene, Professor, Massachusetts Institute of Technology
Bennett, Michael V. L., Director, Division of Cellular Neurobiology, Albert Einstein College of Medicine
Bishop, Stephen H., Associate Professor, Iowa State University
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Brinley, Floyd J., Jr., Professor, Department of Physiology, University of Maryland, School of Medicine
Brodwick, Malcolm S., Assistant Professor of Physiology and Biophysics, University of Texas, Medical Branch
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Burdick, Carolyn J., Associate Professor of Biology, Brooklyn College, The City University of New York
Burger, Max M., Chairman of the Biocenter, University of Basel, Switzerland
Burkart, Werner R., Postdoctoral Research Fellow, Biocenter, University of Basel, Switzerland
Burns, Roy Gordon, Lecturer, Imperial College of Science and Technology, London, England
Casaday, George, B., Postdoctoral Fellow, Cornell University
Chang, Donald C., Assistant Professor, Baylor College of Medicine
Chappell, Richard L., Associate Professor, Hunter College, The City University of New York
Chung, Su-yun, Research Fellow, Harvard University
Cohen, Jonathan B., Assistant Professor of Pharmacology, Harvard Medical School
Cohen, Lawrence B., Associate Professor, Yale University, School of Medicine
Cohen, Seymour S., Professor, State University of New York at Stony Brook
Cohen, William D., Associate Professor of Biological Sciences, Hunter College, The City University of New York
Cooperstein, Sherwin J., Professor of Anatomy, University of Connecticut
Crandall, Edward D., Assistant Professor of Physiology and Medicine, University of Pennsylvania, School of Medicine
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Dentler, William L., Assistant Professor, University of Kansas
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De Weer, Paul, Associate Professor of Physiology and Biophysics, Washington University, School of Medicine
DiPolo, Reinaldo, Associate Investigator, Instituto Venezolano de Investigaciones Cientificas, Venezuela
Dowling, John E., Professor of Biology, Harvard University
DuBois, Arthur B., Director, The John B. Pierce Foundation Laboratory
Dunham, Philip B., Professor of Biology, Syracuse University
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Eckberg, William R., Graduate Assistant Professor, Howard University

Edwards, Donald H., Jr., Postdoctoral Fellow, Stanford University
Ehrenstein, Gerald, Research Physicist, National Institutes of Health
Eisner, Thomas, Jacob Gould Schurman Professor of Biology, Cornell University
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Fishman, Harvey M., Professor of Physiology and Biophysics, University of Texas, Medical Branch at Galveston
Flavin, Martin, National Institutes of Health
Fletcher, Donald J., Postdoctoral Fellow, Emory University
Fohlmeister, Jurgen, Lecturer, University of Minnesota
Freeman, Gary, Associate Professor of Zoology, University of Texas, Austin
Furshpan, Edwin J., Professor of Neurobiology, Harvard Medical School
Fussell, Catherine P., Associate Professor, Pennsylvania State University
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Gelperin, Alan, Associate Professor of Biology, Princeton University
Gibson, Jane, Associate Professor of Biochemistry, Molecular and Cell Biology, Cornell University
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Glantz, Raymon M., Associate Professor of Biology, Rice University
Glusman, Silvio, Research Associate, Harvard Medical School
Goldsmith, Paul K., Biologist, National Institutes of Health
Gould, James L., Assistant Professor, Princeton University
Gould, Robert M., Senior Research Scientist, Institute for Basic Research in Mental Retardation in New York
Greenbaum, Elias, Research Scientist, Corporate Research Laboratory, Union Carbide Corporation
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Humphreys, Tom, Professor of Biochemistry, University of Hawaii
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Kunz-Ramsay, Yvette, University College, Ireland
Kusano, Kiyoshi, Professor, Illinois Institute of Technology
Landis, Story, Instructor, Harvard Medical School
Landowne, David, Associate Professor, University of Miami
Lasek, Raymond J., Associate Professor, Case Western Reserve University
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Menzel, Randolf, Professor, Freien Universitat, Berlin, Germany
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Metz, Charles B., Professor, University of Miami

Miller, Richard S., Professor, Yale University
Mitchell, Ralph, Gordon McKay Professor of Applied Biology, Harvard University
Moore, John W., Professor of Physiology, Duke University
Moore, L. E., Professor, University of Texas, Medical Branch
Mooseker, Mark S., Research Fellow, Harvard Medical School
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Nagano, Toshio, Professor and Chairman of the Department of Anatomy, Chiba University, School of Medicine, Japan
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Nakajima, Shigehiro, Professor, Purdue University
Nakatsuji, Norio, Research Associate, Massachusetts Institute of Technology
Narahashi, Toshio, Professor and Chairman, Northwestern University
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Nelson, Margaret C., Research Fellow in Neurobiology, Harvard Medical School
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Pant, Harish C., Senior Staff Fellow, National Institutes of Health
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Parmentier, James L., Medical Research Assistant Professor, Duke University
Person, Philip, Medical Investigator, Veteran's Administration Hospital
Pethig, Ronald, Lecturer, University of Wales, Bangor, United Kingdom
Pichon, Yves, Maitre de Recherche, Centre National de la Recherche Scientifique, Paris, France
Pierce, Sidney K., Associate Professor of Zoology, University of Maryland
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Quigley, James P., Associate Professor of Microbiology and Immunology, State University of New York, Downstate Medical Center, Brooklyn
Ramon, Fidel, Assistant Professor, Duke University
Rebhun, Lionel I., Professor of Biology, University of Virginia
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Reingold, Stephen C., Postdoctoral Research Fellow, Princeton University
Requena, Jaime, Assistant Investigator, Centro de Biofisica Y Bioquimica, Caracas, Venezuela
Reynolds, George T., Professor of Physics, Princeton University
Rheuben, Mary B., Assistant Professor, Pennsylvania State University
Rickles, Frederick R., Veteran's Administration Hospital and University of Connecticut Health Center
Ripps, Harris, Professor of Ophthalmology and Physiology, New York University, School of Medicine
Rose, Birgit, Research Associate Professor, University of Miami, School of Medicine

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RUSHFORTH, NORMAN B., Professor and Chairman, Department of Biology, Case Western Reserve University
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RUSSELL-HUNTER, W. D., Professor of Zoology, Syracuse University
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SEGAL, SHELDON J., Vice President and Director, Biomedical Division, The Population Council
SEJNOWSKI, TERRANCE J., Research Fellow, Princeton University
SEYAMA, ISSEI, Associate Professor, Hiroshima University, Japan
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SHIELDS, DENNIS, Assistant Professor of Anatomy, Albert Einstein College of Medicine
SHRIVASTAV, BRIJ B., Medical Research Assistant Professor, Duke University
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SIMPSON, TRACY L., Professor, Department of Biology, University of Hartford
SMITH, JANIE E., Biologist, National Institutes of Health
SPECK, WILLIAM T., Associate Professor of Pediatrics, Case Western Reserve University
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SPIEGEL, MELVIN, Professor of Biology, Dartmouth College
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STUNKARD HORACE W., Research Associate, American Museum of Natural History
SUGIMORI, MUTSUYUKI, Research Assistant Professor, New York University Medical Center
SUMMERS, JESSE W., Member, Institute for Cancer Research
SUMMERS, ROBERT G., Associate Professor of Anatomy, State University of New York at Buffalo
SUSSWEIN, ABRAHAM J., Research Fellow, Albert Einstein College of Medicine, Yeshiva University
SZENT-GYÖRGYI, ANDREW G., Chairman, Department of Biology, Brandeis University
SZENTKIRALYI-SZENT-GYÖRGYI, EVA M., Research Associate, Brandeis University
TABATA, MITSUO, Research Fellow, Laboratory of Biophysics
TAMM, SIDNEY, Associate Scientist, University of Wisconsin
TAMM, SIGNHELD, Research Associate, University of Wisconsin
TASAKI, ICHIJI, Chief, Laboratory of Neurobiology, NIMH, National Institutes of Health
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TERAKAWA, SUSUMU, Visiting Associate, National Institutes of Health
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VAN RAALTE, CHARLENE, Instructor, Hampshire College

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WAGNER, RICHARD W., Postdoctoral Fellow, University of Miami, School of Medicine
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WEBER, ANNMARIE, University of Pennsylvania, School of Medicine
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WHITTAKER, J. RICHARD, Associate Professor, The Wistar Institute
WIERCINSKI, FLOYD J., Professor, Department of Biology, Northeastern Illinois University
WILLIAMS, T. P., Professor of Biological Sciences, Florida State University
WILSON, DARCY B., Professor of Pathology, University of Pennsylvania, School of Medicine
WOLF, DON P., Associate Research Professor, University of Pennsylvania, School of Medicine
WOOLEY, JOHN C., Research Fellow in Biochemistry, Harvard University
WORGUL, Basil V., Research Associate, Department of Ophthalmology, Columbia University
WU, CHAU H., Assistant Professor of Pharmacology, Northwestern University
YEH, JAY Z., Assistant Professor, Northwestern University
ZIGMAN, SEYMOUR, Professor of Ophthalmology and Biochemistry, University of Rochester, School of Medicine and Dentistry
ZIRKIN, BARRY R., Associate Professor, Division of Reproductive Biology, The Johns Hopkins University

Lillie Fellow, 1978

LE DOUARIN, NICOLE, Centre National de la Recherche Scientifique, Institut D'Embryologie, France

Alexander Forbes Lecturer, 1978

KRNJEVIC, KRESIMIR, Department of Research in Anaesthesia, McIntyre Medical Sciences Building, Canada

Rand Fellow, 1978

MENZEL, RANDOLF, Institut fur Tierphysiologie, Freien Universitat, Berlin, West Germany

Grass Fellows, 1978

ASHCROFT, FRANCES M., Grass Foundation Fellow, Department of Zoology, Cambridge University, United Kingdom
DAY, JOHN W., Albert Einstein College of Medicine
EDWARDS, DONALD H., Postdoctoral Fellow, Stanford University
FRAZIER, DONALD, Professor, University of Kentucky, School of Medicine
GOLDBERG, MARK T., Grass Fellow, Memorial University of Newfoundland, Canada
LUND, ALBERT E., Postdoctoral Fellow, Northwestern University Medical School
MEYER, DAVID J., Grass Fellow, Health Sciences Center, State University of New York at Stony Brook
NASS, MENASCHE M., Research Fellow in Biology, California Institute of Technology
PELLMAR, TERRY, Research Fellow, National Naval Medical Center, Bethesda
QUANDT, FREDERICK N., Postdoctoral Fellow, University of California, Los Angeles
SATTERLIE, RICHARD A., Grass Fellow, University of California, Santa Barbara
SCOTT, SHERYL A., Postdoctoral Fellow, Carnegie Institution of Washington
SULLIVAN, ROBERT E., Postdoctoral Fellow, University of Hawaii at Manoa
WATSON, WINSOR H., III, Research Associate, University of Massachusetts
ZOTTOLI, STEVEN J., Research Scientist II, Research Institute on Alcoholism

Macy Scholars, 1978

ARMSTRONG, EARLENE, Assistant Professor, University of Maryland
HILL, ANITA V., Assistant Professor of Biology, Grambling State University

Hogan, James C., Jr., Department of Anatomy, Howard University College of Medicine
Howze, Gwendolyn B., Assistant Professor, Texas Southern University
Ivens, M. Sue, Associate Professor, Natural Sciences, Dillard University
Porter, Charles W., Associate Professor of Biology, San Jose State University
Walker, Dorothy G., Associate Professor, Howard University

Summer Research Scholarships, 1978
(Steps Toward Independence)

Armett-Kibel, Christine, Assistant Professor of Biology, University of Massachusetts
Bourne, George B., Assistant Professor, The University of Calgary, Canada
Chung, Su-yun, Research Fellow in Chemistry, Harvard University
Fujiwara, Keigi, Assistant Professor of Anatomy, Harvard Medical School
Hoffman, Richard J., Assistant Professor of Biological Sciences, University of Pittsburgh
Hufnagel, L. Assistant Professor of Microbiology, University of Rhode Island
Kass-Simon, M. Assistant Professor of Zoology, University of Rhode Island
Langford, George M., Assistant Professor of Anatomy, Howard University, College of Medicine
Lewis, Larry M., Assistant Professor of Biology, Millersville State College
O'Melia, Anne F., Assistant Professor of Biochemistry, Louisiana State University Medical Center
Rudy, Bernardo, Assistant Professor of Physiology, Eastern Pennsylvania Psychiatric Institute
Salzberg, Brian, Assistant Professor of Physiology, University of Pennsylvania
Treistman, Steven N., Assistant Professor of Biology, Bryn Mawr College
Young, Lily Y., Assistant Professor of Environmental Microbiology, Stanford University

Research Assistants, 1978

Anderson, David J., The Rockefeller University
Anstrom, John, State University of New York at Buffalo
Antonellis, Blenda, University of Rochester School of Medicine
Aryan, Peter, Yale University
Augustine, George J., Jr., University of Maryland
Barnes, Edward S., Columbia University
Batra, Ranjan, Institute for Sensory Research, Syracuse University
Behrman, Amy, Swarthmore College
Bennett, Holly V. L., The Albert Einstein College of Medicine
Bodick, Neil, Columbia University
Bosler, Robert B., Harvard University Medical School
Boyle, Mary B., Yale University
Brandon, Christopher, Baylor College of Medicine
Bush, James, Rutgers College
Byers, H. Randolph, Harvard Medical School
Calle, Paul, University of Pennsylvania
Capco, David George, University of Texas
Chan, Yue-Wah Steven, Baylor College of Medicine
Chelsky, Daniel, University of Oregon
Cheney, Clarissa M., University of Toronto
Cohen, Richard, Hunter College
Colucci, Barbara Ann, Herbert H. Lehman College, The City University of New York
Crawford, William, University of Hawaii
Critz, Anne McElroy, University of Pennsylvania
Croop, Robert, University of Pennsylvania
Dahl, Robert F., Princeton University
Dandekar, Pramila, State University of New York at Buffalo
Davidson, Dorothy E., University of Delaware
Drake, Peter F., Bryn Mawr College
Eagle, Jane S., University of Connecticut Health Center
Eigner, E. Ann, University of New Mexico School of Medicine
Falkow, Stephen, New York University, School of Medicine

Field, Nancy, Wesleyan University
Fine, Alan, University of Pennsylvania
Finkel, Toren, University of Pennsylvania School of Medicine
Fitt, William K., University of California, Santa Barbara
Fleming, Judy, University of Colorado
Frizzell, Karen L., University of Massachusetts
Galvin, Patric N., University of Colorado at Denver
Gilly, William F., University of Pennsylvania
Glicksman, Marcie, Brown University
Goncalves, Mark A., University of Colorado
Gordon, Doria, Oberlin College
Green, Jeffrey D., State University of New York at Buffalo
Greenwalt, Dale, Iowa State University
Grega, Debra S., University of Kentucky
Grupp, Stephen, University of Cincinnati
Harris, Andrew L., The Albert Einstein College of Medicine
Harris, Vicki Kay, Emory University
Heinrichs, Stefan
Henry, Jonathan J., University of Hawaii
Herlands, Louis, Yale University
Hersch, Steven M., Boston University
Ho, Simon Ming Hung, University of Toronto, Canada
Holmes, Don J., Washington University Medical School
Howard, Louisa, Dartmouth College
Hurst, Terry W., University of Texas, Medical Branch
Huse, William, Albert Einstein College of Medicine
Huttner, Susanne, University of California, Los Angeles
Hylander, Bonnie, State University of New York at Buffalo
Imhof, Ruth, Biozentrum University of Basel, Switzerland
Jaeger, Richard R., Hunter College, The City University of New York
Janus, Todd, J., Northwestern University
Jaslove, Stewart, Duke University Medical Center
Jimenez, Ramon J., University of Guadalajara, Mexico
Jumblatt, James E., University of Basel, Switzerland
Katayama, Robert N., University of Pennsylvania
Kendall, Debra A., Northwestern University
Kiehart, Daniel P., University of Pennsylvania
Kirk, Mark D., Rice University
Koumjian, Lauren, Mount Holyoke College
Laufer, Marc R., University of Pennsylvania
Leibowitz, David H., Columbia University
Lesher, Sarah, Yale University
Levinthal, Adam
Lewis, Stephen, University of Cincinnati
Littlefield, Peter, University of Maryland
Livingstone, M. S., Harvard Medical School
Luebbert, Jo Ann, State University of New York at Stony Brook
Luttinger, Karl M., Columbia University
Marsden, J. Ellen
Meedel, Thomas H., The Wistar Institute
Meyer, Michael A., University of North Carolina at Chapel Hill
Mir, Francoise, University of Basel, Switzerland
Montanaro, George D., University of Iowa
Morello, Robert S., University of Rochester
Morgan, Charles R., Emory University
Morgan, Gina, Howard University
Morris, James R., Case Western Reserve University
Narahashi, Taro, Northwestern University

Necles, Nicholas, Columbia University
Nemhauser, Iris, Hunter College, The City University of New York
Neubig, Richard R., Harvard Medical School
Pelley, Chiquita, University of Oregon
Porter, Mary E., University of Pennsylvania
Ramos, Talia, The Johns Hopkins University School of Hygiene and Public Health
Redmann, Greg A., University of Texas Medical Branch
Reiss, Paul, University of Maryland
Render, Jo Ann, University of Texas, Austin
Rho, Jai-Hyon, The Johns Hopkins University
Robertson, Lola E., American Museum of Natural History
Rollins, Sharon, Cornell University
Rosenkranz, Pnina, Princeton University
Rosman, Gary, New York University
Rumer, Nina, University of Delaware
Samson, Douglas A., The Johns Hopkins School of Hygiene and Public Health
Saunders, Mary J., University of Massachusetts
Scarborough, Ann, Louisiana State University
Schenck, Kathleen, Princeton University
Schmidt, Jeff, University of California, Los Angeles
Schmidt, Madelyn R., Harvard Medical School
Schneider, Mark Alan, Amherst College
Scruggs, Virginia
Shure, Michael S., Yale University
Siegel, Ruth E., Harvard Medical School
Simon, Sanford, New York University Medical Center
Socci, Robin R., Rutgers, The State University of New Jersey
Solomon, Dennis J., Massachusetts Institute of Technology
Solway, Alan, Wayne State University, School of Medicine
Stamler, John F., University of Iowa
Steele, Joy Ann, University of Alberta, Canada
Stich, Thomas J., University of Maryland
Subyak, Sharon E., Suffolk University
Suh, Kyungsun, Columbia University
Swan, Michael C., University of California, Los Angeles
Swenson, Randolphe P., University of Pennsylvania
Talian, John, Carnegie-Mellon University
Taylor, Christopher E., The Johns Hopkins School of Hygiene and Public Health
Timpe, Leslie C., Harvard Medical School
Travis, Mark A., Greenville College
Tyner, Emily M., Vassar College
Von Hippel, David F.
Waldrop, Brian, Rice University
Walton, Kerry, New York University Medical Center
Warren, Mary K., University of Maryland
Weeks, Janis C., University of California, San Diego
Weiss, Kevin, University of Pittsburgh
Westerfield, Monte, Max Planck Institute fur Psychiatrie, Germany
Wheatley, Richard, Columbia University
Wick, Susan M., University of Massachusetts
Williams, Carolyn H., Emory University
Williams-Arnold, Lois D., University of Hawaii
Wodlinger, Harold M., University of Toronto, Canada
Yonemoto, Wes, University of Hawaii
Yulo, Teresa, University of Rochester School of Medicine and Dentistry
Zakevicius, Jane, New York University School of Medicine
Zukowski, Anthony J., University of Hawaii

Library Readers 1978

Adelberg, Edward A., Professor of Human Genetics, Yale University
Allen, Nina Stromgren, Assistant Professor, Dartmouth College
Allen, Robert Day, Professor and Chairman, Dartmouth College
Anderson, Everett, Professor of Anatomy and Associate Director, Laboratory of Human Reproduction and Reproductive Biology, Harvard University Medical School
Bean, Charles P., Biophysicist, General Electric Company
Bourne, Donald, Marine Research Inc.
Candelas, Graciela C., Professor, University of Puerto Rico
Carriere, Rita, Assistant Professor, State University of New York, Downstate Medical Center
Child, Frank M., Professor of Biology, Trinity College
Clifford, Sister Adele, Professor of Biology, College of Mount St. Joseph on the Ohio
Cole, Kenneth S., Research Biophysicist Emeritus, National Institutes of Health
Condouris, George A., Professor and Chairman, New Jersey Medical School
Copeland, Donald Eugene, Professor of Biology, Tulane University
Cornwell, Anne Christake, Assistant Professor of Ophthalmology, Montefiore Hospital and Medical Center
Couch, Ernest F., Associate Professor of Biology, Texas Christian University
Davis, Bernard D., Professor, Harvard Medical School
Dehn, Paula F., Graduate Assistant, University of Southern Florida
Dettbarn, Wolf-D., Professor of Pharmacology, Vanderbilt University, School of Medicine
Dudley, Patricia L., Professor of Biological Sciences, Barnard College, Columbia University
Dundas, Ian, Associate Professor, University of Bergen, Norway
Ebert, James D., President, Carnegie Institute of Washington
Edds, Louise L., Associate Professor, Zoology and Microbiology, Ohio University
Elder, Howard A., Professor of Medicine, Albert Einstein College of Medicine
Eisen, Herman N., Professor of Immunology, Massachusetts Institute of Technology
Englander, Sol Walter, University of Pennsylvania
Fischman, Donald A., Professor and Chairman, State University of New York, Downstate Medical Center
Fisher, Saul H., Clinical Professor of Psychiatry, New York University, School of Medicine
Franzini-Armstrong, Clara, Associate Professor of Anatomy, University of Pennsylvania
Gabriel, Mordecai L., Professor of Biology, Brooklyn College, City University of New York
Gagne, Gerard D., Doctoral Candidate, University of Maine, Orono
Galatzer-Levy, Robert M., Lecturer, Associate Attending Psychiatrist, University of Chicago and Michael Reese Hospital
Goldman, Robert D., Professor of Biological Sciences, Carnegie-Mellon University
Goldstein, Moise H., Jr., Professor, The Johns Hopkins University
Goudsmit, Esther M., Associate Professor, Oakland University, Rochester
Gould, Stephen Jay, Professor of Geology, Harvard University
Grant, Philip, Professor of Biology, University of Oregon
Haubrich, Robert, Professor of Biology, Denison University
Henley, Catherine, National Eye Institute, National Institutes of Health
Hillman, Peter, Associate Professor, The Hebrew University of Jerusalem, Israel
Hinsch, Gertrude W., Associate Professor, University of Florida
Hochstein, Shaul, Lecturer, The Hebrew University of Jerusalem, Israel
Huberman, Michael H., Intern—Internal Medicine and Pre-doctoral Fellow, New York University
Huettner, Robert J., Assistant Professor, Columbia University, School of Dentistry
Hunter, R. Douglas, Assistant Professor of Biological Sciences, Oakland University, Rochester
Ilan, Joseph, Professor, Case Western Reserve University, School of Medicine
Inoue, Sadayuki, Assistant Professor, McGill University, Canada
Ireland, Leonard, Associate, Bermuda Biological Station
Isenberg, Irvin, Professor of Biophysics, Oregon State University
Isselbacher, Kurt J., Mallinckrodt Professor of Medicine, Chief of the Gastrointestinal Unit, Harvard Medical School and Massachusetts General Hospital
Issidorides, Marietta R., Research Professor in the Department of Psychiatry, University of Athens, Medical School, Greece

JAVITT, NORMAN B., Professor of Medicine, Head, Division of Gastroenterology, Cornell University Medical College
JOHNSON, WILLIAM H., Professor of Biology, Rensselaer Polytechnic Institute
KALTENBACH, JANE C., Professor of Biological Sciences, Mount Holyoke College
KARUSH, FRED, Professor of Microbiology, University of Pennsylvania, School of Medicine
KEAN, EDWARD L., Associate Professor, Case Western Reserve University
KEOSIAN, JOHN P., Author
KIRSCHENBAUM, DONALD M., Associate Professor, State University of New York, Downstate Medical Center
KLEIN, MORTON, Professor of Microbiology, Temple University Medical School
KOBAYASHI, MAKOTO, Professor of Physiology, Hiroshima University, Japan
KOGUT, MARGOT, Lecturer, King's College, London, England
KRNJEVIC, K., Professor and Director, Department of Anaesthesia Research, McGill University, Montreal, Canada
LADERMAN, AIMLEE, Doctoral Candidate, State University of New York at Binghamton
LAZAROW, JANE K., Senior Research Analyst, Minnesota Department of Health
LE DOUARIN, NICOLE, Professeur, Directeur de l'Institut d'Embryologie du C. N. R. S. et du College de France, Centre National de la Recherche Scientifique
LEFEVRE, MARIAN E., Associate Scientist, Medical Department, Brookhaven National Laboratory
LEIGHTON, JOSEPH, Professor and Chairman, Department of Pathology, Medical College of Pennsylvania
LEVITAN, HERBERT, Associate Professor, University of Maryland, College Park
LIEBERMAN, MICHAEL W., Associate Professor of Pathology, Washington University, School of Medicine, St. Louis
LINCK, RICHARD W., Assistant Professor, Harvard Medical School
LINEAWEAVER, THOMAS H., III, Author
LUX, HANS DEITER, Max Planck Institute of Psychiatry, Germany
MARSLAND, DOUGLAS, Research Professor Emeritus, New York University
MASLAND, RICHARD H., Assistant Professor of Physiology
MITCHELL, JAMES B., Associate Professor of Biology, Moravian College
MIZELL, MERLE, Professor of Biology, Tulane University
MORRELL, FRANK, Professor of Neurology, Rush Medical College
NEWBURY, THOMAS, Assistant Professor, University of Hawaii
OJAKIAN, GEORGE, Assistant Professor, State University of New York, Downstate Medical Center
PALMER, JOHN D., Chairman and Professor of the Zoology Department, University of Massachusetts, Amherst
PEARLMEN, ALAN L., Associate Professor of Physiology and Neurology, Washington University, School of Medicine
PLOCKE, DONALD J., Associate Professor and Chairman of Biology, Boston College
ROSENBAUM, JOEL L., Professor of Biology, Yale University
ROSENBERG, EVELYN, Professor, Jersey City State College
SALMON, EDWARD D., Assistant Professor, Zoology Department, University of North Carolina, Chapel Hill
SANGER, JOSEPH W., Associate Professor, University of Pennsylvania
SAUNDERS, JOHN W., Professor, State University of New York at Albany
SCHLESINGER, WALTHER R., Professor and Chairman, Department of Microbiology, Rutgers Medical School, College of Medicine and Dentistry of New Jersey
SCOTT, GEORGE T., Professor of Biology, Oberlin College
SHEMIN, DAVID, Professor and Chairman, Department of Biochemistry and Molecular Biology, Northwestern University
SHEPRO, DAVID, Professor, Boston University
SMITH, MICHAEL A., Research Associate, Hunter College, The City University of New York
SONNENBLICK, B. P., Professor Emeritus, Rutgers, The State University of New Jersey
SPECTOR, ABRAHAM, Professor of Ophthalmic Biochemistry, Department of Ophthalmology, Columbia University
SUGDEN, BILL, Assistant Professor, University of Wisconsin
SZAMIER, R. BRUCE, Assistant Professor, Harvard Medical School
TRAGER, WILLIAM, Professor, The Rockefeller University

Wainio, Walter, Professor and Chairman, Department of Biochemistry, Rutgers College, Rutgers, The State University of New Jersey
Waksman, Byron H., Professor of Pathology, Yale University
Webb, H. Marguerite, Professor of Biological Sciences, Goucher College
Weiss, Leon, Chairman, Department of Animal Biology; Professor of Cell Biology, University of Pennsylvania, School of Veterinary Medicine
Wheeler, George E., Professor of Biology, Brooklyn College, Biology Department
Wilson, Thomas H., Professor of Physiology, Harvard Medical School
Wittenberg, Jonathan B., Professor of Physiology, Albert Einstein College of Medicine
Wolf, Jason, Associate Professor, Wesleyan University
Yntema, C. L., Professor Emeritus, Upstate Medical Center, State University of New York
Zacks, Sumner I., Professor and Chairman, Section of Pathology, Brown University; Pathologist-in-Chief, Laboratory Director, The Miriam Hospital

Students, 1978

All students listed completed the formal course programs. Asterisk indicates those completing post-course research programs.

Summer Programs 1978

MARINE ECOLOGY

*Baxter, Molly
Bronson, Becky
Chen, Celia Y.
*Copley, Nancy Jeanette
*Day, Margaret Elisabeth
DeMuth, Robert Emerson
Donn, Theodore E., Jr.
*Fagan, Joanne
Fredericq, Suzanne
*Jensen, Cynthia Lund
Katz, Lawrence C.
*Kirchman, David Louis
Lechleitner, Richard A.
*Mogan, Arthur
*Rietsma, Carol S.
Roslansky, Louise
Silberhorn, Eric Martin
Skinner, Carol Ann
*Talbert, Jean E.

EMBRYOLOGY

Anderson, Kathryn Virginia
Brown, Kenneth Michael
Chilton, Beverly Sue
Edwards, Mary Kaye
Flaster, Murray S.
Gibson, Barbara Louise
Guidice, George J., Jr.
Johnson, Thomas Eugene
Kornher, John Stephen
Kosofsky, Barry Evan
Landzberg, Joel Serge
Marusich, Michael F.
Miller, Carol Lynn
Muneoka, Ken
Rice, Douglas
Salik, Jane Anne
Sandridge, Paul Timothy
Schulz, Stephanie
Szaro, Ben Gregory
Tansey, Terese R.
Wanek, Nancy Lynn
Watanabe, Michiko
Wehrmaker, Alfred
Wise, Lawrence David
Young, Nevin Dale Young

NEURAL SYSTEMS AND BEHAVIOR

*Bleisch, William Virgil
Condon, Timothy
Coquelin, Arthur
David, William S.
Epel, Debra Lynn
Furbish, Dean Russell
Holz, George G., III
Hurlbert, Anya
Hurwitz, Jodie Linda
Javitt, Daniel C.
Kuwada, John
*Levy, Daniel Jonathan
Marcotte, Ronald R.
McManus, Mary Ellen

Mehler, Bruce Leo
*Miller, Mark W.
Moiseff, Andrew
Olson, Leah M.
Orr, Dominic Ping-Yim
*Ready, Neal Edward
Rose, Barry
*Rose, Robert D.
Roselli, Charles Eugene
Schessel, David
Schildberger, Klaus
*Schmitt, Brian C.
*Solon, Michael H.
Tanelian, Darrell Lee
Tompkins, Laurie
*Trevarrow, William W.
Turner, Jeffrey Scott
Wei, Aguan Daniel

EXPERIMENTAL MARINE BOTANY

Ashendorf, Debra S.
Berenberg, Cathy Jo
Boczar, Barbara A.
*Dolyak, Barbara Lorraine
Dunlap, Julie J.
Favreau, Mitchell R.
Fitter, Mindy Sue
*Graves, Joseph L., Jr.
Johnson, Patricia A.
Kaufman, Lon
Monar, Kenneth
Pearce, Robert Elliott
Sheline, Jonathan Lee
Whittaker, Carla J.

NEUROBIOLOGY

Dodd, Jane
Fukada, Keiko
Garcia-Arraras, Jose E.
Lemos, Jose Ramon
Marcey, David James
Pine, Jerome
Roof, Dorothy J.
Schneiderman, Anne M.
Sejnowski, Terrence J.
Stockbridge, Norman Lander
Thomas, William Eric
Yodlowski, Marilyn L.

PHYSIOLOGY

Barr, Alan Howard
*Beckwith, Kirk D.
Beher, Michael Gary
Carman, Alice Blanche
Chang, Jane
Collins, Elizabeth T.
*Collins, Mary
Cooper, John A.
*Cutler, Miriam Ruth
*Dayton, Andrew Imbrie
*Gershon, Nahum David
*Grifo, James Anthony
Hintze, T.
Jaffe, Laurinda Ann
Lieber, Michael R.
Marquez-Sterling, Numa R.
Mattson, Joan Crawford
*McCarthy, Robert Alan
*Mulholland, Joyce I.
*Otter, Timothy
*Pickett, Cecil
*Pine, Richard Ira
*Proffitt, John Houston
*Rausch, Steven
*Rebers, John Eric
*Robinson, Margaret S.
*Selick, Harold E.
*Shahin, Roberta D.
Sullivan, Charles Henry, II
*Sullivan, Susan Jean
*Suprenant, Kathy Ann
*Travis, Jeffrey L.
*Wood, Steven Chenault
*Wu, Chao-ting
*Wyban, James Allen

MICROBIAL ECOLOGY

*Amy, Penny S.
*Bellas, Christine Marie
*Goldfarb, David Scott
Graham, Julia Bell
*Kaster, Allan Gerard
Paster, Bruce Jay
Potrikus, Catherine J.
Reed, William Michael
Siscoe, Peggy Jean
Ward, Bess B.

January Programs 1978

BEHAVIOR

Ashkenas, Linda R.
Beni, Janine E.
Brown, Adolf Maximilian
Bryant, Bruce P.
Clark, Stephen J.
Derby, Charles Dorsett
DuWaldt, Sarah
Elgin, Randall Hull
Hernandez, Teresa
Jones, Maurice, Jr.
Miller, Deborah Lynn
Obin, Martin S.
Poethke, David John
Ragland, Harry Craig
Stratton, Andrea Wood
Williams, Isabelle P.
Woodbury, Patrick B.

DEVELOPMENTAL BIOLOGY

Barbacci, Joseph J., Jr.
Brodell, George Kline
Bryar, Betsy A.
Burke, Daniel J.
Evarts, Jonathan Harold
Ford, William C.
Gillespie, Laura Lee
Gold, Albert Mark
Gustafson, Kirk Robert
Holcomb, Cherie
Howell, Margaret A.
McGimsey, William Claywell
McManus, Geraldine A.
Manning, Michael Scott
Milani, Susan
Patterson, Nancy
Perry, Gail E.
Rhinehart, Mary Ellen
Riddell, Deborah Christie
Smiley, Laura Ellen
Toth, Leslie Ellen
Turnbull, Sarah W.
Vowell, Joanna Lynn

ECOLOGY

Aboya, Ellen Gwen
Behl, Ann
Bergantz, James M.
Bixler, Robert P.
Coupard, Michele
DePelteau, Audrey Marie
Detwiler, Ralph Paul
Farrell, Thomas A.
Feldman, Ira Robert
Greene, George D.
Groff, Joseph Matthew
Hochberg, Ann Patricia
Kelley, Julia Crocker
Kellogg, Dean Lundt, Jr.
Larson, Wendelin Mae
Lawrence, Viki Ann
Leaman, Danna Jo
MacFarlane, Linda Louise
MacWhorter, Susan E.
McGowan, Michael F.
Naughton, Darlene A.
Perfetti, Patricia Ann
Steele, Pamela Curtis
Theby, Janet Elaine
Walker, Sharon Leslie

NEUROBIOLOGY

Aaby, Trygve Gene
Apataoff, Brian R.
Baggott, Brian Burke
Bodenstein, Lawrence E.
Carr, Catherine Emily
Chu, Ka Hou
Dillsaver, Margaret
Dolber, Paul Christian
Garber, Nancy D.
Haber, Michele A.
Heineke, Eric Wayne
Higgins, Gerald Arthur
James, Demetria Elizabeth
Kent, Karla S.
Krasnow, Mark Alan
Lopez, Jose R.
Machlis, Lee Ellen
Mornay, Sharon Marie
Moulton, John Freeman, III
Ogonowski, Michael M.
Steinberg, Alan B.
Valentich, John D.
Verrett, Joyce M.
Weinberg, Bradley Adam
Rind, Jeff
Sladovich, Hedy E.
Feigenbaum, David L.

COMPARATIVE PATHOLOGY OF MARINE INVERTEBRATES

Burns, Cary DeWitt
Cardenosa, Gilda
Carlson, George A.
Chorney, Michael J.
Cooper, Keith Raymond
Couch, David Robert
Deakins, Lynn Wheeler
Goldberg, Harry R.
Gulka, Gary J.
Heatfield, Barry Mark
Keller, Thomas Earl
Keyt, Bruce Alan
Logue, Maureen Denise
Lombardi, Rosemarie
Miller, Timothy K.
Mulvey, Margaret E.
Newman, Michael C.
Seifert, Ronald A.
Sonstegard, Ronald A.
Sypek, Joseph P.
Wallace, James L.
Yeaton, Robin L. W.

BOSTON UNIVERSITY MARINE PROGRAM

Aaronson Rebecca
Ashkenas, Linda
Besse, Sheila
Botero, Leonor
Bryant, Bruce
Buchsbaum, Robert
Cole, James
Cole, Timothy
Cushman, Mary
Davis, Cabell
Derby, Charles
Dojiri, Masahiro
Dourdeville, Theodore
Duncan, Thomas
Eder, Susan
Eisen, Judith
Fabrizio, Mary
Foreman, Kenneth
French, Kathleen
Giblin, Anne
Gibson, Daniel
Hill, Russell
Howes, Brian
Jordan, Thomas
Kent, Karla
Kipp Katrina
Kolba, Clifford
Koumjian, Lauren
Langbauer, William
Maciolek, Nancy
Ogonowski, Mark
Pascoe, Natalie
Piotrowski, Michael
Poole, Alan
Reid, Robert
Walthall, Walter
Werme, Christine
Wier, Susan
Williams, Isabelle
Wilson, John

YEAR-IN-SCIENCE

Spring 1978

William B. Bowden
Sarah M. Mills
James T. Morris
James P. Reed
Mark N. White

Summer 1978

William B. Bowden
Amy Friedlander
Richard Grant
James T. Morris
James P. Reed
Mark N. White

Winter 1978

William B. Bowden
Michael S. Manning
Charles P. McClaugherty
James T. Morris
James L. Olds
James P. Reed

3. Scholarships, 1978

Bio Club: Mary Ellen McManus

Gary H. Calkins: Dominic Orr
Robert Rose

Lucretia Crocker: Barbara Boczar
David Kirchman

Arthur Klorfein: William Bleisch
Daniel Levy

Toshio Nagano
Neal Ready
Robert Rose
Christopher Taylor

Josiah Macy, Jr.:

January	*Summer*
Sharon Mornay	Cecil Pickett
Joyce Verrett	Patricia Chase
William Ford	Carol Bagnell
Margaret Howell	Patricia Johnson
	William Thomas
	Joseph Graves, Jr.
	Paul Sandridge
	Carla Whittaker

Society of General Physiologists:

John Cooper
Miriam Cutler
Andrew Dayton

4. Tabular View of Attendance, 1974–1978

	1974	*1975*	*1976*	*1977*	*1978*
Investigators—Total	508	511	535	501	522
Independent	302	301	312	280	268
Library Readers	75	81	93	82	106
Research Assistants	131	129	130	139	148
Students—Total	158	212	249	212	250
Summer Courses					
Ecology	14	18	18		
Embryology	21	24	24	25	25
Experimental Invertebrate Zoology	30	34	36	33	
Experimental Marine Botany	11	14	16	16	14
Marine Ecology				19	22
Neural Systems and Behavior					31
Neurobiology	12	12	12	12	12
Physiology	40	33	41	34	34
January Courses					
Behavior		17	17	20	17
Biosphere		17			
Comparative Pathology of Marine Invertebrates					22
Developmental Biology	30	20	32	14	21
Ecology			29	17	25
Neurobiology		23	24	22	27
Trainees—Total	41	31	9	10	11
Total Attendance	707	754	793	723	783
Less persons represented in two categories	0	0	1	0	0
	707	754	792	723	783
Institutions Represented—Total	222	237	234	226	196
Foreign Institutions Represented	31	26	33	33	30

5. Institutions Represented, 1978

Albert Einstein College of Medicine
American Museum of Natural History
Amherst College
Baylor College
Boston College
Boston University
Boston University School of Medicine
Brandeis University

Brookhaven National Laboratory
Brooklyn College, The City University of New York
Brown University
Bryn Mawr College
California Institute of Technology
California, University of, Davis
California, University of, Irvine
California, University of, Los Angeles
California, University of, San Diego
California, University of, Santa Barbara
Carnegie Institution of Washington
Carnegie-Mellon University
Case Western Reserve University
Case Western Reserve University, Medical School
Chicago, University of
Cincinnati, University of
College of Mount St. Joseph on the Ohio
Colorado, University of
Columbia University
Columbia University, College of Physicians and Surgeons
Columbia University, School of Dentistry
Connecticut, University of
Connecticut, University of, Health Center
Cornell University
Cornell University Medical College
Dartmouth College
Delaware, University of
Denison University
Dillard University
Duke University
Duke University Medical Center
Eastern Pennsylvania Psychiatric Institute
Emory University
Florida, University of
Florida State University
General Electric Company
Goucher College
Grambling State University
Grass Foundation
Greenville College
Hahnemann Medical College
Hampshire College
Hartford, University of
Harvard Medical School
Harvard University
Hawaii, University of
Herbert Lehman College, The City University of New York
Howard University
Howard University, College of Medicine
Hunter College, The City University of New York
Illinois, University of
Illinois, University of, College of Medicine
Illinois Institute of Technology
Institute for Basic Research in Mental Retardation
Institute for Cancer Research, The
Iowa, University of
Iowa State University
Jersey City State College
John B. Pierce Foundation Laboratory
Johns Hopkins University, The
Johns Hopkins University, The School of Hygiene
Johns Hopkins University, The School of Medicine
Kansas, University of
Kent State University
Kentucky, University of
Kresge Eye Institute
Laboratory of Biophysics, NINCDS-NIH
Louisiana State University
Louisiana State University Medical Center
Maine, University of, Orono
Manchester Community College
Maryland, University of
Maryland, University of, School of Medicine
Massachusetts, University of
Massachusetts General Hospital
Massachusetts Institute of Technology
Miami, University of
Miami, University of, School of Medicine
Millersville State College
Minnesota Department of Health
Minnesota, University of
Miriam Hospital, The
Mount Holyoke College
Montefiore Hospital & Medical Center
Moravian College
National Institute of Mental Health, NIH
National Naval Medical Center
New Jersey Medical School
New Mexico, University of, School of Medicine
New York University
New York University Medical Center
New York University School of Medicine
North Carolina, University of, at Chapel Hill
North Carolina State University, Raleigh
Northeastern Illinois University
Northwestern University
Notre Dame, University of
Oakland University
Oberlin College
Ohio State University
Ohio University
Oregon, University of
Oregon State University
Pennsylvania, University of, School of Medicine
Pennsylvania, University of, School of Veterinary Medicine
Pennsylvania State University
Pittsburgh, University of
Population Council, The

Princeton University
Public Health Research Institute of The City of New York, Inc.
Purdue University
Michael Reese Hospital
Rensselaer Polytechnic Institute
Research Institute on Alcoholism
Rhode Island, University of
Rice University
Rochester, University of
Rochester, University of, School of Medicine & Dentistry
Rockefeller University, The
Rush Medical College
Rutgers—The State University of New Jersey
Rutgers University Medical School
San Jose State University
South Florida, University of
Stanford University
Stanford University School of Medicine
State University of New York, Downstate Medical Center
State University of New York, Upstate Medical Center
State University of New York at Albany
State University of New York at Binghamton
State University of New York at Buffalo
State University of New York at Stony Brook
Suffolk University
Swarthmore College
Syracuse University
Temple University Medical School
Texas Christian University
Texas Southern University
Texas, University of, Austin
Texas, University of, Medical Branch
Trinity College
Tulane University
Union Carbide Corporation
Vanderbilt University
Vassar College
Veterans' Administration Hospital, Brooklyn
Virginia, University of
Virginia, University of, School of Medicine
Washington University School of Medicine
Wayne State University
Wesleyan University
Wisconsin, University of
Wistar Institute
Woods Hole Oceanographic Institution
Yale University
Yale University School of Medicine
Yeshiva University

Foreign Institutions Represented, 1978

Aarhus, University of, Denmark
Alberta, University of, Canada
Athens, University of, Medical School, Greece
Basel, The University of, Biocenter, Switzerland
Bergen, University of, Norway
Bermuda Biological Station
Calgary, University of, Canada
Cambridge, University of, England, U.K.
Centre National de la Recherche Scientifique, France
Centro de Biofisica Y Bioquimica, Caracas, Venezuela
Chiba University, Japan
College de France, Paris, France
Free University, Berlin, Germany
Guadalajara, University of, Mexico
Hebrew University, The, of Jerusalem, Israel
Hiroshima University, Japan
Imperial College of Science & Technology, London, England, U.K.
Instituto Venezolano de Investigaciones, Venezuela
King's College, London, England, U.K.
Laval, Université, Canada
Max Planck Institute for Psychiatry, Munich, West Germany
Memorial University of Newfoundland, Canada
National Cancer Center Research Institute, Japan
North Wales, University of, Wales, U.K.
Ottawa, University of, Canada
Puerto Rico, University of
Toronto, University of, Canada
University College, London, England, U.K.
University College, University of Dublin, Ireland
Wales, University of, Bangor, U.K.

6. Friday Evening Lectures, 1978

June 30

J. Richard Whittaker, The Wistar Institute ..... Egg cytoplasmic determinants of tissue differentiation

July 7

BEATRICE MINTZ ..... Putting mutant genes into mice
Institute for Cancer Research
Ninetieth Anniversary Lecture

July 13

Thursday
KRESIMIR KRNJEVIC ..... Intraneuronal factors that influence excitability
McGill University 1) Regulation of neuronal firing by internal Ca^{2+}
Alexander Forbes Lecturer
at the MBL

July 14

KRESIMIR KRNJEVIC ..... 2) Intracellular actions of synaptic transmitters

July 21

DANIEL MAZIA ..... Mitotic centers
University of California, Berkeley

July 28

ROBERT H. BURRIS ..... Omnipresent N_2—how organisms convert it to
University of Wisconsin NH_3

August 4

STEPHEN JAY GOULD ..... Ontogeny and phylogeny
Harvard University

August 11

HARRIS RIPPS ..... A ray of light on night blindness
New York University
Medical Center

August 18

ROBERT H. WHITTAKER ..... Theory of species diversity
Cornell University

August 25

DANIEL ALKON ..... A neural basis for associative learning
Laboratory of Biophysics,
NINCDS, at the MBL

7. Members of the Corporation, 1978

Including Action of 1978 Annual Meeting

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Kingwell, The Rev. and Mrs. Wilbur J.
Kinnard, Mr. and Mrs. L. R.
Kivy, Dr. and Mrs. Peter
Koelsch, Mr. and Mrs. Herbert
Kohn, Dr. and Mrs. Henry I.
Koller, Dr. and Mrs. Lewis R.
Kris, Dr. and Mrs. Anton O.
Kuffler, Mrs. Stephen W.
Laderman, Mr. and Mrs. Ezra
Lash, Dr. and Mrs. James
Laster, Dr. and Mrs. Leonard
Laufer, Dr. and Mrs. Hans
Lawrence, Mr. Frederick V.

Lawrence, Mrs. William
Lazarow, Mrs. Arnold
Lemann, Mrs. Lucy B.
Lenher, Mr. and Mrs. Samuel
Levine, Dr. and Mrs. Rachmiel
Leventhal, Ms. Monika Meyer
Lewis, Mr. T. Hohn
Lillie, Mrs. Karl C.
Lilly, Mr. and Mrs. Josiah K., III
Lobb, Prof. John
Loeb, Mrs. Robert F.
Long, Mrs. G. C.
Lorand, Mrs. Laszlo
Lowengard, Mrs. Joseph
Lowe, Dr. and Mrs. Charles W.
Luria, Dr. and Mrs. S. E.
Mackey, Mr. and Mrs. William K.
MacLeish, Mrs. William
MacNary, Mr. B. Glenn
MacNichol, Dr. and Mrs. Edward F., Jr.
Maher, Miss Anne Camille
Marks, Dr. and Mrs. Paul A.
Marsland, Dr. and Mrs. Douglas
Martyna, Mr. and Mrs. Joseph
Marvin, Dr. Dorothy H.
Maser, Dr. and Mrs. Morton
Mastroianni, Dr. and Mrs. L., Jr.
Mather, Mr. and Mrs. Frank J., III
Matthiessen, Mr. and Mrs. G. C.
Mayor, Mrs. James W., Sr.
McCusker, Mr. and Mrs. Paul T.
McElroy, Mrs. Nella W.
McGillicuddy, Dr. and Mrs. J. J.
McLane, Mrs. T. Thorne
Meigs, Mr. and Mrs. Arthur
Meigs, Dr. and Mrs. J. Wister
Melillo, Dr. and Mrs. Jerry
The Mellon Foundation
Metz, Mrs. Charles B.
Meyers, Mr. and Mrs. Richard
Miller, Dr. Daniel A.
Mixter, Mr. and Mrs. W. J., Jr.
Montgomery, Dr. and Mrs. Charles H.
Moore, Mr. John W.
Morse, Mr. and Mrs. Charles L., Jr.
Morse, Mr. and Mrs. Richard S.
Moses, Mr. and Mrs. George L.
Moul, Mrs. Edwin T.
Neuberger, Mrs. Harry H.
Newton, C. H., Builders, Inc.
Newton, Miss Helen K.
Nichols, Mrs. George
Nickerson, Mr. and Mrs. Frank L.
Norman, Mr. and Mrs. Andrew E.
Normandie Foundation
O'Herron, Mr. and Mrs. Jonathan
Olmsted, Mr. and Mrs. Christopher
Ortins, Mr. Armand
Pappas, Dr. and Mrs. George D.
Park, Mr. and Mrs. Franklin A.
Park, Mr. and Mrs. Malcolm S.
Parmenter, Miss Carolyn L.
Parmentier, Mr. George L.
Patten, Mrs. Bradley M.
Pecan, Ms. Erene V.
Pendergast, Mrs. Claudia
Pendelton, Dr. and Mrs. Murray E.
Pennington, Miss Anne H.
Perkins, Mr. and Mrs. Courtland D.
Person, Dr. and Mrs. Philip
Peterson, Mr. and Mrs. E. Gunnar
Peterson, Mr. and Mrs. E. Joel
Philippe, Mr. and Mrs. Pierre
Porter, Dr. and Mrs. Keith R.
Prosser, Mrs. C. Ladd
Putnam, Mr. Allan Ray
Putnam, Mr. and Mrs. W. A., III
Ratcliffe, Mr. Thomas G., Jr.
Raymond, Dr. and Mrs. Samuel
Read, Ms. Lee
Redfield, Dr. and Mrs. Alfred C.
Renek, Mr. and Mrs. Morris
Reynolds, Dr. and Mrs. George
Reynolds, Mr. and Mrs. James T.
Reznikoff, Dr. and Mrs. Paul
Riggs, Mr. and Mrs. Lawrason, III
Rhina, Mr. and Mrs. John R.
Robb, Ms. Alison A.
Robertson, Mrs. C. Stuart
Robertson, Dr. and Mrs. C. W.
Robinson, Dr. and Mrs. Denis M.
Rogers, Mrs. Julian
Root, Mrs. Walter S.
Ross, Mrs. John
Rowe, Mrs. William S.

Rubin, Dr. Joseph
Rugh, Mrs. Roberts
Russell, Mr. and Mrs. Henry D.
Ryder, Mr. and Mrs. Francis C.
Saunders, Dr. and Mrs. John W.
Saunders, Mrs. Lawrence
Savery, Mr. Rober
Sawyer, Mr. and Mrs. John E.
Schlesinger, Mrs. R. Walter
Scott, Mrs. George T.
Scott, Mrs. Norman
Sears, Mr. and Mrs. Harold B.
Segal, Dr. and Mrs. Sheldon
Shapiro, Mrs. Harriet S.
Shemin, Dr. and Mrs. David
Sheprow, Dr. and Mrs. David
Sherman, Dr. and Mrs. Irwin
Simkins, Mrs. Willard S.
Slater, Mr. David
Smith, Mr. and Mrs. Dietrich C.
Smith, Mrs. Homer P.
Smith, Mr. and Mrs. Robert I.
Smith, Mr. VanDorn C.
Snider, Mr. Eliot
Sonnebend, Mr. and Mrs. Paul
Stracher, Dr. and Mrs. Alfred
Steinbach, Dr. and Mrs. H. B.
Stetten, Dr. and Mrs. Dewitt, Jr.
Stone, Dr. and Mrs. William
Stuart, Ann
Stunkard, Dr. Horace
Swanson, Dr. and Mrs. Carl P.
Swope, Mr. and Mrs. Gerard L.
Swope, Mr. and Mrs. Gerard, Jr.
Swope, Miss Henrietta H.
Tanner, Dr. and Mrs. Harvey A.
Tartakoff, Dr. Helen
Taylor, Dr. and Mrs. W. Randolph
Tietje, Mr. and Mrs. Emil D., Jr.
Tittler, Mrs. Sylvia
Todd, Mr. and Mrs. Gordon F.
Tolkan, Mr. and Mrs. Norman N.
Tompkins, Mrs. B. A.
Trager, Mrs. William
Troll, Dr. and Mrs. Walter
Tully, Mr. and Mrs. Gordon F.
Valois, Mr. and Mrs. John
Veeder, Mrs. Ronald A.
Vincent, Mrs. Walter S.
Waksman, Dr. and Mrs. Bryon H.
Ware, Mr. and Mrs. J. Lindsay
Warren, Dr. and Mrs. Shields
Watt, Mr. and Mrs. John B.
Weisberg, Mr. and Mrs. Alfred M.
Wengren, Mr. Richard
Wexler, Robert H. Foundation
Wheatley, Dr. Marjorie A.
Wheeler, Dr. and Mrs. Paul S.
Wheeler, Dr. and Mrs. Ralph E.
Whitney, Mr. and Mrs. Geoffrey G., Jr.
Wichterman, Dr. and Mrs. Ralph
Wickersham, Mr. and Mrs. A. A. Tilney
Wickersham, Mrs. James H., Jr.
Wilhelm, Dr. Hazel S.
Wilson, Mr. and Mrs. Robert E., Jr.
Witmer, Dr. and Mrs. Enos E.
Wolfinsohn, Mr. and Mrs. Wolfe
Woodwell, Mrs. George
Yntema, Dr. and Mrs. Chester L.
Zinn, Dr. and Mrs. Donald J.
Zwilling, Mrs. Edgar

VI. REPORT OF THE LIBRARIAN

This year we received some relief in the crowded stack area. Dr. Gross approved the purchase of 300 additional metal shelves to be added to the top stack in the wing. This stack was not filled to capacity when originally built, so we were able to fit nine banks of shelving along each side and five banks at the back without crowding the Reserve Desks on the top floor. This new shelf space will hold approximately three years' growth in journal acquisitions. Before the end of that period we expect to have additional space in the Lillie Building made available to the Library.

Relief for Library salaries was also achieved this year. Specific MBL employee salaries were presented to the Members of the Board and Executive Com-

mittee. The result was increased salaries for many, somewhat comparable to those at the Woods Hole Oceanographic Institution.

A thorough inventory of the book section revealed a total of 25,000 volumes. When added to the journal collection, the complete volume count is now 180,000.

For this annual printed record, I would like to include a part of the preface of Dr. Stephen J. Gould's new book, "Ontogeny and Phylogeny," Harvard University Press, 1977:

> ". . . and, above all, to an institution that has its own humanity and seems to me more an organism than a place—the Library of the Marine Biological Laboratory at Woods Hole. Where else would an idiosyncratic worker like me find a library open all the time, free from the rules and bureaucracy that stifle scholarship and "protect" books only by guarding them from use. It is an anomaly in a suspicious and anonymous age. May it survive as it is, despite all the improbabilities."

VII. REPORT OF THE TREASURER

I am pleased to report that the Marine Biological Laboratory made progress in 1978 in reducing its operating deficit. In 1978, the operating loss was $35,454, a reduction from $85,414 in 1977. Operating results excluded approximately $282,000 of depreciation charges recorded in each year.

Total revenues in 1978 amounted to $2,502,077, an increase of $420,583 over the prior year. The principal sources of the increased revenues were higher tuition as the result of expanded programs and increased overhead recovery due to a 5% increase in the overhead rate, coupled with increased year-round occupancy. In addition, unrestricted gifts increased by almost $100,000.

Total 1978 expenses amounted to $2,537,531, an increase of $370,623 over 1977. This increase was principally due to performing critical plant repairs which had been deferred in prior years for budgetary reasons, expansion of the Continuing Education programs begun in late 1977, the addition of two new course offerings, and a significant increase in the volume of the Chemical Room. Of this $2,537,531, recurring operating expenses represented $2,418,697, an increase of $251,789, or 12%, over 1977. In our report last year, we mentioned that our 1978 budget anticipated that such expenses would increase only 5% from the 1977 level. This unanticipated and unfavorable variation in the expense budget is mainly attributable to the additional Continuing Education course offerings and greater volume in the Chemical Room—both of which generated additional offsetting revenues.

Investment income amounted to $341,846 in 1978, an increase of 6% over 1977. Total investments at December 31, 1978 were valued at $5,605,947 at market and $5,739,745 at cost. The return, as a percentage of average market value, was 6.2%.

As was mentioned to you last year, methods of improving the rate of return on investments were to be examined during 1978. Accordingly, based on the recommendation of the Investment Committee, the Executive Committee, at its February 1979 meeting, appointed Standish, Ayer & Wood, Inc. of Boston to be the investment advisor for all of the Laboratory's investment portfolios.

We have budgeted in 1979 for a further reduction in the operating deficit and our objective for 1980 is to record a modest surplus.

The following is a statement of the auditors:

To the Trustees of Marine Biological Laboratory, Woods Hole, Massachusetts:

We have examined the balance sheets of Marine Biological Laboratory as of December 31, 1978 and 1977, and the related statements of current funds revenues, expenditures, and other changes and changes in fund balances for the years then ended. Our examinations were made in accordance with generally accepted auditing standards and, accordingly, included confirmation from the custodians of securities owned at December 31, 1978 and 1977, and such tests of the accounting records and such other auditing procedures as we considered necessary in the circumstances.

As more fully described in Note B to the financial statements, the Laboratory excludes certain costs of buildings and equipment from the balance sheet. In our opinion, generally accepted accounting principles require that such costs be included as investment in plant in the financial statements.

In our opinion, except for the effects on the financial statements of the matter discussed in the preceding paragraph, the aforementioned financial statements present fairly the financial position of Marine Biological Laboratory at December 31, 1978 and 1977, and its current funds revenues, expenditures and other changes and the changes in fund balances for the years then ended, in conformity with generally accepted accounting principles applied on a consistent basis.

Our examinations were made primarily for the purpose of rendering an opinion on the basic financial statements (pages 94 to 99, inclusive), taken as a whole. The summary of investments (page 102) although not considered necessary for a fair presentation of the financial position at December 31, 1978 and 1977, current fund revenues, expenditures and other changes, and the changes in fund balances for the years then ended in accordance with generally accepted accounting principles, is presented primarily for purposes of supplementary analysis. This additional information has been subjected to the audit procedures applied in the examination of the basic financial statements and, in our opinion, is fairly stated in all material respects in relation to the basic financial statements taken as a whole.

Boston, Massachusetts
March 30, 1979

COOPERS & LYBRAND

MARINE BIOLOGICAL LABORATORY

Balance Sheets

December 31, 1978 and 1977

Assets	*1978*	*1977*
Current Funds:		
Unrestricted:		
Cash, including deposits at interest	$ 382,396	$ 517,513
Accounts receivable, net of allowance for uncollectible accounts of $4,500 in 1978 and $12,955 in 1977	699,708	540,743
Other assets	15,367	15,996
Due to restricted current funds	(278,075)	(294,485)
Due from invested funds	103,248	105,133
Total unrestricted	922,644	884,900
Restricted:		
Cash	18,973	87,013
Investments, at cost; market value: 1978—$1,297,551; 1977—$1,118,917 (Note A, Schedule 1)	1,297,530	1,112,683
Due from unrestricted current fund	278,075	294,485
Due from invested funds	260,967	173,191
Total restricted	1,855,545	1,667,372
Total current funds	$ 2,778,189	$ 2,552,272
Invested Funds:		
Cash	6,982	102,031
Investments, at cost; market value: 1978—$3,950,184; 1977—$3,799,434 (Note A, Schedule 1)	4,084,003	3,822,798
Due to unrestricted current fund	(103,248)	(105,133)
Due to restricted current funds	(260,967)	(173,191)
Total invested funds	$ 3,726,770	$ 3,646,505
Plant Fund:		
Land, buildings and equipment at cost (Note B)	12,421,929	12,374,587
Less accumulated depreciation	4,060,407	3,778,545
Total plant fund	$ 8,361,522	$ 8,596,042

The accompanying notes are an integral part of the financial statements.

MARINE BIOLOGICAL LABORATORY

Balance Sheets

December 31, 1978 and 1977

Liabilities and Fund Balances	*1978*	*1977*
Current Funds:		
Unrestricted:		
Accounts payable and accrued expenses	$ 316,657	$ 195,418
Deferred income	61,432	62,131
Fund balance	544,555	627,351
Total unrestricted	922,644	884,900
Restricted:		
Fund balances:		
Unexpended gifts and grants	1,777,677	1,597,841
Unexpended income of endowment funds	77,868	69,531
Total restricted	1,855,545	1,667,372
Total current funds	$ 2,778,189	$ 2,552,272
Invested Funds:		
Endowment funds	2,174,027	2,172,160
Quasi-endowment funds	934,143	934,143
Retirement fund (Note C)	618,600	540,202
Total invested funds	$ 3,726,770	$ 3,646,505
Plant Fund:		
Invested in plant	8,361,522	8,596,042
Total plant fund	$ 8,361,522	$ 8,596,042

The accompanying notes are an integral part of the financial statements.

MARINE BIOLOGICAL LABORATORY

Statements of Current Funds Revenues, Expenditures, and Other Changes

for the years ended December 31, 1978 and 1977

	Unrestricted		*Restricted*		*Total*	
	1978	*1977*	*1978*	*1977*	*1978*	*1977*
Revenues:						
Instruction:						
Tuition	$ 236,391	$ 164,495	$ 43,100	$ 35,200	$ 279,491	$ 199,695
Grants and contracts:						
Government	7,360	7,468	215,759	128,197	223,119	135,665
Private			70,446	33,258	70,446	33,258
Research:						
Laboratory rentals	503,576	424,631			503,576	424,631
Grants and contracts:						
Government	206,741	166,970	861,515	646,497	1,068,256	813,467
Private	90,550	84,917	390,692	457,896	481,242	542,813
Dormitory	320,020	285,285			320,020	285,285
Dining Hall	157,270	134,454			157,270	134,454
Library	135,028	126,652			135,028	126,652
Biological Bulletin	86,171	85,430			86,171	85,430
Support departments:						
Research Services	187,741	142,420			187,741	142,420
Marine resources	79,822	85,131			79,822	85,131
Investment income	159,703	158,361	41,417	39,359	201,120	197,720
Gifts	240,563	158,850	132,864	101,013	373,427	259,863
Other	91,141	56,430			91,141	56,430
Total revenues	2,502,077	2,081,494	1,755,793	1,441,420	4,257,870	3,522,914

	Unrestricted		*Restricted*		*Total*	
	1978	*1977*	*1978*	*1977*	*1978*	*1977*
Operating expenditures:						
Instruction	194,993	123,037	219,587	71,349	414,580	194,386
Research	3,813	1,386	1,264,631	1,104,393	1,268,444	1,105,779
Scholarships and stipends			216,958	216,736	216,958	216,736
Dormitory	135,769	140,358			135,769	140,358
Dining Hall	149,249	130,349			149,249	130,349
Library	235,401	208,149	22,757	22,043	258,158	230,192
Biological Bulletin	87,273	77,165			87,273	77,165
Support departments:						
Research services	354,485	305,847	25,400	20,415	379,885	326,262
Marine resources	218,794	216,278	34	255	218,828	216,533
Administration	514,204	462,479	4,263	4,048	518,467	466,527
Plant operation	643,550	501,860	2,163	2,181	645,713	504,041
Total expenditures	2,537,531	2,166,908	1,755,793	1,441,420	4,293,324	3,608,328
Excess of expenditures over revenues	(35,454)	(85,414)	—	—	(35,454)	(85,414)
Transfers and additions:						
Excess of restricted gifts, grants and investment income received over amounts expended			188,173	99,834	188,173	99,834
From plant fund—proceeds of sale of plant assets		3,000				3,000
To plant fund—donated unrestricted real estate	(47,342)				(47,342)	
From quasi-endowment fund		335,469				335,469
Net transfers and additions	(47,342)	338,469	188,173	99,834	140,831	438,303
Net increase (decrease) in fund balances	$ (82,796)	$ 253,055	$ 188,173	$ 99,834	$ 105,377	$ 352,889

The accompanying notes are an integral part of the financial statements.

MARINE BIOLOGICAL LABORATORY

Statements of Changes in Fund Balances

for the years ended December 31, 1978 and 1977

	Current Funds		*Invested Funds*			
1977	*Unrestricted*	*Restricted*	*Endowment*	*Quasi-Endowment*	*Retirement*	*Plant Fund*
Balances at December 31, 1976	$ 374,296	$ 1,567,538	$2,147,067	$1,260,509	$488,245	$8,848,333
Increases:						
Unrestricted current fund revenues	2,081,494					
Grants and gifts		1,622,821				29,000
Realized net gains on sale of investments			25,093	9,103		
Investment income		142,588			20,966	
Proceeds of sale of equipment						3,000
Addition to pension fund					50,150	
Tuition		35,200				
Decreases:						
Instruction, research and general expenditures	(2,166,908)	(1,441,420)				
Indirect costs		(259,355)				
Payments to pensioners					(19,159)	
Depreciation						(281,291)
Transfers—additions (deductions):						
Proceeds of sale of equipment	3,000					(3,000)
Transfer to current funds	335,469			(335,469)		
Balances at December 31, 1977	627,351	1,667,372	2,172,160	934,143	540,202	8,596,042

1978	Current Funds		Invested Funds			Plant Fund
	Unrestricted	Restricted	Endowment	Quasi-Endowment	Retirement	
Increases:						
Unrestricted current fund revenues	2,502,077					
Grants and gifts		2,055,387				
Realized net gains on sale of investments			1,867			
Investment income		150,130			32,013	
Addition to pension fund					65,573	
Tuition		43,100				
Decreases:						
Instruction, research and general expenditures	(2,537,531)	(1,755,793)				
Indirect costs		(304,651)				
Payments to pensioners					(19,188)	
Depreciation						(281,862)
Transfers—additions (deductions):						
Transfer to plant fund	(47,342)					47,342
Balances at December 31, 1978	$ 544,555	$ 1,855,545	$2,174,027	$ 934,143	$618,600	$8,361,522

The accompanying notes are an integral part of the financial statements.

MARINE BIOLOGICAL LABORATORY

Notes to Financial Statements

A. *Significant Accounting Policies:*

Basis of Presentation—Fund Accounting

In order to ensure observance of limitations and restrictions placed on the use of resources available to the Laboratory, the accounts of the Laboratory are maintained in accordance with the principles of "fund accounting." This is the procedure by which resources are classified into separate funds in accordance with activities or objectives specified. In the accompanying financial statements, funds that have similar characteristics have been combined.

Externally restricted funds may only be utilized in accordance with the purposes established by the source of such funds. However, the Laboratory retains full control over the utilization of unrestricted funds. Restricted gifts, grants, and other restricted resources are accounted for in the appropriate restricted funds. Restricted current funds are reported as revenue when expended for current operating purposes. Unrestricted revenue is reported as revenue in the unrestricted current fund when received.

Endowment funds are subject to restrictions requiring that the principal be invested and only the income utilized. Quasi-endowment funds have been established by the Laboratory for the same purposes as endowment funds, however, any portion of these funds may be expended.

Investments

Investments purchased by the Laboratory are carried at cost. Investments donated to the Laboratory are carried at fair market value at date received. For determination of gain or loss upon disposal, cost is determined based on the specific identification method.

Investment Income and Distribution

The Laboratory follows the accrual basis of accounting except that investment income is recorded on a cash basis. The difference between such basis and the accrual basis does not have a material effect on the determination of investment income earned on a year-to-year basis.

Investment income includes income from the investments of specific funds and from the pooled investment account. Income from the pooled investment account is distributed to the participating funds on the basis of the market value at the beginning of the quarter, adjusted for the cost of any additions or disposals during the quarter.

Indirect Cost Recovery

The Laboratory recovers indirect costs by charging occupants of laboratory space fees based on a negotiated fixed indirect cost rate for the period the space was occupied. When actual rates are subsequently determined, the difference is reflected in the next negotiated fixed rate.

B. *Land, Buildings and Equipment:*

Following is a summary of the plant fund assets:

Classification	*1978*	*1977*
Land	$ 639,693	$ 639,693
Buildings	10,190,430	10,143,088
Equipment	1,591,806	1,591,806
	12,421,929	12,374,587
Less accumulated depreciation	4,060,407	3,778,545
	$ 8,361,522	$ 8,596,042

The original cost of land, buildings and related initial furnishing equipment is capitalized when the assets are acquired. The cost of subsequent additions and purchases, repairs and remodeling is expensed when incurred. Equipment and remodeling expenditures amounted to approximately $125,000 and $110,000 in 1978 and 1977, respectively.

Depreciation is computed using the straight-line method over estimated useful lives of 40 years for buildings and 20 years for equipment.

C. *Retirement Fund:*

The Laboratory has a noncontributory pension plan for substantially all full-time employees which complies with the requirements of the Employee Retirement Income Security Act of 1974. The actuarially determined pension expenses charged to operations in 1978 and 1977 were $76,374 and $64,277, respectively. The Laboratory's policy is to fund pension costs accrued.

D. *Pledges and Grants:*

As of December 31, 1978 and 1977, the following amounts remain to be received from previous gifts and grants for specific research and instruction programs, and are expected to be received as follows:

	1978	*1977*
1978		$1,697,486
1979	$2,196,201	115,000
1980	28,000	
	$2,224,201	$1,812,486

SCHEDULE I

MARINE BIOLOGICAL LABORATORY

SUMMARY OF INVESTMENTS

December 31, 1978 and 1977

	Cost		*Market*		*Investment Income*	
	1978	*1977*	*1978*	*1977*	*1978*	*1977*
Invested Funds:						
U. S. Government securities	$ 610,661	$ 444,638	$ 594,205	$ 448,295	$ 35,718	$ 40,923
Corporate bonds	689,185	770,012	583,572	676,789	42,969	48,681
Common stocks	2,080,664	2,080,655	2,123,631	2,189,492	131,333	125,462
Commercial paper	—	—	—	—	—	10,990
Preferred stocks	83,032	83,032	54,548	61,072	3,654	4,468
Units in combined pension fund	602,912	426,912	576,679	406,237	33,114	9,394
Real estate	17,549	17,549	17,549 (1)	17,549 (1)		
Total	$4,084,003	$3,822,798	$3,950,184	$3,799,434	246,788	239,918
Less custodian fees					14,867	11,835
					231,921	228,083
Current Restricted Funds:						
U. S. Government securities	1,297,530	1,112,683	1,297,551	1,118,917	90,082	86,748

Current Unrestricted Funds:		
Earned on corporate savings account	19,843	7,084
Net investment income	341,846	321,915
Disposition of investment income:		
Restricted for current use:		
Research	102,538	95,789
Scholarships	25,715	25,289
Library	21,877	21,510
	150,130 (2)	142,588 (2)
Retirement fund	32,013	20,966
Unrestricted—utilized in current operations	159,703	158,361
	$341,846	$321,915

(1) At cost.
(2) $41,417 in 1978 and $39,359 in 1977 was utilized in current restricted operations.

Reference: *Biol. Bull.* 157: 104–111. (August 1979)

CYTOLOGY AND POLYSACCHARIDE CYTOCHEMISTRY OF THE GILL OF THE AMERICAN EEL, *ANGUILLA ROSTRATA*

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Much work has been published on the gills of teleosts, (Vickers, 1961; Munshi, 1964; Steen and Kruysse, 1964; Hughes and Grimstone, 1965; Newstead, 1967; Hughes and Wright, 1970). Morgan and Tovell (1973) and Morgan (1974) described the structure and development of secondary lamellae in gills of trout. Work on eel gills has included cytological studies, (Ogawa, 1962; Yamada and Yokote, 1975), electron microscopic investigations of gill fine structure (Mizuhira, Amakawa, Yamashina, Shirai, and Utida, 1969), and studies of osmotic adaptation of eels to freshwater and seawater for the Japanese eel, *Anguilla japonica*. Keys and Willmer (1932) described chloride-secreting cells in the common eel, *Anguilla vulgaris;* Getman (1950) and Doyle and Epstein (1972) reported on osmotic effects and adaptive changes of chloride cells in the American eel, *Anguilla rostrata*.

The present study describes cytological details of gill filaments and secondary lamellae of freshwater-adapted early juvenile and adult American eels. Arrangement and morphology of epithelium, gill rays, mucous-secreting cells, pillar cells, and blood spaces within secondary lamellae are described and illustrated. Polysaccharide cytochemistry as revealed by periodic-acid–Schiff (PAS) staining as well as Alcian blue reactions at various pH values and with increasing concentrations of $MgCl_2$ of all cell types and connective tissue are described; results are compared with similar tissues in the *Anguilla* species, and in teleosts in general.

Materials and Methods

Fresh water-adapted juvenile and adult eels were obtained from the Aquaculture Laboratories, Mercer Generating Station, Trenton, New Jersey. Animals were killed by decapitation and gills were excised and fixed in Davidson's solution at room temperature for 24 hr. Tissues were processed and embedded in paraffin (Humanson, 1972); sections were cut at 5 μm.

Stains used were hematoxylin and eosin, periodic-acid–Schiff (PAS) (McManus, 1948), Feulgen Picro-Amido-black (modified from Farley, 1969), Alcian blue 8GX (Gurr, London) at pH 0.5 (Lev and Spicer, 1964), Alcian blue pH 2.6, and Alcian blue pH 5.7 with post-treatment in ascending concentrations of $MgCl_2$ (Scott and Dorling, 1965; Mowry, 1970).

Slides were examined and photographed with the Zeiss Photomicroscope II using Kodak SO-410 Monochrome Photomicrography film with a Kodak #66 Wratten gelatin filter.

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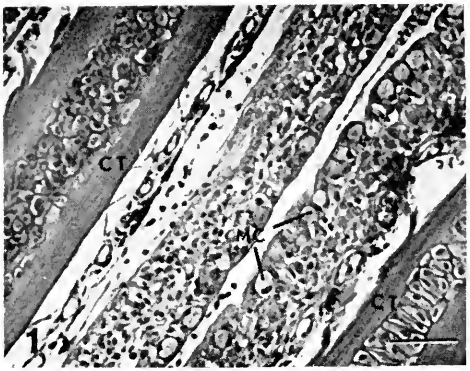

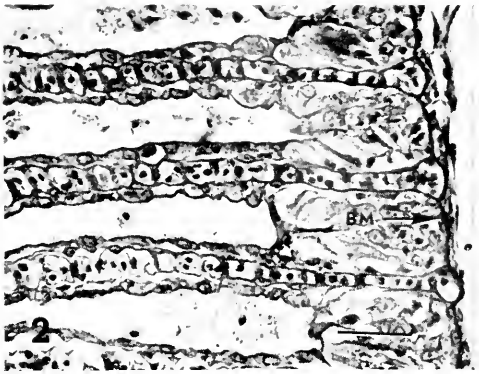

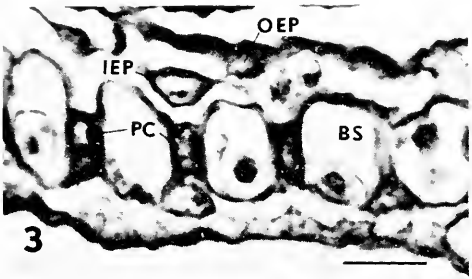

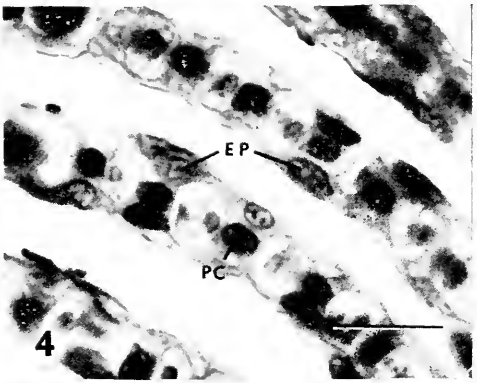

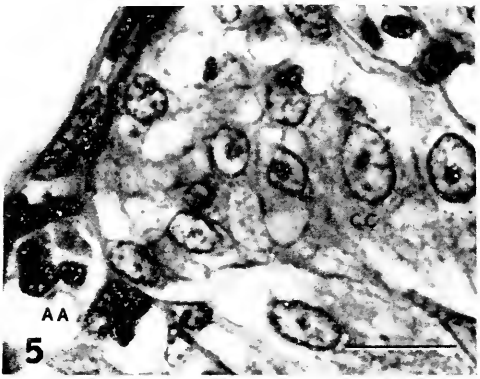

FIGURE 1. Cartilaginous gill rays (CT) support filaments of the eel gill. Mucus-secreting cells (MC) appear at bases of filaments. Hematoxylin and eosin stained; scale bar is 25 μm.

FIGURE 2. Basement membrane (BM) in gill filament stained by PAS. Scale bar is 25 μm.

FIGURE 3. Two-celled layered epithelium of secondary lamella of adult eel. Nuclei of inner cell layer (IEP) shown over pillar cell body (PC); pillar cells delimit blood spaces (BS) in lamellae. Note nucleus of outer layer of epithelium (OEP); scale bar is 10 μm.

FIGURE 4. Secondary lamellae of early juvenile eel, showing single epithelial layer (EP) with nuclei over pillar cells (PC). Blood cells crowd blood spaces between pillar cells. Hematoxylin and eosin stained; scale bar is 10 μm.

FIGURE 5. Chloride cells (CC) at the base of lamellae near afferent lamellar artery (AA). Note the prominent nucleolus, visible with Feulgen Picro-Amido-Black stain; scale bar is 10 μm.

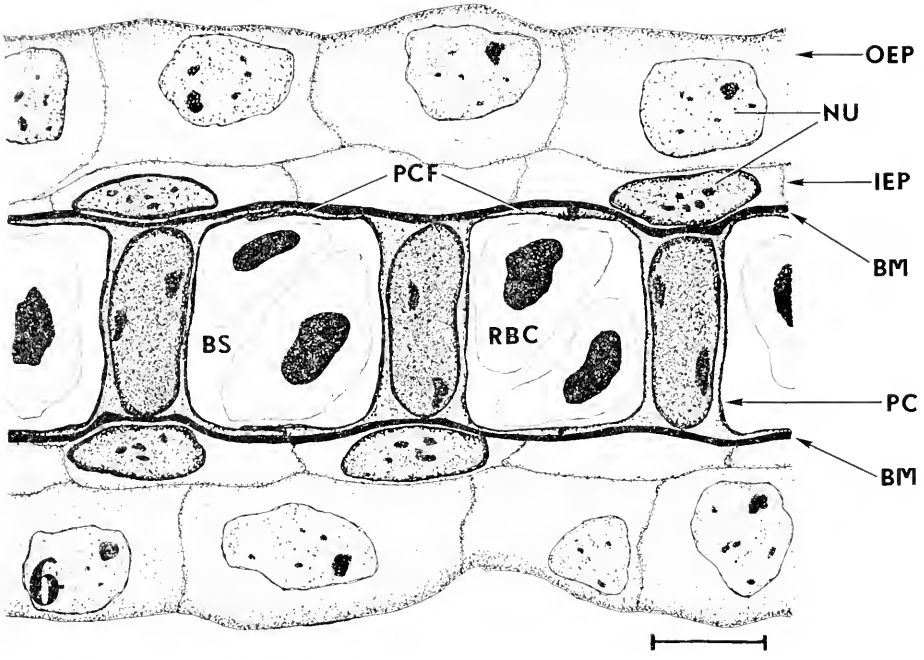

FIGURE 6. Diagram of secondary lamella of an adult eel. Pillar cells (PC) and their cytoplasmic flanges (PCF) delimit blood spaces (BS) within lamella. Two-cell layered epithelium supported by basement membrane (BM) covers secondary lamella. Nuclei (NU) of the inner epithelium (IEP) are more flattened than those of the outer epithelial cells (OEP) and lie over pillar-cell bodies. Scale bar is 5 μm.

RESULTS

General morphology

Gills of the eel consist of four bony gill arches on either side of the pharynx. Gill arches bear two rows of flattened filaments supported for approximately two-thirds of their length by cartilagenous gill rays. Filaments each bear two rows of secondary lamellae.

Cytology and cytochemistry

Gill filaments contain a wide, centrally-located gill ray (Fig. 1) which stains intensely in PAS, Alcian blue pH 0.5, and Alcian blue pH 5.7 with high concentrations of $MgCl_2$ (Table I). Large mucus-secreting cells are found at both base and tip of filaments; cell secretions stain prominently with PAS reactions as well as with Alcian blue pH 2.6. They appear unstained in Alcian blue pH 0.5. A well-defined basement membrane supports the filament epithelium (Fig. 2); this connective tissue encircles afferent lamellar arteries and extends into secondary lamellae as the basement membrane for lamellar epithelia. It is stained intensely by PAS, Alcian blue pH 0.5, and Alcian blue pH 5.7 with concentrations of $MgCl_2$ up to 1.30 M (Table I).

Blood supplied by afferent lamellar arteries flows through blood spaces delimited by pillar cells and their cytoplasmic flanges (Figs. 3, 6). Pillar cell nuclei typically contain two small areas of chromatin material with fine granular material distributed around the margins of the nuclear envelope. Nuclei occupy nearly the entire volume of the cell, with a width of approximately 4 μm and a height of 8 μm.

A two-layered epithelium covers secondary lamellae in adult eels, supported by a basement membrane continuous along pillar-cell flanges. Nuclei of inner epithelial layers frequently lie directly over pillar-cell bodies (Figs. 3, 6), with nuclei flattened against the basement membrane. Several dense areas of chromatin appear near the center of the nucleus, with smaller areas of chromatin adhering to the nuclear envelope. Nuclei of the outer epithelial layer appear more spherical than inner epithelial nuclei; one dark nucleolus is usually visible surrounded by smaller chromatin masses (Fig. 6). Cytoplasm of outer epithelial cells is much less dense than that of inner epithelial cells, with distal portions of cytoplasm staining lightly with Alcian blue pH 2.6. A single-layer epithelium covers secondary lamellae in early juvenile eels (Fig. 4); epithelial nuclei are located over pillar-cell bodies.

Chloride-secreting cells surround afferent lamellar arteries, crowded in the "v" between adjacent secondary lamellae (Fig. 5). Cells are large, with a granular, eosinophilic cytoplasm and a large nucleus with a prominent nucleolus surrounded by small regions of chromatin material (Fig. 5). Crowding of cells in this area of filaments causes them to be elongated, with nuclei typically located at the base of cells. Chloride-cell cytoplasm stains heavily in PAS reactions and exhibits some reaction to Alcian blue pH 2.6, especially at distal portions of cells. In Alcian blue pH 0.5 there is little reaction in chloride cells; further, in Alcian blue pH 5.7, cells reach their extinction point at a critical electrolyte concentration of 0.030 M $MgCl_2$ (Table I).

Discussion

Cartilage supporting gill filaments shows heavy concentrations of sulfated mucopolysaccharides, staining with Alcian blue pH 0.5 (Lev and Spicer, 1964). Polysulfate groups stain selectively as pH is lowered to a point below the pH of carboxyl groups; sulfate groups still dissociated are free to bind the cationic dye. Mowry (1963) reported that, at low pH, hyaluronic acid, heparin, and chondroitin readily stain in Alcian blue; the color reactions are identical but chondroitin comprises only a minor portion of extracellular material. However, the sulfate ester derivatives, chondroitin sulfate A and chondroitin sulfate C, are major structural components of vertebrate cartilage (Lehninger, 1970). In Alcian blue pH 5.7 cartilage reacts intensely when treated with high concentrations of $MgCl_2$ (Table I). Mowry (1970) noted that binding of the cationic dye in 0.30 M or higher concentrations of $MgCl_2$ indicated the presence of sulfated polyanions; the higher the ionic strength of Mg^{2+} the higher the degree of sulfation. Addition of $MgCl_2$ dissolves the stained complexes formed by the polyanion's reaction with Alcian blue (Scott and Dorling, 1965). The critical electrolyte concentration (lowest concentration of Mg^{2+} at which a given polyanion is no longer stainable) is 1.20 M $MgCl_2$ for gill-ray cartilage. The presence of highly sulfated chondroitin

TABLE I

Results of cytochemical tests.

Stain	Mucous cells	Basement membrane	Cartilage	Chloride cells
Hematoxylin and eosin	+	++	+++	+++
PAS	+++++	++++	+++	+++
Feulgen-Picro Amido-black	++	+++	+++	++++
Alcian blue pH 0.5	----	+++++	++++	++
Alcian blue pH 2.6	++++	++	+++	++
Alcian blue pH 5.7 with				
0.00 M $MgCl_2$	+++	+++	+++	++
0.05 M $MgCl_2$	+++	+++	+++	++
0.10 M $MgCl_2$	+++	+++	+++	+
0.20 M $MgCl_2$	---	+++	+++	+
0.30 M $MgCl_2$	---	+++	+++	---
0.40 M $MgCl_2$	---	+++	+++	---
0.50 M $MgCl_2$	---	+++	+++	---
0.60 M $MgCl_2$	---	+++	+++	---
0.70 M $MgCl_2$	---	++++	+++	---
0.80 M $MgCl_2$	---	++++	+++	---
0.90 M $MgCl_2$	---	++++	++	---
1.00 M $MgCl_2$	---	++++	++	---
1.10 M $MgCl_2$	---	++++	++	---
1.20 M $MgCl_2$	---	+++++	++	---
1.30 M $MgCl_2$	---	+++++	---	---

---, No reaction; ++, weak reaction; +++++, strong reaction.

derivatives in cartilage accounts for the intensity of reactions to Alcian blue pH 0.5 and Alcian blue pH 5.7 with high concentrations of $MgCl_2$.

Mucous cells found on filaments at both base and tip have secretions which exhibit properties of acid mucopolysaccharides with vicinal hydroxyl as well as carboxyl groups. Cell secretions stain heavily in Alcian blue pH 2.6, with no staining in Alcian blue pH 0.5. Further, mucous cell secretions reach their extinction point at a critical electrolyte concentration of 0.20 M $MgCl_2$ in Alcian blue pH 5.7 series (Table I). Mowry (1963) stated that basophilia in a 0.30 M or lower concentrations of $MgCl_2$ indicated the presence of polycarboxylates. In addition, Alcian blue is a reliable and sensitive test for carbohydrate polycarboxylates.

Some variation in the exact chemical structure of mucus secretions apparently exists within the *Anguilla* species. Yamada and Yokote (1975) reported the presence of sulfated mucopolysaccharides in mucous cells of the Japanese eel, *Anguilla japonica*, and described staining of cells in a range from 0.10 to 0.60 M $MgCl_2$; those secretions show a higher degree of sulfation than those of *Anguilla rostrata* using similar procedures (Table I). Mucous cells always appear at the base and tip of gill filaments in the eel, as described for trout (Morgan and Tovell, 1973) and many other teleosts (Newstead, 1967; Hughes and Wright, 1970).

Electron microscope studies on epithelial basement membranes in gills from rainbow trout (Morgan and Tovell, 1973) and other teleosts (Hughes and Grim-

stone, 1965; Newstead, 1967) showed a close association of basement membrane and pillar-cell flanges. In the present study, epithelial basement membrane is also in contact with cytoplasmic flanges of pillar cells (Fig. 6); it also surrounds afferent lamellar arteries at bases of secondary lamellae (Fig. 2) and supports gill-filament epithelia. The basement membrane shows heavy concentrations of sulfated mucopolysaccharides, staining intensely in Alcian blue pH 0.5; in Alcian blue pH 5.7 the basement membrane can also be distinguished when treated with concentrations of $MgCl_2$ up to 1.30 M (Table I). Magnesium chloride provides better discrimination of polyanions than other salts (Scott and Dorling, 1965) and the critical electrolyte concentration is a reflection of the type of polyanions present, as well as the concentration. In *Anguilla rostrata,* basement membranes exhibit an even greater degree of sulfation than gill-ray cartilage (Table I).

Blood supplied to secondary lamellae is channeled through blood spaces formed by overlapping pillar cell flanges (Fig. 6). These blood spaces appear to be somewhat similar to capillaries; however, no endothelium could be discerned. Studies on trout (Morgan and Tovell, 1973) and other teleosts (Hughes and Grimstone, 1965; Newstead, 1967; Hughes and Wright, 1970) indicated that blood spaces in secondary lamellae were entirely delimited by extensions of pillar-cell cytoplasm. Morgan (1974) further stated that, developmentally, pillar cells originate directly from mesenchymal cells, and not from cells having affinities to endothelial cells.

Above the pillar cells and basement membrane a two-cell, layered epithelium covers secondary lamellae in adult eels (Fig. 3). Nuclei of inner epithelial cells lie directly over pillar cell bodies (Figs. 3, 6); similar arrangements have been reported to exist in trout (Morgan and Tovell, 1973) and many other teleosts (Newstead, 1967; Hughes and Wright, 1970). Hughes and Grimstone (1965) suggested that location of epithelial nuclei over pillar cell bodies could be adaptive, as little gas exchange would be expected at those points. In the present study, the average water-to-blood distance for adult eels is 5 to 8 μm, but at points where epithelial nuclei are located, the distance is nearly doubled from the free edge to the blood spaces. In early juvenile eels (2–3 g body weight) there is only one epithelial layer in secondary lamellae; nuclei of these cells invariably lie over pillar cell bodies (Fig. 4). Here, the water-to-blood distance is only 3 to 4 μm.

Keys and Willmer (1932) reported only a single layer of epithelium in secondary lamellae of the common eel, *Anguilla vulgaris,* but they gave no information concerning the size of animals used in their study. From the evidence of the two cell layers found in *Anguilla rostrata* and other teleosts, it is apparent that, as the animals mature, a second cell layer appears and the water-to-blood distance increases slightly.

Chloride-secreting cells appear in clusters at bases of secondary lamellae, in close proximity to afferent lamellar arteries (Fig. 5). Cytoplasm of chloride cells exhibits high concentrations of carbohydrate polycarboxylates staining intensely with PAS reactions (Table I) and Alcian blue pH 2.6. In the latter, it is interesting to note that staining is limited to the distal portions of chloride cells; basal portions of cells show no reaction to the stain.

Appearance of chloride cells near afferent lamellar arteries is well known for the Japanese eel, *Anguilla japonica* (Ogawa, 1962; Shirai and Utida, 1970; Utida,

Kamiya, and Shirai, 1971) as well as other teleosts (Vickers, 1961; Munshi, 1964; Newstead, 1967). Getman (1950) suggested that in *Anguilla rostrata,* the location of chloride cells in interlamellar epithelium allows access to a good blood supply and insures exposure to the environment for salt secretion. Shirai and Utida (1970) studied the development and degeneration of chloride cells when eels were adapted to fresh water and to sea water; the secretory mechanism was examined by Utida, *et al.* (1971) to determine the relationship between Na^+-K^+-ATPase and numbers of chloride cells in seawater-adapted animals. In the present study, animals were freshwater adapted, and chloride cells were similar in appearance, location, and stainability to those of freshwater teleosts (Munshi, 1964).

In conclusion, we would like to thank Dr. Joseph A. Vena for his advice and assistance with staining procedures, and Mr. Gerald Nicholls for his suggestions and cooperation on photographic techniques.

Summary

Gills of the American eel were found to be morphologically similar to those of other members of the *Anguilla* species, and to teleosts in general. Gill filaments contain cartilagenous gill rays rich in polysulfates, and stain intensely in PAS and Alcian blue pH 0.5.

Pillar cells delimit blood spaces within secondary lamellae; they were found to be covered by a thin connective tissue supporting a single-layered epithelium in early juvenile animals and a two cell, layered epithelium in adult eels. In the latter, nuclei of the outer layer were much larger and not as densely stained as those of the inner epithelial cells, whose nuclei appeared flattened over pillar-cell bodies.

Basement membranes supporting epithelia of secondary lamellae and gill filaments exhibited heavy concentrations of sulfate groups shown by reactions in Alcian blue pH 0.5 and Alcian blue pH 5.7 with high concentrations of $MgCl_2$.

Chloride cells were found in the interlamellar epithelium, especially surrounding afferent lamellar arteries. They had a granular, eosinophilic cytoplasm with carbohydrate polycarboxylates concentrated in distal portions of cells; nuclei had a prominent, centrally-situated nucleolus surrounded by small chromatin masses.

Results of cytochemical tests for all cell types were reported, and information correlated to previous findings on eel gills in particular, and teleost gills in general.

LITERATURE CITED

Doyle, W. L., and F. H. Epstein, 1972. Effects of cortisol treatment and osmotic adaptation on the chloride cells in the eel, *Anguilla rostrata. Cytobiologie,* **6**: 58–73.

Farley, C. A., 1969. Probable neoplastic disease of the hematopoietic system in oysters, *Crassostrea virginica* and *Crassostrea gigas. Natl. Cancer Inst. Monogr.,* **31**: 541–555.

Getman, H. C., 1950. Adaptive changes in the chloride cells of *Anguilla rostrata. Biol. Bull.,* **99**: 439–445.

Hughes, G. M., and A. V. Grimstone, 1965. The fine structure of the secondary lamellae of gills of *Gadus pollachius. Q. J. Microsc. Sci.,* Pt. 4, **106**: 343–353.

Hughes, G. M., and D. E. Wright, 1970. A comparative study of the ultrastructure of the

water-blood pathway in the secondary lamellae of teleost and elasmobranch fishes—benthic forms. *Z. Zellforsch. Mikrosk. Anat.,* **104**: 478–493.

HUMANSON, G. L., 1972. *Animal Tissue Techniques.* W. H. Freeman and Company, San Francisco, 641 pp.

KEYS, A. B., AND E. N. WILLMER, 1932. "Chloride secreting" cells in the gills of fishes, with special reference to the common eel. *J. Physiol.,* **76**: 368–378.

LEHNINGER, A. L., 1970. Biochemistry. Worth Publishing Incorporated, New York, 838 pp.

LEV, R., AND S. S. SPICER, 1964. Specific staining of sulphate groups with Alcian blue at low pH. *J. Histochem. Cytochem.,* **12**: 309.

MCMANUS, J. F. A., 1948. Histological and histochemical uses of Periodic acid. *Stain Technol.,* **23**: 99–108.

MIZUHIRA, V., T. AMAKAWA, S. YAMASHINA, N. SHIRAI, AND S. UTIDA, 1969. Electron microscopic studies on the localization of sodium ions and sodium-potassium-activated adenosinetriphosphatase in chloride cells of eel gills. *Exp. Cell. Res.,* **59**: 346–348.

MORGAN, M., AND P. W. A. TOVELL, 1973. The structure of the gill of the trout, *Salmo gairdneri (Richardson). Z. Zellforsch. Mikrosk. Anat.,* **142**: 147–162.

MORGAN, M., 1974. Development of secondary lamellae of the gills of trout, *Salmo gairdneri (Richardson). Cell Tissue Res.,* **151**: 509–523.

MOWRY, R. W., 1963. The special value of methods that color both acidic and vicinal hydroxyl groups in the histochemical study of mucins. With revised directions for the colloidal iron stain, the use of alcian blue 8GX, and their combination with the periodic acid-Schiff reaction. *Ann. N.Y. Acad. Sci.,* **106**: 402–423.

MOWRY, R. W., 1970. Analytical methods for carbohydrates. IV. Histochemical methods. Pages 777–807 in W. Pigman and D. Horton, Eds., The *carbohydrates; chemistry and biochemistry,* Vol. 3B, Academic Press, London.

MUNSHI, J. S. D., 1964. "Chloride cells" in the gill of freshwater teleosts. *Q. J. Microsc. Sci.,* Pt. 1, **105**: 79–89.

NEWSTEAD, J. D., 1967. Fine structure of the respiratory lamellae of teleostean gills. *Z. Zellforsch. Mikrosk. Anat.,* **79**: 396–428.

OGAWA, M., 1962. Chloride cells in Japanese common eel, *Anguilla japonica. Sci. Rep. Saitama Univ. Ser. B. Biol. Earth. Sci.,* **4**: 131–137.

SCOTT, J. E., AND J. DORLING, 1965. Differential staining of acid glycosaminoglycans (Mucopolysaccharides) by alcian blue in salt solutions. *Histochemie,* **5**: 221–233.

SHIRAI, N., AND S. UTIDA, 1970. Development and degeneration of the chloride cell during seawater and freshwater adaptations of the Japanese eel, *Anguilla japonica. Z. Zellforsch. Mikrosk. Anat.,* **103**: 247–264.

STEEN, J. B., AND A. KRUYSSE, 1964. The respiratory function of teleostean gills. *Comp. Biochem. Physiol.,* **12**: 127–142.

UTIDA, S., M. KAMIYA, AND N. SHIRAI, 1971. Relationship between the activity of Na^+-K^+-*activated adenosinetriphosphatase* and the number of chloride cells in eel gills with special reference to seawater adaptation. *Comp. Biochem. Physiol.,* Pt. A, **38**: 443–447.

VICKERS, T., 1961. A study of the so-called "chloride-secretory" cells of the gills of teleosts. *Q. J. Microsc. Sci.,* Pt. 4, **104**: 507–518.

YAMADA, K., AND M. YOKOTE, 1975. Morphochemical analysis of mucosubstances in some epithelial tissues of the eel, *Anguilla japonica. Histochemistry,* **43**: 161–172.

Reference: *Biol. Bull.* **157**: 112–124. (August 1979)

FINE STRUCTURE OF MUSCULATURE IN THE COPEPOD *PARANTHESSIUS ANEMONIAE* CLAUS

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Paranthessius anemoniae Claus is a cyclopoid associate of the snakelocks anemone *Anemonia sulcata* (Pennant). First described by Claus (1889) in the Adriatic Sea and later by Bocquet and Stock (1959) from Mediterranean waters, *Paranthessius* has only recently been recorded from British waters (Gotto and Briggs, 1972; Briggs and Gotto, 1973; Briggs, 1973). Other recent studies of *Paranthessius* have described this copepod's general ecology (Briggs, 1976), alimentary canal (Briggs, 1977a), larval development (Briggs, 1977b) and integument (Briggs, 1978). Copepod muscle has been investigated by Hartog (1888), Scott (1901), Lowe (1935), Changeux (1960), Fahrenback (1962) and Park (1966). Ultrastructural studies of *Cyclops* by Bouligand (1962, 1963 and 1964) and of *Macrocyclops albidus* by Fahrenbach (1963) are among the few detailed studies of copepod muscle.

Materials and Methods

Copepods were fixed for 12 hr at 4° C in 5% gluteraldhyde in 0.12 M Millonig buffer (pH 7.4) containing 3% NaCl and 0.1 mM CaCl. Fixed specimens were processed for light and electron microscopy.

Light microscopy

Copepods fixed in gluteraldehyde were dehydrated though ethylene glycol and embedded in glycol methacrylate (G.M.A.) which was polymerized in gelatin capsules at 60° C for 48 hrs. Sections 1 to 2 μ thick were cut on a Reichert OMU2 ultratome and stained on glass slides with mercuric bromo phenol blue (method of Maiza, Brewer, and Alfert, 1953).

Electron microscopy

Fixed copepods were washed in Millonig buffer-wash (2–8 hrs), post-fixed for 2 hrs in 1% osmium tetroxide and the dehydrated through ethanol to propylene oxide and embedded in araldite. Sections were cut with a Reichert OMU2 ultratome, mounted on copper grids, stained with uranyl acetate and lead citrate and examined in an AE1 EM801 electron microscope operating at 60 kV.

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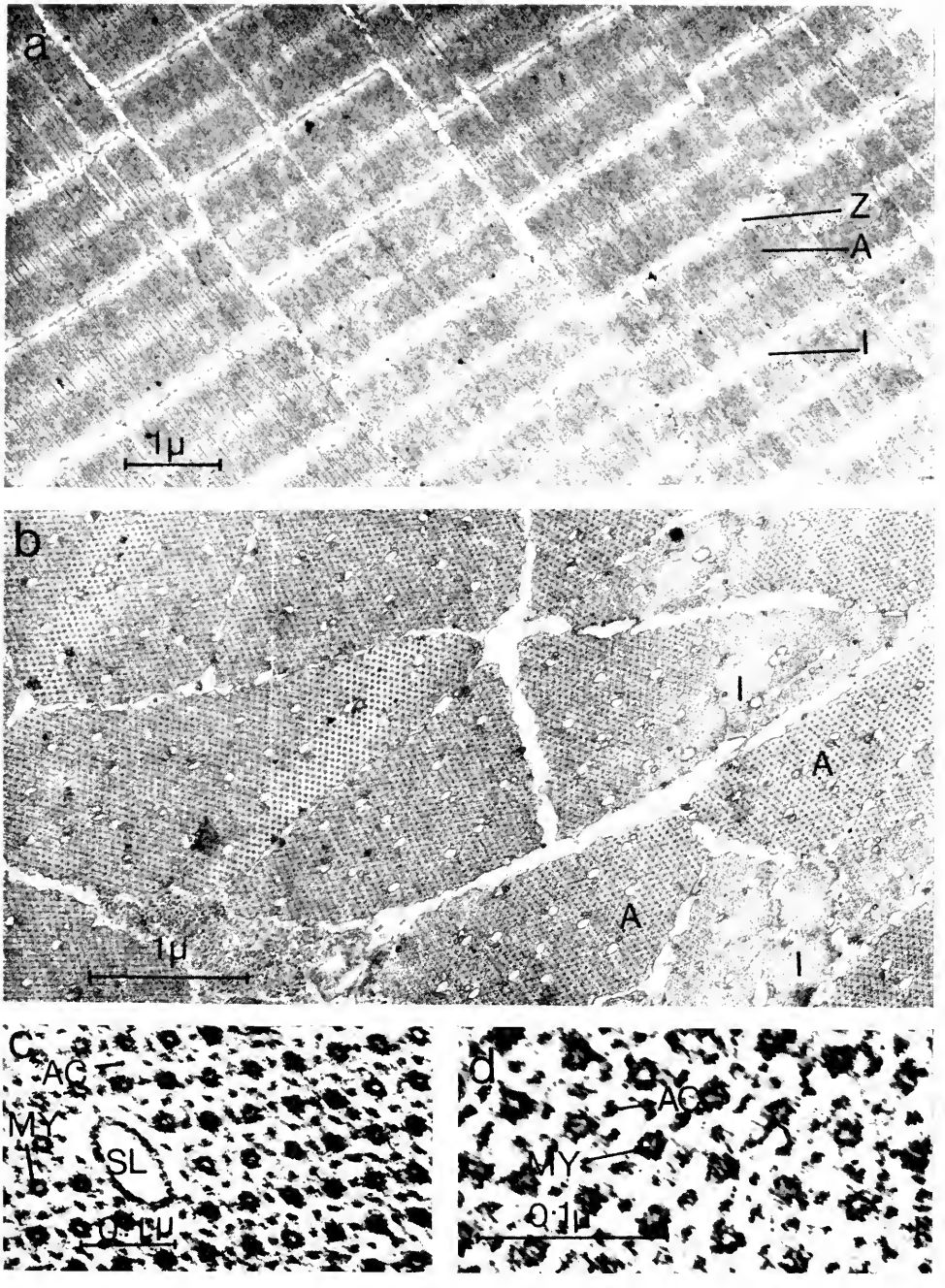

FIGURE 1. Electron micrograph of striated muscle in *Paranthessius anemoniae*. (a) Longitudinal section through muscle. Z line, A and I band are arrowed. (b) Transverse section of muscle fibrils at both A band and I band levels. (c) Transverse section of muscle showing longitudinal sarcoplasmic reticulum element (SL) actin filaments (AC) and myosin. (d) actin (AC) and myosin filaments (MY).

Results

The general body muscle

Light microscopy has shown the longitudinal muscles in *Paranthessius* to be composed of bundles of muscle fibers which pass along either side of the mid-dorsal line. A similar pair of longitudinal muscle bundles are situated in a ventro-lateral position. Treatment with periodic-acid–Schiff (method of McManus, 1946) demonstrated the presence of large amounts of glycogen within the muscle.

Examination of ultra thin sections with the electron microscope shows the muscle of *Paranthesssius* to be striated (Fig. 1a). A and I bands are clearly visible, the former having a well-defined central H zone as is usual for striated muscle (Hanson and Huxley, 1953). The functional units of muscle (sarcomeres) are separated from one another by an electron-dense Z line. Transverse section shows the muscle to be composed of polygonal-shaped myofibrils measuring between 1 and 4 μ in diameter (Fig. 1b). Both thick (myosin) and thin (actin) filaments are present. The myosin filaments, which appear to be hollow, measure 12 nm in thickness with an average length of 1100 nm and are spaced about 25 nm apart. The actin filaments, on the other hand, are on average 4 nm in thickness and are each placed equidistant between two myosin filaments. The sarcomere has an average length of 1200 nm, though this varies with the state of muscle contraction.

Superficial examination of *Paranthessius* muscle reveals an apparent similarity to vertebrate muscle with each myosin filament surrounded by six similar filaments and six actin filaments in hexagonal array (Figs. 1c, d). More detailed examination, however, shows that the actual position of the actin filaments in relation to the myosin filaments is different from vertebrate muscle in that each actin filament does not lie equidistant from three myosin filaments (Fig. 2). If the myosin filaments in *Paranthessius* are imagined to be the apices of an equilateral triangle, then the actin filaments occur in the center of each side (Fig. 2). This arrangement of myofilaments is similar to that described for other copepods, for example Bouligand (1962), Fahrenbach (1963) and Raymont, Krishnaswamy, Woodhouse, and Griffin (1974).

Sections through the muscle show the myofibrils to be surrounded by menbranous material which constitutes the sarcoplasmic reticulum. This is also seen to be regularly distributed within the myofibrils. In transverse section, the interfibrillar sarcoplasmic reticulum appears as circular membranous zones, often paired, measuring 50 nm in diameter. The single or paired units are regularly spaced between 200 nm and 400 nm apart (Fig 3a). Longitudinal sections show the sarcoplasmic reticulum to be in the form of canals measuring an average of 50 nm across and of varying length (Fig. 3b), and appearing elliptical in slightly oblique sections (Fig. 3c).

Examination of a large number of muscle sections has revealed that the sarcoplasmic reticulum is a branching tubular system that runs longitudinally through and around the myofibrils. The intrafibrillar elements link up with those surrounding the fibril in the region of the Z line by means of transverse tubules of sarcoplasmic reticulum. This feature is evident in both transverse and longitudinal sections (Fig. 3d, 4a). The canals surrounding the myofibrils are continuous with the membrane of the sarcolemma surrounding the muscles. This implies the

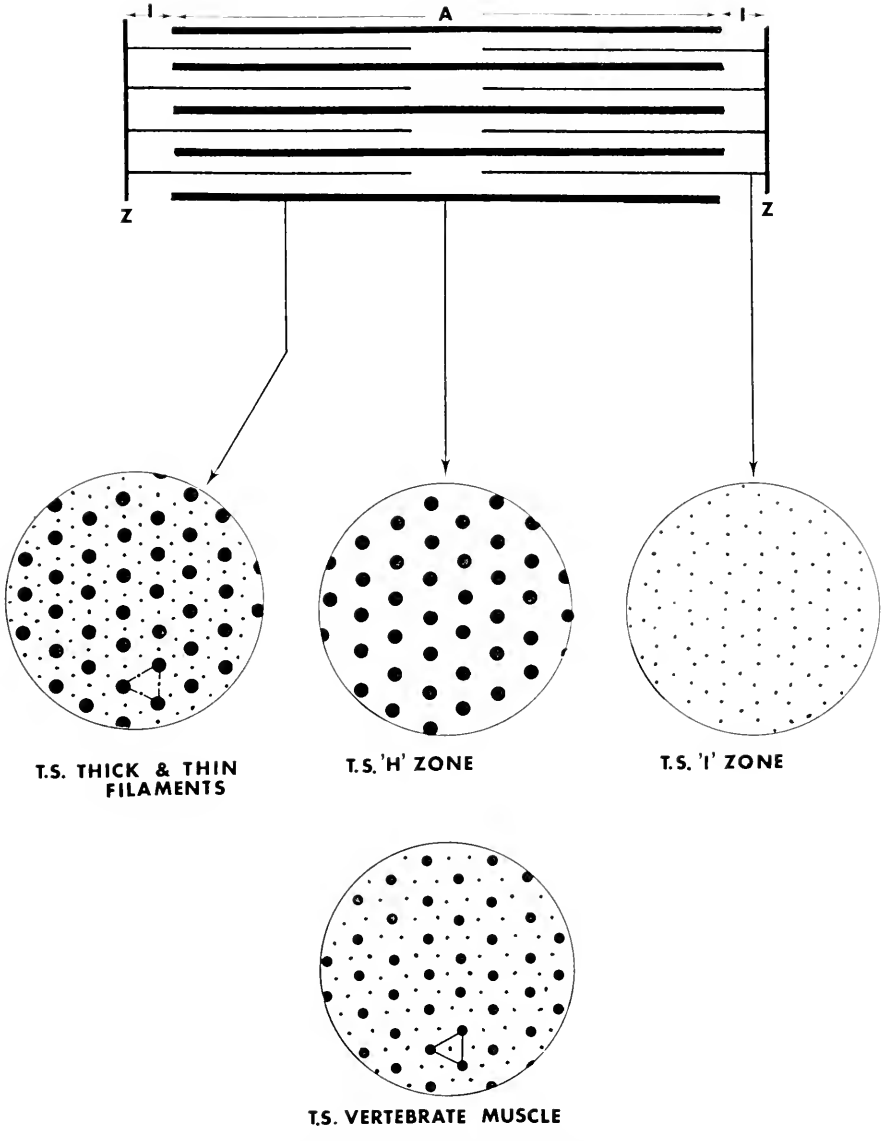

FIGURE 2. Diagrammatic representation of the myofilament arrangement in copepod muscle. Vertebrate muscle is also represented for comparison. If the myosin filaments in copepod muscle are imagined to be the apices of an equilateral triangle, then the actin filaments occur in the center of each side. In vertebrate muscle each actin filament lies equidistant from three myosin filaments.

existence of a continuous system of sarcoplasmic reticulum, leading from the muscle surface and running between the muscle fibers, myofibrils and myofilaments.

The paired nature of some of the longitudinal tubules within the myofibrils seen in transverse section is attributed to elongate membranous vesicles that lie along

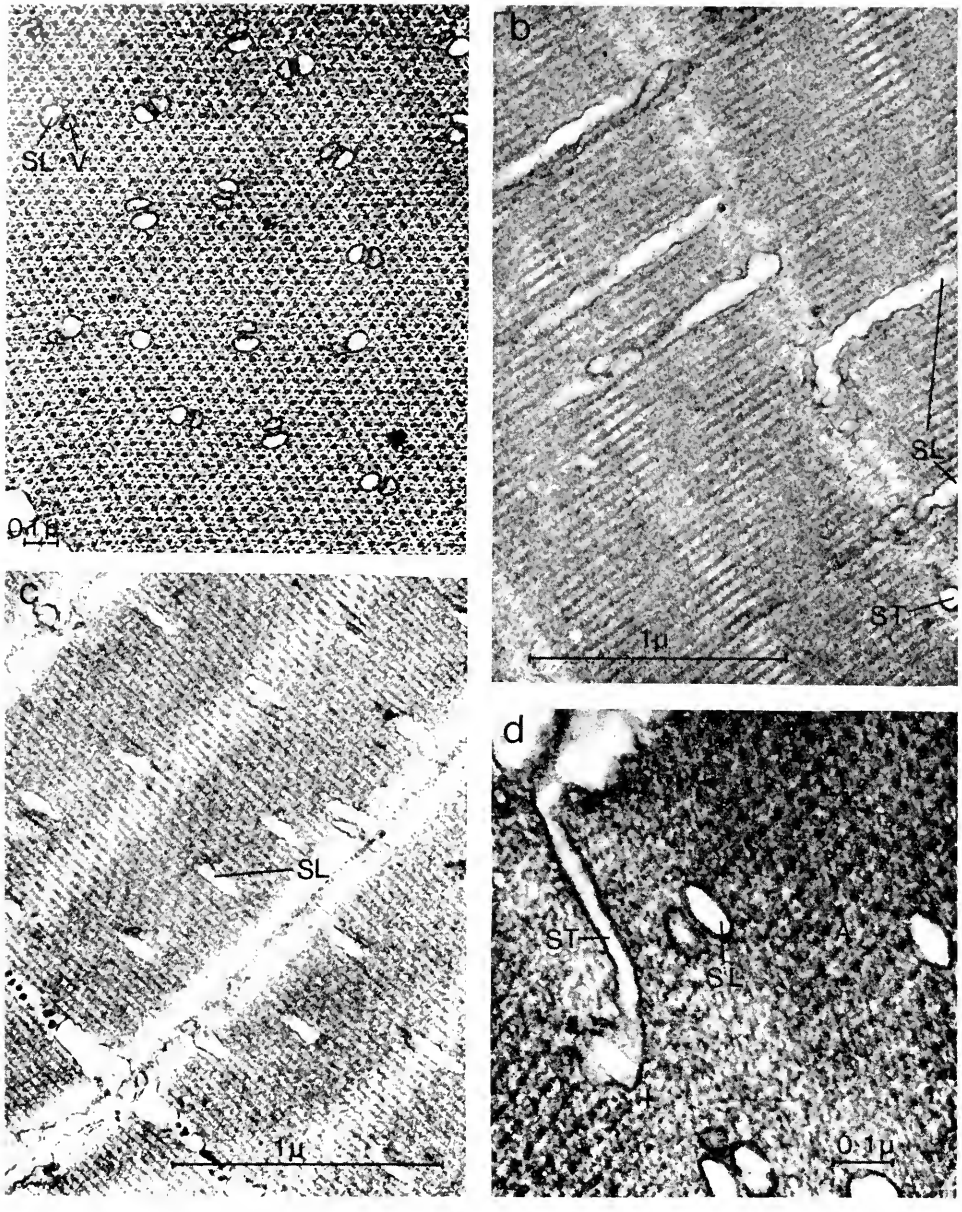

FIGURE 3. (a) Longitudinal sarcoplasmic re iculum elements (SL) and blind ending vesicles (V) in transverse section. The blind ending vesicles have a characteristic granular appearance to their lumen. (b) Longitudinal (SL) and transverse elements (ST) of sarcoplasmic reticulum as seen in longitudinal section. (c) Slightly oblique section through muscle showing longitudinal sarcoplasmic reticulum (SL) elements as oval vesicles. (d) Transverse section of *Paranthessius* muscle showing both longitudinal (SL) and transverse elements (ST) of sarcoplasmic reticulum in the I band (I). No transverse elements were observed in the A band (A).

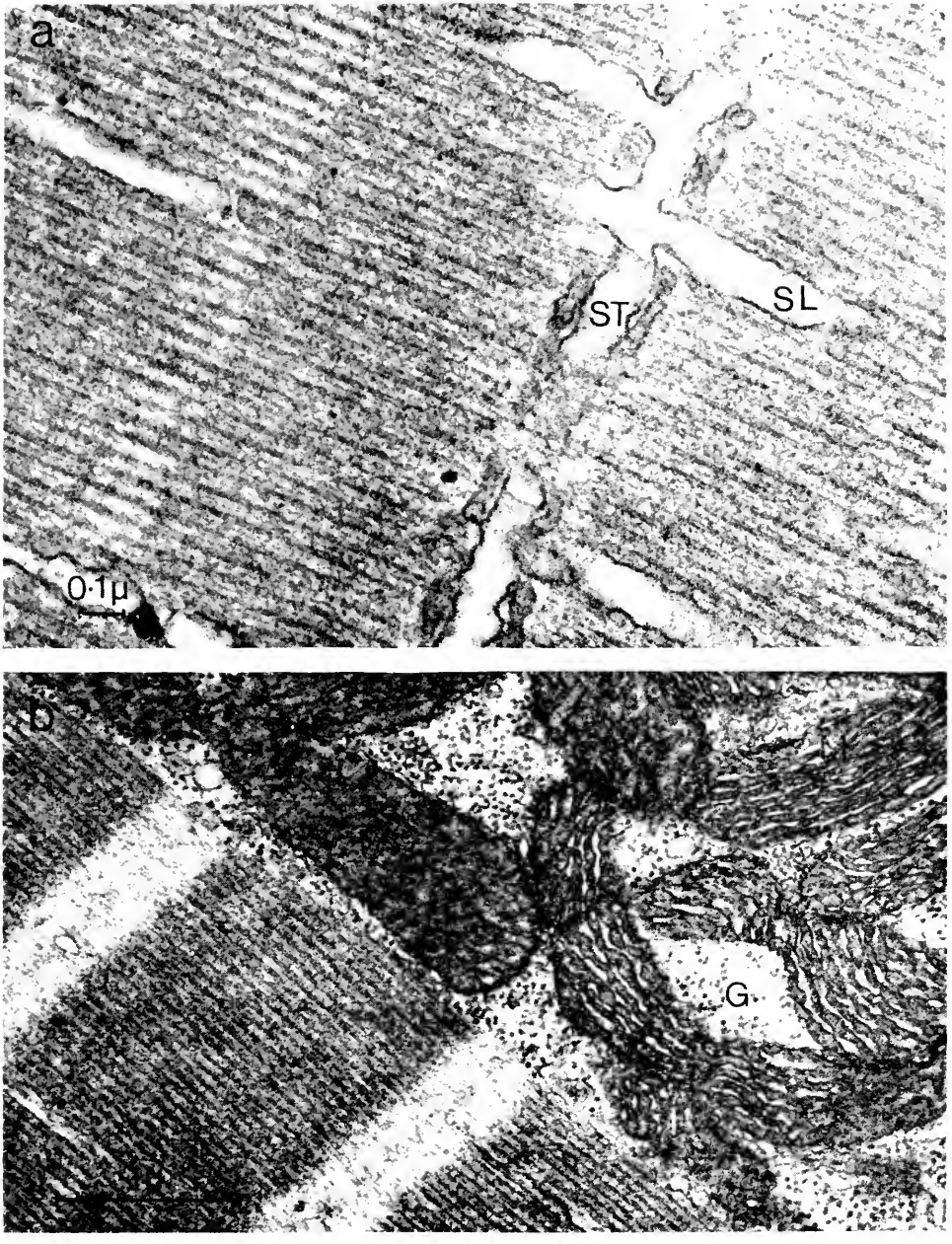

FIGURE 4. (a) Longitudinal section of *Paranthessius* muscle fibril showing junction of transverse sarcoplasmic reticulum (ST) and longitudinal sarcoplasmic reticulum (SL). (b) Mitochondria (M) and glycogen granules (G) on surface of muscle fibril.

longitudinal elements. The lumina of these vesicles have a granular appearance and their length rarely exceeds that of a sarcomere (Fig. 3a, 5). The general irregular nature and the occasional folding and branching of the longitudinal tubules

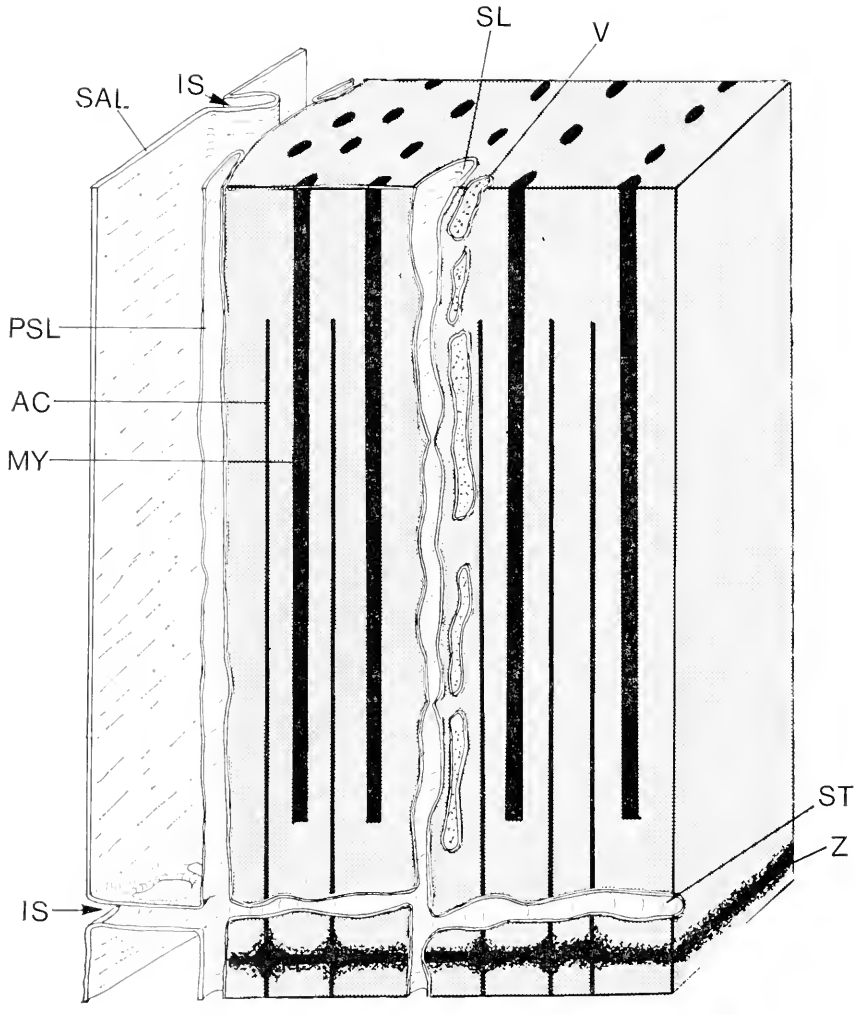

FIGURE 5. Three-dimensional diagram of the sarcoplasmic reticulum system in *Paranthessius anemoniae* muscle showing longitudinal sarcoplasmic reticulum (SL), vesicles (V), peripheral sarcoplasmic reticulum (PSL), transverse sarcoplasmic reticulum (ST), Z line (Z), actin filament (AC), myosin filament (MY), sarcolemma (SAL) and its invaginations (IS).

is probably accounted for by the occurrence of more than two units in some sections examined.

Scattered on the muscle surface under the sarcolemma are numerous mitochondria that may measure up to 2 μ in length, and 0.6 μ in width. The mitochondria of the muscle (or sarcosomes) are characterised by the possession of numerous cristae (Fig. 4b). Granules of glycogen (Fig. 4b) measuring about 25 nm in diameter occur commonly between the myofibrils and are thought to be responsible for the strong positive reaction to the P.A.S. test for carbohydrates

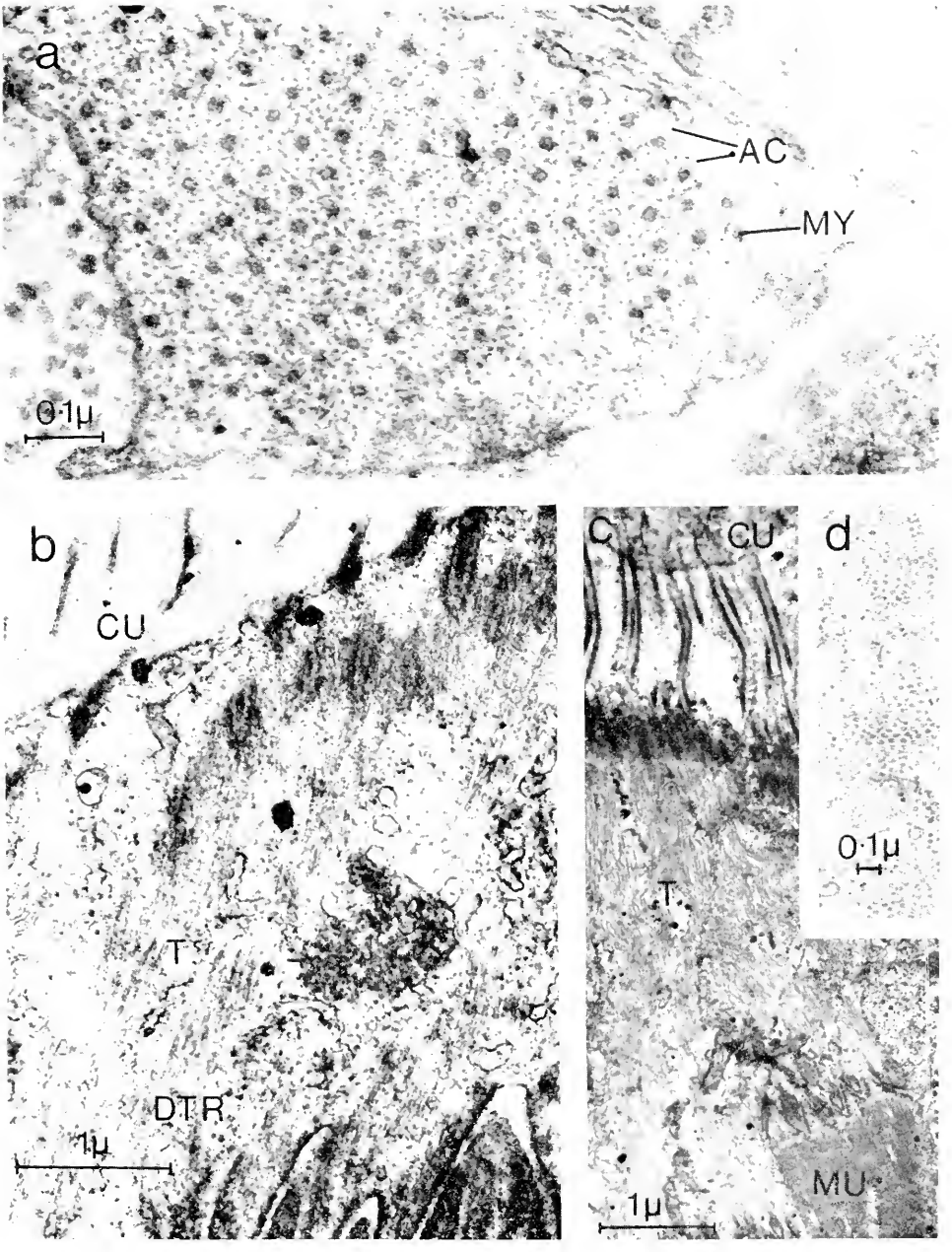

FIGURE 6. (a) Transverse section through muscle of alimentary canal showing both actin (AC) and myosin (MY) filaments. (b) Details of muscle junction with cuticle (CU) showing attachment fibrils (T) and dense terminal region (DTR) of muscle. (c) Further details of attachment of muscle (MU) to cuticle (CU) by tonofibrils (T). (d) Transverse section of the tonofibrils which attach muscle to cuticle in *Paranthessius*.

TABLE I

Dimensions of muscle fibrils in Paranthessius, Cyclops, Macrocyclops *and a vertebrate.*

Species	Myosin			Actin thickness	Reference
	length	thickness	separation		
Paranthessius	1100 nm	12 nm	25 nm	4 nm	
Cyclops	1500 nm	12 nm	25 nm	4 nm	Bouligand (1964)
Macrocyclops	—	15 nm	48 nm	—	Farenbach (1963)
Vertebrate	1500 nm	10 nm	45 nm	6 nm	Threadgold (1967)

observed in light microscope studies. The muscle nuclei are a flattened ovoid shape and have a single nucleolus. They are found in close contact with the muscle surface and were not commonly encountered during these investigations. The longitudinal muscle bundles and those supplying the appendages are constructed as described.

The muscle of the alimentary canal

The alimentary canal was found to be surrounded by muscle of a slightly different structure. There are several isolated bundles or strands of longitudinal muscle measuring 0.5 to 1.0 μ thick lying beneath the basement membrane of the digestive tract epithelium (Fig. 6a). Surrounding these longitudinal muscles is a layer of circular muscle of 1 μ average thickness. The myosin filaments of the gut muscle were seen in transverse section to be surrounded by 10 to 12 actin filaments instead of the six noted in the general body muscle. Although the myosin filaments are about the same distance apart as the general muscle (25 nm) they are somewhat thicker (15 nm). No internal ramification of sarcoplasmic reticulum is seen in this muscle.

The attachment of the muscle to the cuticle

Near the site of attachment to the cuticle the muscle fibrils terminate at an irregular electron-dense line that can be seen in longitudinal sections to traverse the fibril (Fig. 6b). More detailed examination, however, reveals that this electron-dense line is composed of the sarcolemma of the muscle, near to which, at a distance of 30 to 40 nm, is another plasma membrane that gives rise to fine tubules or tonofibrils (Fig. 6c, d). The tonofibrils measure about 20 nm in diameter and seem to pass, in groups, through thickened electron-dense zones, from each of which emerges an electron-dense fiber of roughly 400 nm thickness. These fibers ramify through the cuticle forming a firm attachment. The length of the tonofibrils ranges from 5 to 0.5 μ in different parts of the animal. Attachment is sometimes made to normal cuticle, while in other places the endocuticle invaginates to form an apophysis for muscle attachment (Briggs, 1978).

Discussion

The observations made on *Paranthessius* muscle agree in many respects with those made by Bouligand (1962, 1963, 1964) on *Cyclops* and *Acanthocyclops,* by Fahrenbach (1963) on *Macrocyclops* and by Raymont *et al.* (1974) on *Calanus.* In *Cyclops* the myosin filaments measure 1.5 μ in length compared to 1.1 μ in *Paranthessius. Macrocyclops* has myosin filaments 15 nm thick, which are further apart than those of both *Cyclops* and *Paranthessius.* No detailed measurements are available for *Calanus* muscle. Dimensions of muscle structures given in Table I includes details of typical vertebrate muscle for comparison.

In a study of *Acanthocyclops,* Bouligand (1964) describes zones of double overlapping between the two sets of actin filaments of the sarcomeres when the muscle is contracted. A transverse section through this zone shows twice the number of actin filaments found in other regions of the muscle. In longitudinal sections of contracted muscle these overlapping zones or CM bands are characterised by appearing as a dark band across the muscle in the center of the H zone Examination of many sections of *Paranthessius* muscle did not show this feature to be present. It is possible that the muscle examined here was fixed in the relaxed state. This is considered unlikely, however, since copepods always exhibited strong locomotory movements on encountering fixatives. The probability that all sections cut were of relaxed muscle is, therefore, very low. The general arrangement of the myofibrils is the same as that described for other copepods.

The sarcoplasmic reticulum in *Paranthessius* is very similar to that described by Bouligand (1962, 1963) for *Cyclops,* though it tends to be somewhat less elaborate than that studied by Fahrenback (1962) in *Macrocyclops.* In this species, the blind-ending membranous vesicles found in *Paranthessius* and also in *Cyclops* are more expanded, forming a well developed system of cisternae. As was found with the vesicles of *Paranthessius* muscle, the cisternae do not join with the elements of the sarcoplasmic reticulum, but come to within 10 nm in most regions of the muscle. The term dyad, used by Smith (1961), to describe the association between sarcoplasmic reticulum tubules and the cisternae in the beetle *Tenebrio* is used here to describe similar structures in copepod muscle. In *Paranthessius* a dyad represents the paired membranous tubules seen in a transverse section of muscle myofibrils. This is an association between a sarcoplasmic reticulum tubule and a blind ending vesicle.

The sarcoplasmic reticulum system of copepods may be contrasted with that of vertebrate muscle. In most vertebrates the sarcoplasmic reticulum forms a sleeve around the muscle fibril ending in a number of finger-like projections in the region of the Z line, where it comes into close contact with the invaginated membrane of the sarcolemma. This association forms a triad, composed of two sarcoplasmic reticulum elements (one from each side of the Z line of the fibril) and the membrane of the sarcolemma. The sarcolemma membrane is, therefore, not a continuous tubular system ramifying longitudinally through the myofibrils as is found in copepod muscle.

Fahrenbach (1963) stressed the importance of efficient diffusion of "transmitter substances" (Ca^{++}) in fast acting muscles. Slower contracting vertebrate muscles have the discontinuous triad structure described, in which the sarcolemma membrane

is not connected to the sarcoplasmic reticulum system. The myofilaments in the center of the myofibrils are not brought into such close proximity with a potential impulse-conducting element as is found in the arthropodan continuous dyad system of fast contracting muscle. In most fast muscle studied the distance that calcium ions have to diffuse in order to reach the center of the myofibrils to trigger construction is maintained at a minimum distance of less than 1 μ. Examples include 0.3 to 0.35 μ in the fast muscle of the dragon fly *Aeshna* (Smith, 1961), 0.18 to 0.2 μ in the toadfish *Opsanus* (Fawcett and Revel, 1961), 0.15 to 0.25 μ in the bat *Eptesicus* (Revel, 1962) and 0.2 μ in the copepod *Macrocyclops* (Fahrenbach 1963). The value for *Paranthessius* was found to be on average 0.25 μ. Fahrenbach (1963) proposes that this is the reason why the longitudinal tubules are arranged in a regular hexagonal manner in fast copepod muscle.

Bouligand (1962) suggests that the longitudinal sarcoplasmic reticulum elements of *Cyclops* are regularly arranged, so that their tendency to expand when the muscle contracts, (due to the hydrostatic pressure of their contained fluid) will not disorientate the myofilaments. Bouligand proposes that evidence for this may be gained from observation of true transverse sections of hexagonal array of the tubules. It is visualised that expansion force lines from these tubules would pass through the myosin filaments towards another expanding tubule, which would be exerting a similar force. This implies that the position of the myosin filaments would be undisturbed during muscle contraction. If the expansion forces tended to act between the myosin filaments the latter would be displaced to either side.

Both Fahrenbach's and Bouligand's interpretation of the regular arrangement of sarcoplasmic reticulum are applicable to *Paranthessius* muscle, which has a similar structure to that of the species studied by these authors. Although the sarcoplasmic reticulum is not so complex as that of *Macrocyclops*, all other structural features indicate that the muscle of *Paranthessius* is of a fast contracting type. The muscles of the alimentary canal are probably slower acting, since they have nearly twice as many actin filaments as the general body muscles which is a characteristic of "slower" muscle (Fahrenbach 1967).

Parasitic forms usually have smooth or "slow contracting muscle" (Capart, 1948). It is noteworthy that *Paranthessius* has muscle characteristics of free-living forms, *i.e.*, "fast acting muscle". This is not surprising when it is considered that *Paranthessius* is quite a mobile associated form (Briggs, 1974). It is of survival value for *Paranthessuis* to be capable of rapid swimming in order to regain a position on its host if dislodged. Since the larval instars of *Paranthessius* live freely in the plankton, the infective stage must possess efficient locomotion for host location. The elaborate musculature of the adult may, therefore, represent a legacy from the free living phase of the life cycle.

Apart from the gripping claws of the second antenna and spinal reduction in the mouth parts, *Paranthessius* is relatively unmodified morphologically and bears a strong resemblance to free-living cyclopoids. It is, moreover, associated with the external surface of the host, never being found in the gastrovascular cavity, and is very mobile both on and off the anemone. These features together with the elaborate musculature described here suggest that in an evolutionary context *Paranthessius* is a recent invader of *Anemonia sulcata*. Comparative studies of the musculature in other associated species which exhibit varying degrees of host

dependence and morphological modification might add support to these speculations on the evolution of parasitism in copepods.

I wish to thank Professor Gareth Owen and Dr. R. V. Gotto of the Queen's University of Belfast for their invaluable encouragement and advice during this study, undertaken during the tenure of a postgraduate studentship from the Department of Education for Northern Ireland.

Summary

1. *Paranthessius anemoniae* has striated muscle composed of actin and myosin myofilaments arranged hexagonally, as in free living copepods.

2. The sarcoplasmic reticulum is continuous with the membrane of the sarcolemma in the region of the Z line and forms a continuous system of tubules which ramify through the muscle.

3. Blind-ending vesicles form dyads with the longitudinal sarcoplasmic reticulum tubules.

4. Attachment of the muscle to the cuticle is by tonofibrils.

5. A relatively short sarcomere length, complex sarcoplasmic reticulum and high proportion of myosin to actin filaments indicate the "phasic" nature of the general body muscle in *Paranthessius*.

6. The muscle of the alimentary canal is characteristic of "tonic" muscle.

LITERATURE CITED

Bocquet, C., J. H. Stock, 1959. Copépodes parasites d'invertébrés des côtes de la Marche. VI Redescription de *Paranthessius anemoniae* Claus (Copepoda, Cyclopoida), parasite d'Amemonia, *sulcata* (Pennant). *Arch. Zool. Exp. Gen.*, 98 *Notes et rev.* No. 1 : 43–53.

Bouilgand, Y., 1962. Les ultrastructures du muscle strié et de ses attaches au squelette chez les *Cyclops* (Crustacés Copépodes). *J. Microsc. (Paris)*, 1 : 377–394.

Bouligand, Y., 1963. Les ultrastructures musculairs des Copépodes. II Membrane sarcoplasmique réticulum sarcoplasmique et jonction neuro-musculaire chez les *Cyclops*. *J. Microsc. (Paris)*, 2 : 197–212.

Bouligand, Y., 1964. Les ultrastructures musculaires des copépodes. III Nature de la bande de contraction. Cm des sarcomères. *J. Microsc. (Paris)*, 3 : 697–703.

Briggs, R. P., 1973. *Lichomolgus actiniae* D. V. An associated copepod new to Irish Waters. *Ir. Nat. J.*, 17 : 12.

Briggs, R. P., 1974. Aspects of the biology and structure of the associated copepod *Paranathessius anemoniae* Claus. *Ph. D. Thesis, The Queens University of Belfast*, Belfast, 183 pp.

Briggs, R. P., 1976. Biology of *Paranthessius anemonica* in association with anemone hosts. *J. Mar. Biol. Assoc. U. K.*, 56 : 917–924.

Briggs, R. P., 1977a. Structural observations on the alimentary canal of *Paranthessius anemoniae*, a copepod associate of the snakelocks anemone *Anemonia sulcata*. *J. Zool. (Lond.)*, 182 : 353–368.

Briggs, R. P., 1977b. Larval stages of *Paranthessius anemoniae* Claus (Copepoda, Cyclopida), an associate of the snakelocks anemone *Anemonia sulcata* (Pennant). *Crustaceana*, 33(3) : 248–258.

Briggs, R. P., 1978. Structure of the integument in *Paranthessius anemoniae* Claus, a copepod associate of the snakelocks anemone *Anemonia sulcata* (Pennant). *J. Morphol.*, 156(2) : 293–316.

Briggs, R. P., and R. V. Gotto, 1973. A first record of *Lichomolgus actiniae* Della Valle, 1880 (Copepoda, Cyclopoida) in British water. *Crustaceana*, **24**: 336–337.

Capart, A., 1948. Le *Lernaeocera branchialis* L. *La Cellule*, **52**(2): 159–212.

Claus, C., 1889. Ueber neue oder wenig bekannte halb-parasitische copepoden. *Arb. Zool. Inst. Univ. Wien*, **8**: 327–370.

Chiangeux, J. P., 1960. Contribution à l'etude des animaux associés aux Holothurides. Hermann, Paris, 124 pp.

Fahrenbach, W. H., 1962. The biology of a harpacticoid copepod. *Cellule*, **62**: 303–376.

Fahrenbach, W. H., 1963. The sarcoplasmic reticulum of striated muscle of a cyclopoid copepod. *J. Cell. Biol.* **17**: 629–640.

Fahrenbach, W. H., 1967. The fine structure of fast and slow crustacean muscles. *J. Cell. Biol.*, **35**: 69–79.

Fawcett, D. W., and J. P. Revel, 1961. The sarcoplasmic reticulum of a fast acting fish muscle. The sarcoplasmic reticulum. *J. Biophys. Biochem. Cytol.*, (**4**)**10**: 89–109.

Gotto, R. V., and R. P. Briggs, 1972. *Paranthessius anemoniae* Claus: An associated copepod new to British and Irish waters. *Ir. Nat. J.* **17**: 243–244.

Hanson, J., and H. E. Huxley, 1953. Structural basis of the cross-striations in muscle. *Nature*, **172**: 530–532.

Hartog, M. M., 1888. The morphology of *Cyclops* and the relations of Copepoda. *Trans. Linn. Soc. (Zool.), (series II)*, **5**: 1–146.

Lowe, E., 1935. The anatomy of a marine copepod *Calanus finmarchicus* (Gunnerus). *Trans. R. Soc. Edinb.*, **58**: 561–603.

Mazia, D., P. A. Brewer, and M. Alfert, 1953. The cytochemical staining and measurement of protein with mercuric bromophenol blue. *Biol. Bull.*, **104**: 57–67.

McManus, J. F. A., 1946. The histological demonstration of mucin after periodic acid. *Nature*, **158**: 202.

Park, T. S., 1966. The biology of a calanoid copepod, *Epilabidocera amphitrites*, McMurrich. *Cellule*, **66**: 129–251.

Raymont, J. E. G., S. Krishnaswamy, M. A. Woodhouse, and R. L. Griffin, 1974. Studies on the fine structure of Copepoda. Observations on *Calanus finmarchicus* (Gunnerus). *Proc. R. Soc. Lond.*, (B), **185**: 409–424.

Revel, J. P., 1962. The sarcoplasmic reticulum of the bat Cricothyroid muscle. *J. Cell. Biol.*, **12**: 571–574.

Scott, A., 1901. *Lepeophtheirus and Lernaea*. Liverpool Marine Biology Committee Memoirs, **VI** 62 pp.

Smith, D. S., 1961. Innervation of the fibrillar flight muscles of an insect: *Tenebrio molitar* (Coleoptera). *J. Biophys. Biochem. Cytol.* (**2**)**8**: 447–466.

Threadgold, L. T., 1967. *The Ultrastructure of the Animal Cell.* Pergamon Press, Oxford. 313 pp.

Reference: *Biol. Bull.* 157: 125–137. (August 1979)

RESPIRATORY ADAPTATIONS OF THE ESTUARINE MUD SHRIMP, *CALLIANASSA JAMAICENSE* (SCHMITT, 1935) (CRUSTACEA, DECAPODA, THALASSINIDEA) [1]

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Thalassinid mud shrimps commonly burrow in hypoxic marine sediments, and their success in these habitats seems, at least in part, predicated upon metabolic adaptations. Recent studies of thalassinids from the Pacific coast of North America have identified a number of behavioral and physiological respiratory adaptations to the hypoxic habitats of intertidal species (Farley and Case, 1968; Thompson and Pritchard, 1969; Roxby, Miller, Blair, and Van Holde, 1974; Miller and Van Holde, 1974; Miller, Pritchard, and Rutledge, 1976; Torres, Gluck, and Childress, 1977; Hawkins, 1971, unpublished M.S. thesis, Oregon State University). A rich thalassinid fauna occurs in intertidal and sublittoral habitats along coasts of the western Atlantic, but metabolic regulation among these species has been investigated only in *Upogebia affinis* by Mangum and van Winkle (1973).

The present study concerns *Callianassa jamaicense* (Schmitt) (*Callichirus jamaicense* according to the generic scheme of de Saint Laurent, 1973), a common inhabitant of estuarine mud flats in the northern Gulf of Mexico (Felder, 1978) and other areas of the western Atlantic (Rodrigues, 1971). On the Louisiana coast, dense populations of *C. jamaicense* are found in muddy substrates where low-salinity interstitial water is markedly hypoxic. Tidal exposure of these substrates frequently subjects such populations to extended periods of anoxia. Studies were undertaken to identify respiratory adaptations of *C. jamaicense* to such hypoxic habitats. Specifically, this paper reports (i) survival under aquactic and aerial anoxia, (ii) aerial respiration, (iii) effects of oxygen tension on metabolic rate, and (iv) post-anoxia metabolic rates.

Materials and Methods

Animals were collected from a tidally influenced pond on Grand Terre Island, Louisiana. Methods of collecting, transporting, maintaining and salinity-acclimating animals were the same as previously described (Felder, 1978). Animals were acclimated to a salinity of 20‰ in dark, 25° C incubators; all were maintained at this salinity for nine days before experiments were initiated. Animals were not fed, and aeration was provided during all phases of salinity-acclimation. Only intermolt, uninjured adult males were used in respiration studies. Wet weights were determined by thoroughly blotting animals with tissue and then weighing to the nearest milligram. All sea water used in experimental studies was carefully maintained at a salinity of 20 ± 0.3‰ and temperature of 25 ± 0.2° C.

Anoxic sea water was prepared by gassing sea water with nitrogen. In one

[1] In part adapted from a doctoral dissertation submitted to the Department of Zoology and Physiology, Louisiana State University, Baton Rouge.

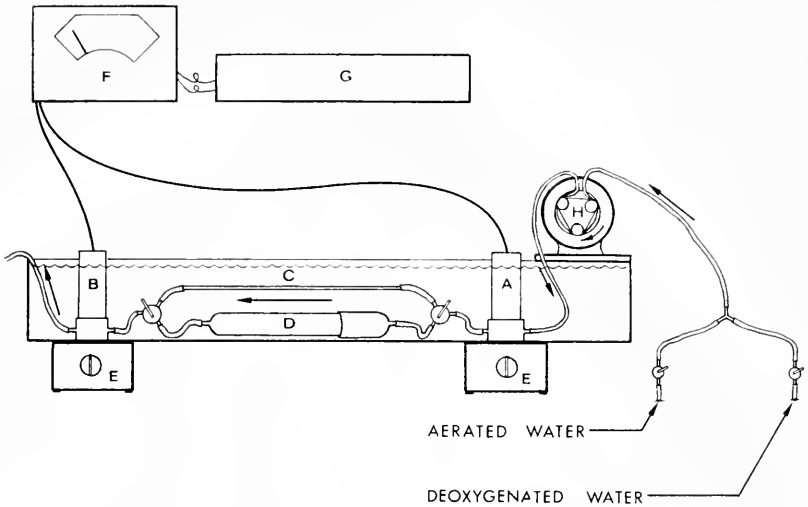

FIGURE 1. Diagrammatic cut-away view of water bath showing components of flow-through respirometer: A, influent oxygen electrode; B, effluent oxygen electrode; C, bypass shunt; D, respiration chamber; E, magnetic stirrers; F, differential oxygen meter; G, integrating chart recorder; H, peristaltic pump. A small bubble trap (not shown) was installed between components H and A.

experiment anoxic sea water was siphoned into BOD (300-ml biochemical oxygen demand) bottles containing one animal each, and bottles were sealed until death occurred. Other animals in individual, perforated vials were placed as a group into 5 liters of anoxic sea water which was replaced daily; whenever the 5-liter jar was opened for removal of dead animals, it was regassed with nitrogen. Control animals were maintained in continuously aerated sea water. Tolerance of aerial anoxia was determined by supporting animals on the rack of a desiccator over water and continuously gassing the water with nitrogen. Control animals in an aerial environment were likewise maintained, but underlying water was gassed with air.

Aerial $\dot{V}_{O_2}$ was measured in a Gilson respirometer with 130-ml respirometry flasks and equivalent ballast. Filter paper wicks and 30% KOH were added to each flask side-arm to absorb CO_2. Each flask contained one animal and one milliliter of sea water to maintain water saturation of air. One hour was allowed for equilibration; thereafter oxygen consumption was read at 30-min intervals.

Oxygen consumption at decreasing oxygen tensions was measured by placing the animal into a 13.5-mm ID (inner diameter) plastic tube with openings at both ends. The tube was wedged vertically against the wall of a BOD bottle, and a small stirring bar was placed at the center of the bottle. An oxygen electrode was fitted snugly into the bottle opening, and depletion of oxygen was recorded with a Beckman oxygen analyzer. The analyzer was calibrated in air-saturated sea water and checked by Winkler titration (Strickland and Parsons, 1972) before each run. Temperature was maintained by a water bath supported over the magnetic stirrer. The stirring rate was set at the lowest speed, which produced maximum deflection of the oxygen meter. The displacement volume of the animal, tube, and stirring bar

was subtracted from the bottle volume. Pleopod ventilatory strokes were counted during 5-min intervals and expressed as mean number min. To minimize effects of handling, each animal was transferred to a plastic tube and placed (anterior end up) into a BOD bottle 30 min before it was sealed; as an additional precaution, the first 30 min of recorded oxygen depletion were discarded.

A flow-through respirometer was assembled from a dual-probe International Biophysics differential oxygen analyzer, a Houston Instrument integrating chart recorder, a peristaltic pump, two magnetic stirrers, a constant temperature water bath, and a 16-mm ID glass respiration chamber (Fig. 1). Flow rate was maintained at *ca.* 10 ml/min and was precisely determined by measuring the volume of effluent; injection of a dye at this flow rate indicated thorough mixing of water as it passed through the respiration chamber. Each animal was placed into the chamber with its anterior toward the influent opening; aerated water was provided for 1 hr before oxygen consumption was read. Prior to each run, the oxygen analyzer was calibrated by Winkler titration and the differential between the electrodes was set to zero. Altered oxygen tensions were achieved by controlled mixing of fully aerated and nitrogen-saturated water. Whenever oxygen content of influent water was altered, 20 min were allowed for the flow-through system to flush before oxygen consumption was read. Anoxic conditions were provided by pumping deoxygenated water into the respiration chamber and then closing valves at either end (Fig. 1). The flow-through respiration chamber was lined with fine-mesh plastic gauze to provide traction for thoracic legs during pleopod beating. Pleopod strokes were counted as previously described.

Field measurements of oxygen in exposed and submerged burrows were made during an afternoon low tide in July 1972. Oxygen concentration of water over-

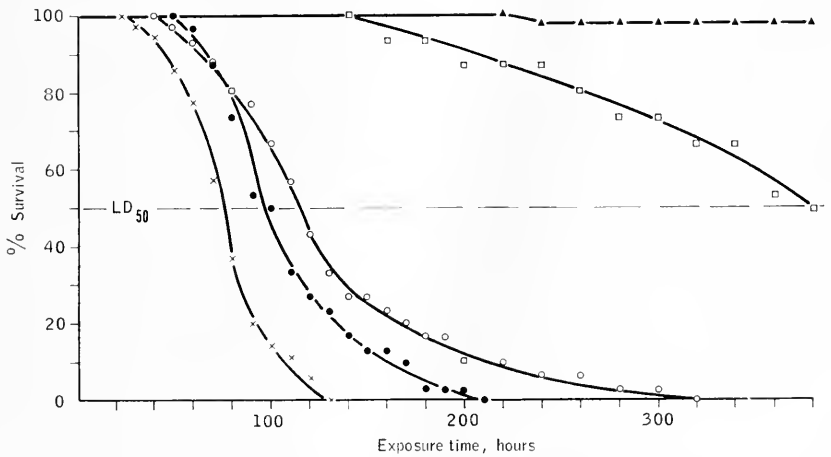

FIGURE 2. Survival among specimens of *Callianassa jamaicense* under aerial and aquatic anoxia compared to survival of controls under normoxia. Experimental conditions include anoxic water with accumulation of metabolic wastes (crosses) (N = 35), anoxic water changed daily (solid circles) (N = 30), aerial anoxia (open circles) (N = 30), aerial normoxia (squares) (N = 15), and aquatic normoxia (triangles) (N = 50); N is the number of animals initially exposed to each condition. Temperature was maintained at 25 ± 0.2° C; salinity was 20 ± 0.3‰.

lying *C. jamaicense* burrows was measured *in situ* with an air-calibrated Yellow Springs Instrument oxygen meter. Water from burrows of *C. jamaicense* was sampled and analyzed as described by Thompson and Pritchard (1969).

Results

Survival under anoxia

Survival of specimens of *C. jamaicense* under aquatic and aerial anoxia is plotted in Figure 2. The LD_{50} (mean lethal dose) was lowest, *ca.* 3.2 days, when anoxic water was not changed and metabolic wastes accumulated for the duration of survival. Under such conditions, with individual animals sealed into 300-ml BOD bottles of anoxic water, ambient pH dropped from an intial level of 8.7 ± 0.3 to 6.9 ± 0.5 at the time of death. When anoxic water was replaced daily, the LD_{50} increased to *ca.* 4 days. The LD_{50} for animals held in aerial anoxia, *ca.* 5 days, exceeded that for animals subjected to aquatic anoxia. Among those animals subjected to aerial anoxia or daily changes of anoxic water, a few survived more than two times the exposures producing LD_{50}'s.

Under normoxic conditions, losses of control animals in a water-saturated environment approached the LD_{50} on the 16th day of exposure. Mortalities among control animals in normoxic water did not exceed 2% within the same 16-day time period.

Aerial oxygen consumption

After 60 min of equilibration in a 25° C Gilson respirometer, oxygen consumption ($\dot{V}_{O_2}$) in water-saturated air was read at 30-min intervals over an additional 2-hr period. Wet weights of the 25 animals used in the aerial respiration experiments ranged from 3.51 to 5.25 g. Mean $\dot{V}_{O_2}$ rates and standard errors over the four successive 30-min time periods were 18.9 ± 1.53, 16.2 ± 1.29, 19.0 ± 1.39, and 18.9 ± 1.12 μl/(g wet wt·hr), respectively. Activity in the respirometer flasks was not quantitatively monitored, but animals were for the most part quiescent during the $\dot{V}_{O_2}$ determinations.

Effects of low oxygen tension on aquatic oxygen consumption

As oxygen was depleted from sealed BOD bottles, specimens of *C. jamaicense* regulated $\dot{V}_{O_2}$ until oxygen tension (P_{O_2}) decreased to *ca.* 20 mmHg (Fig. 3). The critical oxygen tension (P_c) ranged from 10 to 25 mmHg among the 10 animals studied. The slightly higher $\dot{V}_{O_2}$ at 120 mmHg is of questionable significance as it may relate to disturbance of animals when placing them into the BOD bottles at the beginning of the experiment. Mean pleopod ventilatory rates ranged from 20 to 33 strokes/min at oxygen tensions above the P_c. As P_{O_2} fell from 20 to 10 mmHg, pleopod activity increased to near 60 strokes/min; concurrent increases in $\dot{V}_{O_2}$ occurred in some animals and accounted for the large range of $\dot{V}_{O_2}$ at oxygen tensions between 12 and 15 mmHg (Fig. 3). Pleopod activity decreased as P_{O_2} dropped below 9 mmHg and was again near 39 strokes/

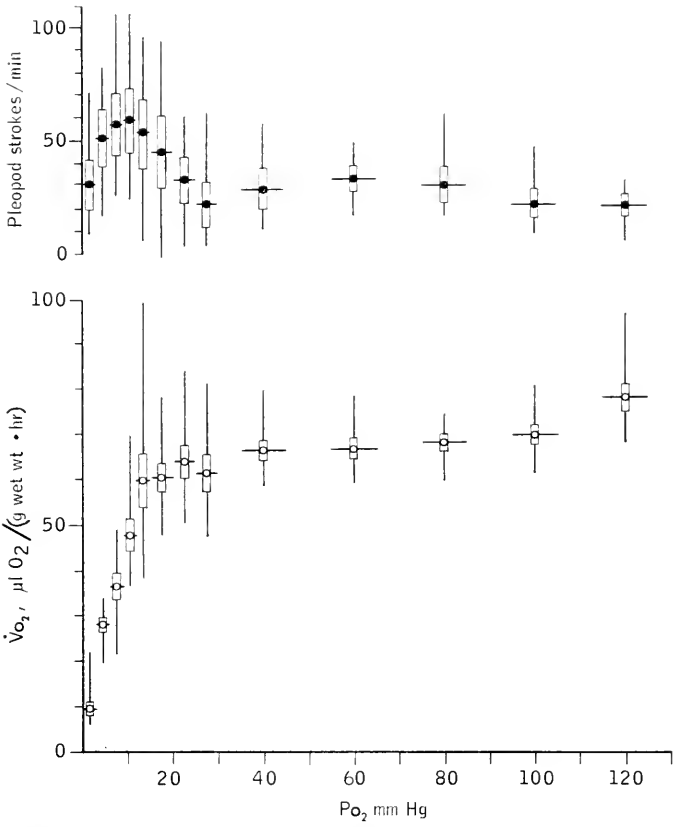

FIGURE 3. Mean oxygen consumption (open circles) and mean ventilatory rate (solid circles) among specimens of *Callianassa jamaicense* as oxygen is depleted from a sealed bottle. Each open circle is mean value for 10 animals and each solid circle is mean value for eight animals. Vertical lines indicate ranges; rectangles indicate standard errors; horizontal lines indicate span of oxygen tension over which means are taken. Wet weights range from 1.45 to 3.82 g. Temperature was maintained at 25 ± 0.2° C; salinity was 20 ± 0.3‰.

min at 0 mmHg. Animals held at complete anoxia continued to decrease pleopod activity and, after 2 to 4 hr, stopped ventilating unless disturbed.

When P_{O_2} was abruptly decreased from normoxia (150 mmHg) to hypoxia (37 mmHg) in a flow-through respirometer, specimens of *C. jamaicense* reduced $\dot{V}_{O_2}$ by more than 50% for 2 to 3 hr (Fig. 4). Oxygen consumption gradually increased after 5 hr of hypoxia and after 9 hr was near 75% of $\dot{V}_{O_2}$ in normoxia. In normoxia, pleopod activity ranged from 14 to 18 strokes/min. As P_{O_2} decreased to 37 mmHg, pleopod activity at first increased slightly but soon subsided to rates less than those in normoxia.

When introduction of hypoxic water (37 mmHg) followed 12 hr of anoxia, the $\dot{V}_{O_2}$ of *C. jamaicense* was initially just above that observed under normoxia (Fig. 5). The $\dot{V}_{O_2}$ in hypoxia decreased slowly from the rates measured shortly after termination of anoxia. Pleopod activity, which was negligible during anoxia,

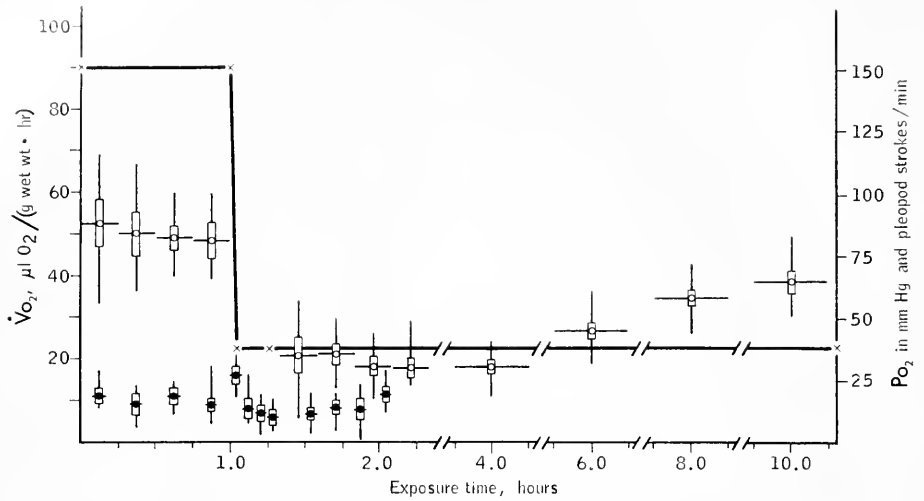

FIGURE 4. Temporal variations in aquatic oxygen consumption (open circles) and pleopod ventilatory rate (solid circles) among specimens of *Callianassa jamaicense* when ambient oxygen tension (crosses on heavy line) is abruptly reduced. Each value is mean rate for five animals. Vertical lines indicate ranges; rectangles indicate standard errors; horizontal lines indicate time spans over which means are taken. Temperature was maintained at $25 \pm 0.2°$ C; salinity was $20 \pm 0.3‰$.

dramatically increased with introduction of hypoxic water. As hypoxic water entered the respiration chamber following 12 hr of anoxia, animals invariably moved to the influent opening of the chamber (Fig. 1) and began rapid ventilation with their pleopods. The accelerated pleopod activity was maintained near 50 strokes/min for *ca.* 30 min after hypoxic water was introduced into the chamber, and animals spent almost all of this time near the influent opening of the respiration chamber. A gradual decrease of pleopod activity paralleled the slowly decreasing $\dot{V}_{O_2}$ which began near the middle of hour 14 and continued through hour 15.

When anoxia was terminated by introducing normoxic (150 mmHg) water, the $\dot{V}_{O_2}$ increased to two times the rates preceding anoxia (Fig. 6). Five hours after anoxia was terminated, $\dot{V}_{O_2}$ approached that observed before anoxia. With the reintroduction of normoxic water, animals moved to the influent opening of the respiration chamber and rapidly ventilated with their pleopods as when hypoxia (37 mmHg) followed anoxia (Fig. 5). However, animals neither remained at the incurrent opening nor maintained accelerated pleopod activity for as long as when hypoxic water followed anoxia.

Field measurements of dissolved oxygen

Burrows of *C. jamaicense* contained very low concentrations of dissolved oxygen when located above the waterline. On Grand Terre Island fluctuating tides exposed numerous burrows along pond margins for periods varying from a few hours to several days. Oxygen tension in water from five active burrows located from 1 to 3 m outside the pond ranged from 0 to 5 mmHg ($\bar{x} = 2.2$ mmHg),

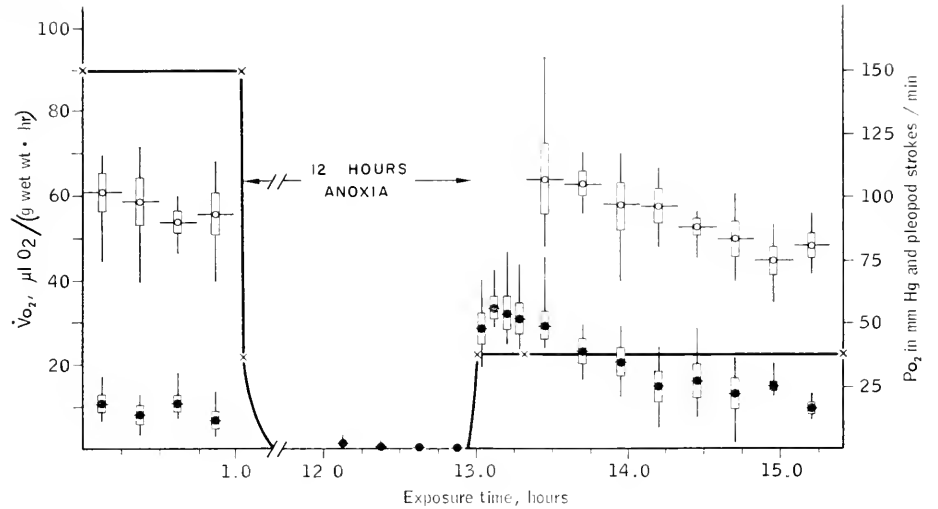

FIGURE 5. Temporal variations in aquatic consumption (open circles) and pleopod ventilatory rates (solid circles) among specimens of *Callianassa jamaicense* when ambient oxygen tension (crosses on heavy line) is dropped to anoxia and then raised to hypoxia. Each value is mean rate for five animals. Vertical lines indicate ranges; rectangles indicate standard errors; horizontal lines indicate time span over which means are taken. Temperature was maintained at 25 ± 0.2° C; salinity was 20 ± 0.3‰.

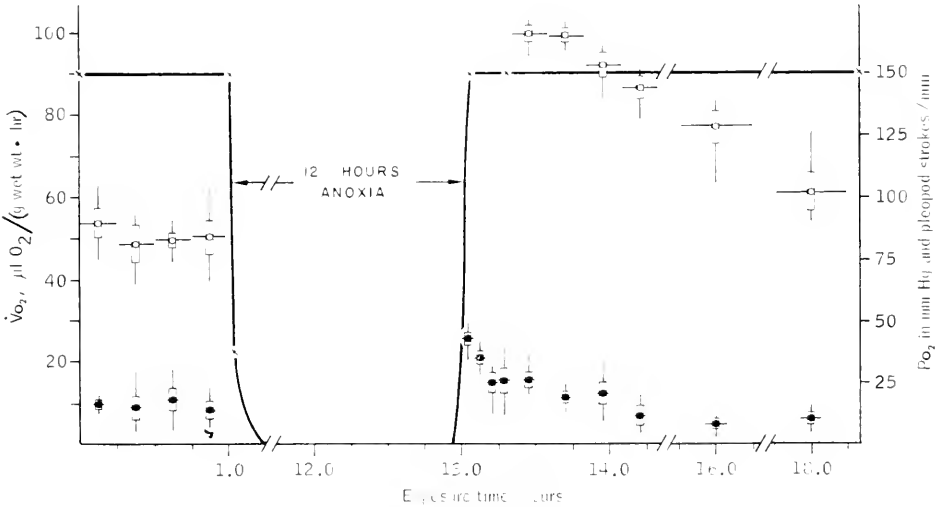

FIGURE 6. Temporal variations in aquatic consumption (open circles) and ventilatory rates (solid circles) among specimens of *Callianassa jamaicense* when ambient oxygen tension (crosses on heavy line) is dropped to anoxia and returned to normoxia. Each value is mean rate for five animals. Vertical lines indicate ranges; rectangles indicate standard errors; horizontal lines indicate time spans over which means are taken. Temperature was maintained at 25 ± 0.2° C; salinity was 20 ± 0.3‰.

which was well below critical oxygen tensions (P_c) established for specimens of *C. jamaicense* in the laboratory. Longer periods of isolation from estuarine pond waters were caused by storm-effected movements of sand which elevated large areas of the pond above water level, sometimes for periods of several months. Excavation of one such area which had been isolated from the pond for 2 months produced numerous living specimens of *C. jamaicense*, although most were moribund; some of these moribund specimens appeared to be occupying portions of burrows above the water table.

Higher and more variable oxygen tensions occurred in burrows at the immediate edge of the pond and just inside the pond. Oxygen tensions in 10 of these burrows, located from 0.2 to 2.0 m inside the pond, ranged from 11 to 119 mmHg ($\bar{x} = 73.4$ mmHg). However, at the time of sampling, oxygen tensions in surface waters were from 151 to 161 mmHg, or well above concentrations in burrows. Oxygen tensions in water of the estuarine pond probably approached these levels only during the periods of photosynthetic activity. Diel cycles of oxygen in water overlying *C. jamaicense* burrows on Grand Terre Island were characterized by decreasing tensions after dark as photosynthesis was replaced by net community oxygen consumption. Diel variations were monitored in October, 1974 (J. Day, personal communication), and P_{O_2} of water overlying *C. jamaicense* burrows remained below the P_c of *C. jamaicense* during a 4 to 5 hr period just before to just after dawn.

Discussion

The limited studies available to von Brand (1946) led him to conclude that decapod crustaceans show little tolerance of anoxia. Extended tolerance of anoxia is, however, one adaptation exhibited by a number of decapods which burrow in potentially hypoxic substrates. For instance, mud-burrowing crayfish survive anoxia four times longer than those inhabiting swift streams (Bovbjerg, 1952). Among the thalassinid decapods, *Upogebia pugettensis* and *Callianassa californiensis* survive anoxia for at least three days (Thompson and Pritchard, 1969) and *Callianassa jamaicense* survives anoxia for three to four days (Fig. 2). These and other thalassinids, such as *Callianassa affinis* in Southern California (Congleton, 1974) and *Callichirus foresti* from west Africa (LeLoeuff and Intes, 1974), are highly specialized for a burrowing existence in shallow, hypoxic, marine substrates, and their tolerance of anoxia is clearly an adaptation to habitat.

Under aquatic anoxia, the longer survival of specimens of *C. jamaicense* when anoxic water is changed daily, compared to survival of specimens when anoxic water is not changed, may reflect the effects of accumulated metabolic wastes. Products of anaerobic metabolism could account for the decrease in pH observed when individuals of *C. jamaicense* are sealed into BOD bottles of anoxic water and left until death occurs. The buffering characteristics of burrow water, particularly in the lower-salinity extremes of *C. jamaicense* habitat, or the ability to exchange anoxic burrow water might thus affect survival of *C. jamaicense* when it is subjected to periods of anoxia in nature. Short-term survival of *C. jamaicense* does not, however, appear to be affected by the physical presence or absence of the burrow, although MacGinitie (1934) reports that a specimen of *Callianassa*

californiensis will soon die if not maintained with its body contacting the wall of a tube. In either anoxia or normoxia, survival of specimens of *C. jamaicense* does not seem to be influenced by whether animals are maintained in plastic tubes or individually in larger bottles and open dishes. The possibility remains, however, that the tube facilitates efficient respiration in hypoxic water.

Survival and oxygen consumption by specimens of *C. jamaicense* in air were investigated despite the lack of direct evidence that this species resorts to aerial respiration in nature. However, on several occasions living animals were collected from substrates which had been exposed for up to 2 months, and on one occasion they were collected from a mud bank more than 1 m above the water table. As negligible concentrations of oxygen are usually found in water of exposed burrows, and as *C. jamaicense* has the ability to survive (Fig. 2) and respire in water-saturated air, occupancy of exposed upper portions of the burrows seems at least a plausible alternative to longterm anoxia. Aerial respiration is best documented among terrestrial and semiterrestrial decapods but is also used on an "emergency" basis by a number of aquatic species (Wolvekamp and Waterman, 1960). For example, *Carcinus maenas* may raise its body and aerially ventilate when stranded in hypoxic ponds (Taylor and Butler, 1973). The humid environment of *Callianassa* burrows fulfills an important requirement for aerial respiration as oxygen diffuses most rapidly across a wet cuticle (Lockwood, 1967).

The lower $\dot{V}_{O_2}$ of *C. jamaicense* in saturated air than in water could be attributed in part to decreased activity under aerial conditions as high P_{O_2} is maintained at the respiratory surface without the need for extensive ventilatory movements. The aerial $\dot{V}_{O_2}$ was less than 40% of aquatic $\dot{V}_{O_2}$ measured in BOD bottles or the flow-through system. This difference in aerial versus aquatic $\dot{V}_{O_2}$ is much greater than that reported for well-adapted semi-terrestrial decapods and suggests that the ability for aerial uptake of oxygen is not particularly well developed. It is not known to what degree *C. jamaicense* depends upon anaerobic pathways under such conditions as at least a partial source of energy. As suggested by Miller *et al.* (1976), it would be interesting to investigate the possible use of anaerobic pathways even at high oxygen tensions.

Within the P_{O_2} range of respiratory independence, $\dot{V}_{O_2}$ of *C. jamaicense* is *ca* 68 μl/(g wet wt·hr) in a stirred BOD bottle from which oxygen is being depleted and 50 to 55 μl/(g wet wt·hr) in a flow-through (10 ml min) respirometer. Both of these methods involve placement of the animal into a small diameter tube which simulates a burrow and allows the animal to brace itself while ventilating. Measurement of $\dot{V}_{O_2}$ in tubes seems to provide the better index of "routine" (*sensu* Fry, 1975) metabolic rates in burrowing thalassinids as this situation most closely approximates the natural mode of respiration. The reported respiration rates for intermolt specimens of *Callianassa californiensis* over the P_{O_2} range of respiratory independence are for animals not in tubes (Thompson and Pritchard, 1969; Miller *et al.*, 1976; Torres *et al.*, 1977), and these rates vary from *ca.* 18 to *ca.* 34 μl (g wet wt·hr). Farley and Case (1968) have shown that pleopod activity is clearly greater when a specimen of *Callianassa californiensis* is placed into a small diameter tube than when it is placed into a tube too large for it to brace against tube walls while countering pleopod strokes. In the present study smaller tubes were used in BOD bottles and this necessitated the

use of smaller animals (1.45–3.82 g) than in the flow-through respirometer (4.55–7.13 g); this size difference, as previously suggested by Torres *et al.* (1974), could account for observed differences in $\dot{V}_{O_2}$. Differences in the stirring or flowing of water during measurements of $\dot{V}_{O_2}$ in BOD bottles and the flow-through system may additionally contribute to a difference in $\dot{V}_{O_2}$ measured by those methods, as flow characteristics can engender adaptive respiratory responses (Mangum and van Winkle, 1973).

Regardless of the method of measurement, the metabolic rates here reported for *C. jamaicense* rank among the lower known for crustaceans at similar temperatures (Wolvekamp and Waterman, 1960) and reflect metabolic adaptation to a hypoxic habitat. Low metabolic rates in *Callianassa californiensis* and *Upogebia pugettensis* at 10° C are also considered adaptations to a similarly hypoxic habitat (Thompson and Pritchard, 1969). Montuori (1913) reports a much higher $\dot{V}_{O_2}$ of 132 μl/(g wet wt·hr) in *Callianassa subterranea* and 368 μl/(g wet wt·hr) in *Gebia litoralis* (*Upogebia litoralis*) at 25° C, but experimental conditions of his study differ too greatly to permit detailed comparisons of data.

The low critical oxygen tension (P_c) for *Callianassa jamaicense* likewise suggests a metabolic adaptation. Metabolic regulation is common to a large number of aquatic crustaceans, and van Winkle and Mangum (1975) note that such regulation is expected where the path of oxygen permeation is restricted to an indirect route by way of circulating body fluids. The P_c between 10 and 25 mmHg for *C. jamaicense* (Fig. 3), like that reported for other thalassinids (Thompson and Pritchard, 1969; Miller *et al.*, 1976; Torres *et al.*, 1977) is well below the P_c for most crustaceans (Wolvekamp and Waterman, 1960). Hypothetical curves of oxygen consumption over decreasing P_{O_2}, as predicted by a polynomial model (Mangum and van Winkle, 1973), suggest a P_c for *Upogebia affinis* similar to that reported for *U. pugettensis* but not as low as those in *Callianassa californiensis* or *C. jamaicense*. It has been suggested that the P_c of crustaceans represents the P_{O_2} at which blood pigment fails to become saturated at the gills (Redmond, 1955) or, more recently, that it reflects the initiation of anaerobiosis (Young, 1973). Regardless, maintenance of aerobic respiration until a very low P_{O_2} is reached would seem a conservative adaptation for burrowers in hypoxic substrates.

Present data do not explain the mechanics involved in metabolic regulation by *Callianassa jamaicense* at low P_{O_2}. Neither pleopod activity (Fig. 3) nor heart rate (Thompson and Pritchard, 1969) shows a linear increase with decreasing ambient P_{O_2}. The findings of Torres *et al.* (1977) additionally show that complete immobilization of the pleopods causes no appreciable change in the P_c. However, scaphognathite ventilation rates were not monitored, and it remains to be seen whether scaphognathite ventilation rate, cardiac output, and circulation patterns undergo proportional increases at lowered P_{O_2}. Studies with totally bled specimens of *Callianassa* suggest that at least part of the ability to regulate $\dot{V}_{O_2}$ is due to respiratory properties of the blood itself (Miller *et al.*, 1976).

The increase in pleopod activity at and just below the P_c may reflect an "escape" reaction; escape from low P_{O_2} in nature could be achieved by rapid pleopod ventilation which would replace low P_{O_2} burrow water with higher P_{O_2} water from overhead. Periodic ventilatory pulses, such as those reported for *Callianassa filholi* when specimens are confined to a glass tube (Devine, 1966), may likewise

be such reactions triggered by depletion of oxygen to a concentration near the P_c. Such reactions and taxic responses exhibited during flow-through respirometry suggest the presence of an internal or external oxygen receptor in *C. jamaicense;* Farley and Case (1968) have previously postulated the existence of such a receptor in *Callianassa californiensis* and *C. affinis,* but direct evidence for an oxygen receptor is still lacking for any thalassinid species.

The drop in $\dot{V}_{O_2}$ following abrupt exposure of specimens of *Callianassa jamaicense* to hypoxic water (Fig. 4) indicates that regulation of metabolic rates in low P_{O_2} is dependent upon how fast hypoxia is approached. The decrease in $\dot{V}_{O_2}$ suggests a partial shutdown of aerobic respiration or loss of metabolic regulatory ability unless hypoxia is approached slowly. Mangum (1970) reports aerobic shutdown in bloodworms rapidly introduced into hypoxic water, and Kushins and Mangum (1971) note that metabolic response of the snail, *Nassarius,* depends upon how rapidly hypoxia is approached. Similarly, Hiestand (1931) reports that a crayfish which normally responds as a metabolic regulator will metabolically conform if placed into a small volume of water where P_{O_2} is reduced rapidly or if depletion of oxygen in a large jar commences at less than air saturation. Because $\dot{V}_{O_2}$ of *C. jamaicense* slowly increases after several hours in hypoxic water (Fig. 4), it appears that a time-dependent internal change, such as descrease in pH or re-establishment of diffusion gradients, is linked to ability to regulate $\dot{V}_{O_2}$. This suggests that some degree of low-P_{O_2} acclimation is induced in *C. jamaicense* after six or more hours of hypoxia.

The lowest ambient oxygen tensions in the *Callianassa jamaicense* habitat occur on occasions when burrows are exposed and animals cannot ventilate by pumping water from overhead. An increase in $\dot{V}_{O_2}$ after termination of anoxia (Fig. 6) suggests the development of an oxygen debt under such conditions; a similar compensatory increase occurs in *Callianassa californiensis* and *Upogebia pugettensis* after exposure to anoxia (Thompson and Pritchard, 1969). Although evidence of oxygen debts among crustaceans is meager (Lockwood, 1967), clear evidence of anaerobic glycolysis in *Callianassa californiensis* tends to support this hypothesis (Hawkins, 1971, unpublished M. S. thesis, Oregon State University). Published field observations of several Pacific coast thalassinids (MacGinitie, 1935) and present observations of *Callianassa jamaicense, C. major,* and *C. islagrande* on the Louisiana coast indicate these animals move to the upper portions of the burrows as high tides flood burrows exposed earlier by low tides. Such behavior would facilitate the most rapid exchange of burrow water and payment of an oxygen debt developed during anaerobiosis. The magnitude and duration of elevated $\dot{V}_{O_2}$ rates in *C. jamaicense* following anoxia are determined by the ambient P_{O_2} provided at the termination of anoxia, as evident in comparing Figures 5 and 6. The rate of oxygen uptake under such circumstances is at least passively affected by the blood-to-water gradient of P_{O_2}, and a pattern for metabolic regulation is temporarily supplanted by metabolic conformation and higher respiratory rates in normoxia. It seems very unlikely that the observed differences in post-anoxia $\dot{V}_{O_2}$ rates can be attributed to activity, because activity is greater and maintained at elevated rates for a longer period during slightly elevated $\dot{V}_{O_2}$ in hypoxic water following anoxia (Fig. 5) than during the greatly elevated $\dot{V}_{O_2}$ in normoxic water following anoxia (Fig. 6).

Oxygen uptake via areas of the integument other than gills has not been investigated in *C. jamaicense*, but as these animals have a thin exoskeleton, such extrabranchial uptake of oxygen seems a strong possibility and could prove advantageous in a hypoxic habitat. Although there is no conspicuous morphological evidence of specialized, extrabranchial respiratory surfaces in *C. jamaicense*, accessory pleopodal gill filaments occur on some thalassinids (de Saint Laurent, 1973). The experiments of Torres *et al.* (1977) show no evidence of extrabranchial uptake in pleopods of *Callianassa californiensis*, but further investigations of accessory uptake are warranted; such studies could possibly explain the low oxygen gradients between prebranchial and postbranchial blood reported by Miller *et al.* (1976).

Summary

Callianassa jamaicense survives exposure to aquatic and aerial anoxia for more than 3 days. In normoxic water-saturated air it survives for *ca.* 16 days. The rate of oxygen consumption ($\dot{V}_{O_2}$) in air is less than 40% of $\dot{V}_{O_2}$ in water. Aquatic $\dot{V}_{O_2}$ is regulated above critical oxygen tensions (P_c) of 10 to 25 mmHg when animals are allowed to slowly deplete oxygen from a sealed bottle. Mean aquatic $\dot{V}_{O_2}$ of animals in a flow-through respirometer or in tubes placed into sealed BOD bottles ranges from 50 to 68 μl/(g wet wt·hr) over oxygen tensions (P_{O_2}) above the P_c.

After a 12-hr exposure to anoxic water, $\dot{V}_{O_2}$ is not regulated; post-anoxia $\dot{V}_{O_2}$ in hypoxic water (37 mmHg) is initially less than $\dot{V}_{O_2}$ measured in normoxic water (150 mmHg) before exposure to anoxia; post-anoxia $\dot{V}_{O_2}$ in normoxic water is initially two times the pre-anoxia $\dot{V}_{O_2}$ and suggests the development of an oxygen debt during anoxia. When P_{O_2} of ambient water is abruptly dropped from 150 to 37 mmHg, specimens of *C. jamaicense* exhibit a partial shutdown of aerobic metabolism, but the $\dot{V}_{O_2}$ begins to recover after 6 hr in hypoxia.

When oxygen tension is slowly decreased, pleopod ventilation rate varies little as P_{O_2} changes from 120 to 20 mmHg. The pleopod ventilation rate increases as P_{O_2} falls 20 to 10 mmHg, but decreases below 10 mmHg and stops after several hours under anoxia. The rapid response of taxis and pleopod activity when *C. jamaicense* is exposed to altered P_{O_2} suggests rapid perception of external oxygen levels and provides further circumstantial evidence of an oxygen receptor in thalassinids.

Tolerance of anoxia, metabolic regulation to a low P_c, low metabolic rates, metabolic responses following anoxia, and taxic response to altered P_{O_2} constitute adaptations to the hypoxic habitat of *C. jamaicense*.

LITERATURE CITED

Bovbjerg, R. V., 1952. Comparative ecology and physiology of the crayfish *Orconectes propinquus* and *Cambarus fodiens*. *Physiol. Zool.*, **25**: 34–56.

von Brand, T., 1946. *Anaerobiosis in Invertebrates*. Biodynamica Monographs, Normandy, Mo., 328 pp.

Congleton, J. L., 1974. The respiratory response to asphyxia of *Typhlogobius californiensis* (Teleostei: Gobiidae) and some related gobies. *Biol. Bull.*, **146**: 186–205.

Devine, C. E., 1966. Ecology of *Callianassa filholi* Milne Edwards 1878 (Crustacea, Thalassinidea). *Trans. R. Soc. N. Z. Biol. Sci.*, **8**: 93–110.

FARLEY, R. D., AND J. F. CASE, 1968. Perception of external oxygen by the burrowing shrimp, *Callianassa californiensis* Dana and *C. affinis* Dana. *Biol. Bull.,* **134**: 261–265.

FELDER, D. L., 1978. Osmotic and ionic regulation in several Western Atlantic Callianassidae (Crustacea, Decapoda, Thalassinidea). *Biol. Bull.,* **154**: 409–429.

FRY, F. E. J., 1957. The aquatic respiration of fish. Pages 1–63 in M. E. Brown, Ed., *The Physiology of Fishes,* Vol. 1. Academic Press, New York.

HIESTAND, W. A., 1931. The influence of varying tensions of oxygen upon the respiratory metabolism of certain aquatic insects and the crayfish. *Physiol. Zool.,* **4**: 246–270.

KUSHINS, L. S., AND C. P. MANGUM, 1971. Responses to low oxygen conditions in two species of the mud snail *Nassarius. Comp. Biochem. Physiol.,* **39A**: 421–435.

LELOEUFF, P., AND A. INTES, 1974. Les Thalassinidea (Crustacea, Decapoda) du Golfe de Guinée, systématique-écology. *Cah. O. R. S. T. O. M. Sér. Occanogr.,* **12**: 17–69.

LOCKWOOD, A. P. M., 1967. *Aspects of the Physiology of Crustacea.* W. H. Freeman and Co., San Francisco, 328 pp.

MACGINITIE, G. E., 1934. The natural history of *Callianassa californiensis* Dana. *Am. Midl. Nat.,* **15**: 166–177.

MACGINITIE, G. E., 1935. Ecological aspects of a California marine estuary. *Am. Midl. Nat.,* **16**: 629–765.

MANGUM, C. P., 1970. Respiratory physiology in annelids. *Am. Sci.,* **58**: 641–647.

MANGUM, C. P., AND W. VAN WINKLE, 1973. Responses of aquatic invertebrates to declining oxygen conditions. *Am. Zool.,* **13**: 529–541.

MILLER, K., AND K. E. VAN HOLDE, 1974. Oxygen binding by *Callianassa californiensis* hemocyanin. *Biochemistry.* **13**: 1668–1674.

MILLER, K. I., A. W. PRITCHARD, AND P. S. RUTLEDGE, 1976. Respiratory regulation and the role of the blood in the burrowing shrimp *Callianassa californiensis* (Decapoda: Thalassinidea). *Mar. Biol.* (Berl.), **36**: 233–242.

MONTUORI, A., 1913. Les processus oxydatifs chex les animaux marins in rapport avec la loi de superficie. *Archs. Ital. Biol.,* **59**: 213–234.

REDMOND, J. R., 1955. The respiratory function of hemocyanin in Crustacea. *J. Cell. Comp. Physiol.,* **46**: 209–247.

RODRIGUES, S. A., 1971. Mud shrimps of the genus *Callianassa* Leach from the Brazilian coast (Crustacea, Decapoda). *Arq. Zool. (São Paulo),* **20**: 191–223.

ROXBY, R., K. MILLER, D. P. BLAIR, AND K. E. VAN HOLDE, 1974. Subunits and association equilibria of *Callianassa* hemocyanin. *Biochemistry,* **13**: 1662–1668.

DE SAINT LAURENT, M., 1973. Sur la systématique et la phylogénie des Thalassinidea: définition des familles des Callianassidae et des Upogebiidae et diagnose de cinq genres nouveaux (Crustacea, Decapoda). *C.R. Acad. Sci. Paris,* **277**: 513–516.

STRICKLAND, J. D. H., AND T. R. PARSONS, 1972. *Bulletin 167, A Practical Handbook of Seawater Analysis (Second Edition).* Fisheries Research Board of Canada, Ottawa, 310 pp.

TAYLOR, E. W., AND P. J. BUTLER, 1973. The behavior and physiological responses of the shore crab *Carcinus maenas* during changes in environmental oxygen tension. *Neth. J. Sea Res.* **7**: 496–505.

THOMPSON, R. K., AND A. W. PRITCHARD, 1969. Respiratory adaptations of two burrowing crustaceans, *Callianassa californiensis* and *Upogebia pugettensis* (Decapoda, Thalassinidea). *Biol. Bull.,* **136**: 274–287.

TORRES, J. J., D. L. GLUCK, AND J. J. CHILDRESS, 1977. Activity and physiological significance of the pleopods in the respiration of *Callianassa californiensis* (Dana) (Crustacea: Thalassinidea). *Biol. Bull.,* **152**: 134–146.

VAN WINKLE, W., AND C. P. MANGUM, 1975. Oxyconformers and oxyregulators: A quantitative index. *J. Exp. Mar. Biol. Ecol.,* **17**: 103–110.

WOLVEKAMP, H. P., AND T. H. WATERMAN, 1960. Respiration. Pages 35–100 in T. H. Waterman, Ed., *The Physiology of Crustacea,* Vol. 1. Academic Press, New York.

YOUNG, R. E., 1973. Responses to respiratory stress in relation to blood pigment affinity in *Goniopsis cruentata* (Latreille) and (to a lesser extent) in *Cardiosoma guanhumi* Latreille. *J. Exp. Mar. Biol. Ecol.,* **11**: 91–102.

Reference: *Biol. Bull.* **157**: 138–152. (August 1979)

AN ANALYSIS OF THE DEFENSIVE MECHANISMS OBSERVED IN THE ANEMONE *ANTHOPLEURA ELEGANTISSIMA* IN RESPONSE TO ITS NUDIBRANCH PREDATOR *AEOLIDIA PAPILLOSA*

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The sea anemone *Anthopleura elegantissima* (Brandt, 1835) is a conspicuous member of mid-intertidal communities along the exposed rocky west coast of the United States (Hand, 1955; Ricketts and Calvin, 1962; Sebens, 1977). Several recent studies (Waters, 1973; Harris, 1973; Edmunds, Potts, Swinfin and Walters, 1975, 1976) have reported that *A. elegantissima* is a preferred prey of the anemone-eating aeolid nudibranch, *Aeolidia papillosa* (Linnaeus, 1767). Edmunds *et al.*, (1976) described behavioral reactions of *A. elegantissima* to attack, including bulging of the column at the site of attack, crawling, and releasing from the substrate. Howe and Sheikh (1975) characterized an alarm pheromone, anthopleurine, from *A. elegantissima* and described the behavioral response it elicited in the anemone. Howe and Harris (1978) demonstrated that *A. papillosa* acquires anthopleurine when feeding on *A. elegantissima* and that leakage of the pheromone caused the alarm response in other individuals. Waters (1973) speculated that *A. papillosa* is evolving to specialize on *A. elegantissima.*

Anthopleura elegantissima has well-developed behavioral responses to attack by *A. papillosa,* but none of these behaviors provides an effective defense in the laboratory. In addition, *A. papillosa* is consistently found associated with one of its least preferred prey, the subtidal anemone *Metridium senile* (Linnaeus, 1767) (Harris, 1973; Brewer, 1977). The ineffectiveness of the defenses of *A. elegantissima* against one of its chief predators under laboratory conditions and the fact that the predator is primarily associated with a less preferred prey suggests that the defenses may be more effective under natural conditions. The purpose of this study was to investigate a series of potential defensive mechanisms which may provide at least partial protection for *A. elegantissima* against *A. papillosa.* The effectiveness of these mechanisms were then evaluated in the context of the environment in which this predator-prey association is found.

Materials and Methods

This study was conducted during the period of January to June 1976, though L.G.H. had been making observations on *A. papillosa* since 1964. The laboratory experiments and observations were conducted at the facilities of Hopkins Marine Laboratory, Pacific Grove, California. Field studies were done at the laboratory and at two nearby locations in Monterey Bay (36° 37′ N, 121° 53′ W).

Some of the specimens of *Anthropleura elegantissima* were collected from a rock

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outcropping on the east side of the laboratory and adjacent to a ramp for an old boat works (Site 1). These rocks face east and are exposed to direct sunlight from sunrise to late afternoon (1500–1600 hr). Most of the specimens of *Anthopleura elegantissima* and some of the specimens of *Aeolidia papillosa* were collected from the rocky intertidal zone under abandoned sardine canneries (Site II) located adjacent to the laboratory; this site is shaded by the buildings and only receives direct sunlight from sunrise until about 1200 hr. Most of the specimens of *A. papillosa* were obtained from a subtidal site under Wharf No. 2 in Monterey (Site III). At this site, specimens of *A. papillosa* of various sizes can be obtained throughout the year, feeding on the large concentrations of *Metridium senile* (Yarnall, 1972; Brewer, 1977). Animals were maintained in running sea water at ambient temperatures (14° C).

It is important to distinguish between two forms of *Anthopleura elegantissima* present in Monterey Bay and farther south. The clonal form of *A. elegantissima* is restricted to the midtide zone, forms clones by binary fission on open rock surfaces, and seldom grows to a column diameter greater than 50 mm. A solitary form of *A. elegantissima* is found in the lower intertidal zone and to depths of 15 m; it attaches in cracks in the rock substrate and has not been observed to reproduce asexually. Individuals of this anemone may attain a column diameter of greater than 80 mm. In this study, we will refer only to the clonal form.

Intensive surveys of two rocky intertidal sites (Sites I and II) were undertaken in March and again in May, 1976, to determine densities of *A. papillosa* associated with *A. elegantissima*. Site II under the canneries faced northeast and received direct sunlight only during the morning hours. Small specimens of *A. papillosa* were common among clones of *A. elegantissima* at this site from January through March, 1976. Sampling was done using a 1 64-m quadrat to determine the relative density of *A. papillosa* compared to the density of *A. elegantissima*. No specimens of *A. papillosa* were ever found associated with clones of *A. elegantissima* at Site I, so no quadrat sampling was done there nor at Site II in June.

Three experiments were conducted to assess the importance of desiccation as an environmental stress on *A. papillosa*. In the first, six specimens of *A. papillosa* and six specimens of *A. elegantissima* were placed in stacking dishes without water for 6 hr. The experiment was done in the laboratory to approximate the time of exposure at neap low tide on a sunless day. In the second and third experiments, groups of *A. papillosa* were placed on rocks among clones of *A. elegantissima* immediately after the water had receded below the clones and were retrieved just prior to reflooding by the incoming tide. At the end of each experiment, animals were placed in dishes containing fresh sea water and held for 24 hr. Those animals which were moribund or unable to hold onto the glass dish after 24 hr were considered lost, while those which were attached and crawling normally were rated as surviving the low tide. The justification was that animals which were too weak to remain attached to the rock would be washed free by wave action. A nudibranch washed off a rock in the midtide may ultimately survive, but it is at least removed from the anemones on which it had been feeding. Controls were left in running sea water during the experiments.

Observations were made on feeding encounters between *A. elegantissima* and *A. papillosa* to determine the sequence of attack, the behavioral responses of *A.*

elegantissima and the preference of *A. papillosa* for particular regions of the anemone. In the first experiment, a number of anemones were removed from rocks and placed in a dish coated with silicone grease which prohibited their attachment. These anemones were left in running sea water for 24 hr and at the time of the experiment a majority of them were open. Fifty nudibranchs (body length 2–3 cm) were released into the dish and allowed to feed for 5 hours. To determine sites of attack by *A. papillosa,* the anemones were then relaxed in 7% MgCl (in fresh water) and surveyed for tissue damage which was obvious by direct observation. In a second experiment, approximately 100 specimens of *A. elegantissima* from a single clone were placed in a running sea-water table and groups of four to five nudibranchs were placed among the anemones; attacks were described and tabulated.

Observations showed that contact of any part of the nudibranch with the column of an anemone caused local swelling of the column at the site of contact. Preliminary experiments showed that the anemones were responding to *A. papillosa* mucus. A series of experiments were conducted to determine the site of receptivity of *A. elegantissima* to *A. papillosa* mucus, the duration of the response and the specificity of this response relative to other nudibranch species.

The tentacles or the column of the anemones were touched with mucus-covered or blank cotton swabs in the first experiment. In experiment two, mucus was obtained from the back or the foot of *A. papillosa,* from the aeolid *Hermissenda crassicornis* (Eschscholtz, 1831) and from the dorid nudibranch *Anisodoris nobilis* (Odhner, 1907). *A. nobilis,* which eats only sponges, served as a control in the second set of experiments. In all tests, nudibranchs were rubbed with a wet cotton swab which was then applied to a part of the body of the anemone. A separate swab was used for each anemone. The behavior of the anemones and duration of any response was described from observations made several times an hour.

Results and Observations

Qualitative sampling of a number of exposed rocky intertidal habitats along the California coast by both authors over several years showed that *A. papillosa* is found in association with clones of *A. elegantissima* throughout the year. The density of nudibranchs tends to be higher in the winter than in the summer, and a majority of the animals seen and/or collected were large—between 30 and 80 mm in length.

At certain times of the year, *A. papillosa* can be very common in association with *A. elegantissima* (Table I). Small specimens of *A. papillosa* (mean length 12 mm; maximum 22 mm) were prevalent at the canneries site (Site II) from January through March. However, the mean length of animals observed and/or collected did not increase over this 3-month period, and they were not present a few hundred meters away at Site I. In late April, a storm pounded the intertidal zone with approximately 2.5-m waves. From late April through July no specimens of *A. papillosa* were observed at either of the intertidal stations, although at Site III all sizes were common in subtidal fouling communities (Table I). *A. papillosa* grows from 1 mm to over 30 mm in about 2 months when feeding on *A. elegantissima* in the laboratory (Harris, in preparation); this suggests that

TABLE I

Comparison of Aeolidia papillosa *density in association with anemone clones at two intertidal and one subtidal (8 m) sites in Monterey Bay in winter and summer. Site I is a rocky outcropping on the beach on the NE side of Hopkins Marine Station. Site II is a rocky ledge under the canneries about 300 m E of Site I. Site III is under commercial Wharf No. 2 in Monterey. Site I and II contain clones of* Anthopleura elegantissima *and Site III is dominated by clones of* Metridium senile. *Where no quadrat numbers are given, a minimum of 5 hr of observations were made at each site during that month.*

	Number *A. papillosa* /m² of anemone clone			
Site	February 1976	April 1976	June 1976	Time of direct exposure to sunlight
I	0 m²	0	0	sunrise to ~1600.
II	24.3 m² (104 1 64 m²)	0	0	sunrise to ~1200.
III	86 m² (10 1 10 m²)	present	9.5 m² (20 1 10 m²)	none

nudibranchs were continuously recruiting to the site under the canneries but that they were not surviving long enough to reach sexual maturity.

Small specimens of *A. papillosa* (< 15 mm length) were typically found within 2 cm of the anemones. Often this was at the edge of a clone or within a scattered aggregate of anemones. A nudibranch might be in a small depression or crack in the rock surface, but the majority of individuals were exposed on flat surfaces, apparently having made no attempt to hide when the tide receded. Large specimens of *A. papillosa* (> 15 mm) may be found farther from their prey and typically seek out cracks in which to hide when not feeding. However, even with intensive

TABLE II

A summary of quadrat analyses done to assess the relative density of Aeolidia papillosa *in relation to the percentage of free space among* Anthopleura elegantissima *clones. The sampling was done at Station II under the canneries on 12 February 1976. The quadrat size was 1 64 m², and 104 quadrats were sampled.*

% Free space	0%	25%	50%	75%	Total
Number of quadrats	22	39	31	12	104
Number of quadrats with *A. papillosa*	2	17	11	4	34
Number of *A. papillosa*	2	18	18	5	44
					Mean
Number of *A. papillosa* per quadrat	0.09[1]	0.46	0.56	0.41	0.42
Density of *A. papillosa* per m² of *A. elegantissima* clone	5.8	29.4	35.8	26.2	24.3
% Quadrats with *A. papillosa*	9%	43%	35%	33%	33%

[1] Significant at <0.01 (*t*-test).

TABLE III

Results of desiccation experiments in which Aeolidia papillosa *was exposed to air for 6 hr and then returned to fresh sea water. Survival was determined after 24 hr. Experiment 1 was done in the laboratory while experiments 2 and 3 were conducted in the field by placing nudibranchs adjacent to clones of* Anthopleura elegantissima *on the receding tide and retrieving them just prior to submergence on the incoming tide. The results were significant at less than 0.01% using chi square.*

Experiment	1	2	3A	3B
Number of *A. papillosa*	6	10	32	33
Number surviving after 24 hr (%)	4 (66%)	4 (40%)	16 (50%)	13 (39%)[1]
Control survival	100%	100%	100%	100%

[1] Eighteen nudibranchs disappeared when the tide washed over the rock containing the animals before they could be retrieved; only 15 animals remained and the 18 missing *A. papillosa* were considered killed.

searching, no nudibranchs larger than 22 mm were found at Site II during the period from January through June 1976.

Results of the quadrat sampling at Site II illustrate the tendency of small nudibranchs to be found in open areas adjacent to groups of *A. elegantissima* (Table II). The quadrats were only placed over areas containing *A. elegantissima,* because numerous observations indicated that *A. papillosa* remains close to its prey. Only two nudibranchs were found within what appeared to be solid masses of anemones while the vast majority occurred at the periphery of clones. Close examination revealed that even in the most tightly packed clones, there was usually bare rock between pedal disks. The sampling data suggest that anemones within tightly packed clones are essentially free from predation by *A. papillosa.* While *A. papillosa* tended to be found at the periphery of clones where there is more free space, two other predators or parasites, the prosobranch *Epitonium tinctum* (Carpenter, 1864) and the pycnogonid *Pycnogonum stearnsi* (Ives, 1892) were common only within these tightly packed aggregations.

While no individuals of *A. papillosa* were collected at Sites I and II during the late spring and summer of 1976, one of us (L.G.H.) had previously collected specimens of *A. papillosa* and egg masses at several locations (Eagle Point, San Juan Islands, Washington; Dillon Beach and Bodega Bay, California) during the months of June and July. These sites are more exposed open coastal locations than the protected environment of Monterey Bay. In each case, the density of *A. papillosa* was well below $1/m^2$ of *A. elegantissima* clone. The majority of the nudibranchs were large (> 40 mm), sexually mature, and were located in tidepools or at the lower end of the anemone's distribution in the intertidal.

Results of tests on the effects of desiccation on *A. papillosa* are recorded in Table III. In both the laboratory and the field experiments, survival of test animals exposed to air for 6 hr was approximately 50%, while control animals maintained in running sea water had 100% survival. In the initial laboratory test, two animals died even though they were sitting in small amounts of residual water and mucus. Most of the nudibranchs were still reactive after 6 hr out of water, but those that ultimately died were unable to hold onto the dish after water was added.

Two smaller nudibranchs (about 20 mm) set out at Site I were so desiccated that they had to be scraped from the rock surface.

In Experiment 3B, (see Table III), 33 specimens of *A. papillosa* were placed among a clone of *A. elegantissima* at Site II. Due to a miscalculation, the nudibranchs were not retrieved until after a few small swells had already washed over the area. Only 15 out of the 33 animals were still attached to the rocks; the other

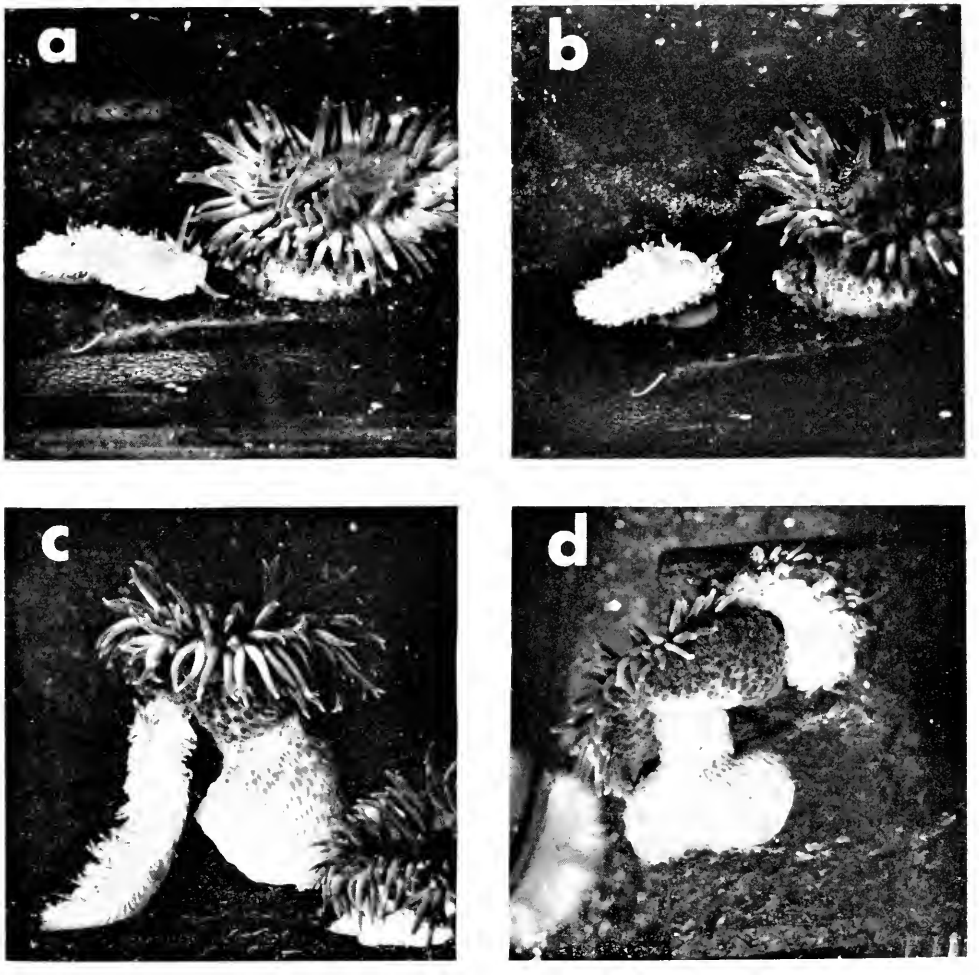

FIGURE 1. Photographic illustration of the initial contact between specimens of *A. papillosa* and *A. elegantissima* and two resulting feeding behaviors and anemone responses: (a) the initial contact involving the nudibranch's rhinophores and the anemone's tentacles; (b) the mutual retraction that typically occurs following first contact; (c) this nudibranch fed on the column for about 3 hr and made numerous attempts to reach the tentacles which were out of reach due to bulging of the column; (d) this nudibranch reached the tentacles before bulging of the column began and was lifted free of the substrate. The nudibranch fed in this position for about 3 hr; note line of mucus and detritus on the glass left by the anemone as it crawled during the attack.

Table IV

Feeding experiments to determine whether Aeolidia papillosa *shows a preference for specific body regions when attacking* Anthopleura elegantissima. *In Experiment A the anemones were not attached and were lying on their sides so an approaching nudibranch had one chance to encounter the oral area first, two chances for the column and one chance for the pedal disk. In Experiment B, the anemones were attached and a nudibranch would typically make contact with a tentacle first, but then it would touch the column even if it attacked the tentacles. The results were significant at less than 0.01% using chi square.*

Experiment	A		B	
	Predicted (%)	Actual (%)	Predicted (%)	Actual (%)
Column	16.5 (50%)	4 (12%)	30.5 (50%)	19 (31%)
Tentacles	8.25 (25%)	18 (54%)	30.5 (50%)	42 (69%)
Pedal disk	8.25 (25%)	11 (33%)	0[1]	0

[1] Anemones were attached so pedal disk was not available.

18 animals had disappeared, presumably washed away by slight swells (about 30 cm). Of the 15 animals remaining, 13 survived.

The results of a separate experiment also suggest the unsuitability of the intertidal for *A. papillosa* in the late spring and summer. On 2 May, 1976, 18 small specimens of *A. papillosa* (mean size 15 mm) were placed among marked clones in protected habitats under the canneries. The animals were placed among clones where there were cracks and algae to provide refuges from desiccation. Three days later, only one nudibranch could still be found in the area where it was placed. By the eighth day, no specimens of *A. papillosa* remained in the vicinity. During the late spring and summer this protected habitat, which is shaded after 1200 hr is apparently too stressful for *A. papillosa*.

The behavior of the nudibranch attack and anemone responses have been described previously (Russell, 1942; Waters, 1973; Harris, 1973; Edmunds *et al.*, 1976), but they will be reviewed because it is relevant to understanding the mechanism behind the bulging behavior described by Edmunds *et al.*, (1976). Figure 1 illustrates the sequence of attack. Initial contact is typically between the nudibranch's rhinophores and the anemone's tentacles (Fig. 1a). The tentacles and nudibranch both retract (Fig. 1b). *A. papillosa* then moves into attack with buccal mass extended, rhinophores retracted and cerata bristled. The oral tentacles touch the column causing it to bulge in the area touched (Figs. 1c, d). The bulging behavior consists of an exaggerated relaxation of the column wall where contact has been made. Waves of contraction run up and down the column and changes in height and shape are frequent during bulging. This behavior continues for up to 6 hr after a single contact with *A. papillosa* mucus. A result of this behavior is to raise the tentacles out of reach of the nudibranch (Fig. 1c). If the *A. papillosa* does reach the tentacles, before bulging is initiated, it may be lifted free of the substrate (Fig. 1d).

Experiments testing for a preferred site of feeding by *A. papillosa* on *A. elegantissima* showed that the tentacles are selected over the column (Table IV). Nudibranchs also select the pedal disc over the column when anemones are not

attached. This preference for the tentacles is even more obvious when the attack sequence is observed; as in Figure 1c, nudibranchs often finally fed on the column, because the anemone's behavioral responses prevented easy access to the tentacles.

The bulging behavior of *A. elegantissima* is elicited by mucus from *A. papillosa* and also from the aeolid *Hermissenda crassicornis* which occasionally attacks anemones (Table V). That mucus from *A. papillosa* caused bulging was first indicated during attempts to observe the alarm response in a tidepool containing several anemones. An *A. papillosa* was placed in the pool to determine if feeding on one anemone would cause the alarm response in other individuals. The nudibranch crawled between two anemones, but did not attack either. Within minutes the contacted anemones had elongated their columns; they remained in this expanded and distinctive posture for over 2 hr. This indicated to us that mucus from the nudibranch may be initiating this behavior. Subsequent experiments (Table V) verified this hypothesis. Mucus from *A. papillosa* caused the column to bulge, and this behavior pattern lasted for 3 to 6 or more hours even without further stimuli from the nudibranch. In addition to inflation of the column on the side touched, the verrucae dropped their attached sand grains and shell bits.

Tests conducted to determine sites of sensitivity in *A. elegantissima* to *A. papillosa* mucus revealed that only the column was responsive. The tentacles contracted when touched by mucus, but did not elicit any inflation behavior by any part of the column and returned to their normal relaxed position within a minute or two. Howe and Sheikh (1975) showed that the tentacles were the site of greatest sensitivity to the alarm pheromone anthopleurine. Mucus from any part of *A. papillosa* and from *Hermissenda crassicornis* elicited column bulging, while mucus from *Anisodoris nobilis* had no observable effect on anemone behavior. Some anemones continued to show bulging reactions for 6 or more hours after a single contact with mucus from *A. papillosa* even in running sea water. Since 6 hr is the approximate time of submergence during a tidal cycle and *A. papillosa* only feeds during high tide, these results suggest a two-part mechanism in which anthopleurine causes a quick and short-term contraction of the tentacles which increases the likelihood that *A. papillosa* will contact the column first. Contact with the column will result in mucus from the nudibranch being

TABLE V

Summary of two experiments to test the effects of Aeolidia papillosa *mucus on column bulging in* Anthopleura elegantissima. *Mucus secreted from glands on the foot and on the cerata was compared. Mucus from two other nudibranchs was also used;* Hermissenda crassicornis *occasionally attacks anemones, while* Anisodoris nobilis *eats only sponges and served as a control.*

Source of Mucus	% Anemones bulging over time in minutes						
	0	min 60	120	180	240	300	360
A. papillosa foot (34)[1]	0%	76	62	53	29	18	6
A. papillosa cerata (37)	0%	81	84	81	62	32	22
H. crassicornis (37)	0%	65	84	78	51	24	22
A. nobilis (40)	0%	2.5	5	7.5	7.5	2.5	0

[1] Total number of anemones used in two replicate experiments.

sensed by the column receptors; this will initiate a slow but long-lasting inflation of the column that will raise the tentacles far above the substrate, often out of reach of the nudibranch.

Verification that *A. papillosa* mucus elicits bulging under field conditions occurred during desiccation experiment 3 (Table III). Nudibranchs were placed in clear areas within scattered clones. Water and mucus from the nudibranchs drained down the rock surfaces and around anemones below the nudibranchs. Thirty minutes after the experiment began, anemones which contacted the fluid draining from *A. papillosa* were bulging; many dropped their sand grain cover and several had released from the rock. The unattached anemones were lying on their sides with the column bulged, but the slope was too gentle for them to fall or roll from their original position without water movement. The next day the anemones were reattached, contracted and recovered with sand grains. However, comparison of photographs taken during the experiment and the following day showed that a number of anemones had moved 2 to 6 cm and four anemones had disappeared.

When attacked by *A. papillosa,* individuals of *A. elegantissima* crawl in the opposite direction. In 3 hr animals may have crawled as far as 4 cm. In the next 24 hr animals continued to crawl for another 3 to 4 cm, although limited observations suggest that in the field they may not crawl as far as they do in the laboratory.

In the laboratory, approximately 10% of the anemones attacked by *A. papillosa* released from the substrate during the attack. We do not know whether the percentage of release is as high in the field where there is active water movement, though both authors have encountered *A. papillosa* in tide pools in the process of attacking unattached anemones. Verrucae of detached anemones are extremely adhesive and attach quickly to any object that they contact. Unless there is active water movement at the time anemone releases, it may ultimately reattach in the same area.

Discussion

The clonal form of *Anthopleura elegantissima* occurs primarily in the midtide zone on the exposed coastline of the west coast of North America (Hand, 1955; Ricketts and Calvin, 1962; Dayton, 1971; Francis, 1973a, b; Sebens, 1977). In this habitat individuals are exposed to wave action and six or more hours of exposure to air twice every 24 hr. Connell (1972) has suggested that the upper limit of a species in the intertidal is due to physical factors, especially physiological tolerance to exposure, and that the lower limits are due primarily to biological factors such as competition and predation. Dayton (1971) showed that *A. elegantissima* cannot survive in more protected areas of the San Juan Islands, because it is incapable of withstanding the long mid-day low tides in the summer. The exposure experiments (Table III) and collecting data suggest that specimens of *A. papillosa* are less able to withstand desiccation than their prey and that affects their ability to hold on to the substrate when the tide returns. The effect of desiccation appears to be greatest in the warmer months of the year and in protected areas such as Monterey Bay, since *A. papillosa* can be collected with *A. elegantissima* in the summer at exposed, open coastal sites (Waters, 1973).

A. papillosa presumably survives better in exposed habitats because spray from waves decreases the threat of desiccation.

The majority of large, reproductive *A. papillosa* found associated with clones of *A. elegantissima* have been at the lower end of the anemone's range in the intertidal. The mean size of *A. elegantissima* is greatest in these habitats (Sebens, 1977). Sebens (1977) has suggested that the longer submersion time increases feeding time for the anemones. A similar phenomenon has been described for the gastropod *Tegula funebralis* (Paine, 1969). The largest specimens of *T. funebralis* were found in the lower intertidal where the increased food supply and time for feeding would be translated into greater reproductive output. However, this was offset by increased threat of predation by the starfish *Pisaster ochraceus*. It is likely that the intertidal distribution of *A. elegantissima* represents at least a partial or seasonal refuge from predation by *A. papillosa* and that the lower limit of the anemone's range is influenced by where the balance between the potential for increased reproductive output and the threat of predation comes out in favor of predation.

Clone formation in *A. elegantissima* has been described as a competitive strategy that allows for rapid space utilization (Sebens, 1977). The fact that *A. elegantissima* has a well developed aggressive response to encroachment by other anemones similar to that reported for corals by Lang (1973) reinforces the likelihood that clone formation is an adaptation for space competition (Francis, 1975b, 1976; Purcell, 1977). Clone formation may also have adaptive significance as a defense against predation. Veligers of *A. papillosa* settle on the rock surface adjacent to their anemone prey (Harris, unpublished observations); therefore, only the periphery of a clone will be available for recruitment of the predator. The circumference of a clone of anemones is less than the sum of the circumferences of the same number of individual anemones were they dispersed, so there is less surface area for settlement. Also, the anemones at the center of the clone will be relatively free from predation (Table II) while those at the periphery of the clone will be exposed to the greatest damage from nudibranch attacks.

Francis (1976) has shown that anemones at the periphery of a clone serve as soliders which expend most of their energy in the production of nematocysts and regenerating wounded areas after aggressive encounters. These soldiers do not contribute directly to asexual or sexual reproductive efforts of the clone. Assuming that clone formation is primarily a competitive strategy similar to encrusting colonial growth as proposed by Jackson (1977), the additional adaptive value of minimizing the threat of predation for a majority of clone-mates should have a synergistic effect in reinforcing selection for cloning.

A. papillosa shows a clear preference for the tentacles of *A. elegantissima* (Table IV). The final feeding site is dependent on several factors including relative sizes of the predator and prey, the initial position of the tentacles, and the reaction of the anemone. If the length of the nudibranch is equal to or greater than the column diameter of the anemone, then the aeolid is large enough to readily attack the tentacles of an anemone in the normal open position (see Fig. 1a). Should the tentacles be raised in an alarm response or chance behavior, then the likelihood of the nudibranch reaching the tentacles is reduced, particularly if the anemone responds to contact with the column by bulging (Figs. 1c, d).

A. papillosa that have previously eaten *A. elegantissima* leak anthopleurine for up to 7 days and anthopleurine leaked from a nudibranch will initiate the alarm response (Howe and Harris, 1978). The alarm response, which involves a quick general contraction of the tentacles, may be of some advantage to an anemone in that it does increase the possibility that *A. papillosa* will not contact the tentacles first. A number of encounters have been observed in which the nudibranch gave up when it failed to reach the tentacles of an anemone. A specimen of *A. papillosa* feeds daily and primarily at high tide. The response to anthopleurine leaking from a nearby nudibranch should be greatest at the beginning of a high tide, since the anemones will have been free of the water-carried signal during low tide; continued exposure to anthopleurine causes fatiguing of the response over time (Howe and Sheikh, 1975).

The alarm response is of short duration and therefore not likely to deter a large, persistent nudibranch. However, raising the tentacles does increase the likelihood that the predator will touch the column. The column responds to *A. papillosa* mucus by swelling and this behavior pattern lasts for several hours (Table V).

There are two possible advantages to bulging behavior. The first is protection of the tentacles. It should be selectively advantageous to keep the feeding structures intact and instead to lose tissue from the column. Attacks to the column typically involve removal of epidermal tissue which will be regenerated, and very seldom result in complete penetration of the body wall. The second advantage is that when a specimen of *A. papillosa* attempts to reach or succeeds in reaching the tentacles, it must release at least partially from the rock surface (Figs. 1c, d) and will be vulnerable to being dislodged by wave action. Being washed off the rock by surge may not kill the nudibranch, but it removes it from further predation on the same clone.

The site of reception for the water-transmitted pheromone, anthopleurine, is the tentacles of *A. elegantissima,* and the alarm response involves short duration, generalized contraction of the tentacles (Howe and Sheikh, 1975). *Acolidia papillosa* mucus is detected by contact, the receptors are in the column and the response is a localized inflation of the column. It is predictable that the receptors for a water-transmitted pheromone would be in the tentacles, for they extend farthest from the central axis of the animal. The generalized alarm response should be most effective if it occurs before contact is made with the predator since no directionality of response is required. It also seems likely that the receptors for a localized response to a slow-moving predator would be at the site of the response as is the case with the receptors for the bulging behavior.

Crawling by an anemone after an attack will potentially decrease the likelihood of a second attack because of the presence of the other members of the clone. Nudibranchs attack the first individual they encounter when foraging. This would spread the damage produced by a nudibranch to several members of a clone and decrease the chances for the loss of an individual and shrinkage of the clone. Another effect of crawling behavior would be to isolate the nudibranch on the rock substrate, increasing the chances of desiccation. This is most likely to occur in young nudibranchs that do not show a strong tendency to hide at low tide.

Approximately 10% of the encounters observed in the laboratory between *A. papillosa* and *A. elegantissima* resulted in the anemone releasing from the substrate. Rosin (1969) reported a similar escape response in *Anthopleura nigrescens* (Verrill) to its predator, the aeolid nudibranch *Herviella* sp. In the field, detaching from the rock substrate will potentially result in the anemone being carried to new habitats which may be viewed as a means of dispersal of the clone, and therefore, a positive side result of this association.

A specimen of *A. papillosa* consumes 50 to 100% of its wet body weight each time it feeds, which is at least once a day (Howe and Harris, 1978). Therefore, the nudibranch becomes an increasing threat as it grows to a point where it is capable of killing an anemone in a single meal; this suggests that the selective value of the defensive mechanisms discussed would be most effective against young nudibranchs. *A. papillosa* is about 0.5 mm in length when it first metamorphoses. Nudibranchs about 1 mm in length grow to about 35 mm and become sexually mature in a little over two months in the laboratory (Harris, in preparation). This suggests that the time and size must be considered in evaluating the selective value of defensive mechanisms since it will be at least 2 months after metamorphosis before a nudibranch is able to cause serious damage during an encounter or before it begins to reproduce.

None of the defensive adaptations described in this paper stop *A. papillosa* veligers from metamorphosing in a clone, nor are they effective in detering predation in any given encounter between a nudibranch and an anemone either in the field or in the laboratory. We suggest that these mechanisms interact in such a way that the predator is killed before it reaches sexual maturity. The overall defensive strategy seems to be to minimize damage to clone members and to increase the likelihood that the predator will be killed or removed by desiccation and/or wave action.

The best evidence for this proposed defensive strategy is the fact that *A. papillosa* is primarily associated with the subtidal anemone *Metridium senile* (Harris, 1973, 1976, and in preparation). A similar pattern occurs in the Atlantic where *A. papillosa* is associated with *Metridium senile* (Stehouwer, 1952), *Sagartia elegans* (C. Todd, University of North Wales, personal communication) and *Cereus pedunculatus* (J. Tardy, University of Poitiers, personal communication); these three anemones were found to be among the least preferred in laboratory studies by Edmunds *et al.* (1975). Swennen (1961) reported large numbers of specimens of *A. papillosa* feeding on *Actinia equina*, a preferred species found in a similar habitat to that of *Anthopleura elegantissima*. Swennen's observations were made during the winter when the threat of desiccation would be least.

To influence the prey preference hierarchy of *A. papillosa*, the defensive strategies of anemones should focus on preventing the nudibranch from reaching sexual maturity and reproducing. Young nudibranchs seldom leave the prey where they have metamorphosed unless the prey is consumed or the nudibranch attains sexual maturity and searches for a mate (Harris, 1973); therefore, the choice of initial prey species takes place at the veliger stage prior to metamorphosis. The primary criterion for the choice of prey species must be survival to sexual maturity and reproduction. The prey preferences reported in the literature (Waters, 1973; Harris, 1973; Edmunds, *et al.*, 1975) are derived from laboratory

choice experiments with adult nudibranchs. Harris (1976) proposed that the prey preference hierarchy for the veliger stage of *A. papillosa* should be limited to relatively few species of anemone in an area and the principle criterion for selection should be survival to reproduction. In contrast, adult nudibranchs should be much less selective in their choice of prey since continued survival and reproduction should be the primary consideration at this stage. *A. papillosa* attains sexual maturity 2 to 3 months after metamorphosis and at a length of about 35 mm; an individual nudibranch is capable of continuing to reproduce for 6 or more months while growing to about 120 mm (Swennen, 1961; Harris, 1973; Clark, 1975). Adult specimens of *A. papillosa* show ingestive conditioning to even non-preferred anemones and will seek out prey they are conditioned to, unless they make contact with a more preferred species, and then they will switch (Harris, 1973; Wood, 1968; Murdoch, 1969). A hungry specimen of *A. papillosa* will attempt to feed on virtually any anemone species and even the corallamorpharian *Corynactis californica* Carlgren, 1936 (Waters, 1973; Harris, in preparation; Edmunds *et al.*, 1975).

In conclusion, the clonal form of *Anthopleura elegantissima* has evolved a series of defensive mechanisms including intertidal position, cloning, the alarm response to the pheromone, anthopleurine, column bulging initiated by nudibranch mucus, crawling and releasing from the substrate. None of these adaptations prevent veligers of *A. papillosa* from metamorphosing in association with *A. elegantissima* nor do they prevent an attack. However, they do combine in the context of the natural environment to form a very effective defensive strategy which increases the chances that the predator will be removed and/or killed by desiccation and/or wave action before it grows to sexual maturity. By preventing reproduction in the majority of nudibranchs which do metamorphose on this species, *A. elegantissima* exerts negative selective pressure on the prey preference hierarchy of the veliger stage. The success of this defensive strategy is illustrated by the fact that *A. papillosa* is primarily associated with one of the least preferred anemones of adult *A. papillosa*.

Mechanisms like cloning and the release of anthopleurine may also serve other functions such as competition or communication between clonemates. The fact that these mechanisms have adaptive value for more than one aspect of the anemone's biology should increase the selection for these traits.

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Summary

1. The defensive mechanisms shown by the west coast, intertidal sea anemone, *Anthopleura elegantissima,* in response to its nudibranch predator *Acolidia*

papillosa are identified and evaluated in the context of the environment where *A. elegantissima* occurs. The defensive mechanisms include intertidal distribution, clone formation, alarm response, bulging of the column, crawling and releasing from the substrate.

2. *A. papillosa* are primarily located at the periphery of clones so that anemones in the interior of the clone have a refuge from predation. Assuming that cloning is an adaptation for space competition in *A. elegantissima,* then the additional advantage derived as a defensive mechanism should increase selection for clone formation.

3. *A. papillosa* was less able than *A. elegantissima* to withstand desiccation from exposure at low tide. This suggests that the intertidal distribution of *A. elegantissima* is a defensive adaptation which reduces the threat of predation by *A. papillosa* at least during the warmer months of the year.

4. The bulging of the column at the site of contact was found to be a localized response of several hours duration. The mucus of *A. papillosa* stimulated the response and the receptors were found to be situated in the column. Mucus from the coelenterate-eating aeolid nudibranch, *Hermissenda crassicornis,* also initiated the response while neither the mucus from the sponge eating dorid, *Anisodoris nobilis,* nor control swabs dipped in sea water caused bulging.

5. None of the defensive mechanism directly protects an anemone from attack by *A. papillosa.* The defensive mechanisms all interact to minimize damage to the clone until the predator is removed by desiccation and or wave action. This strategy is most effective during the 2- to 3-month period between when the veliger metamorphoses and when the nudibranch reaches sexual maturity.

6. This defensive strategy of killing the young nudibranch before it reproduces may negatively influence prey selection by the veliger stage. The evolution of the prey preference hierarchy of the veliger stage should be based on the criterion of survival to sexual maturity. Evidence for the effectiveness of this defensive strategy is that *Aeolidia papillosa* is primarily associated with the subtidal anemone, *Metridium senile,* one of the least preferred prey of adult nudibranchs.

LITERATURE CITED

Brewer, B. A., 1977. The association between an endoparasitic rhabdocoel, Family Fecampidae, and the nudibranch, *Aeolidia papillosa* (Linnaeus, 1761). Masters Thesis, San Jose State University. 43 pp.

Clark, K. B., 1975. Nudibranch life cycles in the Northwest Atlantic and their relationship to the ecology of fouling communities. *Helgol. wiss. Meeresunters.* **27**: 28–69.

Connell, J. H., 1972. Community interactions on the marine rocky intertidal shores. *Ann. Rev. Ecol. Syst.,* **3**: 169–192.

Dayton, P. K., 1971. Competition, disturbance, and community organization: the provision and subsequent utilization of space in a rocky intertidal community. *Ecol. Monogr.,* **41**: 351–389.

Edmunds, M., G. W. Potts, R. C. Swinfen, and V. L. Waters, 1975. The feeding preferences of *Aeolidia papillosa* (L.). (Mollusca, Nudibranchia). *J. Mar. Biol. Assoc. U.K.,* **54**: 939–947.

Edmunds, M., G. W. Potts, R. C. Swinfen, and V. L. Waters, 1976. Defensive behavior of sea anemones in response to predation by the opisthobranch mollusc *Aeolidia papillosa* (L.) *J. Mar. Biol. Assoc. U.K.,* **56**: 65–83.

Francis, L., 1973a. Clone specific segregation in the sea anemone *Anthopleura elegantissima. Biol. Bull.,* **144**: 64–72.

Francis, L., 1973b. Intraspecific aggression and its effect on the distribution of *Anthopleura elegantissima* and some related sea anemones. *Biol. Bull.*, **144**: 73–92.

Francis, L., 1976. Social organization within clones of the sea anemone *Anthopleura elegantissima*. *Biol. Bull.*, **150**: 361–376.

Hand, C., 1955. The sea anemones of central California. Part II. The endomyarian and mesomyarian anemones. *Wasmann J. Biol.*, **13**: 37–99.

Harris, L. G., 1973. Nudibranch associations. Pages 213–314 in T. C. Cheng, Ed., *Current topics in comparative pathobiology*. Academic Press, New York.

Harris, L. G., 1976. Comparative ecological studies of the nudibranch *Acolidia papillosa* and its anemone prey *Metridium senile* along the Atlantic and Pacific coasts of the United States. *J. Moll. Studies.*, **42**: 301.

Howe, N. R., and L. G. Harris, 1978. Transfer of the sea anemone pheromone, anthopleurine, by the nudibranch *Acolidia papillosa*. *Chem. Ecol.*, **4**: 551–561.

Howe, N. R., and Y. M. Sheikh, 1975. Anthopleurine: a sea anemone alarm pheromone. *Science*, **189**: 386–388.

Jackson, J. B. C., 1977. Competition on marine hard substrata: the adaptive significance of solitary and colonial strategies. *Amer. Nat.*, **111**: 743–767.

Lang, J., 1973. Interspecific aggression by scleractinian corals. 2. Why the race is not only to the swift. *Bull. Mar. Sci.*, **23**: 260–279.

Lenhoff, H. M., 1968. Behavior, hormones, and hydra. *Science*, **161**: 434–442.

Murdoch, W. W., 1969. Switching in general predators: experiments on predator specificity and stability of prey populations. *Ecol. Monogr.*, **39**: 335–354.

Paine, R. T., 1969. The *Pisaster-Tegula* interaction: prey patches, predator food preference, and intertidal community structure. *Ecology*, **50**: 950–961.

Purcell, J. E., 1977. Aggressive function and induced development of catch tentacles in the sea anemone *Metridium senile* (Coelenterata, Actiniaria). *Biol. Bull.* **153**: 355–368.

Ricketts, E. F., and J. Calvin, 1962. *Between Pacific Tides, 3rd. Ed.* Stanford University Press, Stanford, California, 516 pp.

Rosin, R., 1969. Escape responses of the anemone *Anthopleura nigrescens* (Verrill) to its predatory aeolid nudibranch *Herviella* (Baba). *Veliger*, **12**: 74–77.

Russell, H. D., 1942. Observations on the feeding of *Acolidia papillosa* (L.) with notes on the hatching of the veligers of *Cuthona amoena* (A. and H.) *Nautilus*, **55**: 80–82.

Sebens, K. P., 1977. Habitat suitability, reproductive ecology and the plasticity of body size in two sea anemone populations (*Anthopleura elegantissima* and *A. xanthogrammica*). *Ph.D. Thesis, University of Washington.* 331 pp.

Stehower, H., 1952. The preference of the slug *Acolidia papillosa* (L.) for the sea anemone *Metridium senile* (L.). *Arch. Neerl. Zool.*, **10**: 161–170.

Swennen, C., 1961. Data on distribution, reproduction and ecology of the nudibranchiate molluscs occurring in the Netherlands. *Neth. J. Sea. Res.*, **1**: 191–240.

Waters, V. L., 1973. Food-preference of the nudibranch *Acolidia papillosa*, and the effect of the defenses of the prey on predation. *Veliger*, **15**: 174–192.

Wood, L., 1968. Physiological and ecological aspects of prey selection by the marine gastropod, *Urosalpinx cinerea* (Prosobranchia, Muricidae). *Malacologia*, **6**: 267–320.

Yarnall, J. L., 1972. The feeding behavior and functional anatomy of the gut in the eolid nudibranchs *Hermissenda crassicornis* (Eschscholtz, 1831) and *Acolidia papillosa* (Linnaeus, 1761). *Ph.D. Thesis, Stanford University.* 126 pp.

Reference: *Biol. Bull.* **157**: 153–165. (August 1979)

REPRODUCTION AND SURVIVAL OF THE PILEWORM *NEREIS SUCCINEA* IN HIGHER SALTON SEA SALINITIES

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The Salton Sea is a large (200 square miles) closed salt lake in a below-sealevel depression in the Colorado Desert of southeastern California. The Sea was formed accidentally from 1905 to 1907 when flood waters of the Colorado River broke through poorly constructed headgates of an irrigation canal. Following repair of the break, the new Salton Sea evaporated to a low of 250 feet below sea level by 1925. Since then the Sea has gradually increased in volume and surface elevation because agricultural waste water inputs exceed the annual evaporation of about 6 feet. In 1977, surface elevation was −228 feet, highest since 1914, and salinities averaged about 37‰ in summer. Early history of the Salton Sea is given by MacDougal (1914), and later history by Walker (1961).

The original biota of the Salton Sea, freshwater organisms from the Colorado River and a few species native to desert springs, died out by the mid-1920's. Attempts to introduce sportfishes did not succeed until the early 1950's, with the establishment of the croaker (*Bairdiella icistia*), sargo (*Anisotremus davidsoni*), and orange-mouth corvina (*Cynoscion xanthulus*), all from the Gulf of California. Several successful introductions of invertebrates have also occurred. The Salton Sea is eutrophic and highly productive (Walker, 1961), but as there are no planktivorous fishes, most plankton die and are converted to benthic detritus (Arnal, 1961). *Nereis* (*Neanthes*) *succinea* Leuckart, a polychaete annelid introduced in 1930, is the most important benthic detritivore and the most important food source for the sportfishes. The quasi-marine ecosystem of the Salton Sea is discussed by Walker (1961) and Young (1970). Ecology and reproductive biology of *N. succinea* in the Salton Sea have been discussed by Carpelan and Linsley (1961a, b) and Walker (1961).

With the salinity of the Salton Sea approaching 40‰ in the early 1970's, much concern has been expressed over the future of the sportfishery, one of the most important in California (Hanson, 1972). Increasing development of local geothermal energy resources may result in spills of highly saline waste brines into the Sea, which may also affect the sportfishes or their food supply (Shinn, 1976). Reproduction by the three sportfishes is limited to salinities below 40‰ (Brocksen and Cole ,1972; Lasker, Tenaza, and Chamberlain, 1972; May, 1975, 1976). There is little published information on high salinity adaptations in polychaetes (Bayly, 1972; Oglesby, 1978), and only one published study on the effects of higher salinities on *N. succinea* in the Salton Sea. Hanson (1972) observed that pileworms survived 96-hr exposures to salinities as high as 67.5‰, but speculated

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that "reproduction of the pileworm would probably be adversely affected at lower salinities."

It is the purpose of the present study to establish upper salinity limits for reproduction and survival of *N. succinea* in Salton Sea waters, in order to assess the possibility that pileworm failure due to increased salinity from either evaporation or brine spills may adversely affect the sportfishery.

Materials and Methods

Pileworms were collected from the northeastern Salton Sea, at Mecca Beach in the Salton Sea State Recreational Area. Worms were picked by hand from shallow sediments, particularly from barnacle shell rubble cemented with gypsum crystals. While a few heteronereids could be collected from the benthos in this fashion, it was necessary to collect by night-lighting in order to secure adequate numbers for experiments on fertilization and development.

In the laboratory, worms were maintained in glass tubes in finger bowls, covered to reduce evaporation. For most experiments worms were maintained at 21 to 23° C; for some experiments, worms were kept at 34° C, somewhat warmer than summer water temperatures in 1976–77, but not as warm as maximum water temperatures of 36° C recorded by Walker (1961).

Salton Sea water was collected from Mecca Beach. Higher salinities were made by evaporating 36‰ Salton Sea water at about 50° C until it reached a concentration of about 90‰. This was then mixed with 36‰ Salton Sea water to provide a full range of salinities. During the concentration process, calcium salts precipitated. Thus, in the final mixtures, calcium may have been somewhat undersaturated. For some experiments media were made up from Instant Ocean, an artificial salt mixture resembling ocean sea water in ionic ratios. All media were routinely filtered through activated charcoal in Whatman Grade 3 filter paper before use. Media were changed in the culture dishes daily. No antibiotics were used.

Laboratory fertilizations were carried out according to the procedures detailed in Costello, Davidson, Eggers, Fox, and Henley (1957) and Smith (1964). Water was changed daily, involving some loss of developmental stages by decanting. In some cultures, bacterial or protozoan contamination was present; however, this did not pose a severe problem experimentally. Since no supplemental food was supplied to the developing larvae, cultures died at a late trochophore or early 3-setiger stage.

Salinities in the field and during ordinary laboratory operations were measured with an American Optical total solids refractometer, calibrated against known solutions measured with a Hewlett-Packard vapor pressure osmometer and with a Buchler-Cotlove chloridometer. Chlorinities were converted to ‰ Salton Sea water using the ion ratios given in Carpelan (1958), Walker (1961), and Young (1970).

Results

Effects of higher Salton Sea salinities on survival of atokous worms

Experiments on survival of large atokes (immature worms) involved either direct transfers to the full range of salinities, or gradual increases of salinity in

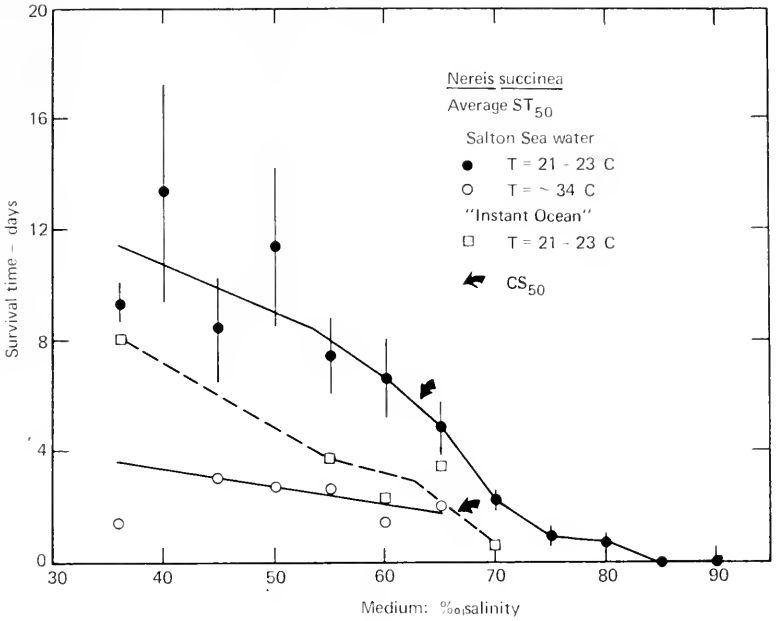

FIGURE 1. Survival time of *Nereis succinea* in increasing concentrations of Salton Sea water, expressed as average ST_{50}. Points are averages of all trays (initially 10 worms each) in each salinity, with the standard error indicated. Arrows indicate the Critical Salinity (CS_{50}) where survival is reduced to 50% of that in 36‰ Salton Sea water.

increments of 10‰ until the final test salinity was reached. Calculation of the time for 50% survival (ST_{50}) of a test group of worms (usually four trays with 10 worms each) began with the day the worms first were placed in the final test salinity. Graphs of ST_{50} as a function of the final salinity were used to determine the Critical Salinity (CS_{50}), the salinity where survival is reduced to 50% of the survival in 36‰ Salton Sea water. This calculation of CS_{50} is possibly biased in those experiments involving graduated salinity increases, since it ignores the mortality of worms in lower salinities during the adaptation period. However, otherwise comparable experiments in which worms were transferred directly to the final salinities gave similar results. Results of both types of experiments are combined for the following analyses.

Results for all experiments on survival of atokes are summarized in Figures 1 and 2. These are averages and calculations based on over 280 worms in 36‰, over 140 worms in each salinity between 45 and 60‰, and between 50 and 100 worms in all higher salinities. Figure 1 shows the average survival time (ST_{50}) for all worms in all salinities, with the standard error indicated. Survival was high at salinities of 50‰ and lower, but declined in higher salinities. The critical salinity CS_{50} is estimated to be slightly lower than 65‰. Figure 1 also provides the results of two related experiments: the effects of elevated temperature (34° C rather than the usual 21–23° C), and the effects of transfer to Instant Ocean. In both cases, overall survival seems to be diminished as compared with worms in

Salton Sea water in 21 to 23° C, though the general pattern is similar. For worms in Instant Ocean, CS_{50} is estimated to be somewhat higher than 65‰. It was not possible to provide an estimate of CS_{50} for the worms at 34° C, for it was higher than the highest salinity used in this experiment, 65‰. It appears that the Critical Salinity is not lowered for Salton Sea pileworms exposed either to ocean sea water or to temperatures only slightly lower than maximum summer water temperatures in the Salton Sea.

Much of the variability in the estimates of average ST_{50} (Fig. 1) is caused by the fact that in all salinities, all the worms in a given tray often would die soon after the first worm died, presumably because of the accumulation of toxic materials before the medium was changed. Not only is variability increased by this phenomenon, which may not be salinity-related, but also the average ST_{50} is decreased. Die-off of entire trays of 10 worms was particularly a problem at 34° C, due to increased bacterial activity. In order to provide an estimate of survival that avoids this problem, Figure 2 shows the survival time for the longest surviving worm in each salinity. At least some pileworms survived longer than the length of the experiments in nearly all salinities of 65‰ and lower, over 4 weeks for the worms in Salton Sea water at 21 to 23° C, and over 2.5 weeks for the worms in Instant Ocean and in 34° C. At higher salinities maximum survival time declined rapidly. No worms survived even a day at salinities higher than 85‰ at 21 to 23° C, nor higher than 75‰ in Instant Ocean. In both these experiments, CS_{50} was 70‰. No salinities higher than 65‰ were used in the experiments at 34° C, and since at least one individual lived as long as 8 days in this salinity, it is not possible to estimate CS_{50}, which must be higher than 65‰.

Most worms in the laboratory did not survive more than a week or two, even when maintained in 36‰ Salton Sea water. Carpelan and Linsley (1961a) indicated that *N. succinea* takes over a month to reach sexual maturity in the Salton Sea, so presumably pileworms must live well over a month in their natural habitat waters. Diminished survival in the laboratory may be a consequence of accumulation of metabolites, to lack of adequate food, or to other artificial causes. For these reasons, the survival times of pileworms in these laboratory experiments should not be taken as an exact counterpart of survival in the Salton Sea as salinity there increases. Rather, these laboratory results should be taken as an index of survival.

Both indices of survival (Figs. 1, 2) indicate that atokous pileworms survive increased Salton Sea salinities up to 60 to 65‰. Survival is considerably diminished at salinities in excess of 70‰, with no survival above 85‰, a concentration which is well over twice the present concentration of the Salton Sea. Even high summer water temperatures of 34° C do not decrease the survival limits of the pileworms. There is no indication that pileworms live longer in ocean water than in Salton Sea water.

Effects of higher Salton Sea salinities on heteronereid development

Studies of sexual development and spawning indicate that while *N. succinea* breeds year-round in the Salton Sea, reproductive activity is somewhat depressed during the summer months of high water temperature (Carpelan and Linsley,

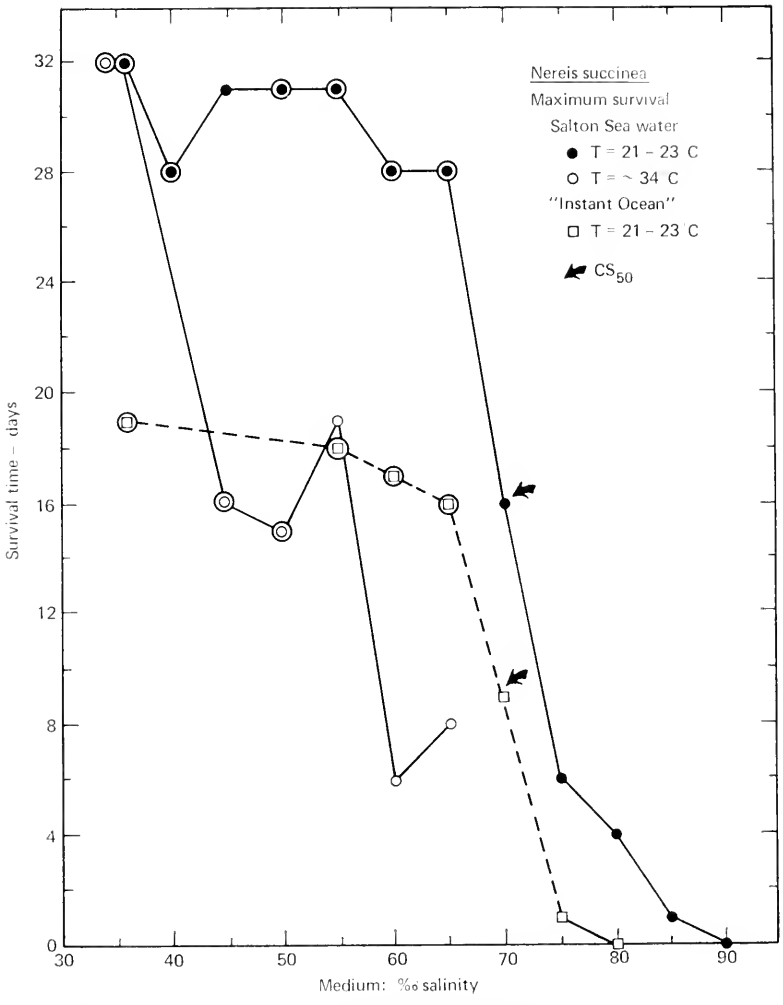

FIGURE 2. Maximum survival time of *Nereis succinea* in increasing concentrations of Salton Sea water. Circled points are those where worms were still alive by the end of the experiment.

1961a, b). Neverthless, heteronereids (sexually mature adult worms) were collected throughout the summers of 1976 and 1977. The sex ratio of worms collected from the benthos at Mecca Beach was about one-third males and two-thirds females. As noted by Costello, *et al.* (1957) and Carpelan and Linsley (1961a), the sex ratio of swarming heteronereids is strongly reversed, males outnumbering females by about 10 to 1. This was also the case in our own collections. Heteronereids regularly developed in laboratory cultures, with the sex ratio comparable to that encountered in benthic worms during collection, about 2 : 1 females to males.

Heteronereid development in the laboratory was strongly influenced by salinity

(Fig. 3). About 15% of the pileworms maintained in low salinities (36 and 40‰) matured as heteronereids during the summer of 1977. A somewhat lower percentage (just under 12%) matured in 50‰, but in higher salinities maturation of heteronereids was greatly depressed, and there was no maturation in salinities of 70‰ or higher. There may be a differential effect of higher salinities on maturation of the two sexes, development of males being more strongly depressed than that of females. This observation may be an artifact of very small numbers of hetero-

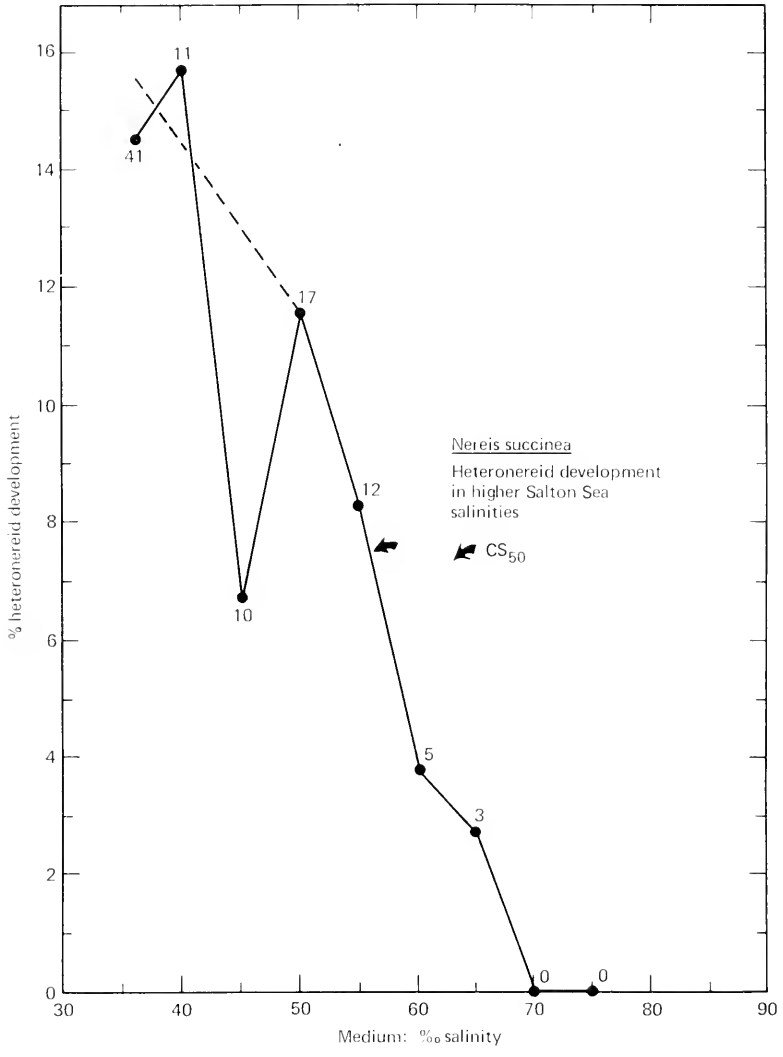

FIGURE 3. Effect of increasing Salton Sea salinities on heteronereid production by *Nereis succinea*. Actual numbers of heteronereids produced are indicated. Arrow indicates CS_{50} where heteronereid production is reduced to 50% of that in 36‰ Salton Sea water.

nereids in the higher salinities. The estimated CS_{50} for heteronereid development is about 55‰ (Fig. 3).

Effects of higher Salton Sea salinities on fertilization and larval development

Laboratory fertilizations, using heteronereids either collected directly from the Salton Sea or produced in laboratory cultures, were readily accomplished. Development of larvae in 36‰ Salton Sea water appeared normal, and had the same time course as in previous studies on Salton Sea worms (Carpelan and Linsley, 1961a, b) and on this species at Woods Hole (Costello, *et al.*, 1957). In all these studies, temperatures were similar, about 20 to 25° C.

Fertilization in various salinities. Fertilization of eggs of *N. succinea* could be accomplished at salinities as high as 50‰, although the percentage of successful fertilizations was much reduced at 50‰. In one experiment, there was less than 5% successful fertilization at 55‰, and in a second experiment, no success at this salinity. In these experiments, eggs from females maintained in 36‰ were initially transferred to culture dishes containing 100 ml of the experimental salinity, and sperm from males opened in 36‰ added. Eggs transferred to salinities of 45‰ and higher shrank from osmotic water loss immediately after the transfer. Shrinkage was particularly noticeable in 50‰ and higher, many eggs becoming greatly distorted. It is estimated that the CS_{50} for fertilization is 45‰. While there was a small proportion of apparently successful fertilizations in 55‰ in one experiment, there was no further development even to the first cleavage. The highest salinity in which any development takes place following fertilization is 50‰. These results are summarized in Figure 4.

Fertilization in 36‰ Salton Sea water, followed by transfers to various salinities. Smith (1964) demonstrated that later developmental stages of *N. diversicolor* were less sensitive both to lower and to higher salinities than early stages. To determine if this is the case with *N. succinea*, several experiments were conducted in which eggs were fertilized in 36‰ Salton Sea water ,and embryos transferred to higher salinities at several different times during development.

In the first group of experiments, eggs were transferred as soon as possible after fertilization into a full range of salinities. There was excellent survival and subsequent development in salinities up through 45‰, although in 45‰ there was some evidence of shrinkage, and a few zygotes cleaved abnormally. These abnormally cleaving embryos (such as "dumbells" which cleaved no further, and irregular cell clusters) did not develop successfully. There was poor survival of embryos transferred to 50‰, with abnormal cleavages and much shrinkage, but a few normal trochophores developed at this salinity. At 55 and 60‰ there was temporary survival, a high proportion of abnormal cleavage patterns, and no successful development. Embryos transferred to salinities higher than 60‰ shrank excessively and showed no type of cleavage. These early cleavage stages seem as sensitive to higher salinities as fertilization itself; it is estimated that the CS_{50} for early cleavage is 45‰, with no successful development higher than 50‰. These results are summarized in Figure 4.

This same experiment was conducted at 34° C to determine if summer water temperatures were more stressful on development than 21 to 23° C. There were

no observed differences in survival and developmental success between 34° C and 21 to 23° C, other than a considerable acceleration of cleavage at the higher temperature. There seems to be no additional stress on development in temperatures as high as summer water temperatures in the Salton Sa. On the other hand, in either temperature regime, development in salinities of 45‰ was markedly delayed as compared with development at lower salinities.

In a second group of experiments, fertilized eggs were allowed to develop in 36‰ Salton Sea water until the early trochophore stage, about 8 to 10 hr. These early trochophore stages were then transferred to salinities up to 75‰. There was excellent survival and development at all salinities up through 50‰. No shrinkage of trochophores was noticed at 45‰. In 50‰ there was some shrinkage, and trochophore cilia ceased beating temporarily. At 55‰ these shock effects were more severe, but there was considerable survival and subsequent development. At 60‰ there was no recovery of ciliary motion following transfer, and while it was estimated that perhaps 5% of the early trochophores survived the transfer, there was no subsequent development. There was no survival at higher salinities. Thus, early trochophores are less sensitive to high salinities than earlier cleavage stages. The estimated CS_{50} is 50‰, but there was no development above 55‰. These results are summarized in Figure 4.

In the final experiment, fertilized eggs were allowed to develop in 36‰ Salton Sea water until the swimming trochophore stage, about 24 hr. Swimming trochophores were then transferred to salinities up to 75‰, and survival and subsequent development monitored to the beginning of segmentation about 18 hr later. Survival was not as good as in the experiments involving transfers at earlier stages, but there was some survival and development up through 55‰. Swimming trochophores transferred to 50 and 55‰ showed some shrinkage, and ciliary activity temporarily stopped. In 60‰ these shock effects were more severe, and survival was low; however, some trochophores were still alive after 18 hours, though development had not progressed. In higher salinities there was little or no survival following transfer, shock effects were very severe, and there was no subsequent development. It appears that swimming trochophores have about the same sensitivity to higher Salton Sea salinities as do early trochophores. These results are summarized in Figure 4.

Discussion

The present experiments supplement and extend the results to Hanson (1972) in showing that atokous *N. succinea* can survive for extended periods of time in very high Salton Sea salinities, at least as high as 65‰, with perhaps some reduction of survival in 70‰. There is short term survival in salinities as high as 80‰, more than twice the present salinity of the Salton Sea. There are reports of nereid and other polychaetes in high salinities in lagoons and estuaries, but few reports are for salinities in excess of 50‰ (Bayly, 1972; Oglesby, 1978). There have been no experiments on any of these other polychaetes to determine actual upper salinity tolerances, or if they actually breed in such high salinities.

Hanson's (1972) prediction that reproduction of the pileworm in the Salton Sea would be adversely affected at salinities lower than the limit for adult sur-

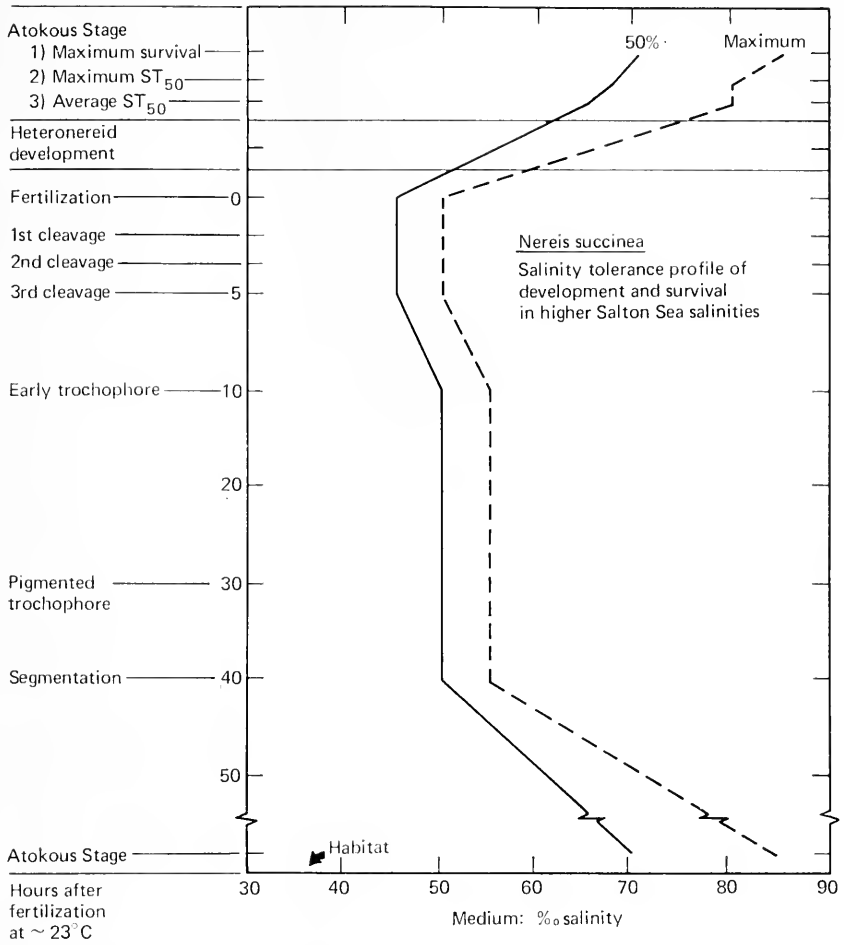

FIGURE 4. Salinity tolerance profile of development and survival of *N. succinea* in higher Salton Sea salinities. Stages and times are approximate for 21 to 23° C. The solid line indicates the Critical Salinity (CS_{50}), where survival and development are reduced to 50% of that in 36‰ Salton Sea water. The dashed line indicates the maximum salinity at which any survival or development occurred.

vival is confirmed by these experiments, for there was no successful fertilization or cleavage in salinities higher than 50‰, and reasonable success was limited to salinities of 45‰ and lower. Early and late trochophores could tolerate somewhat higher salinities, up to 50‰. Assumption of the high salinity tolerance of the atokes must come later in development than the appearance of segmentation, the termination of the present experiments.

Table I summarizes the literature on the effects of low salinities on development of *N. succinea*. Taken together, these reports suggest that *N. succinea*, like many other estuarine and marine polychaetes, cannot reproduce at salinities below the horohalinicum of 5 to 8‰ (Oglesby, 1978), even though atokous *N. succinea* can

survive at considerably lower salinities (Oglesby, 1965; Hogue and Oglesby, unpublished results). The report by Foster (1972) that *N. succinea* can survive in fresh water is not supported by published data (Oglesby, 1965, 1978). The studies summarized in Table I, combined with the present results, provide a picture that is comparable to *N. diversicolor* (Smith, 1964), showing a "bottleneck" of salinities above and below which cleavage is blocked (Fig. 4). That is, while adult and atoke survival limits range from about 1‰ to as high as 80‰, development is successful only between 10 and 45‰.

It is premature to conclude that when the Salton Sea finally exceeds 45 to 50‰, reproduction of *N. succinea* will be blocked. As Smith (1964, 1977) has discussed, at least some of the reason for lack of reproductive success at extreme salinities may be mechanical, due to osmotic swelling in low salinities and osmotic shrinkage in high salinities. Shrinkage was very apparent in the present experiments at 50‰ and higher in eggs and embryos transferred from 36‰. It would have been desirable to attempt fertilizations with eggs and sperm taken from adults adapted to much higher salinities, to avoid the initial osmotic problem. However, even though heteronereids were produced in the laboratory in salinities as high as 65‰ (Fig. 3), never were a male and a female mature at the same time in a salinity higher than 36‰. Smith (1964) reported that some populations of *N. diversicolor* in northern Europe reproduce in salinities greater than the upper salinity limit for reproduction of other populations of the same species. It may

TABLE I

Effects of lower salinities on development of Nereis succinea*

Stage and treatment	Observed effects on development	Reference
Unfertilized eggs	Survival down to 9.6‰.	Just (1928)
Fertilized eggs	Very low resistance to dilute sea water.	Just (1928)
Development in 91% SW after 1 hr exposure to dilute SW	Normal development above 16‰; diminished success at 11–14‰, though some eggs develop normally.	Just (1930a)
Development in varied SW after fertilization in 91% SW	Normal development above 16‰; diminished success at 14‰, but some normal development; cleavage but not trochophores in 11, 13‰.	Just (1930b)
Development in varied SW after fertilization in 100% SW	Normal development of nectochaeta larva in 14–35‰; normal development of trochophore in 8‰; no cleavage in 2–6‰.	Kinne (1954)
Development in varied SW and temperatures after fertilization at 20‰	Development at 10, 15, 20‰, except none at 10‰ at 10° C. Development accelerated at higher salinity and higher temperature.	Dean and Mazurkiewicz (1975)

**Nereis limbata* Ehlers, the name used by Just (1928, 1930a, b) is synonymous with *N. succinea* Leuckart. Just (1928, 1930a, b) did not provide the actual concentration of his "100% SW." Salinities presented in this table are based on data of Cole (1940) for summer water salinities at Woods Hole. Since there are no significant fresh-water inflows in the area, it is reasonable to assume that Woods Hole summer sea water averages about 32‰, or 91% of 35‰ salinity.

be that as the salinity of the Salton Sea gradually rises, there will be genetic selection for *N. succinea* with higher limits for reproductive success.

Developmental events were somewhat slowed at higher salinities, but at salinities below 45 to 50‰ development was otherwise normal. Smith (1964) observed that low salinities also delayed development, particularly cleavage, in *N. diversicolor*.

The present experiments indicate that temperatures as high as 34° C do not have any marked effect on adult survival and reproduction in *N. succinea*. At the present time there is year-round reproduction in the Salton Sea, and this pattern should continue as long as the overall salinity itself does not become too high.

Interestingly, Salton Sea pileworms do not seem to have reduced survival in Salton Sea water as compared with ocean water (Figs. 1, 2). May (1976) found that eggs and larvae of the croaker *Bairdiella* survived well in sea water, but had very poor survival in Salton Sea water of the same salinity. He suggested that this poor survival of the fish was related to the unusual ionic composition of the Salton Sea. This seems not to be a problem with *N. succinea*.

Spills of geothermal waste brines of 5 to 10 times the salinity of the Salton Sea (California Department of Water Resources, 1970; Shinn, 1976) would be expected to eliminate pileworms from the affected area, as well as other benthic and pelagic organisms. These adverse effects could be caused not only by the excessively high salinity *per se*, but also by concomitant elevated temperatures or reduced oxygen concentrations. Pileworm larvae are in the Salton Sea plankton all year (Carpelan and Linsley, 1961a, b), and so there would be a constantly available source for recolonization. Only if there were contamination by heavy metals would there be a long-term problem (Reish and Carr, 1978). Thus, it is unlikely that a spill of even highly saline geothermal waste brines would have any more than a temporary and localized effect on the population of *N. succinea* in the Salton Sea.

The present experiments indicate that reproduction of *N. succinea* in the Salton Sea will continue with undiminished success at salinities at least as high as 45‰, and probably as high as 50‰. This means that gradually increasing salinities of the Salton Sea will not adversely affect the pileworm until some years after the collapse of the sportfishery, which seems sensitive to salinities no higher than 40‰.

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Summary

The polychaete annelid *Nereis* (*Neanthes*) *succinea* is the major benthic detritivore in the Salton Sea, an inland salt lake in southeastern California, and is critical in the trophic chain leading to the sportfishery. In view of the increasing salinity of the Salton Sea, laboratory experiments were conducted to determine critical upper salinity limits for reproduction and survival of pileworms. Atokous (immature) pileworms can survive for extended periods in Salton Sea salinities at least as high as 65‰, with some reduction of survival in 70‰, and with only short term survival in 80‰, more than twice the present salinity of the Salton Sea (36‰). Heteronereid production is depressed by salinities higher than 50‰. Reproduction of *N. succinea* is successful at salinities at least as high as 45‰, and probably as high as 50‰. Fertilization and early cleavage stages are less tolerant of elevated salinities than are later development stages such as trochophores.

LITERATURE CITED

Arnal, R. E., 1961. Limnology, sedimentation, and micoorganisms of the Salton Sea, California. *Geol. Soc. Am. Bull.*, **72**: 427–478.

Bayly, I. A. E., 1972. Salinity tolerance and osmotic behavior of animals in athalassic saline and marine hypersaline waters. *Annu. Rev. Ecol. Syst.*, **3**: 233–268.

Brocksen, R. W., and R. E. Cole, 1972. Physiological responses of three species of fish to various salinities. *J. Fish. Res. Board Can.*, **29**: 399–405.

California State Department of Water Resources, 1970. Geothermal wastes and the water resources of the Salton Sea area. *DWR Bulletin* No. 143-7. 123 pp.

Carpelan, L. H., 1958. The Salton Sea. Physical and chemical characteristics. *Limnol. Oceanogr.*, **3**: 373–386.

Carpelan, L. H., and R. H. Linsley, 1961a. The pileworm, *Neanthes succinea* (Frey and Leuckart). *Calif. Dep. Fish Game Bull.*, **113**: 63–76.

Carpelan, L. H., and R. H. Linsley, 1961b. The spawning of the pileworm *Neanthes succinea* in the Salton Sea. *Ecology*, **42**: 189–190.

Cole, W. H., 1940. The composition of fluids and sera of some marine animals and of the water in which they live. *J. Gen. Physiol.*, **23**: 575–584.

Costello, D. P., M. E. Davidson, A. Eggers, M. H. Fox, and C. Henley, 1957. *Methods for Obtaining and Handling Marine Eggs and Embryos.* Marine Biological Laboratory, Woods Hole, Massachusetts. 246 pp.

Dean, D., and M. Mazurkiewicz, 1975. Methods of culturing polychaetes. Pages 171–197 in W. L. Smith and M. H. Chanley, Eds. *Culture of Marine Invertebrate Animals.* Plenum Publ. Co., New York.

Foster, N., 1972. *Freshwater Polychaetes (Annelida) of North America.* Biota of Freshwater Ecosystems Identification Manual No. 4, U. S. Environmental Protection Agency, Stock Number 5501-0368. 15 pp.

Hanson, J. A., 1972. Tolerance of high salinity by the pileworm, *Neanthes succinea*, from Salton Sea, California. *Calif. Fish Game*, **58**: 152–154.

Just, E. E., 1928. Hydration and dehydration in the living cell. I. The effect of extreme hypotony on the egg of *Nereis*. *Physiol. Zool.*, **1**: 122–135.

Just, E. E., 1930a. Hydration and dehydration in the living cell. III. The fertilization of *Nereis* eggs after exposure to hypotonic sea-water. *Protoplasma*, **10**: 24–32.

Just, E. E., 1930b. Hydration and dehydration in the living cell. IV. Fertilization and development of *Nereis* eggs in dilute sea-water. *Protoplasma*, **10**: 33–40.

Kinne, O., 1954. Über das Schwärmen und die Larvalentwichklung von *Nereis succinea* Leuckart (Polychaeta). *Zool. Anz.*, **153**: 114–126.

Lasker, R., R. H. Tenaza, and L. L. Chamberlain, 1972. The response of Salton Sea fish eggs and larvae to salinity stress. *Calif. Fish. Game*, **58**: 58–66.

MacDougal, D. T., Ed., 1914. The Salton Sea. A Study of the Geography, the Geology,

the Floristics, and the Ecology of a Desert Basin. *Carnegie Inst. Wash. Publ.*, **193L**: 1–182.

May, R. C., 1975. Effects of temperature and salinity on fertilization, embryonic development, and hatching in *Bairdiella icistia* (Pisces: Sciaenidae), and the effect of parental salinity acclimation on embryonic and larval salinity tolerance. *Fish. Bull.*, **73**: 1–22.

May, R. C., 1976. Effects of Salton Sea water on the eggs and larvae of *Bairdiella icistia* (Pisces: Sciaenidae). *Calif. Fish Game*, **62**: 119–131.

Oglesby, L. C., 1965. Steady-state parameters of water and chloride regulation in estuarine nereid polychaetes. *Comp. Biochem. Physiol.*, **14**: 621–640.

Oglesby, L. C., 1978. Salt and Water Balance. Pages 555–668 in P. J. Mill, Ed. *Physiology of the Annelida*. Academic Press, London and New York.

Reish, D. J., and R. S. Carr, 1978. The effect of heavy metals on the survival, reproduction, development, and life cycles for two species of polychaetous annelids. *Mar. Pollut. Bull.*, **9**(1): 24–27.

Shinn, J. H., Ed., 1976. *Potential Effects of Geothermal Energy Conversion on Imperial Valley Ecosystems*. Lawrence Livermore Laboratory, University of California, Livermore. UCRL-52196. 77 pp.

Smith, R. I., 1964. On the early development of *Nereis diversicolor* in different salinities. *J. Morphol.*, **114**: 437–464.

Smith, R. I., 1977. Physiological and reproductive adaptations of *Nereis diversicolor* to life in the Baltic Sea and adjacent waters. Pages 373–390 in D. J. Reish and K. Fauchald, Eds. *Essays on Polychaetous Annelids in Memory of Dr. Olga Hartman*. University of Southern California, Allan Hancock Foundation Special Publication.

Walker, B. W., Ed., 1961. The Ecology of the Salton Sea, California, in Relation to the Sportfishery. *Calif. Dep. Fish Game Fish Bull.*, **113**: 1–204.

Young, D. R., 1970. The Distribution of Cesium, Rubidium, and Potassium in the Quasi-Marine Ecosystem of the Salton Sea. *Ph.D. Dissertation, University of California, San Diego*. 213 pp.

Reference: *Biol. Bull.* **157**: 166–181. (August 1979)

BEHAVIORAL RESPONSES OF *BALANUS IMPROVISUS* NAUPLII TO LIGHT INTENSITY AND SPECTRUM

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Although barnacle larvae have been used in numerous classical studies on phototaxis and spectral response, results have been predominantly qualitative in nature. Groom and Loeb (1890), Ewald (1912), and Rose (1925) studied phototaxis in stage I–II nauplii of *Balanus perforatus*. Vischer and Luce (1928) attempted to define the spectral sensitivity of cyprid *Balanus amphitrite* and "*B. improvisus*". Barnes, Crisp, and Powell (1951) demonstrated orientation to light during settlement of cyprid *Semibalanus balanoides* and *Balanus crenatus*. Based on these and other studies, Thorson (1964) characterized barnacles as maintaining positive phototaxis throughout their larval life.

More recent studies have begun to quantify light responses of barnacle larvae. Barnes and Klepal (1972) determined the spectral sensitivity and threshold of photo-response at 522 nm for stage I nauplii of *Elminius modestus* and *S. balanoides*. Crisp and Ritz (1973) studied the effects of dark-adaptation on relative light sensitivity for stage II *E. modestus* and *S. balanoides,* and limiting intensities of white light for photo responsiveness of stage II *S. balanoides* and *B. crenatus* and cyprid *S. balanoides*.

With the exception of a series of studies on the zoea of the estuarine decapod crustaceans (Forward, 1974; Forward and Costlow, 1974; Forward, 1976b, 1977; Forward and Cronin, 1978), quantitative phototactic and spectral studies of larval crustacean groups are sparse (see Forward, 1976a; Aiken and Hailman, 1978).

In this study, short-term phototaxis (direction) and orthophotokinensis (velocity) of *Balanus improvisus* nauplii are investigated using a new method of video-computer quantification which greatly facilitates studies of movement in small organisms (Davenport, Culler, Greaves, Forward, and Hand, 1970). The ability to rapidly quantify and compare movement paramenters makes this system a potentially powerful tool for behavioral biassays (Anderson, 1971; Olla, 1974). Thus this study demonstrates the capabilities of this system and provides a rigorous analysis of the photobiology of barnacle larvae.

Materials and Methods

Experimental animal

Balanus improvisus nauplii were sorted from surface plankton tows taken at Pettaquamscutt River near Narragansett, Rhode Island. During the collection period (24 Oct–28 Nov., 1977) *B. improvisus* nauplii were abundant and easily

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sorted from plankton samples (Lang, 1979). Water temperature and salinity at collection times ranged from 7 to 14° C, 13 to 20‰. Nauplii were immediately transferred to filtered sea water at 15° C, 15‰ and placed in either 15° or 20° C temperature boxes with constant illumination. For experiments conducted at 30‰, the temperature of the 15‰ water was first allowed to equilibrate at 20° C and nauplii were then transferred to 5‰ salinity increments at one hr intervals.

All larvae were maintained overnight (8–12 hr) at specified temperature/salinity before being used experimentally. *Isochrysis galbana* and *Tetraselmis succia* were added as food. The following morning, nauplii of the desired stage were sorted from initial cultures and groups of 20 transferred to 5-ml beakers. Light-adapted nauplii were exposed to room lights supplemented with a 60-W incandescent bulb for at least 1 hr prior to experimentation; dark-adapted nauplii were kept in dark temperature-controlled boxes for at least 1 hr before experimentation. Studies with other arthropods indicate that these times are adequate for light and dark adaptations (*e.g.*, Hamdorf and Schwemer, 1975; Barnes and Goldsmith, 1977). To minimize effects of possible larval diurnal cycles (Singarajah, Moyse, and Knight-Jones, 1967), all experiments were conducted between 13:00 and 16:30 hr. All nauplii were tested within 26 hr of capture.

Light stimulus and video system

A microscope and closed circuit television system were used to monitor and record swimming behavior of nauplii. Dark field substage illumination, interference filtered to 830 nm (about 15 nm half width), provided light for a Cohu 4400 television camera mounted on a Wild M-5 microscope body (Lang, Lawrence, and Miller, 1979). Larvae were placed in a 1.2 × 1.2 × 1.0 cm lucite cuvette filled to 0.5 cm depth. Movement was monitored in the horizontal plane.

A light stimulus presented horizontally and perpendicular to the cuvette wall was provided by two sources. For initial studies on light response of nauplii, a grating monochromater with 150-W xenon short arc lamp (Oriel Corporation) was used. For spectral and intensity studies a slide projector with 300-W tungsten bulb and thin film absorption filters (Ditric Optics) ranging from 440 to 640 nm in 20-nm intervals (about 7 nm half-width) was used (Latz and Forward, 1977). In both cases light intensity of quantum levels were regulated by neutral density filters. Intensity was measured by a YSI model 64A radiometer.

Experimental procedure

Experiments were conducted in a temperature-controlled, darkened room. A preparation of 20 nauplii was transferred from a 5-ml beaker to the test cuvette; the cuvette was then aligned on the dark field stage. Light-adapted nauplii were transferred under room lights; dark-adapted nauplii were transferred under dim light (interference filtered at 700 nm). All lights were extinguished and nauplii were allowed at least 30 sec prior to experimentation to recover from movement of the test cuvette during placement on stage.

For initial studies on naupliar response to light stimulus, five intensities of 480-nm light ranging from 27 to 0.0027 W/m^2 were used. Replicate preparations of nauplii were tested at each intensity in ascending order with 45-sec intervals

between 2.5 sec stimuli. The response of nauplii 2.5 sec prior to stimulation, during stimulation, and 3.0 sec following stimulation were analyzed by computer.

For spectral response, the 830-nm substage light was turned on 5 to 15 sec prior to light stimulus, a 2.5-sec stimulus applied, and the substage light extinguished. After 1 minute, a second wavelength stimulus (in ascending order) was applied. After four exposures the preparation of dark-adapted nauplii was changed. For both spectral and intensity studies, computer analysis was limited to naupliar response during the latter 2.0 sec of the 2.5-sec stimulus.

The general procedure for testing response to light intensities was similar. One preparation of nauplii was exposed to a complete sequence of seven intensities of 480-nm light starting with an intensity estimated as subthreshold. All stimuli were 2.5 sec in duration with 30- to 40-sec intervals between each stimulus.

At least three replicates were run with each plankton sample. With the exception of stage III nauplii, at least two different plankton samples were represented.

Computer analysis

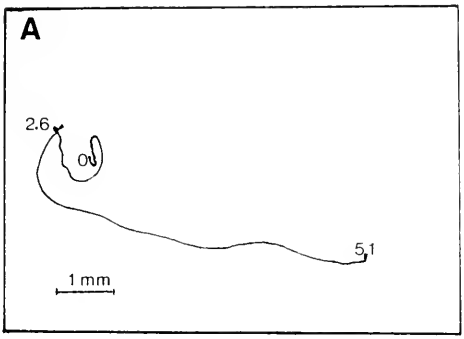
Video recordings of naupliar response to light stimuli were played back through a video-to-digital processor, the "Bugwatcher" (Greaves, 1975). Outlines of each nauplii within the camera field of view are delimited as X–Y coordinates. For this study, video tapes were analyzed by computer at 10 frames per second; every sixth frame of the normal 60 f/sec recording was fed into a Data General Eclipse S/200 computer. Time series of X–Y coordinates (video files) indicating displacement of nauplii at 0.1-sec intervals were generated for each replicate sample. A tone generator synchronized with the light stimulus shutter control marked periods of stimulation; a tone detector in the Bugwatcher determined light stimulus duration to the nearest 0.1 sec on video files.

Video files were analyzed using second generation programs developed by Wilson and Greaves (1979). Processed video files (see Greaves, 1975) yielded a time-scaled computer track of naupliar movement (Fig. 1A). About 10 to 15 nauplii were tracked simultaneously. Linear velocity (Fig. 1B) and direction of travel (Fig. 1C) were calculated for each 0.1-sec interval of the tracks; mean values for individual nauplii during a given time interval were determined (Fig. 1D). Mean direction of travel (DOT) and mean linear velocity (MLV) for all nauplii of an experimental group were then pooled to calculate sample means. Sample DOT distributions were tested using a Chi-square test (Batschelet, 1965). Standard SPSS (Nie, Hull, Jenkins, Steinbrenner, and Bent, 1975) and SAS (Bar, Goodnight, Sall, and Helwig, 1976) analysis of variance programs were used to test sample MLV data.

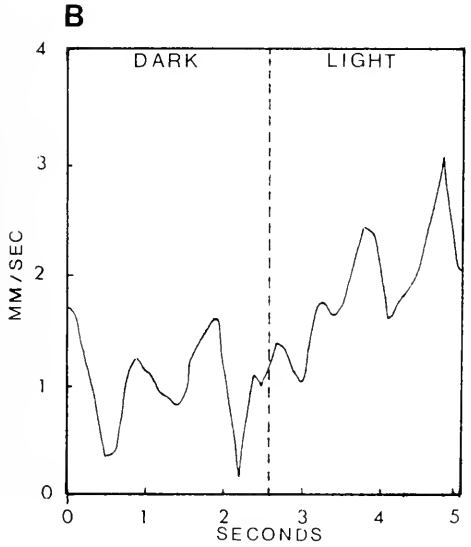
Results

Initial studies on stage II naupliar light response

Preliminary observations indicated that stage II *B. improvisus* nauplii were strongly positively phototactic to lower light intensities (about 10 W/m^{-2}), particularly near 500 nm. The following experiments were devised to further characterize the responses of light-adapted nauplii.



D	MLV mm/sec	MEAN VECTOR θ	r
DARK 0-2.6 sec	1 01	170	0.2
LIGHT 2.6-5.1 sec	1 84	329	0.8



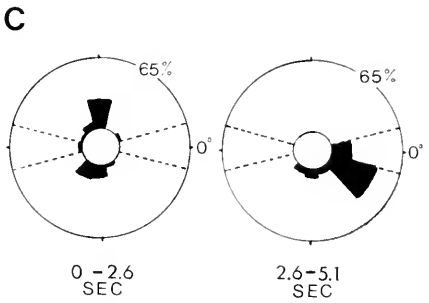


FIGURE 1. An example of computer analysis of a single *Balanus improvisus* nauplius. Digital processing of a video tape produces a computer tracking of the nauplius (A). A light stimulus (480 nm, 0.06 W/m²) was applied from 2.6 to 5.1 sec. Linear velocity (B) and direction of travel (C) are calculated at 0.1-sec intervals (stimulus at 0°) and mean values for desired time intervals (D) determined.

Light-adapted stage II nauplii (15° C, 20‰) were exposed to 480 nm light at five intensities ranging from 27 to 0.0027 W/m². The initial MLV of nauplii exposed only to 830-nm darkfield illumination does not vary significantly ($P = 0.05$) between sample group (Table I). During light stimulation, the MLV for groups at the upper three light intensities increases significantly; recovery to MLV statistically equal to control levels does not occur within the three sec following stimulation. No significant change in MLV occurs at the lower two light intensities

(Table I). Plotting the running mean velocities of each sample group at 0.1-sec intervals shows the time sequence of naupliar photokinetic response (Fig. 2). The change in MLV is delayed in onset and return to normal, relative to light stimulus duration.

A change in MLV (orthophotokinesis) is observed only above 0.027 W/m². However a directional response to light (phototaxis) occurs at all intensities (Table I, Fig. 3). DOT distributions during the initial "dark" interval do not vary significantly ($P = 0.05$) from a theoretical uniform distribution. During the "light" interval, nauplii at all light intensities exhibit nonrandom distributions and a significant difference from initial distributions. Computer plotted histograms (Fig. 3) illustrate a directed response toward the light source.

The individual nauplii paths (*e.g.* Fig. 1A) and time analysis of MLV responses (Fig. 2) indicate that a delay of about 0.5 sec often occurs between stimulation and naupliar response. The DOT distribution for the entire 2.5-sec light stimulus interval clearly indicates a general positive phototactic movement (Fig. 4A). If the initial 0.5-sec "orientation period" at light stimulation is omitted from DOT determinations, a strong directed response becomes evident (Fig. 4B). For the following experiments, mean naupliar direction and MLV during the latter two sec of a 2.5-sec light stimulus are presented. Positive phototaxis is considered a mean direction of travel ± 15° of the light source; negative phototaxis is a DOT ± 15° in the opposite direction. Comparative results using a ± 45° "windows" in respective directions are also included.

To correlate with other laboratory studies (Lang, *et al.*, 1979; Lang, Miller, Lawrence, Marcy, and Clem, in progress), light intensity and spectral experiments were conducted at 20° C, using a tungsten light source and filters. Essentially the same positive phototaxis to 480-nm light was found using the new light source and at the higher temperature.

TABLE 1

Mean linear velocity and χ^2 directional response comparison of light-adapted stage II Balanus improvisus *nauplii (15° C 20‰) during initial "dark" interval (830-nm substage light) at 0–2.5 sec, 480-nm light stimulation at 2.5 to 5.0 sec, recovery "dark" interval at 5.0 to 8.0 sec. A null hypothesis that the distribution of direction of travel means* (60° intervals) *for each sample group of 30 to 38 nauplii is equal to a theoretical uniform distribution was tested using the χ^2 test. χ^2 values for $P \leq 0.05$ are indicated by an asterisk where P represents the probability of rejecting the null hypothesis when actually true.*

Intensity W/m² at 480 nm	Mean linear velocity mm/sec			Direction of travel Chi-square: experimental *vs.* uniform distribution		
	Initial "dark"	Light stimulation	Recovery "dark"	Initial "dark"	Light stimulation	Recovery "dark"
27	1.35 ± 0.35	2.13 ± 0.65	1.97 ± 0.63	7.14	33.67*	8.80
2.7	1.54 ± 0.55	2.29 ± 0.66	1.91 ± 0.61	4.43	64.14*	5.00
0.27	1.25 ± 0.39	1.82 ± 0.52	1.66 ± 0.55	7.86	29.81*	15.29*
0.027	1.46 ± 0.36	1.60 ± 0.45	1.55 ± 0.48	5.00	61.50*	8.74
0.027	1.46 ± 0.31	1.51 ± 0.38	1.50 ± 0.36	4.42	19.60*	8.47

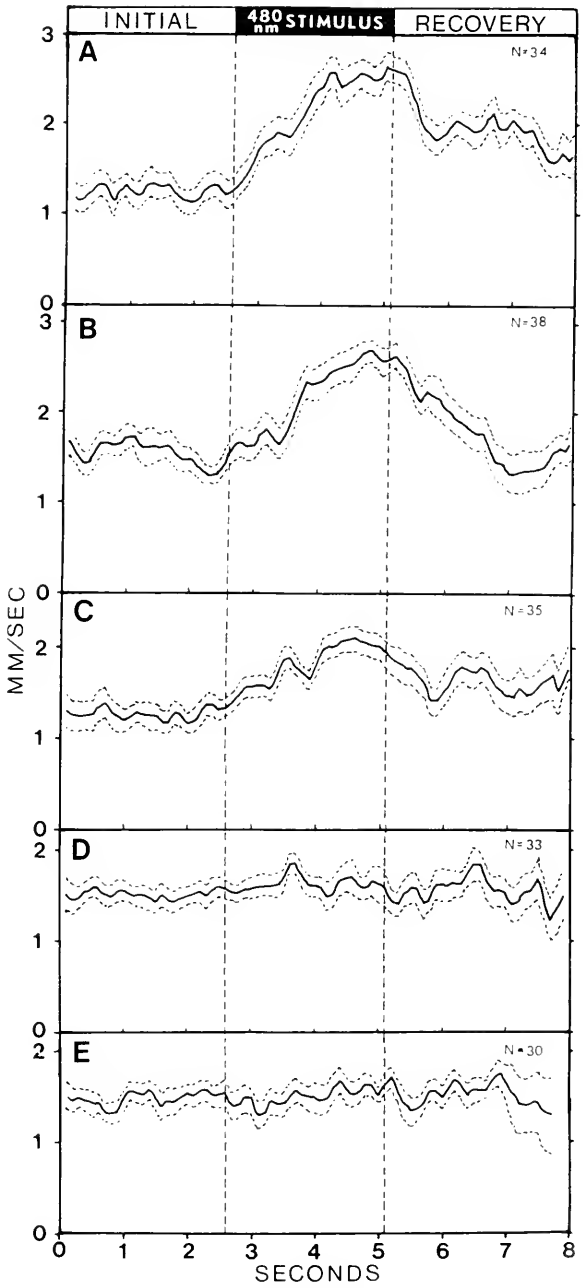


FIGURE 2. The running mean linear velocity ± variance of stage II *Balanus improvisus* nauplii in response to a 480-nm light stimulus applied at 2.6 to 5.1 sec: (A) 27 W/m²; (B) 0.27 W/m²; (C) 0.027 W/m²; (D) 0.0027 W/m²; (E) 0.00027 W/m². The number of naupliar paths analyzed (N) is indicated. Initial and recovery represent "dark" periods of 830-nm substage illumination only.

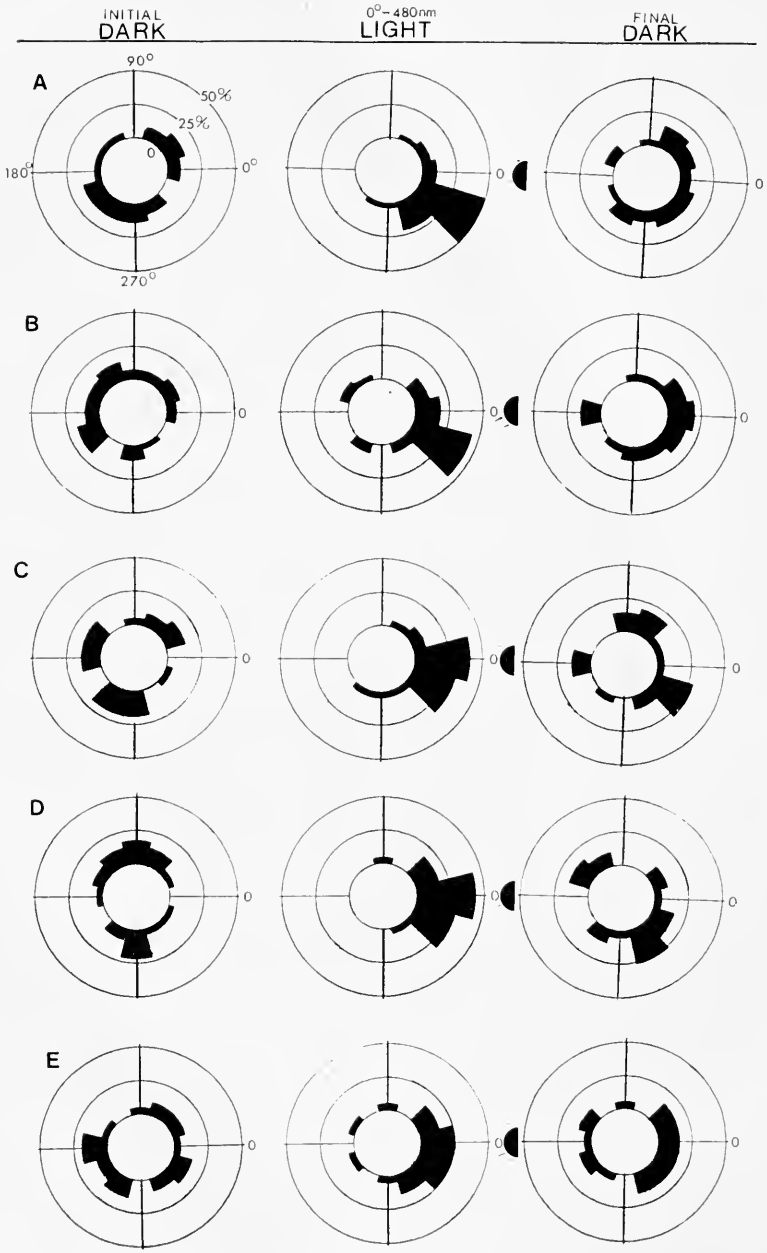


FIGURE 3. Distribution of mean direction of travel of stage II *Balanus improvisus* during initial 2.5-sec "dark" interval, 2.5 sec 480-nm light stimulus at 0°, and final 3.0-sec "dark" interval: (A) 27 W/m^2; (B) 2.7 W/m^2; (C) 0.27 W/m^2; (D) 0.027 W/m^2; (E) 0.0027 W/m^2. Sample numbers and "dark" condition are as in Figure 2. Significantly ($P \leq 0.05$) nonrandom distributions are indicated in Table I.

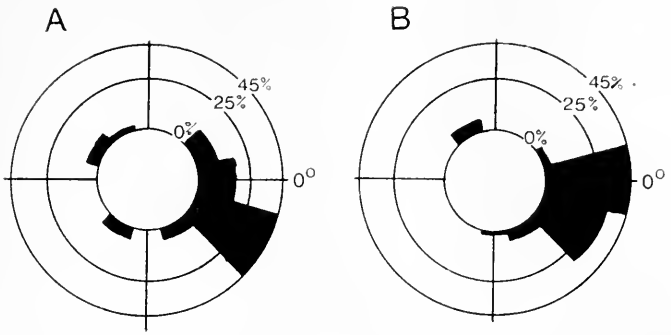


FIGURE 4. Distribution of mean direction of travel for 38 stage II *Balanus improvisus* exposed to a 480 nm, 2.7 W/m² light stimulus at 0° : (A) directional response during full 2.5-sec stimulus; (B) directional response during the latter 2.0 sec of the same stimulus.

Stage II response spectrum

Dark-adapted stage II nauplii (20° C, 15‰) were exposed to filtered light from 440 to 640 nm in 20-nm increments. Quantal intensity was balanced to approximately 0.07×10^{16} quanta m^{-2} sec at each wavelength (calculated values ranged

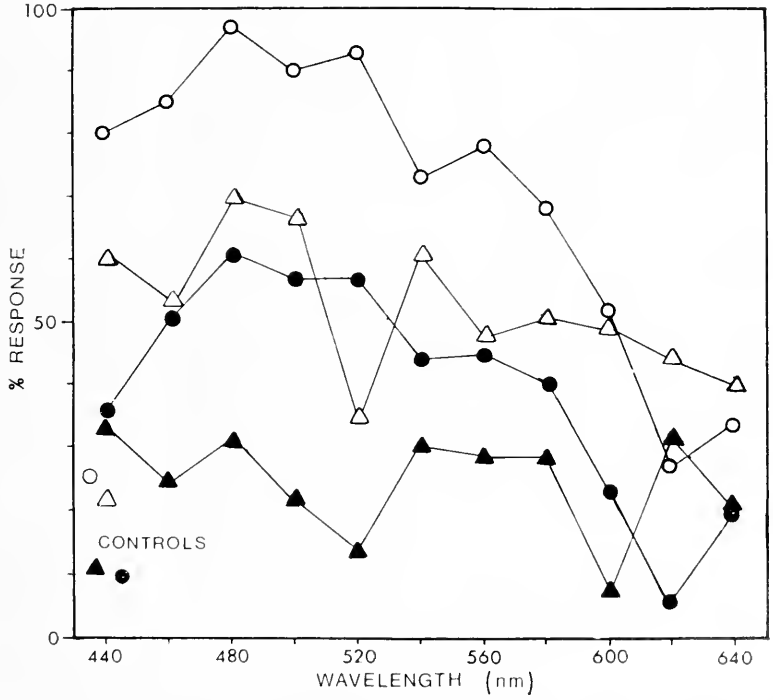


FIGURE 5. Response spectrum for positive phototaxis (% response). Open circle, stage II *Balanus improvisus*; response ± 45° of light source; closed circle; ± 15° of light source. Open triangle, stage VI *B. improvisus*; ±45° of light source; closed triangle, ±15° of light source. See Table II for sample numbers.

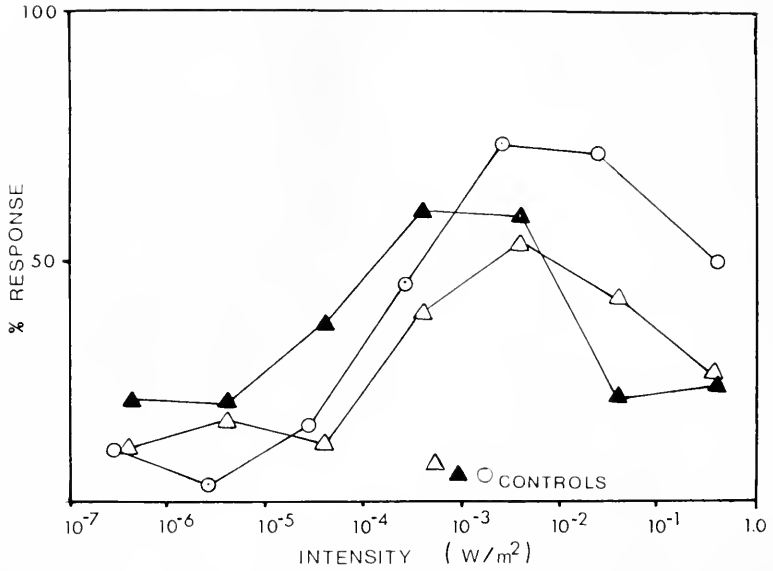


FIGURE 6. Intensity response for positive phototaxis of stage II *Balanus improvisus* at 480 nm. Closed triangle, dark-adapted at 20° C, 15‰ (N = 26–47); open triangle, light-adapted at 20° C, 15‰ (N = 53–60); open circle, light-adapted at 20° C, 30‰ (N = 22–29).

from 0.062–0.088 × 10^{16}). This quantal intensity was chosen because at 480-nm light it produced a clear positive phototactic response, but did not evoke a maximal response.

Analysis of DOT for nauplii indicates a broad response spectrum (Fig. 5). Increasing the ± 15° "window" for positive response to ± 45° produces a similar curve at response levels 30 to 40% higher. The peak percentage of positive phototaxis occurs at 480 nm (60% ± 15°; 97% ± 45°), however similar strong responses are evident at 500 to 520 nm. A significant negative response did not occur at any wavelength.

Although peak swimming speeds were found between 460 and 520 nm, distinct differences in MLV relative to wavelength are absent. One way analysis of variance followed by the Duncan test ($P = 0.05$) yields three broadly overlapping homogenous subsets, where the difference in the means of any two groups within a subset is not significant (Table II).

Intensity response

Light- and dark-adapted stage II nauplii (20° C, 15‰) were exposed to seven intensities of 480-nm light. In both groups, response to light was either positive or not evident; negative phototaxis did not occur significantly above random predictions (Fig. 6).

In close agreement with initial results, light-adapted nauplii exhibit a significant MLV increase at light intensities above 10^{-3} W/m^2 (Table III). Dark-adapted nauplii significantly increase MLV above 10^{-4} W/m^2 and, in contrast to

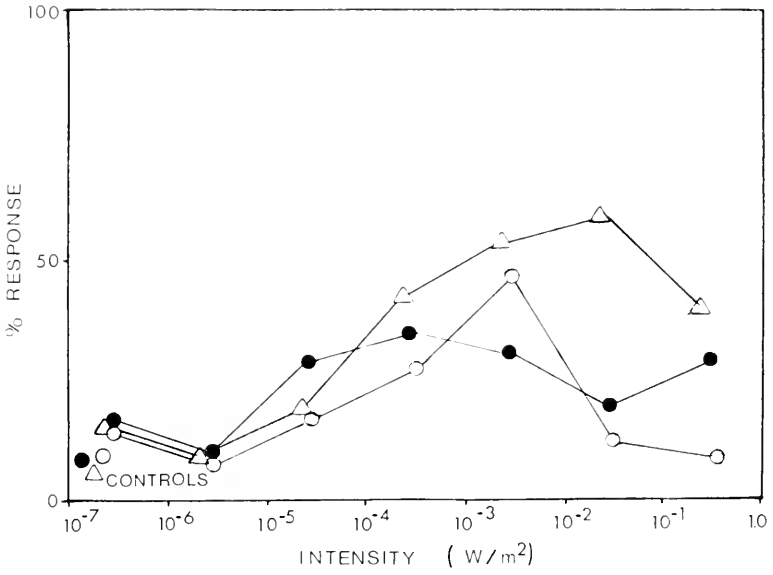


FIGURE 7. Intensity response for positive phototaxis of stage III and stage VI *Balanus improvisus* at 480 nm. Open triangle, light-adapted stage III at 20° C, 30‰ (N = 22–29); open circle, light-adapted stage VI at 20° C, 30‰ (N = 25–42); closed circle, dark-adapted stage VI at 20° C, 30‰ (N = 46–56).

light-adapted larvae, dark-adapted larvae show no increase in MLV at full intensity (Table III).

Directional response results further demonstrate the effects of dark adaptation. Light-adapted nauplii show a plateau of strong positive phototaxis from 10^{-3} to 10^{-1} W/m^2; a similar pattern occurs from 10^{-4} to 10^{-2} W/m^2 for dark-adapted nauplii (Fig. 6).

A significant positive phototactic response is present at all intensities tested for dark-adapted larvae. For light-adapted larvae, no significant response occurs below 10^{-4} W/m^2. In respect to percent response and MLV, dark-adapted nauplii appear to have a somewhat "stronger" response to favorable light intensities relative to light-adapted nauplii (Fig. 6).

Some light-adapted stage II nauplii were also investigated at 30‰, 20° C, to permit comparison with later stage nauplii tested at higher salinity. A similar, but perhaps enhanced response, relative to results at 15‰ was observed (Fig. 6). The change in salinity does not alter the basic pattern of response in stage II nauplii.

Light response in later stage nauplii

Smaller numbers of stage III and stage VI nauplii were tested for response to light, in addition to stage II nauplii. The later stage *B. improvisus* larvae are likely to be carried into increased salinity water (Bousfield, 1955). Hence, these initial studies were conducted at 30‰.

Phototactic response of light-adapted stage III naupli (30‰, 20° C) is similar to stage II nauplii (Fig. 7). The control MLV of stage III nauplii tends

TABLE II

Mean linear velocities of stage II and stage IV Balanus improvisus *nauplii in response to 2.5-sec stimuli of light at different wavelengths. Homogeneous subsets as determined by the Duncan's multiple range test ($P = 0.05$) are indicated (see Nie,* et al., *1975).*

Wavelength $7.0–9.0 \times 10^{11}$ quanta/m²	Stage II			Stage VI		
	N	MLV ± s.d. mm/sec	subset	N	MLV ± s.d. mm/sec	subset
440	26	1.75 ± 0.66	BC	35	3.72 ± 1.35	A
460	46	1.97 ± 0.77	AB	33	3.21 ± 1.19	AB
480	40	1.85 ± 0.83	ABC	35	3.36 ± 1.38	AB
500	45	2.02 ± 0.99	AB	35	3.26 ± 1.49	AB
520	45	2.13 ± 0.79	A	40	2.94 ± 1.07	AB
540	40	1.92 ± 0.62	ABC	37	2.83 ± 1.51	B
560	34	1.69 ± 0.75	BC	34	2.80 ± 1.37	B
600	50	1.56 ± 0.60	C	36	3.05 ± 1.37	AB
620	34	1.81 ± 0.60	ABC	37	3.16 ± 1.18	AB
640	55	1.78 ± 0.66	BC	48	2.89 ± 1.18	B

to be greater than stage II (Table III). Stage III nauplii show no significant increase in MLV during any light stimulus tested (Table III) therefore the photokinetic response seen in stage II nauplii is absent.

Both the response spectrum and responses to different intensities were tested for stage VI nauplii. Photo-responsiveness of stage VI larvae is generally less than that observed for stage II and III larvae; individual variation in nauplii is noticeably increased.

The spectral response function of stage VI nauplii using a ± 15° "window" for positive photo-response yields a generally low response of about 25 to 35% at all wavelengths, except 520 and 600 nm (Fig. 5). Increasing the window to ± 45° produces a curve similar to stage II with peak response at 480 nm, however, responsiveness is 20% or more below stage II results (Fig. 5). Decreased responsiveness of stage VI nauplii at 520 nm and increased responsiveness above 600 nm relative to stage II larvae is evident (Fig. 5). Unlike stage II results, increasing the window to ± 45° changes the basic shape of the response curve for stage VI nauplii and is perhaps indicative of less precise orientation to the light stimulus during the 2.5-sec test interval.

The response of stage VI nauplii to different intensities of 480-nm light is most interesting in respect to dark adaptation. Light-adapted stage VI nauplii first show a significant phototactic response above 10^{-4} W/m² (Fig. 8), which is in agreement with stage II results (Fig. 7). However, unlike stage II nauplii, the positive phototaxis of stage VI nauplii rapidly diminishes above 10^{-2} W/m². Dark adaptation of stage VI nauplii, although enhancing phototaxis between 10^{-5} and 10^{-4} W/m² (Fig. 7), does not appreciably alter the threshold of response. Finally, in marked contrast to stage II (Fig. 7), dark adaptation does not decrease responsiveness of stage VI nauplii to higher light intensities (Fig. 7).

The MLV of stage VI nauplii did not vary significantly as a function of light intensity or wavelength (Table II, III). The MLV for all samples (3.05 mm/sec) was significantly above stage II and III results.

TABLE III

Mean linear velocities of Balanus improvisus *naupliar stages II, III, and VI in response to 2.5 sec 480-nm light stimuli of different intensities. Control represents swimming speeds with 830-nm substage illumination. Homogeneous subsets as determined by the Duncan's multiple range test ($P = 0.05$) are indicated for each naupliar test group (see Nie, et al., 1975).*

Intensity	Dark-adapted II			Light-adapted II		
	N	MLV ± s.d.	subset	N	MLV ± s.d.	subset
6.2×10^{-1}	32	1.63 0.84	BC	60	1.91 0.81	A
$\times 10^{-2}$	33	2.22 0.96	A	59	1.92 0.88	A
$\times 10^{-3}$	26	2.11 1.10	A	58	1.77 0.65	AB
$\times 10^{-4}$	37	1.94 0.73	AB	59	1.58 0.60	BC
$\times 10^{-5}$	36	1.55 0.588	C	60	1.44 0.51	C
$\times 10^{-6}$	47	1.54 0.51	C	54	1.63 0.66	BC
$\times 10^{-7}$	39	1.41 0.57	C	53	1.54 0.53	BC
Control	40	1.52 0.55	C	55	1.48 0.49	C
Intensity	Light-adapted III			Dark-adapted VI		
	N	MLV ± s.d.	subset	N	MLV ± s.d.	subset
4.5×10^{-1}	24	2.14 0.86	A	50	2.94 1.52	A
$\times 10^{-2}$	29	2.40 0.70	A	50	3.00 1.61	A
$\times 10^{-3}$	25	2.32 0.81	A	49	3.03 1.74	A
$\times 10^{-4}$	22	2.24 0.87	A	47	3.15 1.84	A
$\times 10^{-5}$	24	2.18 0.90	A	46	2.60 1.29	A
$\times 10^{-6}$	24	2.23 0.88	A	56	3.00 1.50	A
$\times 10^{-7}$	23	2.08 0.96	A	49	3.08 1.79	A
Control	23	2.20 0.73	A	50	2.98 1.25	A

DISCUSSION

The photophysiology of zooplankton has been recently reviewed by Forward (1976b). In general, zooplankton living in coastal and fresh water tend to have their primary spectral maximum in the 500- to 600-nm region, the wavelengths best transmitted in these waters. Visscher and Luce (1928) noted maximal response of *B. amphitrite* and "*B. improvisus*" cyprids at 530 to 545 nm (although reported as *B improvisus* cyprids, the time of collection and similarity in size to *B. amphitrite* cyprids suggest *Balanus eburcus* as the more probable species tested (McDougall, 1943; Lang, 1979)). In more precisely controlled experiments using stage I *E. modestus* and *S. balanoides* nauplii, Barnes and Klepal (1972) found maximal spectral sensitivity between 520 and 530 nm, with a marked shoulder of strong responses at 450 to 530 nm.

Stage II *B. improvisus* nauplii have a maximal spectral sensitivity at 480 to 520 nm, somewhat shorter wavelengths than expected considering their estuarine habitat and other previous findings for barnacle larvae. Strong photo response occurs within this spectral range with maximal positive phototaxis at 480 nm (Fig. 6) and maximal MLV response to light stimuli at 520 nm (Table II).

Light response studies of stage VI nauplii are hindered by high individual variability of responsiveness; some nauplii appear highly sensitive to light stimuli while

others are unresponsive. Those stage VI nauplii which do respond show strong sensitivity to 440 to 500 nm, are curiously less sensitive to 520-nm light, and appear to be more sensitive to wavelengths at and above 540 nm relative to stage II (Fig. 6). Considerable structural and, presumably, physiological changes occur in stage VI nauplii in preparation for metamorphosis to cyprid (Walley, 1969). This includes development of compound eyes under the dorsal cephalic shield. Stage VI nauplii may have, depending on their age, only a single median naupliar eye, or two well pigmented compound eyes (Kaufmann, 1965; Lang, 1979). Since the larval samples tested in this study included all phases of stage VI development, variability of responsiveness is not unexpected. Increased sensitivity to higher wavelengths is perhaps related to development of compound eyes; studies on specific phases of stage VI development and, more importantly, the cyprid stage, are needed to verify this hypothesis.

Contrasting qualitative observation of photokinetic responses in barnacle larvae have been reported in the literature. Ewald (1912) noted a charatceristic sinking reaction in stage II specimens of *Balanus perforatus* following a sudden dark-to-light transition. Similarly Crisp and Ritz (1973) saw decreased activity in *S. balanoides* cyprids exposed to sudden light increase and conversely, increased activity following light intensity reduction. Essentially opposite reactions were observed in our laboratory for stage II *Balanus venustus, B. improvisus*, and *B. amphitrite;* MLV sharply increased following sudden exposure to bright white light and MLV sharply decreased following removal of the light stimulus (Lang, *et al.,* 1979). Photokinetic responses were found absent in stage II nauplii of *S. balanoides* and *E. modestus* (Crisp & Ritz, 1973) and in *Chthamalus fragilis* (Lang, *et al.,* 1979).

The present study shows that stage II nauplii of *B. improvisus* consistently increases MLV following exposure to a specific range of light intensities at 480 nm, the range being determined, in part, by the initial state of naupliar light adaptation. Dark-adapted nauplii show photokinetic response at reduced light intensities relative to light-adapted nauplii. The upper intensity, if any, which inhibits increased MLV in light-adapted nauplii was above the maximum tested (27 W/m^2). An increase in MLV appears to represent a second aspect in stage II light response. At detectable, but suboptimum intensities, stage II nauplii exhibit positive phototaxis but show no significant change in MLV. Only within a narrower range of light intensities inducing maximal response does MLV increase. A similar change in MLV does not occur in light-adapted stage III nauplii or light- and dark-adapted stage VI nauplii. Results indicate at least two possibilities; either stage II nauplii exhibit a "stronger" response to light incorporating both directional and kinetic reactions or, later stages normally swim at or near their potential MLV and are incapable of further MLV increase in response to light stimulii.

Earlier studies (Lang, *et al.,* 1979) showed that *B. improvisus* nauplii will briefly stop or reduce MLV when a strong white light source was removed. Removal of the 480-nm light stimulii in present studies did not induce this response. We assume this response occurs only with more intense and/or longer light stimulation.

The level of previous adaptation to light has been shown to significantly effect the photoresponse of zooplanktons (Forward, 1976b). Crisp and Ritz (1973)

demonstrated relative reductions in barnacle naupliar light responsiveness following exposure to strong light. Continual exposure to light of sufficient intensity will induce a photonegative response in barnacle nauplii (Groom and Loeb, 1890).

A change in responsiveness to light following dark-adaptation is clearly seen here in *B. improvisus* stage II nauplii. The threshold for positive phototaxis in light-adapted nauplii is at least an order of magnitude greater relative to dark-adapted naupliar responses. Conversely, light-adapted phototaxis is significantly less suppressed at higher light intensities (Fig. 6). Positive phototaxis for at least 10% (above control) of dark-adapted stage II nauplii tested was seen at 6.2×10^{-7} W/m^2 at 480 nm. A consistent, strong response (*i.e.*, 30% or more of the test population) occurred above 10^{-5} W/m^2. These values bracket the same order of magnitude for light responsiveness reported by Barnes and Klepal (1972) and Crisp and Ritz (1973) for three barnacle species of naupliar stages I–II.

A similar clear shift in responsiveness of light- and dark-adapted stage VI nauplii was not seen. In particular, dark-adapted nauplii remained equally or more responsive to higher light intensities as compared to light-adapted nauplii (Fig. 7). Cyprid larvae of some barnacles are known to exhibit at least two fundamental light responses; orientation at fixation and shade-seeking during exploration (Crisp and Ritz, 1973). As with spectral results, we would eventually like to demonstrate whether idiosyncrasies in stage VI light behavior relate to light responses in *B. improvisus* cyprids.

The degree of light-adaptation, although it affects the intensity sensitivity of *B. improvisus* nauplii, does not alter the basic types of response. Under the stable and presumably favorable salinity-temperature conditions tested, the immediate reaction of *B. improvisus* to light stimulation is either positive phototaxis or no response. A characteristic "shadow response" with negative phototaxis seen in light-adapted brachyuran zoea (Forward, 1976a, 1977) tested under similar light stimuli is absent.

In his classic field study, Bousfield (1955) showed that stage II *B. improvisus* nauplii maintained an average water column position near the surface, while later naupliar stages were found at progressively lower average depths. The strong positive photo response of stage II nauplii and marked decline of photo responsiveness in stage VI nauplii seen here correlates well with these field observations. However, a discussion of ecological implications of larval photobehavior is best deferred until further studies are complete.

This study most importantly demonstrates a new research technique. Video-computer systems decidedly enhance the ability to convert visual (video) records into quantified data. With this ability comes a new potential to initiate studies on locomotory behavior involving various parameters and to measure responses with a resolution previously not readily obtainable.

Additional software development for analysis of phototactic responses was provided by Dr. Robert Wilson. Technical assistance for Bugwatcher operations was provided by Dr. John Greaves. Martha Marcy and Sally Lawrence contributed significant assistance in larval maintenance and data analyses.

Summary

A video-computer behavioral analysis system—the "Bugwatcher"—was found to be capable of rapidly and accurately analyzing the phototactic movements of stage II, III, and VI nauplii of the barnacle *Balanus improvisus*. Under the test conditions these larvae only display a positive phototactic response; a negative response was not observed. The response spectrum of dark-adapted stage II shows a plateau of strongest positive phototaxis between 480 and 520 nm with about 60% of test larvae swimming ± 15° toward the light stimulus. In contrast, stage VI nauplii are generally less phototactic. The response spectrum changes to have a depression at 520 nm and enhanced responsiveness to longer wavelengths of 540 to 580 nm.

Responsiveness to different light intensities changed upon light adaptation. Upon stimulation with 480 nm lights, dark-adapted stage II nauplii show a significant positive phototaxis at 6.2×10^{-7} W/m^2. Peak response occurs between 10^{-4} and 10^{-2} W/m^2. A significant increase in mean linear velocity (MLV) accompanies the maximal phototactic response. In contrast, upon light adaptation, stage II nauplii show a significant positive phototaxis only to intensities above 10^{-4} W/m^2, 480 nm. Peak response occurs between 10^{-3} and 10^{-1} W/m^2. A significant increase in MLV occurs from about 10^{-3} to at least 27 W/m^2. Light-adapted stage III nauplii show intensity sensitivity similar to stage II nauplii; however, no increase in MLV occurs during light stimulation. Stage VI nauplii show a reduced percent phototactic response at all intensities as compared to stage II–III. Light- or dark-adaptation does not result in clear shifts in intensity sensitivity as evident in stage II responses. Differences in stage VI naupliar light responses may be related to impending metamorphisis to cyprid. A study of cyprid light response is needed to clarify this.

LITERATURE CITED

Aiken, R. B., and J. P. Hailman, 1978. Positive phototaxis of the brine shrimp *Artemia salina* to monochromatic light. *Can. J. Zool.*, **56**: 708–711.

Anderson, J. M., 1971. II. Sublethal effects and changes in ecosystems. Assessment of the effects of pollutants on physiology and behaviour. *Proc. R. Soc. Lond., B. Biol. Sci.*, **177**: 307–320.

Barr, A. J., J. H. Goodnight, J. P. Sall, and J. T. Helwig, 1976. *A User's Guide to SAS*. SAS Institute Inc., Raleigh, North Carolina, 329 pp.

Barnes, H., D. J. Crisp, and H. T. Powell, 1951. Observations on the orientation of some species of barnacles. *J. Anim. Ecol.*, **20**: 227–241.

Barnes, H., and W. Klepal, 1972. Phototaxis in stage I nauplius larvae of two cirripedes. *J. Exp. Mar. Biol. Ecol.*, **10**: 267–273.

Barnes, S. N., and T. H. Goldsmith, 1977. Dark adaptation, sensitivity and rhodopsin level in the eye of the lobster, *Homarus*. *J. Comp. Physiol.*, **120**: 143–159.

Batschelet, E., 1965. *Statistical Methods for the Analysis of Problems in Animal Orientation and Certain Biological Rhythms*. American Institute of Biological Sciences, Washington, D. C., 57 pp.

Bousfield, E. L., 1955. Ecological control of the occurrence of barnacles in the Miramichi Estuary. *Natl. Mus. Can. Bull.*, No. **137**: 1–69.

Crisp, D. J., and D. A. Ritz, 1973. Responses of Cirripede larvae to light. I. Experiments with white light. *Mar. Biol.* (Berlin), **23**: 327–335.

Davenport, D., G. J. Culler, J. O. B. Greaves, R. B. Foreward, and W. G. Hand, 1970. The investigation of the behavior of microorganisms by computerized television. *IEEE Trans. Biomed. Eng., BME*-**17**: 230–237.

Ewald, W. F., 1912. On artificial modification of light reactions and the influence of electrolytes on phototaxis. *J. Exp. Zool.*, **13**: 591–612.

Forward, R. B., 1974. Negative phototaxis in crusteacean larvae: possible functional significance. *J. Exp. Mar. Biol. Ecol.*, **16**: 11–17.

Forward, R. B., Jr., 1976a. A shadow response in a larval crustacean. *Biol. Bull.*, **151**: 126–140.

Forward, R. B., Jr., 1976b. Light and diurnal vertical migration: photobehavior and photophysiology of plankton. Pages 157–209 in K. Smith, Ed., *Photochemical and photobiological reviews*, Volume 1. Plenum Press, New York.

Forward, R. B., Jr., 1977. Occurrence of a shadow response among brachyuran larvae. *Mar. Biol.*, **39**: 331–341.

Forward, R. B., Jr., and J. D. Costlow, 1974. The ontogeny of phototaxis by larvae of the crab *Rhithropanopeus harrisii*. *Mar. Biol.*, **26**: 27–33.

Forward, R. B., Jr., and T. W. Cronin, 1978. Crustacean larval phototaxis: Possible functional significance. Pages 253–261 in D. S. McLusky and A. J. Berry, Eds., *Proc. 12th European Symp. Mar. Biol.*, Pergamon Press, New York.

Greaves, J. O. B., 1975. The bugstystem: the software structure for the reduction of quantized video data of moving organisms. *Proc. Inst. Electrical Electronics Engr.*, **63**: 1415–1425.

Groom, T. T., and J. Loeb, 1890. Der Heliotropismus der Nauplien von *Balanus perforatus* und die periodischen Tiefenwanderungen pelagischer Tierre. *Biol. Zentralbl.*, **10**: 160–177.

Hamdorf, K., and J. Schwemer, 1975. Photoregeneration and adaptation process in insect photoreceptors. Pages 263–289 in A. W. Snyder and R. Menzel, Eds., *Photoreception Optics*, Springer, Berlin.

Kaufman, R., 1965. Zur Embryonal- and Larvalentwicklung von *Scalpellum scalpellum* L. (Crust. Cirr.) mit einen Beitrag zur Autokologie dieser Art. *Z. Morpholo. Ockol. Tiere*, **55**: 161–232.

Lang, W. H., 1979. Larval development of shallow water barnacles of the Carolinas (Cirripedia: Thoracica) with keys to naupliar stages. *NOAA Tech. Rep., NMFS Circ.*, **421**: 1–39.

Lang, W. H., S. Lawrence, and D. C. Miller, 1979. The effects of temperature, light, and exposure to sublethal levels of copper on the swimming behavior of barnacle nauplii. In F. Jacoff, Ed., *Proc. Symp. The State of Marine Environmental Research*, U. S. Govt. Print. Office, Washington, D. C. (in press).

Latz, M. I., and R. B. Forward, Jr., 1977. The effect of salinity upon phototaxis and geotaxis in a larval crustacean. *Biol. Bull.*, **153**: 163–179.

McDougall, K. D., 1943. Sessile marine invertebrates at Beaufort, North Carolina. *Ecol. Mongr.*, **13**: 321–374.

Nie, N. H., C. H. Hull, J. G. Jenkins, K. Steinbrenner, and D. H. Bent, 1975. *Statistical Package for the Social Sciences*, McGraw-Hill, New York. 675 pp.

Olla, B. L., (Ed.), 1974. Behavioral bioassays. Pages 1–31 in G. V. Cox, Chairman, *Marine Bioassays Workshop Proceedings*, Marine Technology Society, Washington, D. C., 308 pp.

Rose, M., 1925. Contribution a letude de la biologie du plancton; le probleme des migrations journalieres. *Arch. Zool. Exp. Gen.*, **64**: 387–542.

Singarajah, K. V., J Moyse, and E. W. Knight-Jones, 1967. The effect of feeding upon the phototactic behaviour of cirripede nauplii. *J. Exp. Mar. Biol. Ecol.*, **1**: 144–153.

Thorson, G., 1964. Light as an ecological factor in the dispersal and settlement of larvae of marine bottom invertebrates. *Ophelia*, **1**: 167–208.

Visscher, J. P., and R. H. Luce, 1928. Reactions of the cyprid larvae of barnacles to light with special reference to spectral colors. *Biol. Bull.*, **54**: 336–350.

Walley, L. J., 1969. Studies on the larval structure and metamorphosis of *Balanus balanoides* (L.). *Philos. Trans. R. Soc. Lond., B. Biol. Sci.*, **256**: 237–279.

Wilson, R. S., and J. O. B Greaves, 1979. The evolution of the bugsystem: recent progress in the analysis of bio-behavioral data. In F. Jacoff, Ed., *Proc. Symp. The State of Marine Environmental Research*, U. S. Govt. Print. Office, Washington, D. C., (in press).

Reference: *Biol. Bull.* 157: 182–188. (August 1979)

THE EFFECTS OF EYESTALK, LEG, AND UROPOD REMOVAL ON THE MOLTING AND GROWTH OF YOUNG CRAYFISH, *PROCAMBARUS CLARKII*

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The effects of eyestalk removal on molt have been studied in decapod crustaceans (Brown and Cunningham, 1939; Abramowitz and Abramowitz, 1940; Smith, 1940), but few crustaceans have been studied during several consecutive molts, probably because of a high mortality. According to Abramowitz and Abramowitz (1940), however, the removal of the eyestalk itself is unlikely to be the reason for the mortality, because some of their blinded crabs survived for 11 weeks after the removal of eyestalks.

In our preliminary experiments (unpublished), 14 of 90 eyestalkless young crayfish survived after more than 10 molts, though the rest died within 4 months. In most cases, the eyestalkless crayfish died at the time of molt or within 1 or 2 days after the molt, probably because of bacterial infection at the time of molt. If this is so, a high mortality might be avoidable to some extent by keeping the container water clean, without disturbing the molting animals.

The molt cycle in Crustacea is thought to be regulated by two hormones, a molt hormone and a molt-inhibiting hormone. It is believed that the X-organ in the eyestalk produces the molt-inhibiting hormone, which is then stored in the sinus gland. Eyestalk removal, therefore, shortens the following intermolt cycle (Brown and Cunningham, 1939; Smith, 1940). The molt-inhibiting hormone inhibits the activity of the Y-organ, which secretes the molt hormone (Passano, 1960). When the eyestalk is removed the Y-organ is no longer inhibited. If the larvae of the prawn *Palaemonetes kadiakensis* are destalked at metamorphosis, they grow larger in size than the untreated ones, without causing any alteration on the duration of the larval instars (Hubschman, 1963).

It is known that leg removal causes precocious molt in land crabs, *Gecarcinus lateralis* (Skinner and Graham, 1970), freshwater shrimp, *Palaemonetes kadiakensis* (Stoffel and Hubschman, 1974), and crayfish, *Procambarus clarkii* (Bittner and Kopanda, 1973). Skinner and Graham (1972) hypothesized that severing a critical number of leg nerves stimulates the precocious molt.

In the present study, the molt interval and the growth rate of the crayfish were studied during five consecutive molts when the eyestalks, the legs or the uropods were removed.

Materials and Methods

Specimens of the crayfish, *Procambarus clarkii*, used in this work were collected from ponds in the suburbs of Yamagata. Only crayfish of 8 to 12 mm in length (the length was measured from the tip of rostrum to the hind margin of cephalothorax carapace) that molted once in the laboratory (initial molt) were

chosen as experimental material. They were kept separately, first in polypropylene containers (Lustro ware of Boden Co., 70 × 85 × 45 mm), then in large containers (105 × 120 × 53 mm) containing dechlorinated tap water at 22 to 23° C when they grew to more than 15 mm in carapace length. They were kept under a photoperiodic light condition of 14-hr light and 10-hr dark. The animals were fed fish food pellets and fallen dead leaves of persimmon. The dead leaves were effective in keeping the animals healthy. When water in the containers was renewed each day, the crayfish were immersed in 1.3% NaCl solution for 20 to 30 sec to prevent infection by bacteria or protozoa.

The crayfish were classified into four experimental groups: (1) untreated intact crayfish as control; (2) crayfish from which a pair of eyestalks was removed; (3) crayfish from which three pairs of the second, third and fourth walking legs were removed, and (4) crayfish from which a pair of uropods was removed. Each group consisted of 23 individuals that had just finished the initial molt in the laboratory, and every operation was performed on the day following this initial molt. The organs were cut off at their bases with scissors. After the third molt following the operation, the regenerated legs or uropods were removed again. Carapace length was measured two days after each molt.

Results

Molting

Molting rate was recorded daily for 230 days after the initial molt. The results are illustrated in Figure 1A–D. The crayfish which died during the course of the experiments were excluded from the data. Four crayfish from the control group, one from the legless group, and one from the uropodless group failed to complete the fifth molt even after 230 days. Since these animals had a reduced fecal output, their failure to molt may have been the result of factors other than hormonal or nervous controls.

Untreated crayfish (controls). Fourteen individuals completed the fifth molt, but three molted four times and one completed only the third molt. The average time required for 50% of the individuals to reach each consecutive molt (T_{50}) was 17 days for the first molt, 50 days for the second, 87 days for the third, 128 days for the fourth, and 164 days for the fifth, after the initial laboratory molt.

Table I

The days of the intermolt cycles (molt to molt) in crayfish, Procambarus clarkii.

Molt	Control (14)	Eyestalkless (11)	Legless (20)	Uropodless (20)
Initial to 1st	19.6 ± 2.7	6.5 ± 0.2	14.1 ± 1.0	19.7 ± 2.3
1st to 2nd	23.8 ± 2.9	7.1 ± 0.3	12.6 ± 0.9	26.9 ± 2.9
2nd to 3rd	33.6 ± 4.2	7.5 ± 0.2	31.5 ± 3.8	33.6 ± 4.2
3rd to 4th	31.3 ± 3.1	8.8 ± 0.2	21.1 ± 1.6	29.9 ± 2.6
4th to 5th	43.2 ± 6.0	9.2 ± 0.4	35.2 ± 4.6	39.0 ± 3.4

The number of individuals is in parentheses. Data were obtained from crayfish which had completed five consecutive molts. The standard error of the mean is shown.

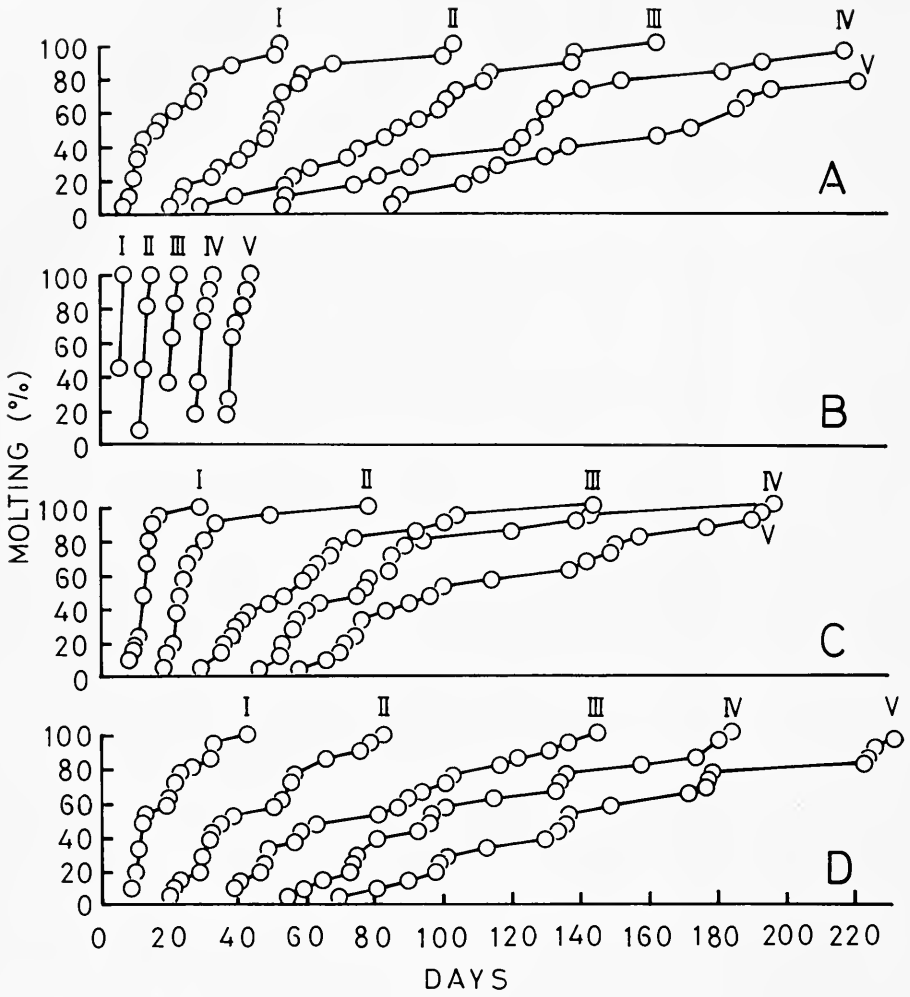

FIGURE 1. Molting percentage of surgically treated crayfish, specimens of *Procambarus clarkii,* at five consecutive molts after the initial laboratory molt. A, untreated crayfish (control); B, both eyestalks removed; C, three pairs of walking legs removed; D, both uropods removed. In the groups where legs or uropods were removed, the regenerated legs or uropods were removed again one day after the third molt. Roman numerals I, II, III, IV and V, show the 1st, 2nd, 3rd, 4th, and 5th molt, respectively.

Large variation, however, was observed among the individuals. For example, in the first molt the earliest crayfish molted on the seventh day but the last crayfish molted on the fifty-third day. As clearly shown in Figure 1, this variation became more pronounced in the later molts.

Eyestalkless crayfish. Twelve of 23 treated crayfish died between the third and fifth molts. The remaining 11 completed all five consecutive molts within 43 days. T_{50} from the first to the fifth molt was 6 (35.3% compared to the value of

control animals), 13 (26.0%), 20.5 (23.6%), 29 (22.7%), and 39 (23.8%) days, respectively. The first molt occurred on the fifth day and all the treated individuals molted by the eighth day. All 23 crayfish completed the second molt between 6 and 8 days after the first molt and finished the third molt between 7 and 8 days after the second molt. Two animals, however, died on the first and fifth day respectively, after the third molt.

The surviving crayfish completed the fourth molt by the intermolt cycle of 7 to 12 days. Seven animals died within two days after the fourth molt. The 14 survivors completed the fifth molt by the intermolt cycle of 8 to 12 days. Three died at the time of the fifth molt.

Legless crayfish. Twenty of 21 animals completed five consecutive molts (one animal completed only four). T_{50} from the first to the fifth molt was 13 (76.5% of the control value), 24 (48.0%), 55 (63.2%), 77 (60.2%), and 99 (60.4%) days, respectively.

A few animals had longer molt cycles than the others. Roughly speaking, the operated animals could complete five molts during the time in which the untreated crayfish completed four molts. The walking legs regenerated to normal size after the second molt. Two crayfish died; one at the time of its third molt and another at its fourth molt.

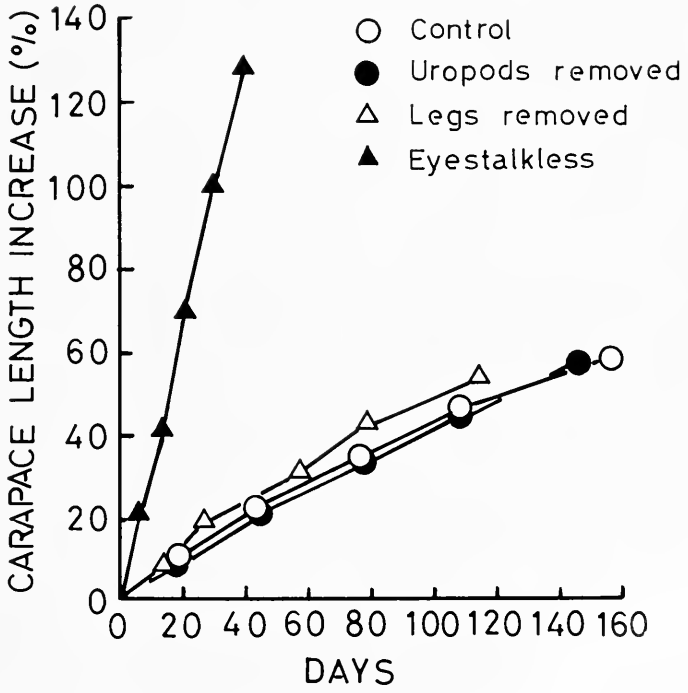

FIGURE 2. The average percentage of increase in carapace length of specimens of the crayfish, *Procambarus clarkii*, to the original length after each of the five consecutive molts. Each point represents the mean days required for each molt following the initial laboratory molt.

TABLE II

The average carapace length (mm) after each molt in crayfish, Procambarus clarkii.

	Initial	1st	2nd	3rd	4th	5th	Mean
Control	11.1 ± 0.2	12.3 ± 0.3 (10.5)	13.5 ± 0.4 (10.4)	14.9 ± 0.5 (10.2)	16.1 ± 0.5 (8.2)	17.5 ± 0.6 (8.5)	(9.6)
Eyestalkless	11.5 ± 0.3	13.9 ± 0.3 (20.7)	16.3 ± 0.4 (17.5)	19.5 ± 0.5 (19.8)	22.8 ± 0.6 (16.8)	26.2 ± 0.7 (14.7)	(17.9)
Legless	11.2 ± 0.3	12.1 ± 0.3 (8.1)	13.3 ± 0.3 (10.0)	14.6 ± 0.4 (10.2)	15.9 ± 0.4 (8.6)	17.1 ± 0.5 (7.8)	(8.9)
Uropodless	11.6 ± 0.3	12.7 ± 0.3 (8.9)	14.1 ± 0.5 (11.3)	15.5 ± 0.6 (9.7)	16.8 ± 0.7 (8.6)	18.3 ± 0.9 (8.6)	(9.4)

The numerals in parentheses are the average percentage of increased carapace length for each molt.

Uropodless crayfish. Twenty of 21 animals completed five consecutive molts, one animal staying at the fourth molt during the experimental period. T_{50} from the first to the fifth molt was 12 (70.0% of the control value), 39 (78.0%), 73 (83.9%) 96 (75.0%), and 136 (82.9%) days, respectively. Regeneration of the uropods was observed on all crayfish after the second molt. Two crayfish died; one at the first molt and another at the third molt.

Growth rates

The mean values of growth rate for crayfish which completed five molts were measured after each of five consecutive molts with special reference to the mean value of the initial carapace length, which was measured after the initial laboratory molt (Fig. 2). As is clear from Figure 2, the rate of increase of carapace length of the eyestalkless group is more than six times greater than that of the control group.

The percentage increases in carapace length from between each premolt and corresponding postmolt stage are shown in Table II. The average percentage of increase in carapace length per molt was 9.6% for untreated group, 17.9% for eyestalkless group, 8.9% for legless group, and 9.4% for uropodless group.

DISCUSSION

The results suggest that the removal of eyestalks, legs, or uropods stimulates molt in the crayfish, *Procambarus clarkii.* The results support the findings of other authors studying eyestalkless crayfish, *Cambarus clarkii* (Smith, 1940), fiddler crabs, *Uca pugilator* (Abramowitz and Abramowitz, 1940) and land crabs, *Gecarcinus lateralis* (Skinner and Graham, 1972); and walking legless crayfish, *Procambarus clarkii* (Bittner and Kopanda, 1973), freshwater shrimp, *Palaemonetes kadiakensis* (Stoffel and Hubschman, 1974) and land crabs, *Gecarcinus lateralis* (Skinner and Graham, 1970). Brown and Cunningham (1939) and Smith (1940) discussed the possible mechanisms concerning the effects of eyestalk removal on the molt, suggesting that the eyestalks contain the inhibiting hormone which delays molting.

Abramowitz and Abramowitz (1940) found that in fiddler crabs both molt and growth were stimulated by eyestalk removal. In our experiments, we found that the average growth rate of carapace length in the eyestalkless crayfish after every molt was about twice that of the intact animals, and that the duration of the molt

cycles of the former was about one fourth of that of the latter. From these data, the mean weight increase of eyestalkless animals was about 15 times larger than the corresponding weight of the control animals 40 days after the operation. On the other hand, the molts of both the leg- or uropod-removed crayfish were stimulated, but the carapace length growth rate after every molt was less than that of the controls. Thus, it is clear that growth was accelerated by eyestalk removal, and that the secretion of molting hormone was induced by the growth of the body.

Weis (1976) found that the removal of seven legs from a fiddler crab did not result in a significant increase in carapace width after molt and regeneration. Krishnakumaran and Schneiderman (1970) found that ecdysterone did not increase DNA synthesis in the epidermis, muscles, nerve cells, and connective tissue of crayfish, *Procambarus clarkii,* although it induced molting. In the present experiments, the carapace length increased after molt in crayfish whose legs or uropods were removed, but the rate of increase was less than that of the untreated control.

It may be safely concluded that the removal of legs or uropods has the same effects on molt and growth in crayfish. This would support the hypothesis of Skinner and Graham (1972) that molt inhibitory factors do not exist in the limbs of Crustacea and that precocious molt is stimulated by the severing of a critical number of nerves. Stoffel and Hubschman (1974) pointed out that the loss of several walking legs stimulates the neurosecretory cells of the X-organ via nervous impulses to stop releasing the molt-inhibiting hormone. According to Holland and Skinner (1976), the removal of one or more limb buds of *Gecarcinus lateralis* inhibited growth of the remaining limb buds until re-regenerates reached an appropriate size. In the present experiment, both legless or uropodless crayfish molted before they grew in body size sufficient for molt. Therefore, if the crayfish is missing legs or uropods, it may be possible that the regenerating buds grow faster than all the other parts of the body, and the regenerating buds stimulate the release of molt hormone. The intensity of stimulus may depend, to some extent, on the number of buds, or on the surface of the cut ends or organs. It is known that land crabs missing many legs prepare for molt sooner than those which are missing one or two legs (Skinner and Graham, 1970).

It has been reported that the mortality is high in destalked crustaceans (Abramowitz and Abramowitz, 1940; Smith, 1940; Skinner and Graham, 1972). According to Abramowitz and Abramowitz (1940), the viability is concerned in some way with the eyestalks. In general, death during molt by intact Crustacea is common. In the present experiments, five crayfish of the control group, two of the legless group, and two of the uropodless group died at the time of molt or within a day after their molt. In the eyestalkless group, twelve individuals died at the time of molt or within a few days after their molt. The mortality of the eyestalkless group, therefore, is higher than the other groups. It may be possible that the eyestalkless crayfish grow too rapidly to prepare properly for molt and this leads to failure at molt.

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Summary

1. Removal of a pair of eyestalks induces precocious molt and accelerates the growth of the crayfish, *Procambarus clarkii*.

2. Removal of three pairs of walking legs or a pair of uropods induces precocious molt without any effects on the growth of the body.

3. The average time required for 50% of the individuals to reach the fifth molt after the intial molt is 39 days for eyestalkless crayfish, 99 days for legless, and 136 days for uropodless crayfish. The untreated crayfish (controls) required 164 days to attain the fifth molt.

4. The average percentage of increase in carapace length at the time of the fifth molt is 9.6% for the untreated crayfish, 17.6% for eyestalkless, 8.9% for legless, and 9.4% for the uropodless group.

5. The mortality during the approximately eight-month experimental period was 5:23 for the untreated group, 12:23 for the eyestalkless group, 2:23 for the legless group and 2:23 for the uropodless group. The eyestalkless crayfish were healthy until the third molt, but experienced great mortality at the time of the fourth and fifth molt. The failure of molt in eyestalkless crayfish may be due to too rapid increase in the body size, imparing preparations for molt.

LITERATURE CITED

Abramowitz, R. K., and A. A. Abramowitz, 1940. Moulting, growth, and survival after eyestalk removal in *Uca pugilator*. *Biol. Bull.*, **78**: 179–188.

Bittner, G. D., and R. Kopanda, 1973. Factors influencing molting in the crayfish *Procambarus clarkii*. *J. Exp. Zool.*, **186**: 7–16.

Brown, F. A. Jr., and O. Cunningham, 1939. Influence of the sinus gland of crustaceans on normal viability and ecdysis. *Biol. Bull.*, **77**: 104–114.

Holland, C. A., and D. M. Skinner, 1976. Interactions between molting and regeneration in the land crab. *Biol. Bull.*, **150**: 222–240.

Hubschman, J. H., 1963. Development and function of neurosecretory sites in the eyestalks of larval *Palaemonetes* (Decapoda: Natantia). *Biol. Bull.*, **125**: 96–113.

Krishnakumaran, A., and H. A. Schneiderman, 1970. Control of molting in mandibulate and chelicerate arthropods by ecdysones. *Biol. Bull.*, **139**: 520–538.

Passano, L. M., 1960. Molting and its control. Pages 473–536 in T. H. Waterman, Ed., *The Physiology of Crustacea. I. Metabolism and Growth*. Academic Press, New York and London.

Skinner, D. M., and D. E. Graham, 1970. Molting in the land crab: Stimulation by leg removal. *Science*, **169**: 383–384.

Skinner, D. M., and D. E. Graham, 1972. Loss of limbs as a stimulus to ecdysis in Brachyura (true crabs). *Biol. Bull.*, **143**: 222–233.

Smith, R. I., 1940. Studies on the effects of eyestalk removal upon young crayfish (*Cambarus clarkii* GIRARD). *Biol. Bull.*, **79**: 145–152.

Stoffel, L. A., and J. H. Hubschman, 1974. Limb loss and the molt cycle in the freshwater shrimp, *Palaemonetes kadiakensis*. *Biol. Bull.*, **147**: 203–212.

Weis, J., 1976. Effects of environmental factors on regeneration and molting in fiddler crabs. *Biol. Bull.*, **150**: 152–162.

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EVOKED RESPONSES TO ELECTRICAL STIMULATION IN THE COLONIAL HYDROID *CLAVA SQUAMATA*: A CONTRACTION PULSE SYSTEM[1]

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Electrical activity has been recorded from several hydrozoans (phylum Cnidaria) representing two distinct classes of polyps—those which produce recurring behavioral events of spontaneous origin, for example, *Corymorpha* (Ball, 1973; Ball and Case, 1973); *Hydra* (Josephson, 1967; Josephson and Macklin, 1969; Passano and McCullough, 1962, 1963, 1964, 1965; Rushforth, 1966, 1971; Rushforth and Burke, 1971); *Millepora* (deKruijf, 1976); *Obelia* (Morin and Cooke, 1971a, b, c); *Tubularia* (deKruijf, 1977; Josephson, 1962, 1965, 1974; Josephson and Mackie, 1965; Josephson and Rushforth, 1973; Josephson and Uhrich, 1969); and those which are behaviorally quiescent, *e.g. Cordylophora* (Josephson, 1961b; Mackie, 1968); *Hydractinia* (Stokes, 1974a, b) and *Proboscidactyla* (Spencer, 1974). Despite these obvious behavioral differences, a review of these studies (Rushforth and Stokes, 1978) showed that there is at least one element of electrically evoked activity which is common to all species of both classes. These common elements are the large (0.5–15 mV), slow conducting (2–20 cm/sec), long duration (20–500 msec) potentials which are correlated with contraction of whole or isolated parts of a polyp. Examples include the Josephson Pulses (JPs) of *Cordylophora*, Hydranth Pulses (HPs) and Neck Pulses (NPs) of *Tubularia*, Stalk Pulses (SPs) and Hydranth Pulses (HPs) of *Corymorpha*, Contraction Pulses (CPs) or Contraction Bursts (CBs) of *Hydra*, Symmetrical Contraction Pulses (SCPs) of *Hydractinia*. Contraction Pulses (CPs) of *Millepora*, Contraction Pulses (KPs) of *Obelia* and the Tentacle Contraction Pulses (TCPs) and Colonial Pulses (CPs) of *Proboscidactyla*.

It has been proposed (Rushforth and Stokes, 1978) that these pulses are homologous representations of a fundamental conducting system termed the 'Contraction Pulse System' (CPS). This system functions to activate muscles of widespread distribution, or isolated blocks of muscles. In an effort to strengthen this proposal, we have investigated the evoked responses of the colonial gymnoblastic hydroid, *Clava squamata*. The purpose of the present study was to examine the electrical activity of this behaviorally quiescent species.

Materials and Methods

Collection and maintenance of animals

The Supply Department of the MBL, Woods Hole, provided all animals for this study. Only colonies growing on *Ascophyllum* were used. Colonies in which the

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polyps appeared unhealthy or damaged, or in which the alga had begun to decay, were discarded. All specimens were placed in an aerated, refrigerated (14° C) Instant Ocean System containing sea water, and were fed daily on newly hatched *Artemia* nauplii. Collected colonies were studied within 2 to 3 days. Glass microscope slide cultures of *Clava* were also made by dissecting two to three interconnected polyps from a colony and attaching them to the slide by fine surgical thread. Such cultures were maintained under conditions similar to those of the freshly collected colonies. New stolons grow out from the transplant, adhere to the glass slide and give rise to new polyps. After about 1 week the original transplant and the surgical thread can be removed, leaving only new growth attached to the slide.

Most experiments used whole colonies on their algal substrate. The alga was trimmed to about 2 to 3 cm length and pinned to the Sylgard bottom of a dish containing 500 ml of sea water. The temperature of the sea water in the dish was also maintained at 14° C by an outer jacket of circulating, refrigerated water. The attached hydroids did not appear to be damaged by this process and survived for several days under such conditions, when appropriately fed. In certain experiments single polyps were used. These polyps were excised from the colony by means of iridectomy scissors at the junction between polyp and stolon.

Stimulation and recording techniques

Suction electrodes were used for recording and stimulation. Plastic Tygon or polyethylene tubing was flame heated and drawn to diameters appropriate for attachment to a specific region of the polyp or stolon, usually 50 to 100 μm. The tubing was then squarely and evenly cut to insure minimal damage to the soft tissue. Glass electrodes modeled after those described by Josephson (1967) were used in experiments on isolated polyps. These electrodes have a bell-shaped tip in which the polyp can be held without damaging the tissues. Both kinds of electrodes can be attached by means of mild suction obtained by manipulation of a hypodermic syringe at the opposite end of the tubing or glass. Micromanipulators served to facilitate movement and placement of the electrodes. Both stimulation or recording could be achieved through these suction electrodes, and in either case, the indifferent electrode was a coil of chlorided silver wire placed in the experimental dish. Electrical stimuli were single or repetitive pulses delivered through a stimulus isolation unit. Conventional capacitor coupled amplifiers and display devices were used.

Results

General organization of the colony

Clava may be found at Woods Hole, Massachusetts in association with an intertidal brown alga, *Ascophyllum*. It is generally located at the branch points of the alga or within damaged flotation sacs, and only rarely along the lengthy exposed parts of the stem. Occasionally one can find *Clava* on rocks or wharf pilings. Colonies growing on *Ascophyllum* are comprised of upwards of 50 to 100 monomorphic polyps; the hydranth of each bears a naked hypostome and terminal

mouth, and some 20 to 30 filiform tentacles. The tentacles are uniformly distributed over the hypostomal region and do not form a distinct ring or rings. The lengthy stalk region below the hypostome is naked in immature polyps. Gonophores bud off from this stalk region 1 to 2 mm below the most proximal tentacles in mature polyps. The polyps vary in length, from about 0.5 to 1.5 cm, and are joined basally by a coenosarc of interconnecting stolons. The sexes are separate and a colony is comprised of all male or all female polyps, probably originating from a single planula. Occasionally, as reported by Föyn (1927), male and female colonies will occur together in the same clump.

General behavior and responses to mechanical stimuli

Spontaneous and rhythmically recurring behavior of individual polyps or groups of polyps does not occur under uniform conditions of illumination in *Clava*. Polyps may bend slowly or reorient individual tentacles, but the general observation is prevailing quiescence. Endogenous pacemaker systems like those responsible for the rhythmic behavior of *Tubularia* (Josephson, 1962; Josephson and Mackie, 1965) or *Hydra* (Passano and McCullough, 1962, 1965) do not appear to be present in *Clava*.

Mechanical stimulation of a polyp may induce weak or vigorous polyp activity depending on the intensity of the stimulus. Pinching an individual tentacle can result in contraction of that tentacle alone; contraction of the tentacle and movement of the hydranth towards the stimulated side; or contraction of the entire polyp. Contraction of the polyp may be graded, but in most cases, particularly colonies growing on *Ascophyllum*, it was not observed to spread to neighboring polyps. In rare cases, vigorous pinching of the hydranth resulted in a vigorous contraction of the polyp, and that contraction spread in a graded fashion to the neighboring polyps. Pinching a stolon also can elicit the same response; however, in no case were more than three polyps involved in this coordinated response.

Long-term electrical recordings from individual polyps show that electrical activity does not occur in *Clava* in the absence of behavioral events. Furthermore, many complex behaviors, particularly those associated with feeding, occur in the complete absence of detectable electrical events. Other complex responses induced by certain amino acids are associated with the generation of electrical potentials; however their role in feeding, if any, is not known.

Following mechanical stimulation of a *Clava* polyp, either single or multiple pulses can be recorded with a short latency (less than 400 msec) which correlate with the visual observation of overall polyp contraction. Weak pinching of any part of a polyp produces single electrical pulses with each contraction, whereas vigorous stimulation produces multiple electrical pulses and a more vigorous polyp contraction. These same results can be obtained by mechanical stimulation of the interconnecting stolons.

Electrical stimulation-correlated behavior and electrical activity

The electrical responses in this study have been recorded extracellularly from rather large blocks of tissue comprised of ectoderm, mesoglea and endoderm.

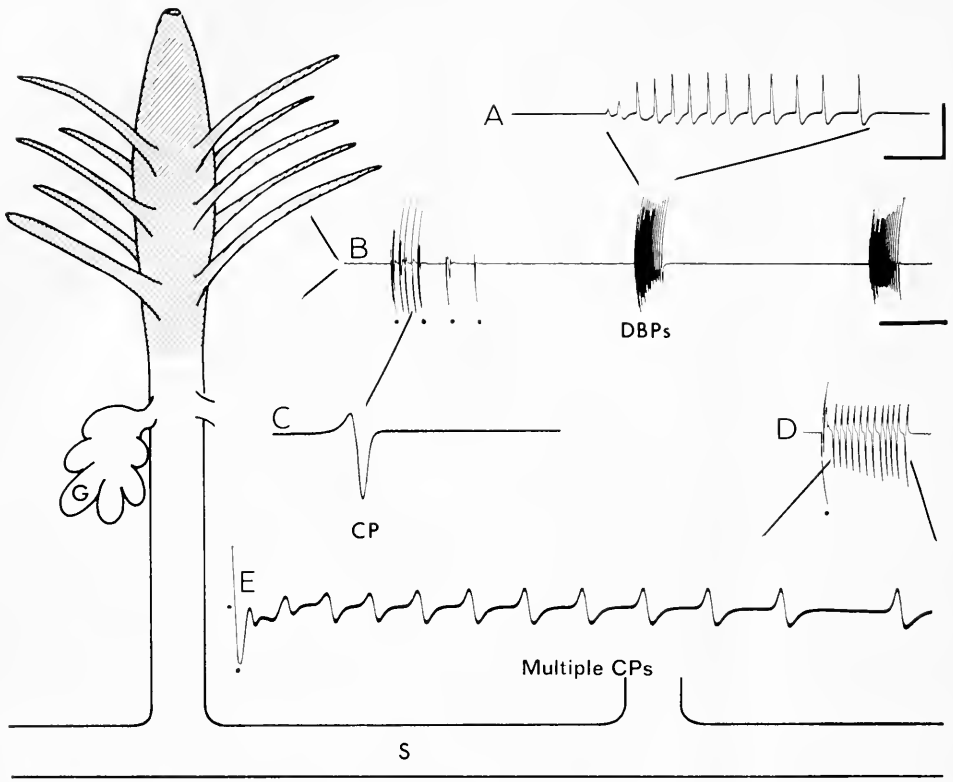

FIGURE 1. *Clava* polyp schematic showing representative kinds of evoked electrical pulses. A, delayed burst potentials similar to those shown in record B, but recorded at faster time base. Stimulus not indicated. B, a sequence of CPs and two bursts of DBPs evoked by four stimuli (dots beneath recording) applied to the burst generator region (hatched region). C, a typical CP evoked by stimulation of any part of the body column or tentacles. D, typical record of multiple CPs recorded from the polyp stalk. E, multiple CPs similar to those recorded in D, but at a faster time base. Dot indicates stimulus artifact. G, gonophores, S, stolon; Vertical scale A applies also to B, C, D, E, =0.5 mV; Horizontal Scale A applies also to C, E, =0.6, 0.2, 0.3 secs, respectively; Horizontal Scale B applies also to D =5.0 sec.

Positive identification of the underlying morphological source of electrical signals generated by these tissues is difficult and hence, for the moment, we cannot identify these events as neural, muscular, or epithelial. Instead the neutral term "conducting system" (Josephson and Mackie, 1965) will be used to define the substrates of these electrical events.

Two distinct kinds of electrical activity can be recorded from *Clava* depending on the stimulus site. Evidence presented here and in a subsequent report (Rushforth and Stokes, in preparation) indicates that these two events represent activity in separate conducting systems. One of these conducting systems is activated only by direct stimulation of the hydranth distal to the gonophore stalks (Fig. 1, hatched region). The electrical events (Figs. 1A, B) occur as a burst or a

TABLE I

Characteristics of electrically stimulated contraction pulses in Clava: *mean ± s.e.*

Pulse type	Latency (msec)	Interpulse interval (msec)	Pulse duration			Pulse amplitude mV	Conduction velocity cm/sec
			Rise time (msec)	+ve to −ve Peak (msec)	Total (msec)		
A. Single contraction pulses	331 ± 109 (3)*		74 ± 26 (2)	48 ± 11 (4)	144 ± 20 (4)	0.53 ± 0.03 (4)	2.6 ± 0.2 (11)
B. Multiple contraction pulse bursts	330 ± 70 (2)	371 ± 19 (9)	73 ± 6 (2)	50 ± 7 (9)	140 ± 8 (9)	0.61 ± 0.10 (9)	2.1 ± 0.3 (5)

* Values in parentheses = N.

program of multiple bursts of pulses after a long latency ($\bar{x} = 19.2 \pm 1.4$ sec, $N = 18$) and are referred to as Delayed Burst Potentials (DBPs). A burst of DBPs on an expanded time scale is shown in Figure 1A. In no case have DBPs been initiated by stimulation of stolons or the stalk region proximal to the gonophores. DBPs correlate with a synchronized depression of the tentacles and, at least to the first few bursts of a program, with a symmetrical shortening of the body stalk. The stalk contraction element is not apparent after the first few bursts of a program. Also, the pulses within a burst appear much more homogeneous following loss of an observable stalk contraction. The conducting system underlying these bursts of pulses, called the Delayed Burst System (DBS) is the subject of a subsequent report (Rushforth and Stokes, in preparation).

A second type of electrical event can be evoked by stimulation of the polyp at any location—tentacles, hypostome, body column or base. Characteristically, these potentials are single (Figs. 1B, C) or multiple pulses (Figs. 1D, E), which are similar in all respects to those evoked by mechanical stimulation. These potentials

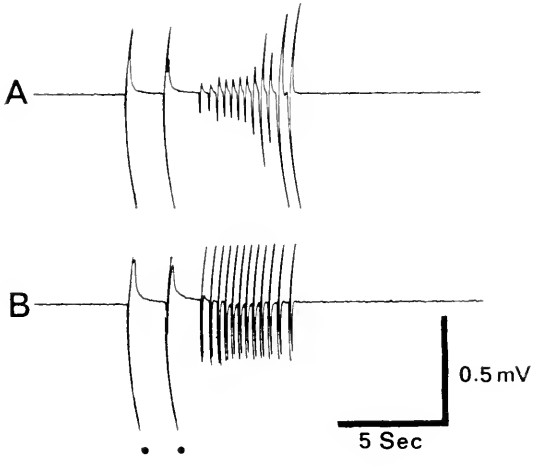

FIGURE 2. Facilitation of CP potentials within a "burst", recorded at two sites (A and B) on a single polyp stalk. Two stimuli were applied (dots) adjacent to site B.

are correlated with a symmetrical, often localized contraction of the polyp and are thus referred to as Contraction Pulses (CPs). The conducting system underlying the CPs is referred to as the Contraction Pulse System (CPS). The threshold for activation of the CPS is generally below that for the DBS. Both single and multiple CPs appear to originate within the tissues beneath the stimulating electrode and have a short latency (about 300 msec) in comparison to the DBPs. Characteristics of the electrical pulses associated with single and multiple CPs are presented in Table I. In summary, both single CP events and individual CPs of a multiple sequence are nearly identical with respect to pulse rise time (75 msec), positive to negative peak (50 msec), total pulse duration (140 msec) and pulse amplitude (0.5–0.6 mV). The refractory period for single CPs was determined to be about 200 to 250 msec, and the interpulse intervals for multiple CPs are on the average about 370 msec. As shown in Figure 1E, the interpulse intervals tend to elongate towards the end of a CP burst. CPs are generally biphasic, although triphasic CPs have also been recorded, usually following the first stimulus of a regime of stimuli given to a fully expanded polyp.

Both single and multiple CPs are nonpolarized conducted events (Table I). The conduction velocity was determined by recording from two Tygon suction electrodes attached to the same side of the poylp while stimulating through a glass holding electrode. From these records, the time delay between negative peaks of the electrical event recorded at the two sites and the measured distance between the recording electrodes were used to compute the conduction velocity. The mean conduction velocity for single CPs is 2.6 cm/sec ($N = 11$) and for multiple CPs 2.1 cm/sec ($N = 3$). For three animals, conduction velocities for single CPs were determined for both the proximal and distal directions. No significant differences were observed ($\bar{x} = 2.3 \pm 0.1$ distally and $\bar{x} = 2.2 \pm 0.3$ proximally). The mean conduction velocity for DBPs is 9.8 cm/sec ($N = 7$).

The responses of a single polyp to electrical stimulation appear to be graded with stimulus intensity. A single threshold shock to a tentacle may result only in contraction of the stimulated tentacle. A small CP is associated with the tentacle contraction. Similarly, threshold stimulation of the hydranth may result only in contraction of the hydranth region, with associated CPs, and in no apparent contraction of the stalk region. Responses of the polyp are more extensive and also more vigorous with increasing numbers and intensity of stimuli. The amplitude and number of recorded CPs decrement with distance from the stimulus site. Multiple CPs of large amplitude occur in regions of vigorous contraction, the number and amplitude diminishing in regions where contraction intensity is also reduced. During repetitive stimulation, contraction spreads to and involves a greater part of the polyp, an observation which appears to correlate with facilitation of CPs to each shock. Single threshold shocks generally give a localized polyp contraction and a single CP. Multiple shocks of threshold intensity or single suprathreshold stimulation of either proximal or distal regions of the polyp stalk can evoke multiple firing of the CP system. Some 30 or more CPs have been recorded to a single suprathreshold stimulus. Multiple firing of the CP system such as that shown in Figures 1D, E is correlated with a prolonged, continuous shortening of the polyp stalk, which often reduces the polyp to a stubby ball. In

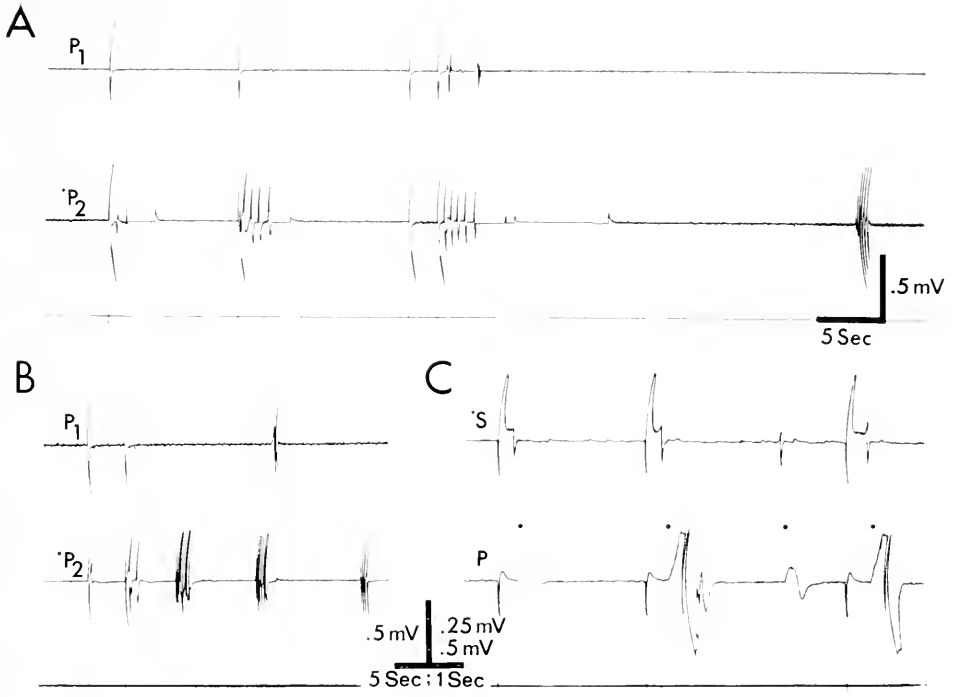

FIGURE 3. Interpolyp communication. A, simultaneous recordings from two interconnected polyps (P_1 and P_2) following application of four stimuli to one of them (asterisk). Largest events (stimulus artifacts) correlated to stimulus record (lower channel). Note that a burst (DBPs) occurs only in the stimulated polyp. B, same as A, but note that a CP occurs in P_1 shortly after a burst of DBPs in P_2. C, simultaneous recordings from a polyp (P) and an attached stolon (S) while stimulating the same stolon (asterisk). Note the small potentials in S correlate with repetitive CPs (dots) in P which facilitate to each stimulus (marked in lower trace).

this condition, the polyp is refractory to further stimulation, and it may take 30 min or more before the polyp fully expands once again.

Dual recordings from a single polyp during multiple CP firing (Fig. 2) also show more vigorous contraction adjacent to the stimulus site and uniformly large CPs (Fig. 2B), while at a distant recording site the contraction may not be apparent initially but becomes increasing more vigorous throughout a CP burst. The CPs in such a burst show a marked facilitation (Fig. 2A). As a wave of contraction passes a recording site, subsequent stimulation can result in a marked reduction and even a defacilitation of CPs within a burst.

Stimulation of the hydranth region of a polyp, as shown in Figure 1B, can evoke both single and multiple CPs in addition to DBPs. In this record, the first of four stimuli initiated some four CPs and the third stimulus a single CP of smaller amplitude. And finally, some 12 sec after the fourth stimulus, the DBS fired a burst of potentials.

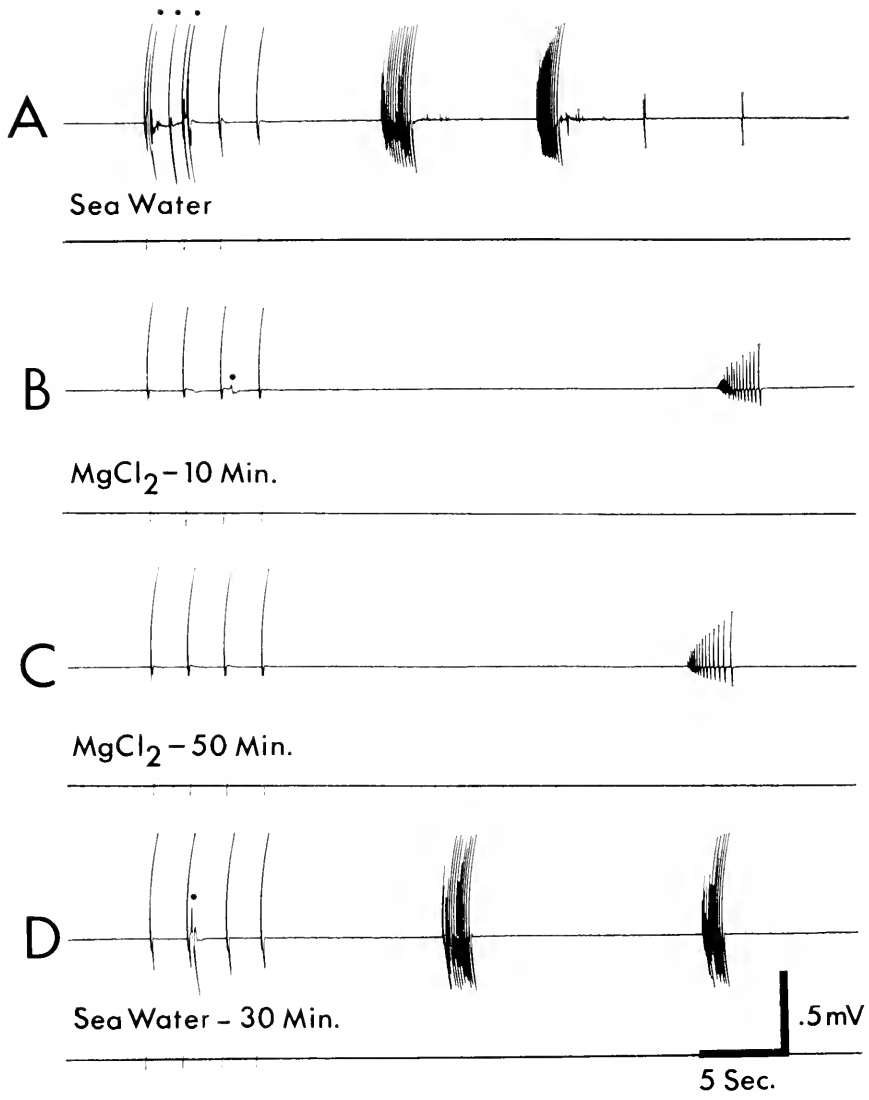

FIGURE 4. Effects of $MgCl_2$. A, recording from an isolated polyp in sea water. Stimuli in this and following records are marked in lower trace. B, same as A, after 10 min in $MgCl_2$. C, same as A, after 50 min in $MgCl_2$. D, same as C, following return of polyp to normal sea water for 30 min.

Interpolyp communication

Communication from polyp to polyp by means of the interconnecting network of stolons has been difficult to demonstrate in wild colonies. In such colonies, the polyps arise from a tangled mass of stolons which makes it difficult to determine

which of the polyps are directly connected. In addition there are numerous symbionts (crustaceans, platyhelminths, protozoans) which live in association with the tangled stolons, many of which have been observed to feed on the soft tissues of the colony. It seems likely that these symbionts disrupt the structural organization of the colony, leaving whole portions of the colony or even individuals functionally isolated from each other. In only a few colonies did mechanical stimulation of one polyp affect the behavior of adjacent polyps. These were young colonies with new growth and no apparent damage to the stolons joining the two polyps. In all cases, very intense mechanical stimulation was necessary to show spread of excitation to an adjoining polyp, and in all cases only two polyps were involved.

Slide culture colonies provide a better preparation for the study of interpolyp communication. The stolons grow out in rather straight formations and give rise to polyps at intervals of about 3 to 5 mm. One can maintain cultures free of symbionts and also easily observe the integrity of the stolon network. Recording from and stimulating stolons and polyps which arise from them is greatly facilitated in culture colonies, and consequently it is easier to demonstrate interpolyp communication. Three examples of such are shown in Figure 3. In record A, recording electrodes were placed on two polyps in such a culture colony. One polyp, P_2, was stimulated with four shocks in the region on the burst generator. CPs were recorded first in P_2 (the polyp stimulated), which eventually, after the fourth stimulus, were conducted through the stolon to P_1 where two small CPs were recorded. P_2 was observed to contract following the first stimulus, P_1 not until after the fourth stimulus. The DBS was activated in P_2 as can be seen by the delayed burst in this record, but the DBPs did not conduct through the stolons to the distant polyp (P_1). In Figure 3B, the polyp stimulated (P_2) gave rise to CPs and DBPs, the second burst of DBPs appears to activate the CP system of the distant polyp. While this is not conclusive evidence for an interaction between the DBS and CPS, it is supportive of additional evidence for such an interaction presented subsequently (Rushforth and Stokes, in preparation). There is no evidence that CPs initiate activity in the DBS.

In Figure 3C, recording electrodes were placed on a stolon (S) and a polyp (P) arising from that same stolon. The stolon was stimulated with three stimuli all of which initiated a small (150 μV), fast spike in the stolon which correlated with CPs in the distant polyp. These CPs facilitated to each stimulus. In one case the small spike fired independently of an electrical stimulus, and it too correlated with a CP in the polyp and contraction of the polyp. DBPs were never initiated by stimulation of the stolon. Repetitive stimulation of a stolon results in a graded spread of excitation involving only the CP system. Spread of excitation to some 8 to 10 interconnected polyps in a colony has been observed, polyps closer to the stimulus site showing more CP activity than those more distant.

Effects of $MgCl_2$

The effects of isosmotic $MgCl_2$ on the mechanical and electrical activity of attached individual polyps are shown in Figure 4. Electrical activity and the visually monitored behavioral responses were first studied in normal sea water and when typically consistent responses were observed, isosmotic $MgCl_2$ was

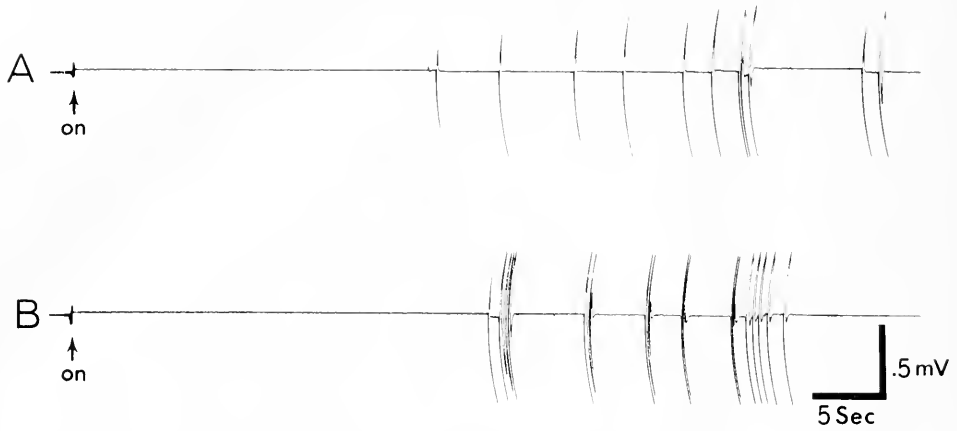

FIGURE 5. Light induced CP activity (A) and multiple CP activity (B) of *Clava* following a 15 min period of dark-adaptation. Onset of illumination is indicated by the arrows.

added to the experimental dish until a final concentration of 40% was obtained. The polyps were stimulated in the burst generator region with four shocks at 2-second intervals; the stimulus burst being repeated at 10-minute intervals. Only polyps giving both CPs and DBPs to two consecutive stimulus tests 10 minutes apart in normal sea water were used. A typical record in normal sea water is shown in Figure 4A. Both visual and electrical records were made following each stimulus regime. The results were similar for five polyps, one from each of five colonies.

Isosmotic $MgCl_2$ abolishes nearly all CP activity as well as the correlated behavioral responses after ten minutes exposure (Fig. 4B). The DBPs, however, persist in somewhat altered form and increased latency, despite the absence of a behavioral response. The bursts appear to consist of uniformly facilitating pulses of somewhat diminished amplitude from that in normal sea water. Delayed bursts have been recorded for up to 3 hours in $MgCl_2$ without further change. Both CPs and typical DBP bursts are restored following exposure to normal sea water for a brief period (10–30 min; Fig. 4D).

Effects of light

Whole colonies of *Clava* respond to sudden sharp increases in illumination after an apparent delay of some several seconds. Polyps contract symmetrically and often in distinct steps in much the same manner as occurs following CP activity. There does not appear to be a systematic co-ordination of polyp contraction throughout the colony; polyps nearest the source of illumination generally contract first, but there is no set order of responses. Synchronized tentacle depression characteristic of DBS activity has not been observed. The latency of the contraction responses coupled with the absence of tentacle depression led to a series of experiments designed to determine whether electrical events result in CP activity, DBS activity, or perhaps an as yet undescribed conducting system.

TABLE II

Responses of dark-adapted Clava *polyps to the onset of illumination. means ± s.e. (N = 5).*

	Number of pulses			Latency* to first pulse
	Before	During	After	
a) Intact polyp				
Midstalk	0.3 ± 0.2	10.9 ± 2.7	0.6 ± 0.3	35.4 ± 4.8
b) Intact polyp				
Hydranth	0.8 ± 0.8	13.5 ± 2.4	1.8 ± 1.8	23.4 ± 5.8
Prox. stalk	1.0 ± 0.7	12.1 ± 2.0	3.1 ± 2.7	24.9 ± 7.0
c) Transected polyp				
Hydranth	0.5 ± 0.5	6.3 ± 4.0	0.6 ± 0.6	10.2 ± 1.2
Prox. stalk	0.6 ± 0.2	11.8 ± 0.7	0.8 ± 0.4	30.4 ± 6.4

* Defined as the time interval in seconds from the onset of illumination to the intitation of electrical activity.

Recordings were made from single *Clava* polyps of a whole colony which had been dark-adapted for 15 min. The results of these experiments showed that polyps are quite sensitive to light, producing both single (Fig. 5A) and multiple (Fig. 5B) electrical pulses during the initial stages of stimulation. The numbers of pulses recorded from single (midstalk) and dual sites (hydranth and proximal stalk) were determined for a control period of 2 min before light stimulation, and a 2-min period following light stimulation. The light source for all experiments was a 6-V bulb from a microscope lamp set about 15 cm from the preparation. Light intensity was similar throughout. The experimental regime was repeated for a mimimum of five trials with a 15-min period of dark-adaptation between successive light exposures, for five polyps. The results for single recordings for all experiments are shown in Table II. The mean number of pulses recorded during the 2-minute light exposures for all experiments were on the order of 10 to 40 times greater than either before or after the light stimulation period. The mean latencies (defined as the time interval from the onset of illumination to the onset of the electrical response) varied from about 10 sec in the isolated hydranth preparation to 35 sec in stalk recordings. Although most of the electrical activity appears in the form of single or doublets of pulses, they are often produced in distinct groups. The mean number of pulses per group is significantly different for the hydranth and proximal stalk regions (5.1 ± 0.8 and 2.0 ± 0.1, respectively). An intermediate value of 3.4 ± 0.1 was obtained from midstalk recordings.

Electrical responses to the onset of illumination have been recorded also from isolated tentacles (three of five preparations) isolated hypostomes (three of five preparations) and hydranth preparations from which all tentacles and the hypostome were removed (six of six preparations). We have not examined isolated pieces of stolon.

Addition of $MgCl_2$ abolishes the characteristic responses to the onset of illumination within a period of five minutes. The only exception occurred in a single isolated hypostomal preparation which produced DBPs that persisted in somewhat modified form for three hr, with no observable behavioral correlate.

Discussion

Evidence from this study indicates that the CP system of *Clava* is another example of a fundamental muscle activating conducting system found in hydrozoan polyps. Electrical potentials from this system are correlated with symmetrical contractions of whole, or regions of whole, polyps, and are similar in pulse characteristics and conduction velocity to the electrical potentials correlated with contractions in other hydroids. Such pulses have the common features of long duration (up to 500 msec), relatively large amplitude (up to 15 mV), and slow conduction velocity (2–21 cm/sec) when recorded externally (Rushforth and Stokes, 1978).

Activation of CPs in *Clava* results always in some degree of symmetrical shortening of the polyp in the direction of the interconnecting network of stolons. The polyp may shorten in one or more steps depending upon the intensity and frequency of stimulation. Following successive contractions, the polyp is reduced to a stubby ball. Furthermore, when contracted, the polyps are shielded between the branches or within the damaged flotation sacs of the alga upon which they naturally occur. These responses are adaptive in that they provide a limited degree of protection for the exposed, softbodied polyp from potentially hazardous environmental stimuli. Similar protective functions of polyp contraction are apparent also in *Hydractinia* where the polyps ultimately contract below a layer of chitinous spines (Stokes, 1974b); in *Millepora*, where the polyp contracts into a calcified skeleton (deKruijf, 1976; and in *Obelia* where the polyp withdraws into a hydrotheca (Morin and Cooke, 1971a). Protective withdrawal, like the escape responses of insects and crustaceans with giant fiber systems, may be the major role of the CP system in *Clava*. Behavioral activities such as those associated with prey capture, feeding, and defecation are more complex and are probably integrated by other conducting systems within the polyp. Multiple conducting systems have been physiologically identified in all hydroid polyps thus far examined, though in no case is the behavior totally attributable to known conducting systems. *Clava* has at least two distinct conducting systems within the polyp. In addition to the CPS, a non-polarized Delayed Burst System (DBS) has been identified which produces programs consisting of bursts of pulses. Such programs are initiated after a long delay (about 20 sec) following stimulation. The DBS can be distinguished from the CPS by its somewhat higher threshold of activation; resistance to Mg^{2+}; restricted location within the hydranth of the polyp; and different behavioral correlates (tentacle depression vs. polyp contraction). The potentials produced by the DBS also have different conduction velocity and pulse characteristics. DBPs have shorter duration (50–60 msec), shorter interpulse intervals (170–230 msec), and faster conduction (8–12 cm/sec) than multiple contraction pulses (*cf.* Table I).

Though the functional significance of the DBS is not known, there is evidence to suggest that it interacts with the CPS. Tentacle depression together with polyp contraction is observed during the initial delayed bursts following DBS activation. These initial delayed bursts often appear to contain CPs interspersed with the DBPs (Stokes and Rushforth, personal observations). The CP elements in the delayed bursts are absent in the latter phase of a long program of bursts.

when the polyp is reduced to a stubby ball and contractions are no longer apparent. In this contracted state the CP system is not evoked by electrical stimulation. In addition, exposure of a polyp to Mg^{2+} eliminates contractions, and results in delayed bursts which appear to consist of a single pulse type, presumably DBPs. These observations suggest that the CP system is excited initially by the DBS and polyp contraction is caused in the early phase of a burst program. However, the system becomes increasingly refractory and CPs drop out in the final stages of the program.

Simultaneous recordings from two polyps connected by a stolon support the hypothesis that the DBS excites the CPS. Activation of the DBS by stimulation of the hydranth of one polyp can trigger CPs in the same polyp which then give rise to a CP in a neighboring second polyp. Very small potentials recorded in the muscle-free stolons interconnecting the two polyps correlate with the observed polyp contractions. These pulses may represent activity in nerve cells of an interconnecting nerve net. Nerve cells have been identified in the stolons of *Hydractinia* (Stokes, 1974a) but have not yet been looked for in *Clava* stolons. Single or multiple CPs of one polyp can also serve to initiate CPS activity in a second interconnected polyp. However, we have observed no case of CP triggering of DBPs. Interactions of the DBS and CPS in individual polyps and interactions of the CPS from polyp to polyp provide for a means of colonial co-ordination. Föyn (1927) was able to identify members of individual, interspersed colonies of *Clava* by pinching one polyp and observing which additional polyps contracted. Co-ordinated responses of polyps comprising a colony have been observed also in *Cordylophora* (Josephson, 1961b) and *Hydractinia* (Josephson, 1961a; Stokes, 1974b) where they are presumed to be protective and co-ordinated by conducting systems underlying muscle contraction. Clearly all members of the colony would be served by advanced notice of a predator attempting to feed on one member of the colony.

The electrical activity evoked by dark-adapted *Clava* polyps which occurs after the onset of illumination has certain features of both DBS and CPS activation. The recorded latencies following the onset of illumination to the initiation of electrical activity are usually quite long, sometimes on the order of 35 sec. The latencies of DBPs evoked by electrical stimulation are often equally as long. However, despite such long latencies, the following evidence suggests that light activates the CPS; removal of the burst generator region by transection of a polyp well below the hydranth does not affect the generation of pulses in the remaining proximal stalk region, clearly demonstrating that the burst generator region is not necessary for the light induced responses. Though patterns of light-induced potentials sometimes consist of programs of burst, such bursts are closer in pulse characteristics to multiple CPs than DBPs. Frequently the light response consists of sets of widely spaced single or double pulses; Mg^{2+} abolishes the light-induced activity and the behavioral responses in the same time course as CPs.

The long latency of the photic responses in *Clava* is similar to that recorded for *Hydractinia* (21–70 sec; Stokes, 1972). Such long latencies may result from a similar mechanism of pulse generation and spread of excitation. In fact, it is a contraction pulse system (the SCP) which is activated by light in *Hydractinia*. The latency of the response may reflect the levels of photosensitive pigments which

have accumulated during the period of dark adaptation (Ballard, 1942). In *Clava* we have preliminary data showing that there is an inverse relationship between the length of the dark adaptation period and the latency to the response. The light receptors, be they photosensitive pigments or some as yet unidentified photoreceptor, would appear to be widespread throughout a polyp. However, since more pulses are induced in the hypostomal region, than in the body column and base of the polyp, there may be relatively more pigment or more photoreceptors in this region. In *Hydractinia* electrical activity occurs only by direct photic stimulation of the basal mat (Stokes, 1972). We have not examined the light sensitivity of *Clava* stolons.

Very little is known of the morphological substrates of the CPS or DBS in *Clava*. Preliminary histological studies utilizing reduced methylene blue show the presence of nerve cells in the stalk region. The burst generator region and the stolons remain to be examined. However, electrical recordings suggest that CPs are not solely a result of neuronal activity. The electrical potentials are too large and of too long in duration to originate from the small nerve cells. Furthermore, they are conducted much more slowly than one would expect for purely neuronal pathways. It has been suggested that similar large potentials from other hydroids are propagated in epithelial sheets via low resistance junctional specializations (Josephson, 1967). Septate junctions have been found connecting epitheliomuscular cells of *Hydra* (Wood, 1959) and *Hydractinia* (Stokes, 1974a) where they have been implicated in contraction responses. Josephson and Macklin (1967) have shown that the CP of *Hydra* is a transepithelial event. On the other hand, as Mackie (1970) suggests, conduction of such large potentials may combine both neuronal and epithelial elements.

In this study we have shown that the colonial hydroid, *Clava squamata* possesses a Contraction Pulse System whose properties are similar to the CP systems of other hydroids. It provides further evidence that the CP system is a common conducting system in hydroid polyps. Studies with other hydrozoans should indicate whether it is a universal feature.

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Summary

1. At least two conducting systems are present in the colonial hydroid, *Clava squamata,* the contraction pulse system (CP system) which initiates symmetrical polyp contraction, and a delayed burst system (DBS) which is correlated with tentacle depression and polyp contraction.

2. The CP system has properties similar to contraction pulse systems of other hydroids; its electrical pulses are of large amplitude (greater than 0.5 mV) and long duration (150 msec), and slow conduction velocity (2–3 cm/sec).

3. The CP system courses through the polyps and their interconnecting stolons.

Electrical stimulation of a single polyp gives rise to CPs associated with contraction of that polyp, which sometimes can be recorded also in adjacent polyps.

4. Isosmotic $MgCl_2$ abolishes CPs and associated column contractions, but does not suppress delayed burst pulses.

5. Light initiates contractions of the polyp and correlated CPs.

6. It is postulated that the CP system of *Clava* is similar to contraction pulse systems previously described for other hydroids.

LITERATURE CITED

Ball, E. E., 1973. Electrical activity and behaviour in the solitary hydroid *Corymorpha palma*. I. Spontaneous activity in whole animals and in isolated parts. *Biol. Bull.*, **145**: 223–242.

Ball, E. E., and J. F. Case, 1973. Electrical activity and behaviour in the solitary hydroid, *Corymorpha palma*. II. Conducting systems. *Biol. Bull.* **145**: 243–264.

Ballard, W. W. 1942. The mechanism of synchronous spawning in *Hydractinia* and *Pennaria*. *Biol. Bull.*, **82**: 329–339.

deKruijf, H. A. M., 1976. Spontaneous electrical activity and colonial organization in the hydrocoral *Millepora* (Milleporina, Coelenterata). *Mar. Behav. Physiol.*, **4**: 137–159.

deKruijf, H. A. M., 1977. Bursting pacemaker activity in the solitary hydroid *Tubularia solitaria*. *J. Exp. Biol.*, **68**: 19–34.

Föyn, B., 1927. Studien Über Geschlecht und Geschlechtszellen bei Hydroiden. I. Ist *Clava squamata* (Müller) eine gonochoristische oder hermaphrodite Art? *Arch. Entwicklungsmech Org.* (Wilhelm Roux), **109**: 513–534.

Josephson, R. K., 1961a. Colonial responses of hydroid polyps. *J. Exp. Biol.*, **38**: 559–577.

Josephson, R. K., 1961b. Repetitive potentials following brief electrical stimuli in a hydroid. *J. Exp. Biol.*, **38**: 579–593.

Josephson, R. K., 1962. Spontaneous electrical activity in a hydroid polyp. *Comp. Biochem. Physiol.*, **5**: 45–58.

Josephson, R. K., 1965. Three parallel conducting systems in the stalk of a hydroid. *J. Exp. Biol.*, **42**: 139–152.

Josephson, R. K., 1967. Conduction and contraction in the column of hydra. *J. Exp. Biol.*, **47**: 179–190.

Josephson, R. K., 1974. Factors affecting muscle activation in the hydroid *Tubularia*. *Biol. Bull.*, **147**: 594–607.

Josephson, R. K., and G. O. Mackie, 1965. Multiple pacemakers and the behaviour of the hydroid *Tubularia*. *J. Exp. Biol.*, **43**: 293–332.

Josephson, R. K., and M. Macklin, 1967. Transepithelial potentials in hydra. *Science*, **156**: 1629.

Josephson, R. K., and M. Macklin, 1969. Electrical properties of the body wall of *Hydra*. *J. Gen. Physiol.*, **53**: 638–665.

Josephson, R. K., and N. B. Rushforth, 1973. The time course of pacemaker inhibition in the hydroid *Tubularia*. *J. Exp. Biol.*, **59**: 305–314.

Josephson, R. K., and J. Uhrich, 1969. Inhibition of pacemaker systems in the hydroid *Tubularia*. *J. Exp. Biol.*, **50**: 1–14.

Mackie, G. O., 1968. Electrical activity in the hydroid *Cordylophora*. *J. Exp. Biol.*, **4**: 387–400.

Mackie, G. O., 1970. Neuroid conduction and the evolution of conducting tissues. *Q. Rev. Biol.*, **45**: 319–332.

Morin, J. G., and I. M. Cook, 1971a. Behavioural physiology of the colonial hydroid *Obelia*. I. Spontaneous movements and correlated electrical activity. *J. Exp. Biol.*, **54**: 689–706.

Morin, J. G., and I. M. Cook, 1971b. Behavioural physiology of the colonial hydroid *Obelia*. II. Stimulus-initiated electrical activity and bio-luminescence. *J. Exp. Biol.*, **54**: 707–721.

Morin, J. G., and I. M. Cook, 1971c. Behavioural physiology of the colonial hydroid *Obelia*. III. Characteristics of the bioluminescent system. *J. Exp. Biol.*, **54**: 723–735.

Passano, L. M., and C. B. McCullough, 1962. The light response and the rhythmic potentials of *Hydra*. *Proc. Natl. Acad. Sci. U.S.A.*, **48**: 1376–1382.

Passano, L. M., and C. B. McCullough, 1963. Pacemaker hierarchies controlling the behaviour of *Hydra*. *Nature*, **199**: 1174–1175.

Passano, L. M., and C. B. McCullough, 1964. Coordinating systems of behaviour in *Hydra*. I. Pacemaker systems of the periodic contractions. *J. Exp. Biol.*, **41**: 643–664.

Passano, L. M., and C. B. McCullough, 1965. Coordinating systems and behaviour in *Hydra*. II. The rhythmic potential system. *J. Exp. Biol.*, **42**: 205–231.

Rushforth, N. B., 1966. An analysis of spontaneous contraction pulse patterns in *Hydra*. *Am. Zool.*, **6**: 524.

Rushforth, N. B., 1971. Behavioral and electrophysiological studies of *Hydra*. I. Analysis of contraction pulse patterns. *Biol. Bull.*, **140**: 255–273.

Rushforth, N. B.,, and D. S. Burke, 1971. Behavioral and electrophysiological studies of *Hydra*. II. Pacemaker activity of isolated tentacles. *Biol. Bull.*, **140**: 502–519.

Rushforth, N. B., and D. R. Stokes, 1978. Contraction pulse systems in hydroids. *Am. Zool.* **18**: 605.

Spencer, A. M., 1974. Behaviour and electrical activity in the hydrozoan *Proboscidactyla flavicirrata* (Brandt). I. The hydroid colony. *Biol. Bull.*, **146**: 100–115.

Stokes, D. R., 1972. Functional organization of conducting systems in the colonial hydroid *Hydractinia echinata* Fleming. *PhD thesis, University of Hawaii*, Honolulu, Hawaii, 319 pp.

Stokes, D. R., 1974a. Morphological substrates of conduction in the colonial hydroid, *Hydractinia echinata*. I. An ectodermal nerve-net. *J. Exp. Zool.*, **190**: 19–46.

Stokes, D. R., 1974b. Physiological studies of conducting systems in the colonial hydroid, *Hydractinia echinata*. I. Polyp specialization. *J. Exp. Zool.*, **190**: 1–18.

Wood, R. L., 1959. Intercellular attachment in the epithelium of *Hydra* as revealed by electron microscopy. *J. Biophys. Biochem. Cytol.*, **6**: 343–352.

Reference: *Biol. Bull.* **157**: 205–220. (August 1979)

AN ANALYSIS OF POPULATION STRUCTURE IN PACIFIC MOLE CRABS (*HIPPA PACIFICA* DANA)

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Hippa pacifica Dana, a hippid mole crab, inhabits the intertidal zone of tropical and subtropical Pacific island beaches. At Oahu, Hawaii, it primarily scavenges *Physalia*, the Portuguese man-of-war which washes onto beaches from the open ocean (Matthews, 1955; Wenner, 1977). At Enewetak Atoll, Marshall Islands, although it sometimes receives an abundance of *Physalia* (S. Smith, personal communication), the mole crab apparently depends more upon lagoon-produced mysids and other zooplankton which wash ashore at night (Wenner, 1977).

A comparison of population samples in this species, both in Hawaii and at Enewetak, provided some unexpected observations. In Hawaii, samples of *Hippa pacifica* suggested a remarkably consistent population structure. During a 5-month period, samples were essentially identical to one another, whether obtained from different beaches or from different parts of the same beach on Oahu. However, the consistent results obtained in Hawaii did not hold for extensive sampling at Enewetak Atoll. Samples from populations on different beaches at that atoll showed wide differences when sex ratio was analyzed as a function of crab size.

Wenner (1972) earlier had examined the question of sex ratio and size for many marine crustaceans. At that time he conjectured that the several distinct patterns found might be species-specific, on the basis of data available. However, this hypothesis failed with the initial comparison of data from Hawaiian and Enewetak mole crab samples. Hawaiian population samples fell into what had been termed an "intermediate" pattern, whereas the first year's samples at Enewetak formed the "reversal" pattern found in protandrous species such as the pandalid shrimps studied by Butler (see Wenner, 1972; Fig. 8).

The second year of sampling at Enewetak yielded conflciting data among various islets and led to a reconsideration of the premise that the sampling method provided representative data, even though this discrepancy among samples was not initially obviously related to sampling method (the same procedure had been used in all cases). To test the efficacy of sampling technique, a beach of limited extent was sampled at Enewetak in the usual manner, except that animals were not returned as had been done earlier. Instead, crabs were removed during a 3-week period until few or no crabs came to the individual bait stakes (sampling with removal). Initial samples, which represented the normal sampling procedure, could then be compared with the larger segment of population removed from the beach. The question became: In what ways did the initial samples represent the larger beach population?

Materials and Methods

The study site at Enewetak Islet was a 250-m long beach which terminated on its eastern end at a solid-walled cargo pier and tapered in the other direction to

a narrow strip of sand among concrete blocks and limestone rubble. Much of the western half of the beach fronted a limestone reef flat. At low tides the sand on that portion of the beach bordered on exposed coral reef.

During a 3-week period in 18 separate sessions (1–2 hr each), a total of 4011 animals was removed from the beach with the use of a baiting procedure described fully by Wenner (1977). Stakes baited with shark meat and placed approximately 10 m apart along the entire stretch of beach during each sampling session attracted the crabs. Thirty to forty stakes were kept in the sand for approximately 20 min and were reset two or three times more during each session.

Mole crabs, apparently reacting to chemical stimuli (Matthews, 1955), scurried toward the bait as it was repeatedly covered by wave action. Successive sets of bait during each sampling session usually yielded progressively fewer animals per bait stake. By the end of each session, even fresh bait placed between the sampling stakes did not generate an increase in catch rate.

Animals were usually collected during the mid-point of an outgoing tide, because that appeared to be the optimal time (on the basis of earlier experience). They were occasionally collected during other parts of the tidal cycle in order to sample sand patches covered by water only at such times. Such attempts, however, never yielded as many animals per unit effort as did those run during outgoing tides. Accordingly, in some comparisons samples were grouped by twos in order to offset those small numbers and to better reveal trends.

After animals were sieved from the sand, they were measured with the aid of an automatic sizing device (Wenner, Fusaro, and Oaten, 1974). Tallies included size, sex, and percentage of females carrying eggs within each size class. Females were returned alive to beaches at the opposite end of the islet. Males were kept in a sea water table and eventually returned alive to the original beach as part of another experiment.

Results

Changes in catch during removal

Animals, when considered by sex and size, did not come uniformly to the bait during the 3-week period. The overall catch of males fluctuated until mid-way through the program and then began tapering off (Fig. 1A). By contrast, female catch was greatest at first, with a rather consistent decline thereafter (Fig. 1B). Small females (those equivalent in size to the male size range—solid bars in Fig. 1B) showed much the same catch pattern as males, however, indicating that size rather than sex was the factor responsible for the differences in catch pattern between males and females.

One prominent feature of the change which occurred during the removal program was the selective catch of large females during the early part of that period (Figs. 1B, C). More than 80% of the 378 females caught in the first two samples were greater than 12.4-mm carapace length (the mean minimum size of egg-carrying females). In the last six samples, however, fewer than 18% of the females caught were at least that large ($N = 319$).

The percentage of those females larger than 12.4-mm carapace length which carried eggs also varied at the removal beach during the 3-week period (Fig. 1D).

In the first two samples combined, 64.2% of the 307 larger females carried eggs, whereas 85.0% of the remaining number of larger females had eggs ($N = 860$). A 9 by 2 χ^2 test for homogeneity failed ($\chi^2 = 77.00$, $P < 0.001$), indicating that the percentage of egg-carrying females changed significantly during the 3-week period. Since egg development time is approximately 20 days (unpublished results), such a change with time would not be unexpected.

Results from the other two islets which had been repeatedly sampled yielded somewhat more consistent data. At Jedrol (David) Islet, where the removal program was started later and run only 10 days, the percentages of large females in berry for the five samples were 87.2, 85.7, 86.0, and 83.6%, respectively, with data for the last two samples combined to eliminate small sample error ($N = 376$, 434, 150, and 116, respectively). Those percentages did not show a significant change with time ($\chi^2 = 1.05$, $P < 0.05$). At Boken Islet, two samples taken 6 days apart had 89.3 and 84.2% of the larger females in berry ($N = 337$ and 505, respectively), a marginal significant difference ($\chi^2 = 4.53$, $P < 0.05$).

Sex ratio fluctuated more markedly than any other variable measured during the removal program. The first three of the 18 samples yielded a total of 923 animals, with each of those three having a low percentage of males (27.7, 28.2, and 30.1%, respectively). In later samples the percentage of males varied widely, from a low of 40.6% males (fifth sample) to a high of 78.8% males (last sample). However, a persistent upward trend in that percentage became evident when the percentage of males was considered as a function of cumulative number of animals caught (Fig. 1E). Beginning with the fourth sample, the overall percentage of males increased uniformly. After 4011 animals had been collected, 48.4% of them were males. When those data were grouped into four blocks of 923, 1127, 896, and 1065 animals, a 4 by 2 χ^2 test yielded a value of 234.36 ($P < 0.001$). When the same test was run with the first group omitted, the data remained heterogeneous ($\chi^2 = 113.00$, $P < 0.001$). This level of significance, coupled with the close fit of the line to the points, indicates that males were under-represented in the first group of three samples and over-abundant in the last group of 1065 animals (last seven samples combined).

It was after the trend shown in Figure 1E began to emerge that a total of 1679 animals was removed from the beach on Jedrol (Rex) Islet, where a parallel set of results emerged (Fig. 1F). Unfortunately, time did not permit additional sampling at that islet.

Two samples from Boken (Irwin) Islet were also obtained. The male percentage there rose from 1.7 to 6.4% during removal of a total of 346 and 456 animals, respectively. Boken Islet differs from the other two in being downwind from the lagoon waters during trade wind conditions and is, presumably, in a more food-rich location (Fusaro, 1978b; Wenner, 1977).

Estimates of population size

Since catch per unit effort generally declined during removal trapping, and since rate of decline is directly related to size of total population and to number removed, the total population size can be estimated by various methods (Southwood, 1966, pp. 181–186). However, not all of the four conditions as listed by

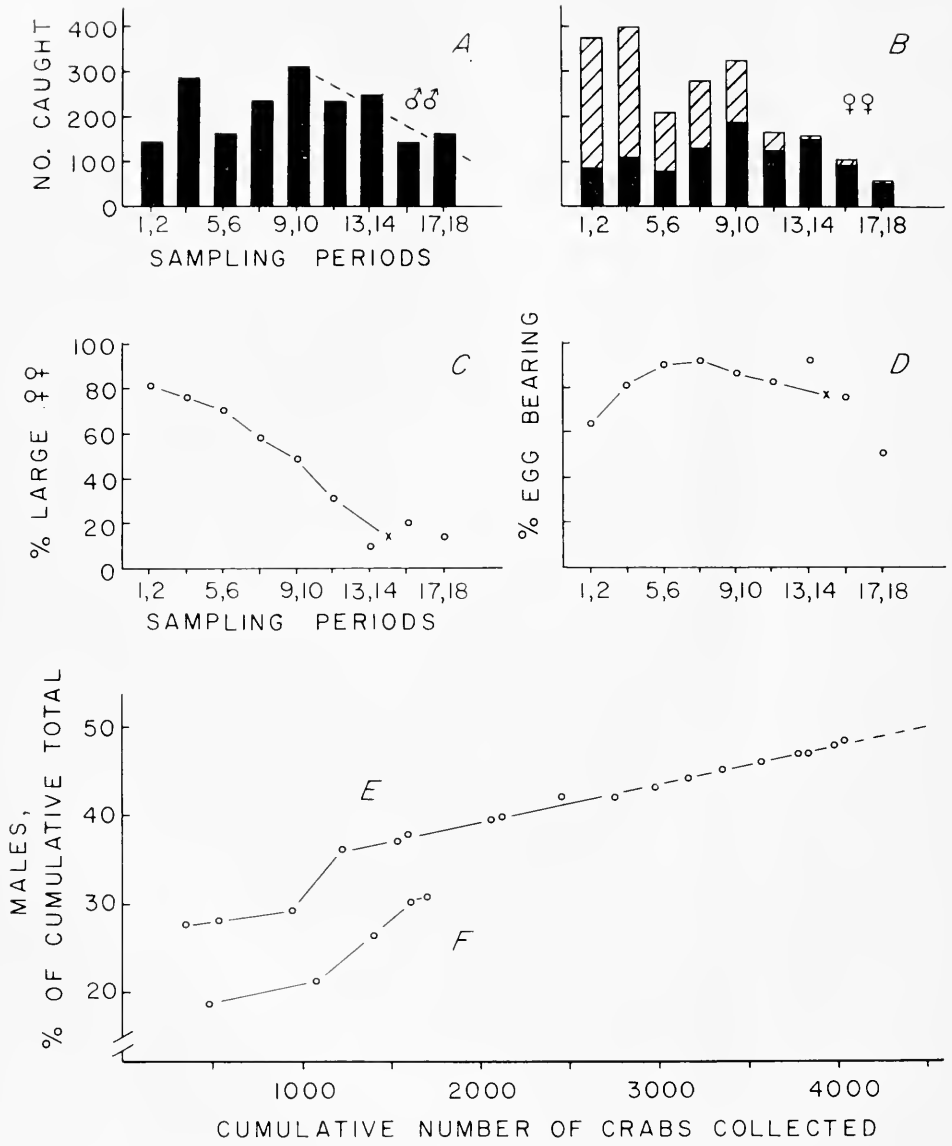

FIGURE 1. Changes in population samples during the three-week removal program. The bars depict sequential male (A) and female (B) catches, with the 18 samples grouped in pairs. Hours spent collecting each pair of samples were: 2, 2, 2, 2.25, 4, 2.5, 2.5, 3 and 3, respectively. The dashed line in Figure 1A indicates a consistent decline in catch per unit effort starting midway through the removal program; the solid portion of each bar in Figure 1B represents small females (females $\leq$12.5-mm carapace length, the mean maximum size of males). The line in Figure 1C shows the decline in percentage of females which were large (females $\geq$12.4-mm carapace length, the mean minimum size at onset of egg-bearing), while Figure 1D illustrates the differences obtained in egg-bearing percentages for those larger females during

TABLE I

Various estimates of the number of animals inhabiting the study beach on Enewetak Islet, determined by different methods based upon changes in catch pattern during removal sampling.

	Estimates of total population size			
	males	Total females	Small females	Total animals
Method				
Max. likelihood (±s.e.)	9575 (±2996)	2374 (±35)	6091 (±6182)	6279 (±208)
Regression	8661	2532	4859	6539
"Corrected Regression"	2661	2389	1271	4658
Time-Unit	2967	2223	1518	4752
1:1 Sex ratio	2250	2250		4500
Total caught	1940	2071	1008	4011

Southwood (1966) were met in this present study. One condition in particular, "The chance of being caught must be equal for all animals" was clearly violated; males and small females were not caught as readily in the first eight sessions as were large females (first four bars in Figs. 1A, B). Nevertheless, a use of the various methods did provide a set of estimates (Table I), from which inferences can be drawn. In each case, estimates were made independently from the data for males, females, small females, and total number of animals, on the basis that the catch patterns differed markedly from one another in those different categories.

The method based on maximum likelihood, which Southwood (1966) called "the most accurate method", yielded the least consistent set of results. The male estimate (9575 ± 2995) exceeded the estimate for total number of animals (6279 ± 208). Likewise, the calculated number for small females exceeded the number estimated for all females.

Use of the regression method initially provided the same lack of consistency as that yelded by the maximum likelihood method; estimates for the number of males and small females were far too high to correspond with the estimates for total numbers of all animals or of all females. By inspection of Figures 1A and 1B, on the other hand, such a result would be expected, since males and small females clearly began a consistent decline in numbers only after the first eight samples (first four bars in those figures) had been removed from the beach.

Alternatively, one can adapt the regression method by applying it to the data

sequential removal. In both Figures 1C and 1D a weighted mean ($\bar{x}$) for the last three paired points compensates for small sample variation. The line in Figure 1E represents the percentage of males as a function of cumulative number of animals caught in the 18 samples at the main removal beach, while the line in Figure 1F shows the same relationship for animals taken from Jedrol Islet.

for the last 10 samples (last five bars). The estimates derived in each case can be added to the numbers caught in the first eight samples. Serious inconsistencies then disappear ("corrected regression" in Table I).

The "time-unit" method for estimating population size (Kono, in Southwood, 1966) relies heavily on data obtained at only three times (as well as on the cumulative catch at those times): the first sample, the mid-point sample, and the last sample. An application of that method to the data yielded estimates relatively close to those produced by the "corrected regression" method, with males and small females again being somewhat over-estimated because of their under-representation in the first sample.

Finally, if one assumes a 1 : 1 sex ratio for the population (the megalopa stage of *Emerita analoga* arrives on the beach in a 1 : 1 sex ratio, Wenner, 1972), an extrapolation of the line in Figure 1E to the 50% mark would yield an estimate of about 4500 animals. The 2250 females so estimated (assuming a 1 : 1 sex ratio) closely matches each of the other estimates for the total number of females.

The various estimates shown in Table I, qualified by the nature of the violation of conditions outlined by Southwood (1966), would indicate that most of the animals were removed from the study beach (between 87% and 93%, particularly if one relies on the quite consistent set of results for the total number of females).

The foregoing analysis now permits an assessment of how well the initial samples represented the larger beach population.

Modal size classes

The data for all samples combined fell into discrete modal size classes (Fig. 2), when separated by the method outlined by Cassie (1954). Data for males separated cleanly into only three modes, with the third mode having a slight inflection above the 95% level. The steep slope of Mode 3 suggests a maximum size for that sex. Female data actually fell into five modes. However, since the third and fourth modes did not differ appreciably, those data were combined and were thereafter treated as Mode 3 (Figs. 2, 3). The data thus reveal that the beach likely experienced four or five periods of recruitment during the year or two prior to sampling. (It should be noted, however, that the mode of largest females is based upon only seven animals caught during the first three sampling periods.)

To illustrate some of the differences in modal size class structure which arose during the removal process, data from the first three samples ($N = 923$) are herein compared to data obtained from the last six samples ($N = 876$). In all cases, modes were again separated by the same method.

Females in the first three samples ($N = 657$) fell primarily into the four or five modes mentioned earlier (Fig. 3A), but females in the last three samples ($N = 319$) provided data for only three distinct modes (Fig. 3B). The large displacement in the two sets of lines in Figure 3 does not represent an appreciable change in the size of animals within each mode. Rather, the displacement reflects a change in relative *percentage* of females which fell into each of the three modal size classes when one compares the beginning and end of the 3-week sampling period. For example, as can be seen in Figure 3, most females in the first three samples were in the third mode (79% of 657 females), whereas the second mode contained

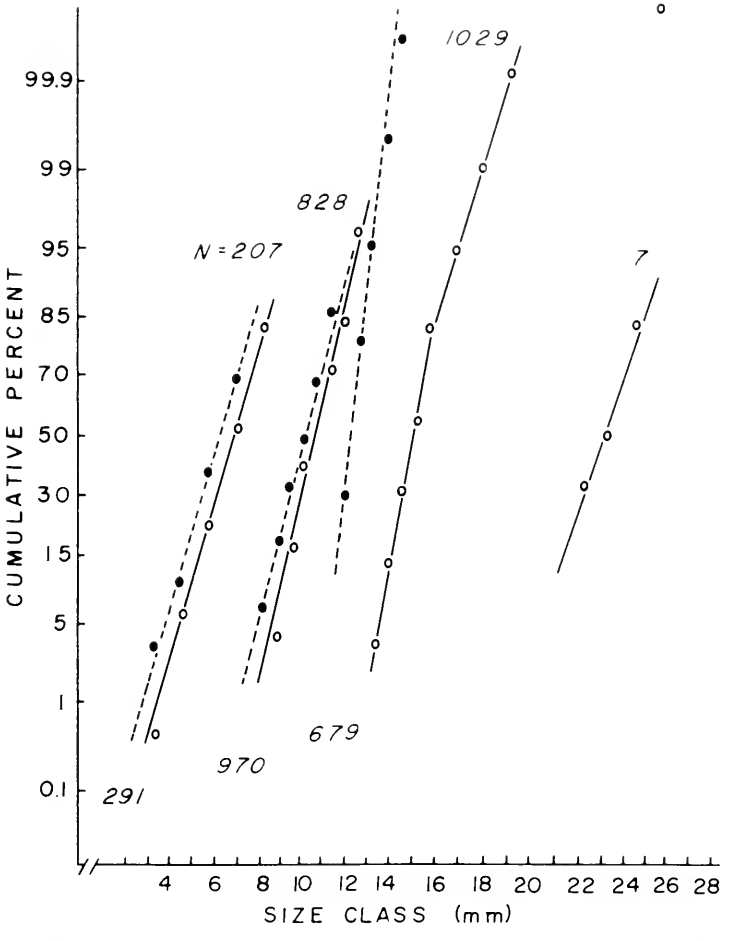

FIGURE 2. Male and female modal size classes for all data (N = 4011). Males (broken lines) fell into three distinct size classes when modes were separated by the method of Cassie (1954). Females (solid lines) continued growth beyond the maximum male size. Approximate numbers within each mode are indicated below each line representaing male modes and above each line representing female modes.

most of the females in the last six samples (72% of 319 females). Thus, two facets of modal size class structure should be distinguished: first, the estimated mean size of animals within each mode and second, the relative percentage of animals within each mode.

The data derived from the captured males (Fig. 4) differed from that for females in some important respects. Only two male modes occurred in the first three samples combined (Fig. 4A), while four or five females modes were evident in those combined samples (Fig. 3A). The smallest and largest females had no counterpart among the males in the first three samples. However, the last six

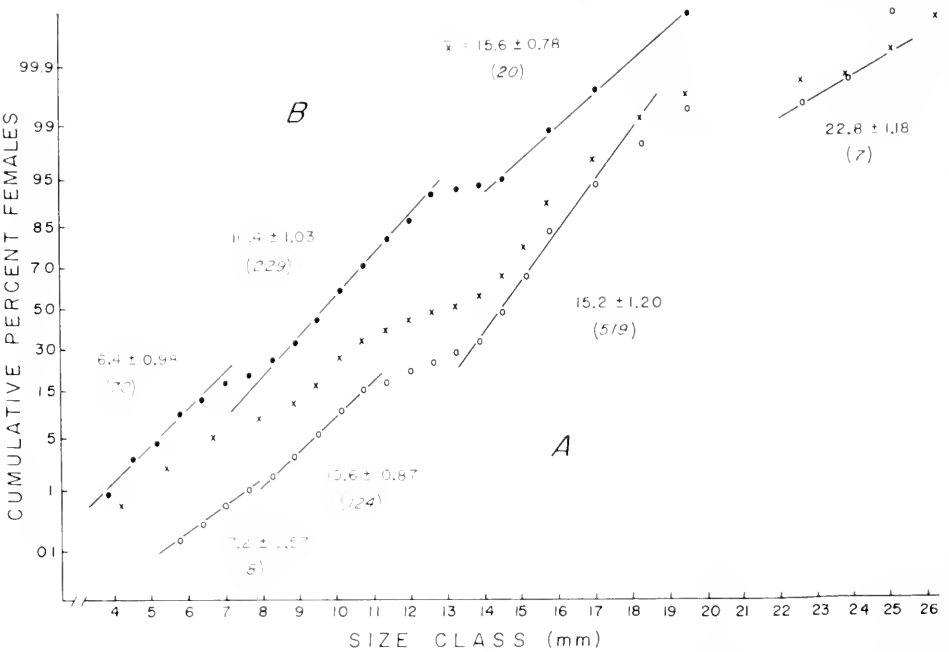

FIGURE 3. Changes in cumulative percentage of females as a function of size during removal. Data for the first three samples combined (open circles) fell into at least four modes (A). Data from the last six samples (closed circles) formed only three discrete modes, displaced upward on the graph due to a later catch of a higher percentage of smaller females (B). The *x* symbols indicate the relationship for all female size data (N = 2071). The sets of numbers below the lines indicate means, standard deviations, and approximate number (in parentheses) of animals within each mode in Figure 3A, and the numbers above the lines represent the same characteristics for Figure 3B. (See Fig. 2 for separation of modes for the total female data.)

samples yielded three modes for the males (Fig. 4B), modes which could be matched quite readily with the three modes representing smaller females in Figures 2 and 3B.

On the other hand, data for males obtained in the first three and in the last six samples (Fig. 4) did not show that same parallel relationship found in the female data (Fig. 3). The lines representing the upper mode for males in first and last samples converged. S. R. Haley (personal communication) and M. Page (personal communication) have concluded from laboratory results that males reach a maximum size and cease growth, though they continue molting. Females in the laboratory, according to Haley and Page, showed no such cessation of growth under similar circumstances.

The relative percentage of males which fell into each mode also differed markedly in the first and last samples (Fig. 4). The upper (third) mode in the first three samples contained approximately 75% of the 266 males, while 61% of the 557 males caught in the last six samples were in the second mode.

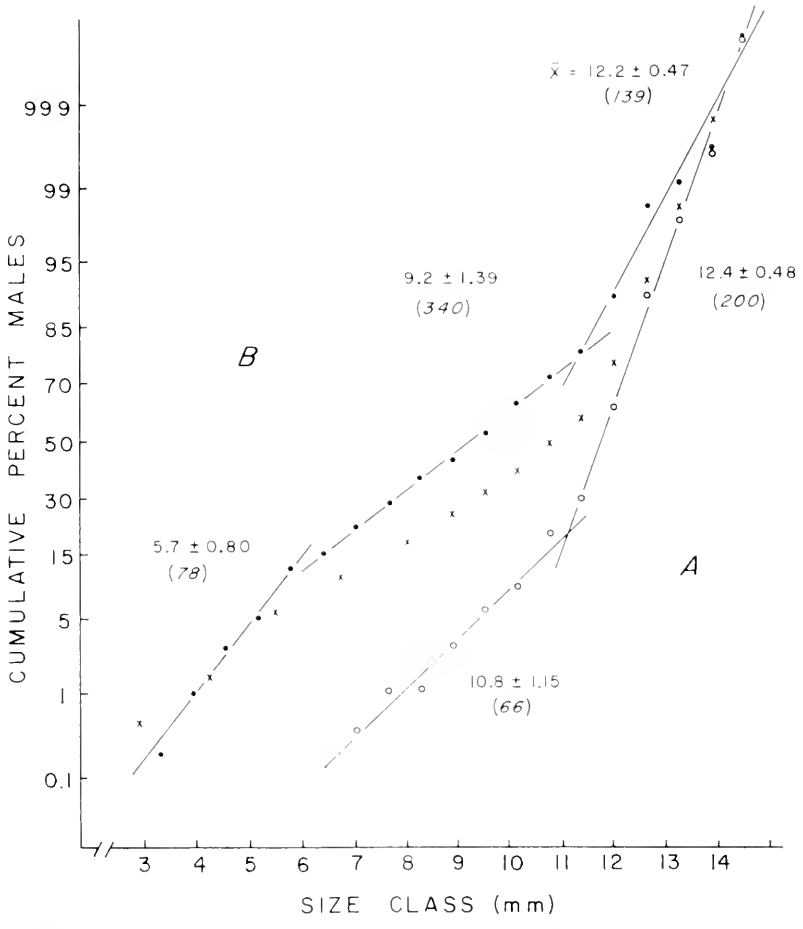

FIGURE 4. Changes which occurred in cumulative percentages of males, as in Figure 3 for females. Initial samples (A) yielded only two modes, as against the four to five modes for females. Final samples (B), however, resulted in three discrete modes for males. The convergence of the two lines representing the largest males agrees well with the concept of of a maximum size reached by that sex. Means, standard deviations, and sample sizes are as shown in Figure 3.

Sex ratio curves

An earlier analysis (Wenner, 1972) revealed that sex ratio may vary with size in marine Crustacea. At the time, such variation was believed to form a pattern characteristic of a species or population. It is clear from more recent results obtained from mole crabs both in Hawaii and Enewetak that the "characteristic pattern" hypothesis is now untenable. The data published in 1972 indicated an "intermediate" sex ratio pattern for *Hippa pacifica* in Hawaii, but data gathered later at Enewetak in 1972 yielded a "reversal" sex ratio pattern for the same species (see Fig. 5A).

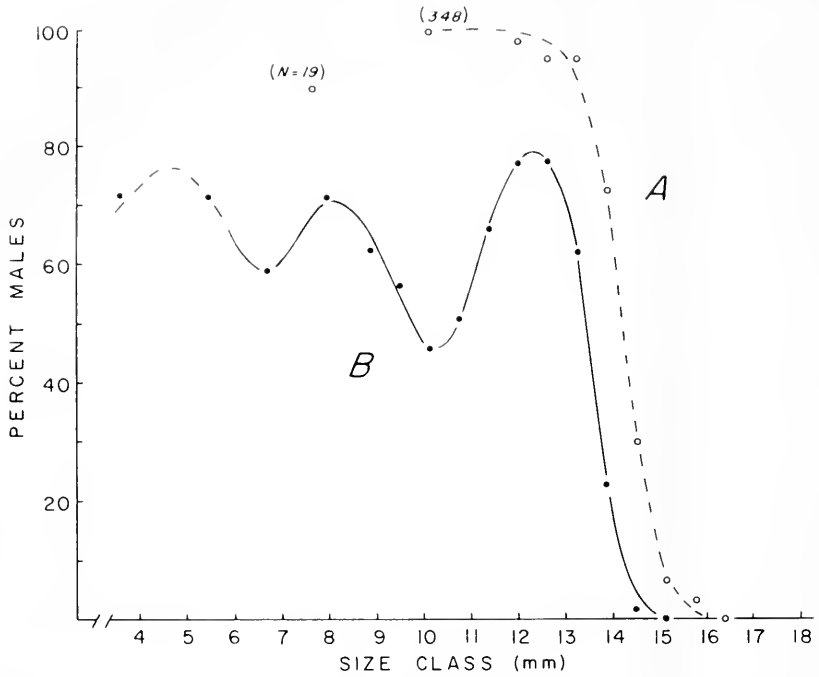

FIGURE 5. A discrepancy between sex ratio curves (sex ratio as a function of size—Wenner, 1972). A sigmoid curve ("reversal pattern") was obtained in 1972 when approximately 2000 animals were collected from six beaches on five different islets (A). A new pattern ("oscillation pattern") emerged when 4011 animals were removed from a single beach in the present study (B). Out of several hundred small animals in 1972, only one was female (the first point in Fig. 1A).

Data for initial samples in the current study yielded neither the Hawaii nor the earlier Enewetak sex ratio patterns; a sex ratio pattern existed which had not been found earlier (Fig. 5B). Instead, the percentage of males oscillated with increase in crab size. It is further evident that the number of oscillations corresponded well with the basic number of modal size classes found for males and females in the comparable size ranges (*i.e.*, Fig. 2).

The "oscillation" sex ratio pattern is what one might expect if the following conditions apply: first, the population consists of different cohorts which have arrived at different times from the plankton (three recent cohorts in this case), second, one sex grows faster than the other (females in this case—see Haley, 1979), and third, one sex reaches a maximum size, while the other sex continues growth beyond that size (females in this case).

For the first three samples of the present study, the sex ratio curve formed by the data for all animals greater than 10-mm carapace length nearly exactly matched that same portion of the curve for the total number of animals collected, as seen in Figure 5B. For the 86 animals in the first three samples which were 10 mm and smaller, however, only 28% were males, a point which falls far below

any of the points on the curve for all animals. This result corresponds with the under-representation of males as seen in Figure 4.

Despite the large discrepancy between sex ratio curves at the two localities and in different years, it should be noted that the sigmoid portions (for animals larger than 12 mm carapace length) of the curves in Figures 5A and 5B differ from one another by only about $\frac{3}{4}$-mm carapace length at the 50% level in the graph. The mean maximum size of males (12.5-mm carapace length) also falls close to that same level. It is also noteworthy that an analysis of *Hippa cubensis* data (from Hanson, 1969) places that portion of the curve for his species between the two curves shown in Figure 5.

Size at onset of egg production

The mean minimum size of egg production can be a useful measure in population studies if one wishes to compare the success of animals which live in one habitat with the success of animals which live elsewhere (Wenner, Fusaro, and Oaten, 1974). It is essential, however, that initial sample data accurately represent the entire population before one makes that comparison. (In decapods the mean minimum size at which females can extrude eggs is one convenient measure of sexual maturity, provided conditions are optimum for egg production, since eggs are usually retained on the pleopods until they hatch.)

Unfortunately, samples of *Hippa pacifica* populations in both Hawaii and at Enewetak taken in earlier years did not permit a determination of the mean minimum size at which egg-bearing occurred because small females were seldom caught. The previous sections document one possible reason for such a failure—samples taken early in the sampling period did not include an accurate representation of small females present in that population.

The removal of a larger percentage of the population in the present study produced a sizeable number of smaller females and permitted the derivation of a curve which represented the mean minimum size of egg production for this particular beach ($\bar{x} = 12.4 \pm 1.02$ mm; N = *ca.* 1000). The curve had a striking similarity to the shape of a comparable curve published earlier for *Emerita analoga* (Wenner, Fusaro, and Oaten, 1974).

A question remained as to whether initial samples would indicate a different size at onset of egg production than would data for the population as a whole. Consequently, data for the first three samples combined were compared to the data for the total 3-week catch. Although the available sample size for the early data was quite small (and although more scatter existed among the points), it was evident that a marked difference did not exist between early samples ($\bar{x} = 12.7 \pm 4.64$ mm; N = *ca.* 150) and total data in this comparison. Only 0.3-mm carapace length difference existed between means for best-fit lines from all data compared to data for only the first three samples.

Discussion

Pacific mole crabs (*Hippa pacifica*) apparently live only in the intertidal zone (Wenner, 1977); the same habit was reported for *Hippa cubensis* by Hanson

(1969). (However, see Borradaile, 1906.) This restricted habitat, together with a behavior of readily coming to bait, makes these animals particularly suitable for studies of crustacean biology. Large numbers of these carnivores can be collected in a relatively short time. In addition, the animals can be measured quite rapidly while still alive by means of a graded sieve (Wenner, Fusaro, and Oaten, 1974). In the present study, these combined attributes permitted measurement and removal of a large percentage of Pacific mole crabs from a beach limited in length. From population estimates (Table I), based on changes which occurred during sampling (Southwood, 1966), it would appear that approximately 90% of the animals which inhabited that short stretch of beach on Enewetak Islet, Enewetak Atoll had been removed.

Some of the contrasting results between first and last samples were unexpected, in the sense that earlier (1970–71) samples had yielded repeatable data. At that time samples from different beaches or islets were very similar, and repeated samples (sampling with replacement) from the same beach matched one another. In retrospect, one might conclude that the sampling bias could have been anticipated, since animals were caught by "trapping" (*e.g.*, Gilbert, Gutierrez, Frazer, and Jones, 1976).

Trawling for animals can apparently lead to problems similar to those posed by trapping with bait. Gotshall (1972), while sampling shrimp during a 4-year period, experienced a bias problem similar to that encountered in the present study.

Small animals were caught less frequently at first and were primarily males; this discrepancy led to an imbalance in sex ratios throughout the sampling program. Hanson (1969; p. 15) earlier found such a discrepancy in his sampling program with *Hippa cubensis*. He wrote: "Length-frequency distributions of the (trapped animal) samples showed close conformation to those obtained by the more exact procedure of sieving sand samples from different levels of the beach, except for the smallest crabs (4–8-mm carapace length). Only 17.8% of the Bellair (trapped) samples were in this size range while 39.2% of the crabs from the Paynes Bay sieved samples were in this range." By contrast, Hanson derived an overall figure of 48.3% males for the animals from the several Paynes Bay beaches from initial samples, identical to the percentage we finally obtained only after removing more than 4000 animals from *one* beach. In addition, the first few samples in this present study were remarkably similar to one another, and by then 23% of the total catch had been removed. It was not until the fourth sample that a marked change in catch pattern occurred.

Although some changes during sampling were appreciable, the overall effort provided a rare opportunity: comparing all of the data from the removal beach with data obtained from the first few samples.

In studies of crustacean populations, a number of measures can assess just how discrete populations differ from one another in response to differences in environmental influences, but one must first have some confidence that initial samples have provided an accurate estimate of population characteristics before comparing populations with one another. Among the measures one can use are: first, modal size classes, including number of each sex in each size class and percentage of animals within each mode; second, size at onset of egg production (Wenner, Fusaro, and Oaten, 1974); third, extent of egg production, including

percentage of females carrying eggs and number of eggs per given size of female; fourth, "instantaneous" growth rate (Fusaro, 1978a), including field molt rate and size increment at molt; and fifth, sex ratio patterns (Wenner, 1972), including shape variations and pattern displacement. Age is generally not used, since that measure is very difficult or impossible to determine directly for crustaceans in nature (Wilder, 1953).

This current study has shown that initial samples provided reliable data for most of the above measures, but not all aspects, as discussed below.

Overall, the percentage of males and females within the different modes in the first three samples did not accurately represent the population structure obtained during the entire sampling program (Figs. 3, 4). Neither small males nor small females were properly represented in those first samples. Also, the seven large females caught at first were apparently the only very large animals on the beach.

However, although the percentage of males and females within each mode changed drastically during removal, the mean size of animals within each mode did not change appreciably (Figs. 3, 4). This means that population samples for this species can be compared to one another through time, if one takes into account the fact that the absolute percentages can vary greatly within each mode.

The first three samples yielded a reliable estimate for mean minimum size of egg production (Wenner, Fusaro, and Oaten, 1974), when compared to that estimate obtained in later samples or to that obtained from the total data (12.4-mm carapace length). Hanson (1969) found a 15-mm carapace length for the same characteristic in *Hippa cubensis*.

It is not yet certain whether the percentage of mature females which bears eggs is reliably determined from initial samples, but this seems to be the case. At the primary removal beach (Fig. 1D), an estimate derived from the first two samples (64.2%) was significantly lower than that obtained from all remaining samples (85.0%). However, data obtained from Jedrol Islet were quite consistent; the percentage of larger females (females greater than 12.4-mm carapace length) bearing eggs did not change during removal. It is therefore possible that an influx of food had occurred just prior to the beginning of sampling at the removal beach on Enewetak Islet (see Wenner, 1977, Table I). If so, it may be that the rising percentage of ovigerous females reflected that particular energy input.

At the removal beach, the sex ratio pattern (sex ratio as a function of size) obtained from initial samples quite clearly did not characterize the species or population as suggested earlier (Wenner, 1972, p. 344) for at least two reasons: first, small animals did not come to the bait in proportion to their numbers in the population when removal was begun; and second, it is now apparent from other work that both shape and position of the sex ratio curve can differ between populations and at different times of the year (see Fusaro, 1977).

A striking contrast between various Hawaii results (Wenner, 1972, Fig. 10; and Haley, 1979, Fig. 2) and Enewetak data (Figs. 5A, B, this study) can now be reconciled. The "intermediate" (Wenner, 1972, Fig. 10), "reversal" (Fig. 5A, this study), and "anomalous" (Haley, 1979, Fig. 2) sex ratio curves obtained from initial samples at various times likely did not accurately represent that aspect of population structure for the small animals. Rather, the differences between these patterns in the lower size classes probably reflect the degree to which

small animals had not been captured or the degree to which they might not have been seen during hand catching (Haley, 1979).

Three out of four of the previously described types of sex ratio patterns (Wenner, 1972) have thus now been obtained for a single species, apparently reflecting both sampling bias and changes in population structure. It is further apparent that each of those curves probably represented part of yet another sex ratio pattern (an "oscillation" pattern, Fig. 5B), a pattern which has now also been found for a confamilial mole crab, *Emerita analoga* (Fusaro, 1977). Furthermore, the number of oscillations in that pattern matches the number of major modal size classes found for males and small females (see Fig. 2), oscillations which may represent the number of major influxes of young in the recent months or years, modified by a differential growth rate between sexes (Haley, 1979).

In retrospect, it would appear that points which deviate from the sex ratio curve for other crustaceans (see Fig. 11 for *Calcinus latens* in an earlier analysis—Wenner, 1972) could well represent real deviations.

The results of this study indicate that males reach a maximum size in this species, a parameter which could be quite valuable for comparing populations one with another. In such comparisons, however, the largest male which can be found in each population is not the best estimate of that parameter (the "largest male size" normally increases with sample size). Rather, one can use the 50, 95, or 99% level in the mode representing the largest males. In Figure 2 the corresponding values would be: 12.5 ± 0.59, 13.8, and 14.3, respectively. Statistical comparisons between populations are feasible, of course, only if one uses the first of these estimates (because one then has an estimate of standard deviation).

The initial three samples provided an accurate estimate of mean maximum size of males when compared to the data for all males. The first three samples combined yielded a mean and standard deviation of 12.4 ± 0.48 mm carapace length (N = 200); the remaining large males had a comparable mean of 12.5 ± 0.52 mm (N = 479).

The fact that males reach a maximum size while females continue growing beyond that size provides a related measure for comparing populations: an arbitrary point on the sigmoid portion of the sex ratio curve, where females first become dominant in the larger size classes (13.7 ± 0.47 mm at the 50% level in Fig. 5B). Since these sigmoid portions of the sex ratio curve are straight lines on probability paper, one can derive an estimate of the mean and standard deviation for that 50% transition point. This transition size was also quite accurately determined from intial samples. The first three samples yielded values of 14.0 ± 0.73 mm carapace length (N = 393), compared to the overall value shown above of 13.7 ± 0.47 mm (N = 745).

Southwood (1966) outlined methods by which some systems might be sampled so as to reduce the amount of bias inherent in estimates of population structure. Gilbert, Gutierrez, Frazer, and Jones (1976, p. 57) updated the approaches outlined and admonished: "The sample must represent the defined 'population' of animals or plants we wish to investigate." The concern of these workers has been supported only in part by the current study. Initial samples did accurately represent some aspects of population structure. It should also be stressed that researchers who would like to understand crustacean biology better are not merely "choosing suit-

able species for ecological work" (Gilbert, Gutierrez, Frazer, and Jones, 1976, pp. 58, 59). Marine forms can often be readily caught *only* by some form of trapping (*i.e.,* bait, nets, etc.). Although there is perhaps no way to know if initial samples contain a bias without going through a removal process, it is also obvious that a bias can be tolerated once one knows its nature.

Other researchers have reported similar discrepancies. In an extreme example, Bolin (1961) found that plankton hauls caught only female lantern fish (*Tarletonbeania crenularis*), while albacore tuna stomachs contained males but no females. The discrepancy became partially resolved when an unexplained event caused a massive death, resulting in millions of these fish being washed onto the beaches in central California. Of 521 specimens examined, 43.25% were males. Bolin speculated that males, being faster than females, could escape plankton nets. Conversely, he felt that females, having no photophores, would not be seen by tuna at that depth and could escape that mode of predation.

In a less extreme but still significant example, Gotshall (1972), who found marked discrepancies in catches of *Pandalus jordani* with respect to some year classes (size classes), suggested possibilities for those anomalous results, and reviewed some similar problems encountered by other researchers.

In the present study, although initial samples did not accurately portray a few of the conventional measures used by population biologists, reasonable estimates were gained of several parameters, as outlined above. The present study thus demonstrates the value of comparing data obtained from initial samples with actual population structure, especially when a biased sampling technique is the only feasible way to capture a sample from a population.

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Summary

1. Essentially all of a population of mole crabs (*Hippa pacifica* Dana) was removed from an isolated beach at Enewetak Atoll, Marshall Islands. Estimates of poplation size, based on catch pattern, indicate that 87 to 93% of the animals were bait-trapped out of their restricted habitat. The sequential trapping permitted a number of comparisons between initial sample data and actual population structure.

2. The first three samples were large and yielded highly consistent data when compared to one another, but some aspects of population structure were nonetheless non-representative. For example, the percentage of crabs within each modal size class in the total population differed markedly from that estimated by initial samples. The location of those modal size classes did not change during the removal process.

3. After the first three samples (where male percentages were about 29%), sex ratio fluctuated wildly in individual samples, apparently as a consequence of size discrepancy in arrival at the bait. Eventually, males comprised about 48.4% of the 4011 animals removed.

4. Several other aspects of population structure were accurately estimated in early samples, including: mean size at onset of egg production, percentage of mature females carrying eggs, the sex ratio-size class relationship (for larger crabs), and the mean maximum size of males.

LITERATURE CITED

Bolin, R. L., 1961. The function of the luminous organs of deep-sea fishes. *Proceedings of the Ninth Pacific Science Congress (1957)*, **10**: 37–39.

Borradaile, L. A., 1906. The Hippidea, Thalassinidea and Scyllaridea. Pages 750–754 in J. S. Gardiner, Ed., *The Fauna and Geography of the Maldive and Laccadive Archipelagoes*. **Vol. 2.** Cambridge University Press, London.

Cassie, R. M., 1954. Some uses of probability paper in the analysis of size frequency distribution. *Aust. J. of Mar. Freshwater Res.*, **5**: 513–522.

Fusaro, C., 1977. Population structure, growth rate, and egg production of the sand crab, *Emerita analoga* in two different environments: a comparative analysis. *Ph.D. Dissertation, University of California at Santa Barbara*, Santa Barbara, California.

Fusaro, C., 1978a. Growth of the sand crab, *Emerita analoga* (Hippidae), in two different environments. *U. S. Fish Wildl. Serv. Fish Bull.*, 76 (**2**): in press.

Fusaro, C., 1978b. Food availability and egg production: A field experiment with *Hippa pacifica* Dana (Decapoda, Hippidae). *Pac. Sci.*, **32**: 17–23.

Gilbert, N., A. P. Gutierrez, B. D. Frazer, and R. E. Jones, 1976. *Ecological Relationships*. W. H. Freeman & Co., San Francisco, 157 pages.

Gotshall, D. W., 1972. Population size, mortality rates, and growth rates of Northern California ocean shrimp, *Pandalus jordani*, 1965 through 1968. *Calif. Dep. Fish Game Fish Bull.*, **155**. 47 pages.

Haley, S. R., 1979. Sex ratio as a function of size in *Hippa pacifica* Dana (Crustacea, Anomura, Hippidae): A test of the sex reversal and differential growth hypotheses. *Am. Nat.*, **113**: 391–397.

Hanson, A. J., 1969. The life-history of the sand crab, *Hippa cubensis* Saussure living on a small island. *M. S. Thesis. University of British Columbia*, Vancouver.

Matthews, D. C., 1955. Feeding habits of the sand crab *Hippa pacifica* (Dana). *Pac. Sci.*, **9**: 382–386.

Southwood, T. R. E., 1966. *Ecological Methods*. Methuen, London. 391 pages.

Wenner, A. M., 1972. Sex ratio as a function of size in marine Crustacea. *Am. Nat.*, **106**: 321–350.

Wenner, A. M., 1977. Food supply, feeding habits, and egg production in Pacific mole crabs (*Hippa pacifica* Dana). *Pac. Sci.*, **31**: 39–47.

Wenner, A. M., C. Fusaro, and A. Oaten, 1974. Size at onset of sexual maturity and growth rate in crustacean populations. *Can. J. Zool.*, **52**: 1095–1106.

Wilder, D. G., 1953. The growth rate of the American lobster (*Homarus americanus*). *J. Fish. Res. Board Can.*, **10**: 371–512.

Continued from Cover Two

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CONTENTS

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Continued on Cover Three

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SALT AND WATER BALANCE IN TWO MARINE SPIDER CRABS, *LIBINIA EMARGINATA* AND *PUGETTIA PRODUCTA*. I. URINE PRODUCTION AND MAGNESIUM REGULATION

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The excretory organs of decapod crustaceans function in magnesium regulation (Robertson, 1957; Lockwood, 1962; Potts and Parry, 1964) and to a lesser extent, in nitrogen excretion (Delaunay, 1931; Binns and Peterson, 1969). Magnesium regulation has been observed in decapods from a variety of habitats (Robertson, 1939, 1949, 1953; Webb, 1940), the usual pattern being a lowered blood concentration and an elevated urine concentration, with respect to the medium. This phenomenon has been investigated extensively in two osmoregulating crabs, *Pachygrapsus crassipes* (Prosser, Green and Chow, 1955; Gross and Marshall, 1960; Gross and Capen, 1966) and *Carcinus maenas* (Riegel and Lockwood, 1961; Lockwood and Riegel, 1969), but little is known about magnesium regulation in osmoconforming crabs.

A problem in the study of magnesium regulation, and other aspects of salt and water balance, is that of making accurate estimates of urine production rates. Cannulation is usually not feasible because of the geometry and delicacy of the excretory duct. Many methods have been used, perhaps the best known being nephropore-occlusion, but only a few estimates have been made for an extended period of time by the continuous collection of urine: *Procambarus clarkii* (Kamemoto and Ono, 1968; Ono and Kamemoto, 1969); *Paranephrops zealandicus* (Wong and Freeman, 1976); *Cancer magister* (Holliday, 1977); *Callinectes sapidus* (Cameron and Batterton, 1978). A technique for the continuous collection of urine has been used in the present study.

Materials and Methods

Specimens of the osmoconforming crab, *Pugettia producta*, were collected near the Bodega Marine Laboratory, Bodega Bay, California, and maintained at 10 to

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12° C, either in running sea water (SW) at the Bodega Marine Laboratory, or in filtered SW (about one crab per 4 liters) at the University of California, Berkeley, California. Intermolt crabs were used except where otherwise noted.

Estimates of urine production were made by continuous collection of urine. A polyester resin cast of the region surrounding the two nephropores was made in order to position two polyethelene tubes. In making the cast, the crab was clamped in a supine position and the periopods secured with rubber bands. The ventral surface was dried with compressed air and those portions of the third maxillipeds distal to the ischia were cut off. The region surrounding the nephropores was lightly swabbed with vaseline which acted as a mold releasing agent. A transverse dam of plasticine, placed distal to the third maxillipeds, prevented the casting material from flowing into the mouth parts. A freshly mixed polyester resin, such as "G-R-R-R-I-P" (Idaho Chemical Industries, Inc.) was applied so that it extended posteriad to the plasticine dam, anteriad to the antennules, and laterad to about 5 mm beyond the nephropores. After 15 min, the cast was removed and the crab returned to SW. This procedure was carried out at least 24 hr before the start of an experiment.

The impressions of the opercula that cover the openings of the nephropores could clearly be seen in the cast. Each impression was used to center a hole of 0.063 inch (1.60 mm) diameter. A drill, larger by about 20% than the diameter of the opercula, was used to countersink the first pair of holes to a depth slightly greater than the diameter of the opercula. This allowed sufficient room for the opercula to open. Polyethelene tubing (P.E.Intramedic 190, Clay Adams) was pressed into the holes in the cast. Silicone grease was carefully applied around the periphery of the cast and between the two holes on its inner face. The crab was replaced in the clamp, the periopods secured with rubber bands, and the region surrounding the nephropores dried with compressed air. The cast, with the attached tubing, was positioned and pressed onto the crab. In most cases it snapped into position and was firmly held in place by a rubber band.

The crab was then suspended in an aquarium so that the top of the carapace was just submerged. The seal between the cast and the animal was checked by blowing into the tubes and watching for air bubbles. The tubes were led over the lip of the aquarium, about 3 to 5 cm above the nephropores, and fed into two vials, positioned at about the same level as the nephropores. The urine was collected under mineral oil.

Urine production was also estimated by blocking the nephropores with Eastman 910 cement (Armstrong Cork Co.) and measuring the change in weight. The Eastman 910 was allowed to set for 10 min before the crabs were returned to SW. During this period, excess water was removed from the branchial chambers, the animals were dried for one minute with compressed air, and weighed.

The concentrations of sodium, magnesium, calcium, potassium, chloride, and ninhydrin positive substances (NPS), and osmotic pressure, in blood, urine and SW were measured. Blood samples were withdrawn by puncturing an arthrodial membrane at a leg base with a drawn-out Pasteur pipet. Samples were centrifuged under mineral oil for 10 min in either an International Clinical Centrifuge at about 6000 rpm at room temperature or a Sorval model RC2-B, at 10,000 rpm at 1° C, the latter being used in the preparation of samples for osmotic pressure

determinations. Urine samples were also collected at the nephropore. A small hook, guided with the aid of a dissecting scope, was used to lift an operculum and the urine was collected in a drawn-out Pasteur pipet. Samples of plasma, urine and medium were stored under mineral oil in polystyrene vials.

Concentrations of cations were measured with a Perkin-Elmer model 290 atomic absorption spectrophotometer. Concentrations of chloride were measured on a Buchler-Cotlove chloridometer. NPS were determined by the method of Fowden (1951). Plasma was deproteinized by the addition of an equal volume of 10% trichloroacetic acid. For uniformity, urine received the same treatment. Samples were read on a Klett colorimeter against glycine standards. Measurements of osmotic pressure were made with an Advanced Instruments "Osmette" osmometer. Measurements of electrical potential difference across the body wall were made with an Analog Devices model 40J operational amplifier (input impedance, 10^{11} ohms) as a preamplifier and voltages were read on a Tektronix oscilloscope. Chlorided silver wires in 3 M KCl were used to make the electrical connections to 3 M KCl-agar bridges. One bridge served as the reference electrode while the other was placed in a hole in the top of the carapace, which remained out of water.

Results

Urine production rates estimated by continuous collection

Figure 1 shows the results of an experiment where urine was collected over a 24-hr period. The sum of the cumulative volume of urine released from both nephropores has been arbitrarily fitted with a fifth order polynomial. The urine production rate, the first derivative of this polynomial, is also included. The rate of urine production was usually greater during the first 12-hr period than during subsequent collecting periods. The rates which will be reported were obtained after a 12-hr lapse from the start of collection. Urine release is intermittent, the interval between successive releases can vary greatly, and urine is usually released simultaneously from both nephropores.

Intermolt specimens of *Pugettia producta* (average weight, 101 g) in 100% SW produced urine at 6.40 ± 3.08 (22) % body weight (bw)/day, mean $\pm$ SD (N). Postmolt crabs (73 g) produced urine at 2.89 ± 3.68 (5) % bw/day and premolt crabs (65 g) produced urine at 29.5 ± 3.98 (3) % bw/day. A single classification analysis of variance indicates that there are significant differences among these means ($P < 0.01$) and *a priori* tests indicate that there are differences between the rates for intermolt and premolt crabs ($P < 0.01$), and intermolt and postmolt crabs ($P < 0.05$). The cause for these differences is not known.

Urine production rates for 10 crabs (114 g) were compared on two successive days. The average rate on the first day was 5.06 ± 3.68% bw/day; on the second day it was 5.48 ± 3.27 % bw/day. These means are not significantly different (paired *t*-test). For one crab, urine production was measured for a 2-week period. During this time, the rate varied from 2 to 6% bw/day, the average being 3.02% bw/day. There was no apparent pattern to the daily fluctuations, and it is possible that the changes could be accounted for by changes in the volume of urine held in the bladders, which in *Pugettia* can exceed 5% bw (Cornell, 1976).

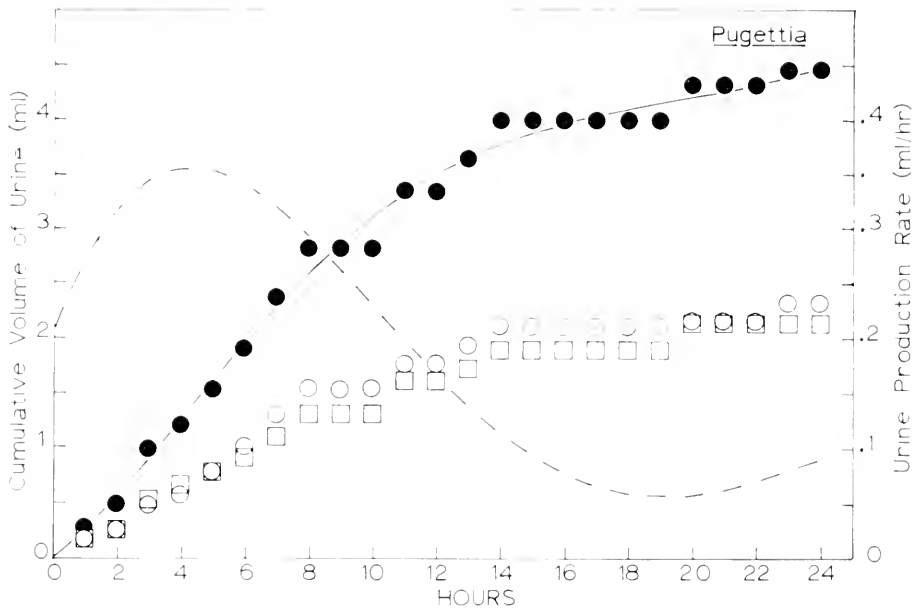

FIGURE 1. Urine production in a 46 g specimen of *Pugettia producta* from the time of placement of the polyester resin cast until the 24th hr. The cumulative volume of urine produced from the left and right nephropores is indicated by the square boxes and open circles, respectively, and can be read on the left ordinate. The sum of the cumulative volume of urine from both nephropores, indicated by solid circles, has been arbitrarily fitted with a fifth order polynomial, indicated by the solid curve. The first derivative of this polynomial, or urine production rate, is indicated by the dashed line and can be read on the right ordinate.

Urine production estimated from weight gain

Intermolt specimens of nephropore-occluded *Pugettia* (43.54 g) gained 3.38 ± 2.10 (12) % bw after 24 hr, while a control group (46.10 g) gained 0.43 ± 0.88 (9) % bw in the same period. Four crabs with blocked nephropores gained an additional 1.0% between 24 and 48 hr. These animals were markedly swollen, the posterior region of the carapace being lifted away from the abdomen. The urine production rate for intermolt crabs determined by weight gain is significantly less than that determined by continuous collection ($P < 0.005$, t-test), suggesting that the rates estimated by weight gain are under-estimates of the true rate, and that significant back pressures can occur.

Some major constituents of blood and urine

Measurements of some major constituents of blood, urine and medium for crabs in 100% SW are presented in Table I. An approximate statistical test (Sokal and Rohlf, 1969, p. 372), analogous to a single classification analysis of variance, was used to test for equality among means since these data are heteroscedastic.

The concentrations of sodium and chloride in the blood were both 98% of

their respective concentrations in the medium. In the urine, the concentration of chloride was about equal to, while sodium was 96% of, the concentration in the medium. The concentrations of magnesium, calcium and potassium in the blood were 88, 118, and 105% of their respective concentrations in the medium. In the urine, these same ions were 135, 124, and 114% of the medium, respectively. NPS were about 10 times more concentrated in the blood than in the urine. The osmotic pressure of the blood was about 2 mosM greater than that of the medium and the urine was isosmotic to the blood, but not statistically different from the medium.

The concentrations of inorganic ions were more variable in the urine than in the blood; in particular, magnesium was the most variable, as judged by the ratio of the standard deviation to the mean. Magnesium concentrations ranging from 48 to 128 mM were observed in the urine. Table II shows two correlation matrices, one each for the blood and the urine. In each matrix, a rank correlation coefficient has been computed for each inorganic ion with every other inorganic ion. There is a significant negative correlation between sodium and magnesium in the urine. There are also significant positive correlations between magnesium and calcium and between magnesium and potassium in the urine.

In the blood, magnesium concentrations were also relatively more variable than the other ions which were measured. A significant negative correlation between magnesium and calcium was found, the reverse of the condition in the urine. Significant positive correlations between magnesium and chloride, and between sodium and potassium, were also found.

TABLE I

Concentrations of some major constituents of the blood and urine of specimens of Pugettia producta *in sea water. Relative concentrations are expressed as a percentage of the medium. Absolute concentrations of inorganic ions, ninhydrin positive substances (NPS) and osmotic pressure (OP) are expressed in mM, mM glycine, and mosM, respectively. F(df) indicates the F ratio of an approximate test with the calculated degrees of freedom. One and two asterisks denote $P < 0.05$ and $P < 0.01$, respectively.*

Solute	Fluid Relative concentration Absolute concentration, mean ± sd (N)			F (df)
	Blood	Urine	Medium	
Cl^-	98.0% 524 ± 12.9 (13)	99.4% 532 ± 27.9 (14)	100% 535 ± 2.73 (6)	4.24* (2, 18)
Na^+	97.8% 450 ± 16.0 (23)	96.1% 442 ± 22.1 (37)	100% 460 ± 5.74 (6)	9.60** (2, 30)
Mg^{2+}	88.3% 46.1 ± 2.48 (23)	135% 70.5 ± 19.2 (37)	100% 52.2 ± 0.65 (6)	75.7** (2, 24)
Ca^{2+}	118% 11.8 ± 0.52 (13)	124% 12.4 ± 1.11 (22)	100% 10.0 ± 0.17 (6)	95.6** (2, 43)
K^+	105% 10.2 ± 0.38 (13)	114% 11.1 ± 1.13 (22)	100% 9.74 ± 0.19 (6)	11.5** (2, 21)
NPS	— 3.72 ± 2.00 (14)	— 0.39 ± 0.12 (5)	— —	38.4** (1, 13)
OP	100.2% 1016 ± 2.47 (10)	100.2% 1016 ± 3.77 (10)	100% 1014 ± 1.19 (10)	3.69* (2, 15)

TABLE II

Correlation matrices (Spearman's rank correlation coefficient, N) for inorganic ions in the blood and urine of Pugettia producta *in sea water. One and two asterisks denote $P < 0.05$ and $P < 0.01$, respectively.*

Blood

Urine	Cl^-	Na^+	Mg^{2+}	Ca^{2+}	K^+	Blood
Cl^-		0.38, 13	0.55, 13*	−0.41 13	0.30, 13	Cl^-
Na^+	−0.03, 14		−0.02, 23	−0.36, 13	0.48, 13*	Na^+
Mg^{2+}	0.19, 14	−0.40, 37*		−0.64, 13*	−0.13, 13	Mg^{2+}
Ca^{2+}	0.20, 14	−0.19, 16	0.81, 16**		−0.23, 13	Ca^{2+}
K^+	−0.40, 14	−0.01, 16	0.50, 16*	0.41, 16		K^+
	Cl^-	Na^+	Mg^{2+}	Ca^{2+}	K^+	

Urine

TABLE III

Magnesium and sodium concentrations in the blood of normal and nephropore-occluded specimens of Pugettia producta *in sea water. See text for statistical tests.*

Ion	Time					
	0 hr		24 hr		48 hr	
	Normal	Occluded	Normal	Occluded	Normal	Occluded
Mg^{2+} (mM) sd, N	47.1 2.05, 7	47.5 2.48, 10	46.8 1.01, 7	48.5 4.33, 10	46.1 2.44, 7	51.4 3.87, 10
Na^{+} (mM) sd, N	448 14.8, 7	454 20.1, 10	450 4.5, 7	462 28.9, 10	446 16.8, 7	473 23.9, 10

The effects of nephropore-occlusion on magnesium regulation

Of the inorganic ions studied, magnesium represents the clearest example of an ion which is regulated by the excretory system. Its concentration in the blood, urine, and medium suggest that it is continuously diffusing down the concentration gradient from medium to blood, and that this gradient is maintained by its active removal by the excretory system. Estimates of the electrical potential difference across the body wall of three crabs indicate a difference of less than 0.1 mV. Thus, the driving force for the diffusion of magnesium from the medium to the blood must be the difference in chemical potential.

Blocking the nephropores should stop the removal of magnesium from the blood, and thus the magnesium level in the blood should rise, reaching equilibrium with the magnesium in the medium. The results of such an experiment appear in Table III. The magnesium concentration in the blood of the experimental animals with blocked nephropores increased; after 48 hr the ratio of the magnesium concentration in experimental crabs to that in control crabs was 1.12. There was also an increase in sodium concentration in the blood of experimental crabs, the above ratio being 1.06. This may indicate that sodium is also normally regulated below its equilibrium concentration. The sodium and magnesium data were separately analyzed by two-way analysis of variance after randomly removing the data for three crabs in the experimental group in order to facilitate the calculations. These analyses indicate that there are significant increases in sodium and magnesium concentrations in the blood of nephropore-occluded crabs ($P < 0.01$ for both ions in the sub-groups Normal and Occluded; for both ions, the F ratios were not significant in the sub-group Time, and for the interaction between sub-groups Normal and Occluded × Time).

In *Pachygrapsus crassipes*, Gross and Capen (1966) demonstrated that the magnesium concentration in the urine is a direct function of the time the urine is held in the bladder. This does not seem to be the case in *Pugettia*. When, after 86 hr, the magnesium concentration in the urine of nephropore-occluded and control crabs were compared, the magnesium concentration in the control crabs, 81.9 ± 26.8 (7) mM, was greater than that in the experimental crabs, 58.4 ± 9.93 (9) mM. This unexpected result seems to have been caused by chance, since the

concentration in the control group was greater than expected. Also, since one of the crabs in the experimental group had died, the remaining animals may not have been in good condition and the transport of magnesium could have been reduced. The experiment was repeated using a paired design and a shorter period of time to minimize the effects of individual variation and nephropore-occlusion. No differences were found in 10 crabs between the magnesium concentrations at 0 hr, 65.3 ± 20.7 mM, and 24 hr, 64.2 ± 18.3 mM, after nephropore-occlusion. These experiments suggest that the bladders of *Pugettia* do not secrete magnesium into the urine.

Discussion

Decapod crustaceans have considerable ability to regulate their internal ionic compositions (Robertson, 1949, 1953). This appears to be independent of the ability to osmoregulate and has been defined by Robertson (1949, p. 182) as the "maintenance in a body fluid of concentrations of ions differing from those of a passive equilibrium with the external medium." Since the blood of decapods contains considerable amounts of non-diffusible, negatively charged protein, the internal/external concentration ratio of an ion can differ passively from unity. This ratio is expected to be less than 1.0 for the anions and greater than 1.0 for the cations. Robertson (1953) found, using a dialysis technique, that it was often 1.03 for the cations, except for calcium for which it was estimated that up to 20% was complexed with proteins. Greenaway (1976) has confirmed these results for calcium.

The present data suggest that *Pugettia producta* hypo-regulates magnesium and sodium; potassium is probably slightly hyper-regulated, while chloride is probably very close to its equilibrium concentration. From these data it is not possible to determine if calcium is regulated, since an unknown amount is complexed with proteins. The comparison of the concentrations of various ions in the blood of *Pugettia* with those of other decapod crustaceans, tabulated by Prosser (1973), indicates that the pattern of regulation in *Pugettia* is similar to that in other marine decapods.

Expressed as mM glycine, the blood and urine of *Pugettia* contain 3.7 and 0.39 mM NPS, respectively. It was the usual policy to use crabs which had not been fed for a week. Thus, NPS in the blood of *Pugettia* fresh from the field could be different. A comparison of these values with those reported for *Carcinus maenas* (Binns, 1969b; Evans, 1972) indicates that the blood concentrations are similar in both crabs when differences in technique are accounted for (see Evans, 1972). The concentration of NPS in the urine of *Pugettia* appears to be about half of that in *Carcinus*, but this can be quite variable and may not represent a true difference. The antennal gland of crustaceans is not a major route for nitrogen loss. Binns and Peterson (1969) estimate that in the spiny lobster *Jasus edwardsi*, 90% of all the soluble nitrogen is excreted extra-renally.

The urine production rate for intermolt specimens of *Pugettia*, determined by continuous collection, was 6.40% bw/day. However, the rate determined by weight gain was less than half of this, 2.95% bw/day after correction for the control value. There is good reason to believe that the former estimate is the more correct, the latter being reduced by back pressure. Although few data exist, there appears

TABLE IV

Magnesium excretion rates and magnesium permeabilities for some decapod crustaceans in sea water. One dagger indicates that C_m and C_b are magnesium concentrations (mM) in medium and blood, respectively. Two daggers: see text for discussion of the effects of the electrical potential on these values. Three daggers denote the references: (1) Gross and Marshall, 1960; (2) urine and blood [Mg^{2+}]—Riegel and Lockwood, 1961; urine production rate—Binns, 1969a; (3) this report.

Animal (reference)†††	Blood Mg^{2+} mM	Urine Mg^{2+} mM	Medium Mg^{2+} mM	Urine flow rate % bw/day	Mg^{2+} excretion rate $\frac{\mu mol}{g \cdot day}$	Mg^{2+} permeability $\frac{\mu mol}{(C_m - C_b)g \cdot day}$†
Pachygrapsus crassipes (1)	20	305	52.0	3.9	11.9	0.37††
Carcinus maenas (2)	31	250	52 (?)	4.4	11.0	0.52††
Libinia emarginata (3)	44	66	49.0	5.1	3.4	0.68
Pugettia producta (3)	46	70	52.2	6.4	4.5	0.72

to be a relationship between urine production and molt stage since premolt crabs produced urine at greater rates, and postmolt crabs produced urine at lesser rates, than intermolt crabs. The cause of this relationship is a matter for speculation.

The urine production rate was also determined by continuous collection for the osmoconforming spider crab *Libinia emarginata* (5.1% bw/day) and the freshwater crayfish *Pacifastacus leniusculus* (6.0% bw/day). At present, it is difficult to explain, considering water permeabilities and osmotic pressures of blood and media, why *Libinia*, *Pacifostacus* and *Pugettia* produce urine at comparable rates. Regardless, it is clear from many studies that most moderate-sized decapods produce urine at 2 to 10% bw/day when tested at salinities representative of normal habitat salinity, the exception being the freshwater brachyurans which produce no more than about 1% bw/day (Shaw, 1959; Thompson, 1970; Harris, 1975).

The present data suggest that the excretory system of *Pugettia* is responsible for the lowered magnesium concentration in the blood since magnesium is concentrated in the urine, and since blocking the nephropores results in elevated levels of magnesium in the blood. Calcium and potassium are also concentrated in the urine, although not to so great a degree as magnesium. There appears to be some relationship among magnesium, calcium, and potassium in the urine, the concentration of the latter two ions being positively correlated with that of magnesium. By contrast, the calcium concentration in the urine of *Carcinus* is independent of the magnesium concentration (Lockwood and Riegel, 1969).

The concentration of magnesium in the urine of *Pugettia* is negatively correlated with that of sodium. Similar relationships between sodium and magnesium have been reported for *Carcinus* (Webb, 1940; Riegel and Lockwood, 1961), *Pachygrapsus crassipes* (Prosser, Green and Chow, 1955; Gross and Marshall, 1960; Gross and Capen, 1966) and *Cancer magister* (Hunter and Rudy, 1975). Thus, some from of Na^+/Mg^{2+} exchange mechanism may exist. Little is known about the mechanisms of magnesium transport. However, Holliday (1978) has found

that the net magnesium flux in the isolated bladder of *Cancer* is inhibited by ouabain and that only a small part of the opposing net sodium flux is associated with the magnesium flux. In the isolated gut of the insect *Hyalophora cecropia,* magnesium transport is independent of sodium and related, in a complex way, to potassium transport (Wood, Jungreis and Harvey, 1975).

The reabsorption of fluid from the urine, as suggested by the urine/blood (U/B) ratio of filtration markers, is insufficient to account for the U/B ratios of magnesium in a number of decapods (Gross and Capen, 1966; Lockwood and Riegel, 1969; Franklin, Teinsongrusmee and Lockwood, 1978). In *Pugettia,* the U/B ratio of magnesium is about 1.5 and the U/B ratio of inulin may approach this value (Cornell, 1976). However, there is some difficulty in the interpretation of inulin U/B ratios in animals with large bladders (see Riegel, Lockwood, Norfolk, Bulleid and Taylor, 1974; and Cornell, 1976). Thus, it seems unwarranted to conclude that fluid reabsorption accounts for the concentration of magnesium in the urine of *Pugettia.*

Gross and Capen (1966) have shown that the magnesium concentration in the urine of *Pachygrapsus* is a function of the time the urine is held in the bladder. However, during nephropore-occlusion the magnesium concentration in the urine did not increase in *Pugettia,* suggesting that magnesium is concentrated in the antennal gland. A similar experiment on *Pachygrapsus* produced an increase in urine magnesium. In Figure 3 of their report, Gross and Capen (1966) presented results which suggest that the fluid entering the bladders contains 38 mM magnesium, about 20 mM higher than the blood. The urine of *Pugettia* is about 24 mM higher in magnesium than the blood. The implication is that the antennal glands of both animals perform equivalent tasks, and that it is the bladders of *Pachygrapsus* which concentrate most of the magnesium, a function which the bladders of *Pugettia* do not seem to perform.

The concentration of magnesium in the blood must be a function of at least three parameters: the permeability of the body wall, the excretion rate, and the electrochemical potential across the body wall. Neglecting for the moment the electrical potential, and dividing the weight-specific magnesium excretion rate by the concentration difference between the blood and the medium, an estimate of the permeability of the body wall can be obtained. In Table IV these quantities have been computed for four decapods. Taking into account the electrical potential does little to change these results. In *Pugettia* and *Libinia,* the potential is negligible, 0 ± 0.1 mV. Potentials of -2.0 mV (inside negative) for *Pachygrapsus* (Rudy, 1966) and *Carcinus* (Greenaway, 1976) have been reported for animals in SW. This is a small potential and for present purposes may be neglected, however, the permeability of *Pachygrapsus* and *Carcinus* may be slightly less than indicated. The data in Table IV suggest that those crabs which have much reduced the magnesium concentration in their blood have done so by lowering the magnesium permeability and by raising the rate of magnesium excretion.

The significance of magnesium regulation in crustaceans, first noted by Robertson (1949, 1953), is that a strong negative correlation exists between the activity of an animal and the magnesium concentration in its blood. Thus, animals with low magnesium levels tend to be highly active, while those with high levels

tend to be lethargic. Robertson (1953) has pointed out that high extracellular levels of magnesium have been found to block neuromuscular transmission in *Carcinus* (Katz, 1936) and to reduce the mechanical response of isolated crayfish legs to electrical stimulation (Waterman, 1941).

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Summary

1. A new technique for the continuous collection of crab urine is described. Estimates of urine production, based on this technique, indicate that specimens of *Pugettia producta* in sea water produce urine at 6.4% body weight (bw)/day. Premolt and postmolt crabs produce 30 and 3.0% bw/day, respectively. Intermolt specimens of *Libinia emarginata* produce 5% bw/day.

2. The urine production rate for specimens of *Pugettia,* estimated by weight gain following 24 hr of nephropore-occlusion, is 3.0% bw/day. This is significantly less than that determined by the continuous collection of urine, suggesting that back pressure can interfere with urine production.

3. Ion regulation was examined in specimens of *Pugettia.* When expressed as a percentage of their concentrations in sea water, the values in blood plasma of chloride, sodium, magnesium, calcium, and potassium are 98, 98, 88, 118, and 105%, respectively, for crabs in sea water. Likewise in the urine, the values for these same ions are 99, 96, 135, 124, and 114%, respectively. Ninhydrin positive substances, measured with glycine standards, are 3.7 and 0.39 mM in blood plasma, respectively. The electrical potential across the body wall of both species of crab is zero.

4. In *Pugettia,* blocking the nephropores causes an increase in the magnesium concentration in the blood, suggesting that the excretory system is mainly responsible for regulating this ion. However, blocking the nephropores causes no change in the magnesium concentration of urine stored in the bladder, which suggests that the antennal gland is mainly responsible for concentrating magnesium in the urine. *Libinia* and *Pugettia* excrete magnesium at a lower rate, and are more permeable to magnesium, than brachyurans which are strong magnesium regulators.

LITERATURE CITED

Binns, R., 1969a. The physiology of the antennal gland of *Carcinus maenas* (L.). II. Urine production rates. *J. Exp. Biol.,* **51**: 11–16.

Binns, R., 1969b. The physiology of the antennal gland of *Carcinus maenas* (L.). IV. The reabsorption of amino acids. *J. Exp. Biol.,* **51**: 29–39.

Binns, R., and A. J. Peterson, 1959. Nitrogen excretion by the spiny lobster *Jasus edwardsi* (Hutton): the role of the antennal gland. *Biol. Bull.,* **136**: 147–153.

Cameron, J. N., and C. V. Batterton, 1978. Antennal gland function in the freshwater blue crab, *Callinectes sapidus:* water, electrolyte, acid-base and ammonia excretion. *J. Comp. Physiol.,* **123**: 143–148.

Cornell, J. C., 1976. Aspects of salt and water balance in two osmoconforming crabs *Libinia emarginata* and *Pugettia producta* (Brachyura: Majidae). *Ph.D. thesis, University of California,* Berkeley, 243 pp. (University Microfilms/Dissertation Abstracts International No. 77-15,646.)

Delaunay, H., 1931. L'excrétion azotée des invertébrés. *Biol. Rev.,* **6**: 265–301.

Evans, P. D., 1972. The free amino acid pool of the haemocytes of *Carcinus maenas* (L.). *J. Exp. Biol.,* **56**: 501–507.

Fowden, L., 1951. The quantitative recovery and colorimetric estimation of amino-acids separated by paper chromatography. *Biochem. J.* **48**: 327–333.

Franklin, S. E., B. Teinsongrusmee, and A. P. M. Lockwood, 1978. Inhibition of magnesium secretion in the prawn *Palaemon serratus* by ethacrynic acid and by ligature of the eyestalks. Pages 173–193 in K. Schmidt-Nielsen, L. Bolis, and S. H. P. Maddrell, Ed., *Comparative physiology: water, ions and fluid mechanics.* Cambridge University Press, New York and London.

Greenaway, P., 1976. The regulation of haemolymph calcium concentration of the crab *Carcinus maenas* (L.). *J. Exp. Biol.,* **64**: 149–157.

Gross, W. J., and L. A. Marshall, 1960. The influences of salinity on the magnesium and water fluxes of a crab. *Biol. Bull.,* **119**: 440–453.

Gross, W. J., and R. L. Capen, 1966. Some functions of the urinary bladder in a crab. *Biol. Bull.,* **131**: 272–291.

Harris, R. R., 1975. Urine production rate and urinary sodium loss in the freshwater crab *Potamon edulis. J. Comp. Physiol.,* **96**: 143–153.

Holliday, C. W., 1977. A new method for measuring the urinary rate of a brachyuran crab. *Comp. Biochem. Physiol.,* **58**A: 119–120.

Holliday, C. W., 1978. Magnesium transport by the bladder of the crab, *Cancer magister. Am. Zool.,* **18**: 577.

Hunter, K. C., and P. P. Rudy, 1975. Osmotic and ionic regulation in the Dungeness crab *Cancer magister* Dana. Comp. Biochem. Physiol., **51A**: 439–447.

Kamemoto, F. I., and J. K. Ono, 1968. Urine flow determinations by continuous collection in the crayfish *Procambarus clarkii. Comp. Biochem. Physiol.,* **27**: 851–857.

Katz, B., 1936. Neuro-muscular transmission in crabs. *J. Physiol.,* **87**: 199–221.

Lockwood, A. P. M., 1962. The osmoregulation of Crustacea. *Biol. Rev.,* **37**: 257–305.

Lockwood, A. P. M., and J. A. Riegel, 1969. The excretion of magnesium by *Carcinus maenas. J. Exp. Biol.,* **51**: 575–589.

Ono, J. K., and F. I. Kamemoto, 1969. Annual and proecdysial variations in urine production in crayfish. *Pacific Sci.,* **23**: 305–310.

Potts, W. T. W., and G. Parry, 1964. *Osmotic and ionic regulation in animals.* Pergamon Press, London, 423 pp.

Prosser, C. L., 1973. Inorganic ions. Pages 79–110 in C. L. Prosser, Ed., *Comparative animal physiology,* 3rd ed. Saunders, Philadelphia.

Prosser, C. L., J. W. Green, and T. J. Chow, 1955. Ionic and osmotic concentrations in the blood and urine of *Pachygrapsus crassipes* acclimated to different salinities. *Biol. Bull.,* **109**: 99–107.

Riegel, J. A., and A. P. M. Lockwood, 1961. The role of the antennal gland in the osmotic and ionic regulation of *Carcinus maenas. J. Exp. Biol.,* **38**: 491–499.

Riegel, J. A., A. P. M. Lockwood, J. R. W. Norfolk, N. C. Bulleid, and D. A. Taylor, 1974. Urinary bladder volume and the reabsorption of water from the urine of crabs. *J. Exp. Biol.,* **60**: 167–181.

Robertson, J. D., 1939. The inorganic composition of the body fluids of three marine invertebrates. *J. Exp. Biol.,* **16**: 387–397.

Robertson, J. D., 1949. Ionic regulation in some marine invertebrates. *J. Exp. Biol.*, **26**: 182–200.

Robertson, J. D., 1953. Further studies on the ionic regulation of marine invertebrates. *J. Exp. Biol.*, **30**: 277–296.

Robertson, J. D., 1957. Osmotic and ionic regulation in aquatic invertebrates. Pages 229–246 in B. T. Scheer, T. H. Bullock, L. H. Kleinholz, and A. W. Martin, Ed., *Recent advances in invertebrate physiology*. University of Oregon Publications, Eugene, Oregon.

Rudy, P. P., 1966. Sodium balance in *Pachygrapsus crassipes*. *Comp. Biochem. Physiol.*, **18**: 881–907.

Shaw, J., 1959. Salt and water balance in the east african fresh-water crab *Potamon niloticus* (M. Edw.). *J. Exp. Biol.*, **36**: 157–176.

Sokal, R. R., and F. J. Rohlf, 1969. *Biometry*. W. H. Freeman and Co., San Francisco, 776 pp.

Thompson, L. C., 1970. Osmoregulation of the fresh water crabs *Metopaulias depressus* (Grapsidae) and *Pseudothelphusa jouyi* (Pseudothelphusidae). *Ph.D. thesis, University of California*, Berkeley, 101 pp. (University Microfilms/Dissertation Abstracts International No. 71–9938.)

Waterman, T., 1941. A comparative study of the effects of ions on whole nerve and isolated single nerve fiber preparations of crustacean neuromuscular systems. *J. Cell Comp. Physiol.*, **18**: 109–126.

Webb, D. A., 1940. Ionic regulation in *Carcinus maenas*. *Proc. R. Soc. London, B. Biol. Sci.*, **129**: 107–136.

Wong, J. M., and R. F. H. Freeman, 1976. Osmotic and ionic regulation in different populations of the New Zealand freshwater crayfish *Paranephrops zealandicus*. *J. Exp. Biol.*, **64**: 645–663.

Wood, J. L., A. M. Jungreis, and W. R. Harvey, 1975. Active transport of mangesium across the isolated midgut of *Hyalophora cecropia*. *J. Exp. Biol.*, **63**: 313–320.

Reference: *Biol. Bull.*, **157**: 234–248. (October, 1979)

DIETARY FATTY ACID AND TEMPERATURE EFFECTS ON THE PRODUCTIVITY OF THE CLADOCERAN, *MOINA MACROCOPA*

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Analyses of the relationships between physical parameters and the quantity and quality of food in relation to zooplankton physiological processes such as ingestion, assimilation (growth) and reproduction have been scarce. These studies are difficult since experimental techniques have lacked means to keep biotic factors constant while modifying a physical factor, or vice versa. As a result, the nutritional quality of a particular food under certain physical conditions has not been evaluated.

The study of interactions between the quantity and quality of food available for a zooplankter and existing physical parameters is very important. The structure of zooplankton communities may be partially determined by the relative abilities of fauna to efficiently utilize and process the available food and thereby satisfy their individual nutritional requirements.

Investigators who have classified the nutritional suitability of a particular food for a predator have observed effects on ingestion, assimilation and reproduction. No study has adequately controlled experimental conditions so that the interrelationships among a physical factor, the chemical composition of diet, and the population dynamics of a zooplankton species could be identified.

Stuart, McPherson, and Cooper (1931) studied the relative value of a variety of bacterial species as food for the aseptic cladoceran, *Moina macrocopa*. They found differences in growth, fertility, and fecundity. Lefevre (1942) demonstrated that the normal growth and reproduction of various cladoceran species were dependent upon the suitability (physical or physiological) of species of fresh water algae. Monoxenic culture of two species of Crustacea, *Artemia salina*, and *Tigriopus japonicus*, an harpacticoid copepod, by Provasoli, Shiraishi and Lance (1959) demonstrated that many unialgal diets either failed to permit growth to adulthood or allowed only a few consecutive generations. Interestingly, a phytoplankton species that was nutritionally good for one species was not always good for the other species. The apparent nutritional deficiencies of some of the unialgal diets could often be rectified by the addition of specific vitamins or other organic compounds (Shiraishi and Provasoli, 1959). Lee, Tietjen, and Garrison (1976) observed a seasonal "switching" of nutritional requirements for *Nitocra typica*, an harpacticoid copepod from salt marsh aufwuchs communities. In these studies the comparative nutritional value of unialgal diets of various species and strains was determined by growth rate and fecundity measurements. In some cases the nutritional adequacy of a particular algal diet was temperature dependent. Guerin and Gaudy (1977) and Gaudy and Guerin (1977) grew the harpacticoid copepod, *Tisbe holothuriae*, on a variety of artificial chemically undefined particulate diets. The dry weight, elementary chemical composition, fecundity, sex ratio, and result-

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ing population dynamics of this species were significantly affected by the quality of food. Schindler (1971) showed that the assimilation efficiencies of the copepods *Diaptomus gracilis, Cyclops strennus,* and the cladoceran, *Daphnia longispina* are dependent upon the type of food eaten. Roman (1978) speculated that the successful association of the blue-green alga *Trichodesmium* and the harpacticoid copepod *Macrosetella gracilis* may be due to the copepod's efficient conversion of the carbon and nitrogen fixed by the alga into secondary production.

A precise understanding of how zooplankton population dynamics are influenced by the chemical composition of the diet can only be realized when proper conditions will permit the experimenter to independently control qualitative and quantitative changes. The axenic culture of a zooplankter on a chemically defined artificial diet would satisfy such controlled conditions. Such a diet must permit not only adequate ingestion, digestion, and assimilation but also continuous reproduction so that large populations and successive generations may be studied.

The biphasic medium for the axenic culture and continuous reproduction of *Moina macrocopa* formulated by Conklin and Provasoli (1978) provides for the precise study of food/zooplankton relationships. The medium consists of a soluble phase and a particulate phase. Water soluble vitamins, nucleic acids, amino acids, and inorganic salts comprise the soluble phase, while coagulated protein-starch and protein-lipid particles comprise the particulate phase. The medium has permitted the first analysis of the nutritional requirements of a crustacean (Conklin and Provasoli, 1977). By employing this medium the experimenter can control the chemical composition of the diet as well as the mode of presentation to the herbivore (*i.e.*, number of particles/ml, amount of chemical compound/particle). Since the particles, unlike natural phytoplankton and bacteria, are inert to the chemical or quantitative changes associated with physical environmental factors, precise relationships between diet composition and some physical variable can be determined.

This study is directed toward analysis of dietary fatty acid and temperature interrelationships and the effects upon the productivity of *Moina macrocopa.* Undoubtedly there are other chemical compounds which affect the normal growth and reproduction of *Moina* (Conklin and Provasoli, 1977). Experimentation with fatty acids was chosen because, for this class of compounds, there are unique compositional differences amongst the orders of algae (Wood, 1974) and because past research demonstrates the essentiality of lipid factors for sustained fertility in *Moina macrocopa* and *Daphnia magna* (Provasoli, Conklin, and D'Agostino, 1970; Viehoever and Cohen, 1938).

The importance of dietary lipids for the Crustacea was also inferred from the research on the phylogenetically related insects; lipids affect their metamorphosis, diapause, fertility, and fecundity (Beck, Lilly, and Stauffer, 1949; Vanderzant, Kerue, and Reiser, 1957; Tamaki, 1961; Dadd, 1960; Nayar, 1964; Adkisson, Bell and Wells, 1963; Bull and Adkisson, 1960; Foster and Crowder, 1976; Ikan, Stanic, Cohen and Shulov, 1970; Chumakova, 1962).

Materials and Methods

The organism employed in this research was *Moina macrocopa americana,* a member of the order Cladocera. Descriptions and illustrations may be found in

Goulden's monograph of the Moinidae (Goulden, 1968). Reproduction occurs primarily by parthenogenesis. At times sexual females and males will appear in populations and sexual reproduction will occur (D'Abramo, in preparation). Parthenogenetic eggs are released from a pair of ovaries into a brood pouch. The neonates are born viviparously and are miniature images of the adults. Neonates pass through four stages of growth (instars) before becoming sexually mature. During the fourth or adult instar a female lays her first brood of eggs. Generation time is temperature-dependent but is rapid compared to many other Crustacea (4–5 days at 26° C).

Axenization

A serial dilution method as described by Conklin and Provasoli (1978) was employed to free the experimental organisms from bacteria. Recently born neonates were washed for 15 min in 15 separate baths composed of 10 ml of DM_7 medium (Provasoli and D'Agostino, 1970) in 6-cm diameter Petri dishes. All baths contained two drops of an antibiotic mix (D'Agostino and Provasoli, 1970). The eighth bath of the series contained a suspension of bacteria-free *Chlamydomonas reinhardii* (GMS^-), to allow for feeding and clearing the gut of bacterial flora. After the entire washing procedure, individual animals were transferred to 20 × 125 mm screwcap test tubes containing 10 ml of DM_7 and a suspension of *C. reinhardii*. After two days growth at room temperature under a bank of cool white fluorescent bulbs, 0.5 ml of the culture medium was dispensed into semisolid DA medium (D'Agostino and Provasoli, 1970) for detection of any bacterial or fungal contaminants. This sterility test was incubated at room temperature for 15 to 20 days.

Once monoxenic cultures were established and found aseptic, young were aseptically transferred to 20 × 125 mm screwcap test tubes that contained 10 ml of a sterile artificial medium, $K_7$33, which is a modification of the artificial medium developed for *Moina* by Conklin and Provasoli (1977) (Table I). Acclimation to the artificial medium occurred within three generations and was determined by visual observation of the number of progeny per brood and of a normal swimming behavior. Culturing of animals fed artificial food was done in the darkness at room temperature. Growth in the dark discourages algal reproduction and all traces of algal cells generally disappeared by the third transfer in artificial media.

Populations of *Moina* were maintained on artificial and natural diets. Maintenance populations on artificial medium $K_7$33 were incubated at 19° C in darkness to prevent photodeterioration of riboflavin. Growth at this temperature slowed population growth rates, thereby increasing the time between transfers. Populations fed *Scenedesmus nacgalii* (Chodat) and *C. reinhardii* (GMS^-) were grown at 19° C with a 16:8 L/D photoperiod. New cultures of natural and artificial diets were usually started every three weeks.

Diet formulation

The artificial media used in the experimentation with *Moina macrocopa* were modifications of the F1 medium of Conklin and Provasoli (1977). Modifications may be found in Table I. The media contained two types of particles, starch-

TABLE I

Modifications of Conklin-Provasoli F1 Medium.

Common basal medium (per cent w or v/v)

Changes: (a) Metal mix P II to metal mix L; Metal mix L, 1 ml = $Na_2EDTA \cdot 2H_2O$, 3.81 g; Zn(as $SO_4^=$), 0.30 mg; B, 0.12 mg; Mn(as Cl^-), 0.087 mg; Fe(as $NH_4SO_4^-$), 0.06 mg; Co(as Cl^-), 0.024 mg; Cu(as $SO_4^=$), 0.024 mg; Mo(as NH_4^+), 0.036 mg.

(b) Liver infusion L25 at 70 mg to defatted liver infusion L25 (lipid extraction by chloroform/methanol (2/1) (v/v) for three hr under N_2) at 40 mg.

Additions: (a) glycogen 10 mg

(b) globulin + 2X crystalline egg albumin, 1 ml, 1 ml = bovine α globulin (Fraction IV) 2.25 mg + 2X crystalline egg albumin (ICN), 0.75 mg. Mixture is formed by dissolving components in water; coagulating this mix by autoclaving; homogenizing (2600 rpm for 5 min) the coagulum to produce particles; autoclaving the particles and rehomogenizing.

Deletions: (a) DF_2

Particles

Changes: (a) SA gel particles: 1 ml = 10 mg rice starch + 4.10 mg 2X crystalline egg albumin.

(b) FV particles: 1 ml = 8 mg 2X crystalline egg albumin + 0.75 egg lecithin + 1 mg BHT (butylated hydroxytoluene) + 0.66 ergocalciferol + 0.25 retinopalmitate + 2 mg dl-αtocopherol + fatty acids in variable quantities and qualities, depending upon experiment. Fatty acids for $K_7$33 medium: 1 mg palmitic acid + 0.3 mg oleic acid + 0.7 mg linoleic acid + 1 mg α-linolenic acid.

Artificial medium

Basal medium 96 ml + 3 ml SA gel + 1 ml FV particles, pH = 8.0, Particle concentration = 540×10^3 ml

protein and lipid-protein. All inorganic and organic additions were made from prepared stock solutions. Generally inorganic stock solutions were stored at room temperature while organic solutions or suspensions were stored either at 8° C or frozen. To prevent bacterial contamination, 0.5 ml of a volatile preservative solution (Hutner and Bjerknes, 1948) was added to all stock solutions. The volatile preservative vaporizes during autoclaving. Stock solutions were usually renewed within a four-month period. All fatty acids used in the diets were 99+% pure and were stored frozen under a N_2 atmosphere. Prepared media varied in the qualitative and quantitative composition of fatty acids absorbed onto the protein particles. Qualitative fatty acid additions were made to simulate the unique proportional differences found amongst four orders of algae, Cyanophyceae, Cryptophyceae, Chlorophyceae, and Bacillarophyceae (Table II).

Since the work involved the effect of quantitative and qualitative changes in dietary fatty acids upon the productivity of *Moina*, concern developed regarding the possible differential uptake of particular fatty acids by the albumin. To insure that results could be genuinely attributed to particular diets, particles prepared 2 months previously were subjected to a lipid compositional analysis by thin layer and gas chromatographic techniques. These analyses were kindly performed by Dr. David H. Beach of the Department of Microbiology, State University New York, Upstate Medical Center at Syracuse. Lipid analysis of the particles revealed

TABLE II

Fatty acid mixtures simulating average percent composition of representative algal classes (total fatty acids = 3 mg).

Fatty acids (mg)	Mixtures			
	Cyanophyceae[1]	Chlorophyceae	Cryptophyceae	Bacillariophyceae
14:0 (myristic)	—	—	0.30	—
16:0 (palmitic)	1.25	1.00	0.45	0.75
16:1 (palmitoleic)	0.80	0.10	—	1.20
18:0 (stearic)	0.12	—	—	—
18:1ω9 (oleic)	0.30	0.25	0.30	0.15
18:2ω6 (linoleic)	0.40	0.35	0.30	—
18:3ω3 (α-linolenic)	0.13	1.30	0.90	—
20:5ω3 (eicosapentaenoic)	—	—	0.45	0.90
22:6ω3 (docosahexaenoic)	—	—	0.30	—

[1] The 18:4 (octadecatetraenoic acid) which comprises from 15 to 30% of the cryptophyceae fatty acids was not incorporated into the mixture because of the lack of a conveniently available and highly pure (99 + %) source.

that the experimentally intended dietary differences, both qualitative and quantitative, were genuine. The lipophilic albumin had no tendencies toward differential absorption of the fats and vitamins. Uptake was complete.

Determination of particle size and particle concentration—optical transmission relationships

The number of particles in media of different particle concentrations was determined through the use of a Coulter Counter (Model Z_B, Counter Electronics, Hialeah, Florida). The counter was calibrated with the use of 10.2-μ diameter pollen. Counts of artificial particles were performed using a 100-μ aperture, an amplification of four and an aperture current equal to one half. The lower and upper threshold settings were 1 and 40, respectively. The threshold factor measured as the average volume of the known system divided by the lower threshold dial setting at half count equalled 16.8186. Particle size frequency distribution for a sample was determined using a Coulter Channelyzer (base channel threshold = 1, window width = 100) and an X-Y Recorder II for automatic plotting. The size of the particles was determined by employing the formula, Channel number × window width/100 + Base Channel Threshold × Threshold Factor = cubic microns.

Aliquots of variable volumes from samples of percent transmissions ranging from 45 to 85% were diluted to 20 ml by means of a special electrolyte solution, Isoton II (Curtin Matheson Scientific, Inc.). From these diluted suspensions ten separate 500-μl samples were counted, from which an average was computed. Counts for these samples ranged from 10,000 to 30,000 per 500 μl. Background counts did not exceed 100 per 500 μl. The derived average particle concentration (number per 500 μl) was then multiplied by the dilution factors to obtain the number of particles per ml. A standard curve relating particle concentration (number/ml) to per cent transmission was then constructed. Particle concentration within the media was altered by additions from the stock mixtures. All prepared media were

adjusted to pH 8.0 with a pH meter and were then dispensed with a macropipette (Macroset-Oxford Laboratories) as 10 ml aliquots into 20 × 125-mm screw cap culture tubes (Pyrex # 9825). The media in the culture tubes were then autoclaved, and, after cooling to room temperature, were stored at 8° C until use. Experiments involving the comparative quality of diets were all performed with media containing the same initial number of particles. Normal concentration was 540×10^3 particles/ml.

Preparation of inoculum and productivity determinations

To determine the relative nutritive quality of diets with each particular fatty acid composition, 10 to 12 first instar females were inoculated into separate culture tubes containing the same variable. These inocula were neonates of the first and second broods of single females that had been previously isolated from maintenance cultures and grown at 19° C in 20 × 125-mm screw cap culture tubes containing 10 ml of K_733 media. From these, replicate inocula populations were allowed to develop by parthenogenesis at various temperatures and defined times. The particles of the artificial media were kept in suspension by daily mixing with a Vortex-genie mixer (Scientific Industries, Inc.). Particle sedimentation rates were slow and most of the particles (*ca* 70%) would remain in suspension over a 24-hr period. From the 10 to 12 growing populations a set of three or four culture tubes were harvested at three different predetermined particle concentrations (60–70%, 70–80%, 80%+ optical transmission at 650 nm). Optical transmission was measured by an instrument similar to a Spectronic 20 (Bausch and Lomb) but modified to accept culture tubes.

The sequential harvesting at different particle concentrations permitted an analysis of the changing structure of the *Moina* populations through time. Animals were killed by the addition of 0.5 ml of ethyl alcohol and then were transferred by pipette to a modified Bogorov counting tray (Wickstead, 1965). With the aid of a binocular stereoscope (36×) each individual comprising a population was counted, sized, and categorized. Categories included female instar I-IV, male instar I-IV, adult females, adult males, ephippial females, and ephippia. Measurements of the animals were made with the use of an ocular micrometer. Measurements were made anteroposteriorly, from a point just distal to the eye to the caudal tip of the carapace.

Average dry weights of the four female instars were determined by selecting a sufficient number of animals of a particular instar and placing them on a pretared aluminum dish. These samples were then dried at 60° C for 24 hr in a laboratory oven (Model 10–200C, Grieve-Hendry, Co., Chicago, Ill.), cooled for one hr in a desiccator, and weighed immediately on a Cahn gram electrobalance (Model G-Cahn Instrument Co., Paramount, California). Additional weighings of a sample were performed until no change in weight could be detected. Average dry weight of a particular instar was determined by dividing the biomass in the dish by the number of instars which comprised the sample. Three samples of well-fed animals were taken for the average weight determination of each instar. Each sample was derived from a population growing on a different diet. A $\log_{10}$-$\log_{10}$ plot of average length of an instar *vs.* average dry weight indicated that

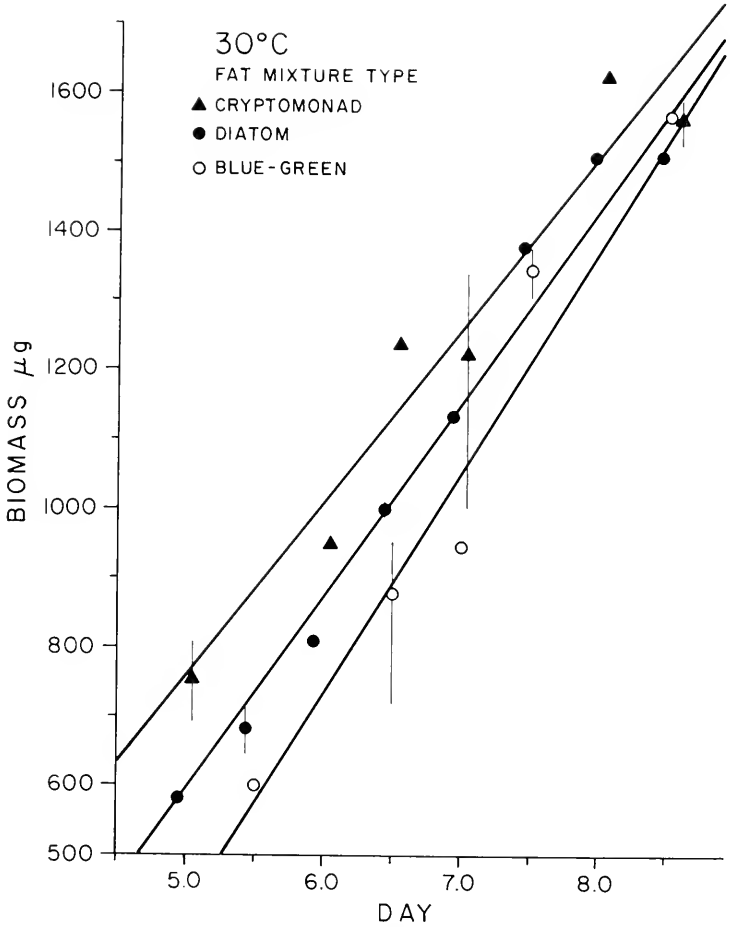

FIGURE 1. Fatty acid composition dependent qualitative effect. 30° C; diets containing cryptomonad, diatom, and blue green fatty acids at 3 mg%.

the dry weight of *Moina* increases as the cube of the body length. The equation is: dry weight = 1.1 body length^3; corr. = 0.9992. The total biomass of a population of *Moina* was then determined by converting lengths of each individual comprising the population into dry weight and summing the total.

Productivity was determined by dividing the total biomass of an harvested population by the total number of days since the appearance of the first brood of the original female inoculum. The day when the inoculum's first brood appeared was considered day one in order to eliminate some of the observed individual variability associated with time to maturity and thereby provide comparable data among samples of the same medium.

Many of the populations analyzed contained males and ephippial females but their presence did not affect productivity calculations since they invariably had not attained sexual maturity when the populations were harvested.

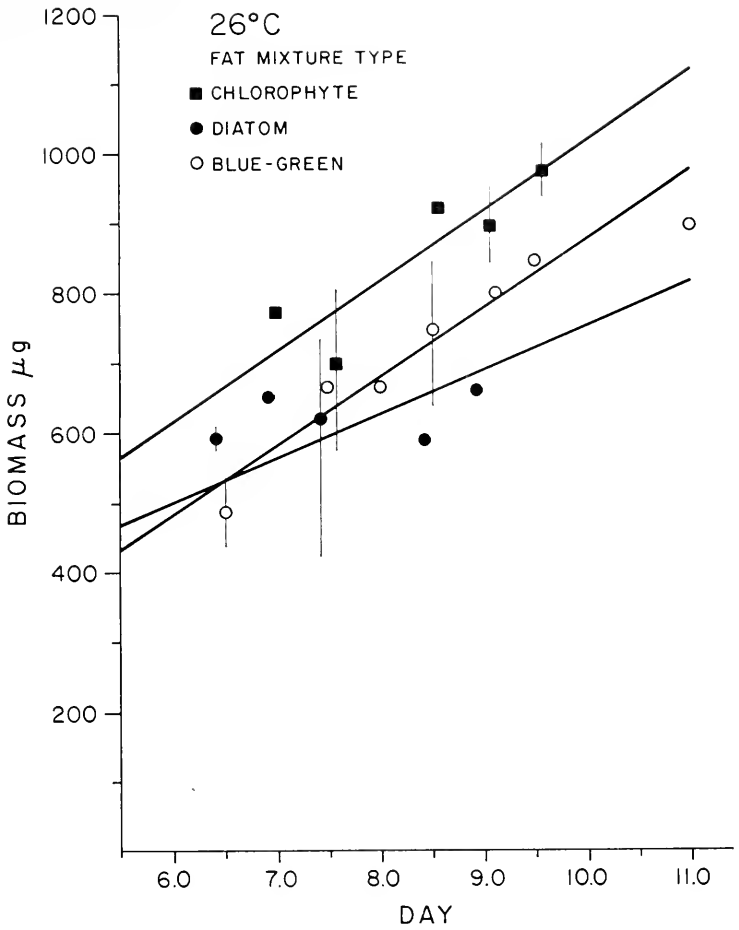

FIGURE 2. Fatty acid composition dependent quantitative effect. 26° C; diets containing chlorophyte and blue green fatty acids at 3 mg% and diatom fatty acids at 1.5 mg%.

All experimental populations were incubated in darkness. This procedure was used to eliminate the photoperiod factor which has been associated with the transition to sexuality in some species of Cladocera and to prevent the photodegradation of riboflavin, a required vitamin present in all media. Exposure to light was minimal and occurred only when cultures were shaken or when optical transmission measurements were taken. The source of light was indirect, consisting of a 10 watt light bulb fitted into a photographic safelight stand which was covered with red acetate film. Within the optical transmission apparatus red acetate material covered the slit (passage) between the light source and the area where the culture tube was positioned for a measurement. Light transmitted by the red acetate material was 600 to 700 nanometers. Past research indicates that Cladocera are minimally responsive to wavelengths in this region in both visual and nonvisual sensitivity (Scheffer, Robert, and Medioni, 1958).

Results

Particles

Two types of particles were used as food in all media. It was important, therefore, to determine their size distribution. Although formulated separately, starch-protein and lipid-protein particles did not differ in size distribution. Particle size ranged from 2 to 20 μ^3 with the majority of particles (80%) in the 2 to 10 μ^3 range. Because of the similar size distributions it was assumed that there was no different sedimentation rate which could affect the probabilities of ingestion of the two types of particles.

Effects of dietary fatty acids and temperature on productivity

A series of experiments was done to define the effect of varying the quantity and quality of dietary fatty acids at different temperatures. Results of the experiments are shown in Figures 1, 2, and 3. Points of graphs represent either a single determination or an arithmetic average of multiple (2–4) determinations of biomass of populations developed on particular diets at selected days of harvest. Points derived from multiple observations include the range of values (vertical bars). Computed regression lines are included. Mortality within populations did not affect productivity determinations since, in most cases, the maximum length of an experimental period never exceeds the life span of *Moina*. If mortality was observed the population was discarded.

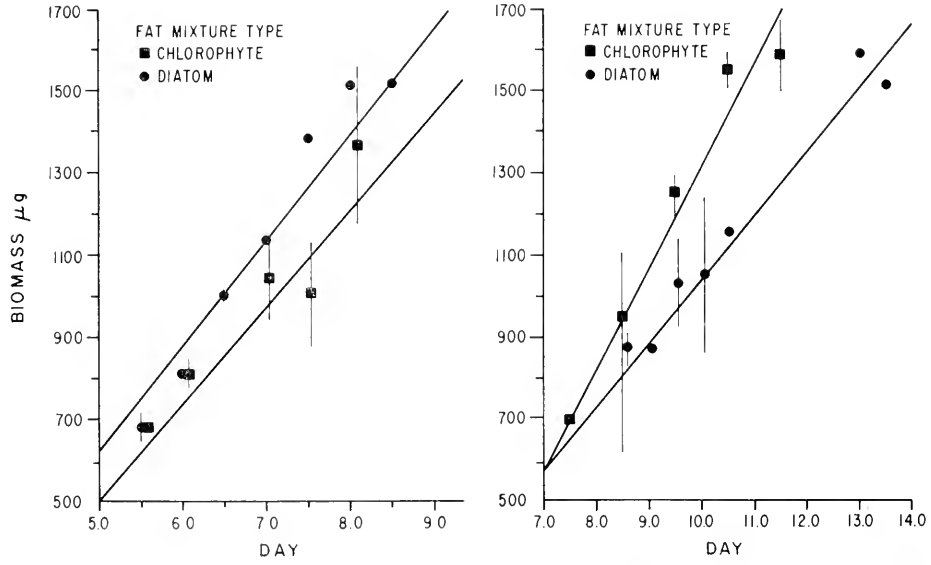

Figure 3. Fatty acid composition dependent temperature effect. Diets containing chlorophyte and diatom fatty acids at 3 mg%, 30° C and 22° C.

TABLE III

Productivity (μg/day, $\bar{x} \pm$ s.d.)—temperature relationships; diets containing green or cryptomonad fatty acids at 3 mg%. Initial particle concentration = 540 × 10^3/ml, (N) = number of observations.

Diet	Fatty acid concentration	Temperature				
		30° C	26° C	22° C	18° C	14° C
Cryptomonad	3 mg%	173.4 ± 22.4 (11)	163.4 ± 31.1 (13)	122.4 ± 32.5 (10)	58.0 ± 7.1 (12)	49.2 ± 8.0 (12)
Blue green	3 mg%	155.0 ± 31.6 (11)	151.3 ± 27.3 (10)	108.4 ± 19.6 (12)	51.4 ± 5.5 (11)	44.5 ± 7.0 (12)

TABLE IV

The relationship of productivity (μg/day, $\bar{x} \pm$ s.d.) and concentration of dietary fatty acids. Diets containing chlorophyte fatty acids, 3 and 6 mg% at 22° C. Diets containing cryptomonad fatty acids at 1.5 mg%, 3.0 mg% and 6 mg% at 26°, 22°, and 14° C. Initial particle concentration = 540 × 10^3/ml, (N) = number of observations.

Diet	Fatty acid concentration	Temperature		
		26° C	22° C	14° C
Cryptomonad	1.5 mg%	89.2 ± 12.7 (13)	89.4 ± 15.7 (11)	36.0 ± 7.7 (13)
Cryptomonad	3.0 mg%	163.4 ± 31.2 (13)	122.4 ± 32.5 (10)	49.2 ± 8.0 (12)
Cryptomonad	6.0 mg%	127.0 ± 14.7 (11)	93.1 ± 10.3 (10)	35.8 ± 6.4 (11)
Chlorophyte	3.0 mg%		125.1 ± 20.1 (11)	
Chlorophyte	6.0 mg%		113.4 ± 14.0 (13)	

Figure 1 illustrates the qualitative effect of dietary fatty acid composition. At 30° C and 3 mg% total fatty acids, a diet containing cryptomonad-type fatty acids is more productive than those of diatom and blue green type fatty acids. Figure 2 depicts the quantitative effect of dietary fatty acid composition. In this experiment the three diets were supplied at one-half the normal total particle concentration and the content of total fatty acids per particle was varied. At 26° C the diatom type fatty acid diet containing 1.5 mg% of fatty acids is as productive as the blue green type diet which contains 3.0 mg% fatty acid. The chlorophyte fatty acid diet at 3.0 mg% fatty acid concentration is more productive than both. Hence at 26° C the qualitatively poor blue green diet barely equalled the productivity of the qualitatively better diatom diet even with twice the total calorific content.

Figure 3 exemplifies the effect of temperature in relation to the quality of dietary fatty acids. At 30° C and 3 mg% fatty acid concentration, the diet containing diatom fatty acids is slightly more productive than the diet containing the chlorophyte fatty acids. This relationship is inversed at 22° C.

One way ANOVA indicates that productivity differences between chlorophyte (3 mg%) and diatom (1.5 mg%) fatty acid diets at 26° C, the chlorophyte (3 mg%) and blue green (3 mg%) fatty acid diets at 26° C and the chlorophyte (3 mg%) and diatom (3 mg%) fatty acid diets at 22° C are significant ($P < 0.05$).

The productivity of every diet decreased as the incubation temperature decreased. This relationship is illustrated in Table II where productivity values of a cryptomonad fatty acid diet at 3 mg% fatty acid and a blue green fatty acid diet at 3 mg% fatty acids are listed at five different temperatures, 30°, 26°, 22°, 18°, and 14° C. One way ANOVA indicates that differences between these diets at 22° and 18° C are significant ($P < 0.05$).

Increasing the total amount of fatty acids per particle in diets while keeping the starch-protein ratio constant at 1.5 : 1 had an effect on productivity at the three different temperatures studied. Three diets were made to contain cryptomonad-type fatty acids in concentrations of 1.5, 3.0 and 6.0 mg% respectively. Table III shows that at those temperatures investigated, 22°, 18°, and 14° C, productivity increased from 1.5 to 3.0 mg%. However, an increase to 6.0 mg% reduced productivity, indicating that a too high dietary fat content is inhibitory. An increase in total fatty acids from 3.0 to 6.0 mg% in the chlorophyte type fatty acid diet was also inhibitory at 22° C.

Discussion

Axenic culture of *Moina macrocopa* in almost chemically defined biphasic media has allowed a precise analysis of some dietary factors affecting its population dynamics, and particularly a better understanding of the combined effects of abiotic and biotic factors. It has been established that the chemical composition of food, particularly the type and content of fatty acids, can directly affect productivity. Qualitative fatty acid differences in the diets were formulated in an attempt to simulate the general composition that is unique to each of four orders of algae, Cyanophyceae, Chlorophyceae, Bacillarophyceae, and Cryptophyceae. Variations

in productivity amongst the diets can most probably be attributed to the combined effects of differences in generation time, brood size, and time between broods. The comparative quality of a particular diet can also be temperature dependent.

The poor quality, in general, of those diets containing Cyanophyceae-type fatty acids may partially explain the observed poor nutritional value of some species of blue-green algae (Arnold, 1971). A partial explanation of the different nutritiousness of various species of algae for zooplankton species may in fact be the qualitative and quantitative content of dietary fatty acid. Though no fatty acid analyses were performed on *Moina* organisms grown on particular diets, the notable differences in productivity of the various diets suggest that body fatty acids are derived entirely from dietary sources. The qualitative fatty acid composition of the lipids of *Moina* has been found to be significantly affected by diet (Watanabe, Arakawa, Kitajima, Fukusho, and Fujita, 1978). It appears that the zooplankter's capacity for efficient lipid biosynthesis or inter-conversion is poor. Fatty acids were found to be essential to fertility in *Moina* (Conklin and Provasoli, 1977).

The results of the research conducted with increased fatty acid quantities (1.5 mg%-3 mg%-6 mg%) suggest that subtle interrelationships can exist amongst macronutrient dietary components. Little or no increase in the productivity of *Moina* was attained by increasing the total cryptomonad fatty acids concentration from 3 to 6 mg% at the different temperatures. The same results occurred for a chlorophyte type fatty acid diet at 22° C. Protein and carbohydrate concentrations remained constant in these experiments. Such observations add to the convincing evidence that crustaceans cannot tolerate high levels of dietary lipid. Andrews, Sick, and Baptist (1972) demonstrated that a dietary lipid supplement (1/3 beef tallow, 1/3 corn oil, and 1/3 menhaden oil) at levels >10% adversely affected growth and survival in the shrimp *Penaeus setiferus*. Forster and Beard (1973) supplemented a shrimp meal based diet for *Palaemon serratus* with 7.5 and 15% levels of cod liver and corn oil. At the 15% level significant growth inhibition was observed for both lipid sources. The inhibitory effects of increased fatty acids in the diet may also partially explain the nutritional inadequacy of senescent algal cells. Twenty-five percent of the total dry weight of these cells is known to be fat (Fogg, 1965).

Optimal productivity of *Moina macrocopa* requires the presence of macronutrients in the proper proportions. Provasoli and D'Agostino (1969) showed that optimal starch-protein ratios for the growth of *Artemia salina* were 5 : 1 and 10 : 1. A 1 : 1 ratio was inhibitory. Hence, the potential quality of food sources cannot be entirely evaluated from considerations of calorific content.

The interesting physiological responses of *Moina* to varied nutrition permit some speculation. It seems plausible to assume that given the same food, the growth and reproductive capacities of species of zooplankton can be entirely different. Zooplankton community structure in an aquatic system may in part be determined by competitive processes whose outcome is based upon the satisfaction of nutritional requirements and/or the most efficient systems of biochemical assimilation and conversion.

The subtle nutritional interrelationships between predator and prey must be

identified if successful continuous culture of other phagotrophic invertebrates like *Moina* is to be realized. With a proper understanding of nutritional requirements and efficient energy budgets, the potential husbandry of marine particulate feeders such as lobsters, oysters, scallops, and shrimp can be greatly enhanced. Knowledge of crustacean nutrition may permit the use of cladoceran cultures as an alternative secondary sewage treatment. Filter feeding of particulates by the Cladocera would eliminate much of the potential biological oxygen demand (BOD) of primary treatment effluent and resulting populations could be harvested and used as fish food.

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Summary

1. The cladoceran, *Moina macrocopa americana* was cultured axenically on an artificial diet consisting of a particulate and soluble phase. The effect of changes in the quantitative and qualitative dietary fatty acid composition was investigated.

2. Qualitative fatty acid differences were made to simulate the unique proportional differences found among four orders of algae, Cyanophyceae, Chlorophyceae, Cryptophyceae, Bacillarophyceae.

3. The quality of dietary fatty acids available to *Moina* exerts an effect upon productivity and the nutritional value of a particular diet in relation to fatty acid composition can be temperature dependent.

4. Increased levels of fatty acids in the diet of *Moina* reduces productivity.

5. The cladoceran, *Moina macrocopa americana,* may be entirely dependent upon diet for its source of fatty acids.

6. A partial explanation for the differential nutritiousness of particular species of algae may be their qualitative and quantitative fatty acid content.

LITERATURE CITED

Adkisson, P. L., R. A. Bell, and S. G. Wells, 1963. Environmental factors controlling the induction of diapause in the pink bollworm, *Pectinophora gossypiella* (Sanders). *J. Insect Physiol.*, **9**: 299–310.

Andrews, J. W., L. V. Sick, and G. P. Baptist, 1972. The influence of dietary protein and energy levels on growth and survival of Penaeid shrimp. *Aquaculture* **1**: 341–347.

Arnold, D. E., 1971. Ingestion, assimilation, survival, and reproduction by *Daphnia pulex* fed seven species of blue green algae. *Limnol. Oceanogr.*, **16**: 906–920.

Beck, S. D., J. H. Lilly, and J. F. Stauffer, 1949. Nutrition of the European corn borer, *Pyranasta nubilalis* (Mubn.) *Ann. Entomol. Soc. Am.*, **42**: 483–496.

Bull, D. L., and P. L. Adkisson, 1960. Certain factors influencing diapause in the pink bollworm, *Pectinophora gossypiella*. *J. Econ. Entomol.* **53**: 793–798.

Chumakova, B. M., 1962. Significance of individual food components for the vital activity of mature predatory and parasitic insects. *Voprosy Ekologii i Biotsenologii,* **8**: 133–134. (From Biol. Abstr. 45, 44, 502, 1964).

Conklin, D. E., and L. Provasoli, 1978. Biphasic particulate media for the culture of filter feeders. *Biol. Bull.,* **154**: 47–54.

Conklin, D. E., and L. Provasoli, 1977. Nutritional requirements of the water flea, *Moina macrocopa*. *Biol. Bull.,* **152**: 337–350.

Dadd, R. H., 1960. The nutritional requirements of locusts. *J. Insect Physiol.,* **4**: 319–347.

D'Agostino, A. S., and L. Provasoli, 1970. Dixenic culture of *Daphnia magna,* Straus. *Biol. Bull.,* **139**: 485–494.

Fogg, G. E., 1965. *Algal cultures and phytoplankton ecology*. University of Wisconsin Press, Madison, Wis. 126 pgs.

Forster, J. R. M., and T. W. Beard, 1973. Growth and experiments with the prawn *Palaemon serratus* Pennant fed with fresh and compounded diets. *Fish Invest., Ser.* II., **27**(7): 16 pp.

Foster, D. R., and L. A. Crowder, 1976. Fatty acids of diapause and nondiapause pink bollworm larvae, *Pectinophora gossypiella* (Saunders). *Comp. Biochem. Physiol.,* **55B**: 519–521.

Gaudy, R., and J. P. Guerin, 1977. Dynamique des populations de *Tisbe holothuriae* (Crustacea: Copepoda) en evelage sur trois regimes artificiels differents. *Mar. Biol.* **39**: 137–145.

Goulden, C. E., 1968. The systematics and evolution of the Moninidae. *Trans. Am. Philos. Soc.,* New Series, **58**: 511–522.

Guerin, J. P., and R. Gaudy, 1977. Etude des variations du peids sec et de la constitution chimique elementaire de *Tisbe holothuriae* (Copepoda: Harpacticoida) eleve sur differents regimes artificiels. *Mar. Biol.,* **44**: 65–70.

Hutner, S. H., and C. A. Bjerknes, 1948. Volatile preservatives for culture media. *Proc. Soc. Exp. Biol. Med.,* **67**: 393–397.

Ikan, R., V. Stanic, E. Cohen, and A. Shulov, 1970. The function of fatty acids in the diapause of the Khapra beetle *Trogoderma granarium* Everts. *Comp. Biochem. Physiol.,* **37**: 205–214.

Lee, J. J., J. H. Tietjen, and J. R. Garrison, 1976. Seasonal switching in the nutritional requirements of *Nitocra typica,* an harpacticoid copepod from salt marsh aufwuchs communities. *Trans. Am. Microsc. Soc.,* **95**(4): 628–637.

Lefevre, M., 1942. L'utilisation des algues d'eau douce par les Cladoceres. *Bull. Biol. Fr. Belg.,* **76**: 250–276.

Nayar, J. K., 1964. The nutritional requirements of grasshoppers. *Can. J. Zool.,* **42**: 11–22.

Provasoli, L., D. E. Conklin, and A. S. D'Agostino, 1970. Factors inducing fertility in aseptic Crustacea. *Helgol. Wiss. Meeresunters.,* **20**: 443–454.

Provasoli, L., and A. S. D'Agostino, 1969. Development of artificial media for *Artemia salina*. *Biol. Bull.,* **139**(3): 434–453.

Provasoli, L., K. Shiraishi, and J. R. Lance, 1959. Nutritional idiosyncrasies of *Artemia* and *Tigriopus* in monoxenic culture. *Ann. N.Y. Acad. Sci.,* **77**: 250–261.

Roman, M. R., 1978. Ingestion of the blue green alga Trichodesmium by the harpacticoid copepod, *Macrosetella gracilis*. *Limnol. Oceanogr.* **23**(6): 1245–1247.

Scheffer, D., P. Robert, and J. Medioni, 1958. Reactions oculomotrices de la Daphnie (*Daphnia pulex* DeGeer) en response a des lumicres monochromatiques a egale energie. Sensibilite visuelle et sensibilite dermatopique. *C.R. Seances Soc. Biol. Fil.,* **152**: 1000–1003.

Schindler, J. R., 1971. Food quality and zooplankton nutrition. *J. Anim. Ecol.,* **40**: 589–595.

Shiraishi, K., and L. Provasoli, 1959. Growth factors as supplement to inadequate algal food for *Tigriopus japonicus*. *Tohoku J. Agri. Res.,* **10**: 89–96.

Stuart, C. A., M. McPherson, and H. J. Cooper, 1931. Studies on bacteriologically sterile *Moina macrocopa* and their food requirements. *Physiol. Zool.,* **4**: 87–100.

Tamaki, Y., 1961. Studies on nutrition and metabolism of the smaller tea tortix *Adcrophyes orana*. II. An essential factor for adult emergence. *J. Appl. Entomol. Zool.,* **5**: 58–63.

VANDERZANT, E. S., D. KERUE, AND R. REISER, 1957. The role of dietary fatty acids in the development of the pink bollworm. *J. Econ. Entomol.*, **50**: 606–608.

VIEHOEVER, A., AND J. COHEN, 1938. The response of *Daphnia magna* to vitamin E. *Am. J. Pharm.*, **110**: 297–315.

WATANABE, T., T. ARAKAWA, C. KITAJIMA, K. FUJUSHO, AND S. FUJITA, 1978. Nutritional quality of living feed from the viewpoint of essential fatty acids for fish. *Bull. Jap. Soc. Sci. Fish.*, **44**(11): 1223–1227.

WICKSTEAD, J. H., 1965. *An introduction to the study of tropical zooplankton.* Hutchinson and Co., Ltd., London, 160 pp.

WOOD, B. J. B., 1974. Fatty acids and saponifiable lipids. Pages 236–265 in W. D. P. Stewart, Ed., *Algal Physiology and Biochemistry*, Blackwells, Oxford.

Reference: *Biol. Bull.*, **157**: 249–257. (October, 1979)

DIFFERENCES IN STARFISH OOCYTE SUSCEPTIBILITY TO POLYSPERMY DURING THE COURSE OF MATURATION[1]

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It is known that the eggs of marine invertebrates such as the sea urchin and starfish lose the capability to fertilize or to develop normally when they are maintained in sea water for too long a period after being shed (Goldforb, 1935; Clark, 1936). In starfish oocytes, the optimum period for insemination is between germinal vesicle breakdown and the formation of the first polar body (Fol, 1879; Delage, 1901); insemination after the formation of the first polar body results in a decreased rate of subsequent normal development (Lillie, 1915).

Some earlier investigators reported that starfish oocytes, when inseminated in sea water for several hours, lose their capacity to resist polyspermy (Chambers, 1923; Clark, 1936). Thus, the decreased rate of normal development after the formation of the first polar body may be related to polyspermy. On the other hand, Chambers and Chambers (1949) have suggested that during the course of oocyte maturation, there is a specific period of ripening of cytoplasm when insemination would result in normal fertilization and development; after this period there is a decline in proper functional interrelation between the sperm and cytoplasm.

The present paper reports findings in support of the work of Chambers (1923) and Clark (1936).

Materials and Methods

Oocytes from *Asterina pectinifera* and *Asterias amurensis* were used. The starfish were collected at the seashore near the Marine Biological Station of Tohoku University, Asamushi. Specimens of *Asterina pectinifera* were collected in September and kept in an aquarium supplied with circulating cold sea water at 10 to 13° C for two months. Specimens of *Asterias amurensis* were collected in April and maintained in running sea water tanks at 7 to 9° C for a month in the laboratory.

Asterina pectinifera. All but a few of these oocytes showed no conspicuous change when isolated from the ovary in sea water. The isolated full-grown oocytes with germinal vesicles were prepared by tearing the ovaries with forceps in sea water and washed three times with sea water. The oocytes were kept for at least 1 hr to make sure that they did not undergo spontaneous maturation.

Asterias amurensis. These oocytes usually undergo spontaneous maturation when

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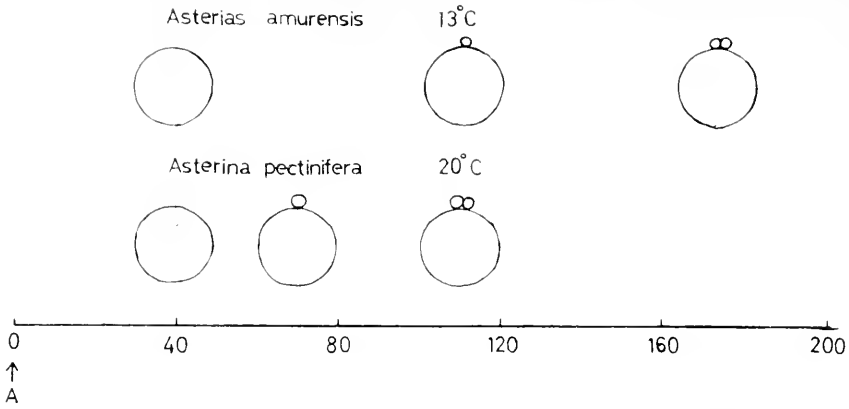

FIGURE 1. The time course of oocyte maturation in *Asterina pectinifera* and *Asterias amurensis*. This shows the time (min) when germinal vesicle breakdown occurs, and the first and second polar body form. 1-Methyladenine was added at A.

isolated in sea water. To obtain oocytes, ovaries were directly immersed in 1-methyladenine sea water. Full-grown oocytes which began germinal vesicle breakdown were spawned and these oocytes were used.

1-Methyladenine. 1-Methyladenine (Sigma) dissolved in deionized water at a concentration of 10^{-3} M was served as stock solution, and diluted with sea water to a concentration of 10^{-6} M before use.

Schedule of oocyte maturation. As shown in Figure 1, in an oocyte of *Asterina pectinifera*, the germinal vesicle disappears completely 40 min after being placed in 1-methyladenine sea water (10^{-6} M) and the first polar body forms at 65 to 75 min and the second polar body at 105 to 115 min, respectively at 20° C. In *Asterias amurensis*, oocytes which began germinal vesicle breakdown were released from ovary 40 min after ovary was immersed in 1-methyladenine sea water (10^{-6} M). The first polar body forms at 105 to 115 min and the second polar body at 170 to 180 min at 12° C.

Spermatozoa. To obtain a sperm suspension, an isolated testis was torn with forceps in an empty Petri dish. Before use, 10 μl of semen was sucked up into a micropipette and diluted into 10 ml of 10^{-4} M histidine sea water to increase the sperms' motility. These sperm suspensions were diluted from 10-fold to 100,000-fold with fresh sea water and the number of spermatozoa was counted with haematometer. Inseminations were carried out by mixing 9 ml of oocyte suspension with 1 ml of sperm suspension.

Cytological procedures. Examined oocytes were fixed with Carnoy's solution and were sectioned by the usual paraffin method. The sections, 10 μ in thickness, were stained with Feulgen's reaction and counterstained with fast green.

Experimental procedures. The capacity of oocytes for normal development was investigated as follows; oocytes of *Asterina pectinifera* were used. Isolated immature oocytes were immersed in 1-methyladenine sea water (10^{-6} M) at 20° C and then, one part of these oocytes was transferred to another Petri dish containing fresh sea water and inseminated at one period, the other part was transferred to another Petri dish and inseminated at another period, respectivly, so on. Oocytes were 2000 to 2500 per 10 ml sea water and concentration of sperm was finally 2×10^6 per ml. The number of the oocytes which formed fertilization membranes was counted 5 min after each insemination and then these oocytes were kept for 20 hr at 20° C. The oocytes were allowed to develop to early gastrula and the number was counted under a light microscope.

The occurrence of normal first cleavage was checked as follows: the ovaries of specimens of *Asterias amurensis* were directly immersed in 1-methyladenine sea water (10^{-6} M) and one part of released oocytes (2000–2500) was transferred to Petri dishes containing 10 ml fresh sea water at 40, 110, 150, and 180 min after the immersion in 1-methyladenine sea water and inseminated respectively. The concentration of sperm used was finally 8×10^5 per ml. Formation of fertilization membrane and first cleavage were observed 2.5 hr after each insemination. This experiment was performed at 12° C.

The relation between occurrence of abnormal cleavage and sperm concentration was examined as follows: oocytes of *Asterias amurensis* (2000–2500) were transferred to Petri dishes containing 10 ml fresh sea water at 40 and 180 min after addition of 1-methyladenine sea water (10^{-6} M) and inseminated with various sperm concentrations which were from 8×10^1 to 8×10^6 per ml, respectively. Formation of fertilization membrane and first cleavage were checked at 2.5 hr after each insemination. This experiment was done at 12° C.

Results

Oocytes of *Asterina pectinifera* were immersed in 1-methyladenine sea water for various periods ranging from 40 to 180 min. The oocytes were inseminated in fresh sea water, at various intervals, after being placed in 1-methyladenine sea water. The formation of fertilization membrane was checked 5 min after insemination, and then the oocytes were continuously kept in sea water for 20 hr; at the end of the experiment the number of oocytes which developed to early gastrula was counted under a light microscope.

When inseminations were performed between 40 and 80 min after being placed in 1-methyladenine sea water, 73% of the oocytes developed to early gastrula. Forty-five per cent developed to gastrula, when inseminated at 120 min (period of the formation of second polar body) and only 2% at 180 min, respectively (Fig. 2).

Usually, germinal vesicle breakdown occurs spontaneously in a few per cent of the oocytes after isolation in sea water without 1-methyladenine application in *Asterina pectinifera* oocytes. In order to synchronize the course of oocyte maturation, isolated oocytes were kept in sea water for 1 hr and then spontaneously matured oocytes were checked. In this experiment, the mean rate of spontaneous maturation was 19%. For these spontaneously matured oocytes, about 100 min had

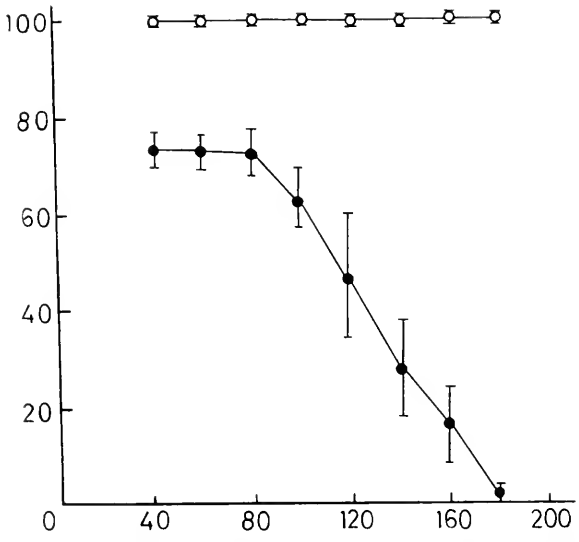

FIGURE 2. The percentage of fertilization membrane formation and normal early gastrula in *Asterina pectinifera* oocytes. Abscissa: time (min) after 1-methyladenine application; ordinate: the rate (%) of fertilization membrane and normal early gastrula formation. Open circles, fertilization membrane formation; closed circles, normal early gastrula. Each point shows mean ± of five experiments.

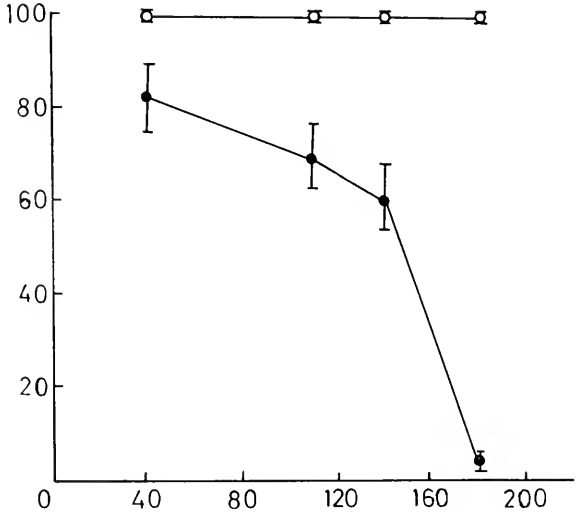

FIGURE 3. The percentage of fertilization membrane formation and normal first cleavage in *Asterias amurensis* oocytes. Abscissa: time (min) after 1-methyladenine application; ordinate: the rate (%) of fertilization membrane formation and normal first cleavage. Open circles, fertilization membrane formation; closed circles, normal first cleavage. Each point shows mean ± of four experiments.

already elapsed after isolation in sea water, before insemination, and these spontaneously matured oocytes developed abnormally upon insemination. Subsequently, the maximum rate of oocytes which developed to early gastrula did not exceed 73% even in case of the insemination at 40 min.

Next, the occurrence of first cleavage was checked 2.5 hr after insemination. In this experiment, oocytes of *Asterias amurensis* were used. After being placed in 1-methyladenine sea water for various times, oocytes were transferred to fresh sea water and inseminated. Although the formation of fertilization membrane occurred in all oocytes, the rate of normal first cleavage decreased with increased intervals after being placed in 1-methyladenine sea water; 82%, when inseminated at 40 min, 69% at 110 min (period of first polar body formation) and only 4% at 180 min (period of second polar body formation) (Figs. 3, 4).

In addition, these abnormal cleavages occurred even if 1-methyladenine was completely removed by washing with fresh sea water after occurrence of germinal

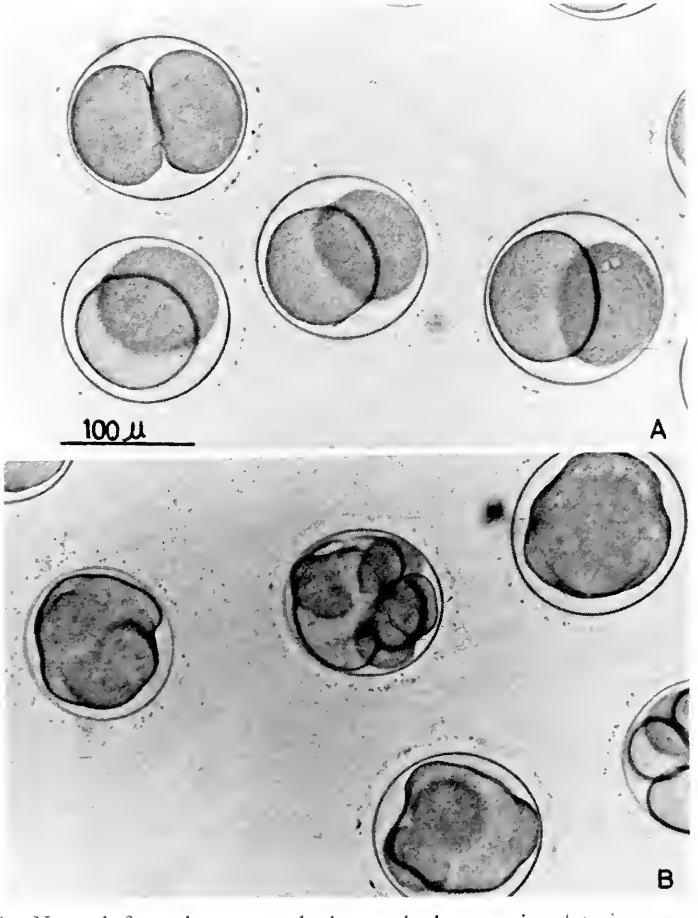

FIGURE 4. Normal first cleavage and abnormal cleavage in *Asterias amurensis* oocytes when inseminated at 40 min (A) and 180 min (B) after 1-methyladenine application.

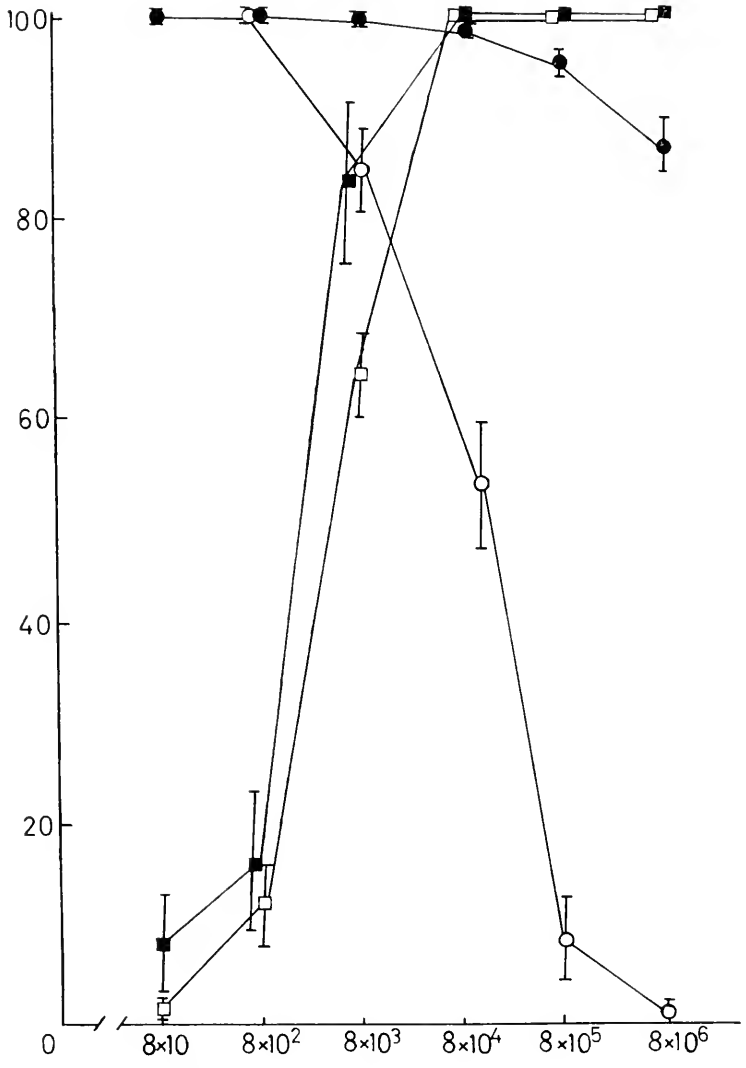

FIGURE 5. Relation between sperm concentration and normal first cleavage. Abscissa: sperm concentration (per ml); ordinate: the rate (%) of fertilization membrane formation and normal first cleavage. Solid squares, fertilization membrane formation upon insemination at 40 min; open squares, fertilization membrane formation upon insemination at 180 min; closed circles, normal first cleavage upon insemination at 40 min; open circles, normal first cleavage upon insemination at 180 min. Each point shows mean ± of five experiments.

vesicle breakdown. Therefore, this abnormality was not due to the excess of 1-methyladenine.

Next, oocytes of *Asterias amurensis* were inseminated at 40 and 180 min after being placed in 1-methyladenine sea water, with various sperm concentrations ranging from 8×10^3 to 8×10^6 per ml. When inseminations were performed

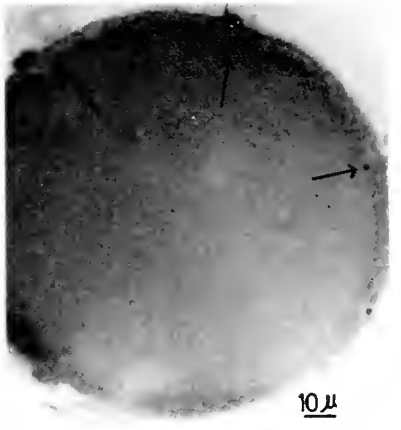

FIGURE 6. Section through an *Asterina pectinifera* oocyte which was inseminated at 180 min after 1-methyladenine application. Arrows show sperm nuclei.

40 min after being placed in 1-methyladenine sea water, the rate of fertilization membrane formation increased with the increased sperm concentrations. The rate of normal first cleavage showed only a slight decrease with higher sperm concentrations (Fig. 5); 87% of these oocytes cleaved normally when inseminated with heavy sperm concentration (8×10^6 per ml). On the other hand, when inseminations were performed at 180 min (period of second polar body formation), the rate of normal first cleavage showed a sharp decrease accompanying the increased sperm concentrations, although the rate of fertilization membrane formation showed a similar curve seen at 40 min; the rate of normal first cleavage was 53%, with moderate sperm concentration (8×10^4 per ml) which brought normal cleavage for almost all oocytes that were inseminated at 40 min, 8% with 8×10^5 per ml and only 1% with heavy sperm concentration (8×10^6 per ml). These results showed that the increase of abnormality in the oocytes inseminated at 180 min was due to the increased sperm concentration. Therefore, it seemed that polyspermy occurred in the oocytes inseminated at 180 min. This probability was confirmed cytologically (Fig. 6). Oocytes of *Asterina pectinifera* were inseminated 120 min after being placed in 1-methyladenine sea water with a moderate sperm concentration (5×10^5 per ml), and 5 min after insemination they were fixed with Carnoy's solution. Microscopic examination showed that most oocytes contained more than two sperm nuclei.

In addition, when insemination was performed with moderate sperm concentration (8×10^4 per ml) at the period of second polar body formation, the rate of fertilization membrane formation did not attain one hundred per cent (Fig. 5), but about 50% of the fertilized oocytes showed normal first cleavage and most of them developed to early normal bipinnaria.

DISCUSSION

During the course of starfish oocyte maturation, the optimum period for insemination is between germinal vesicle breakdown and first polar body formation

(Fol, 1879; Delage, 1901). Insemination after first polar body formation results in a tendency toward a decline of normal development; abnormality becomes more pronounced when oocytes are inseminated 30 to 60 min after second polar body formation (Lillie, 1915). But in these studies, the cause for occurrence of abnormality was not clarified. In our results, when inseminations were performed between germinal vesicle breakdown and first polar body formation, fertilization membrane formed normally in all cases and the first cleavage occurred normally in most oocytes. However, when inseminations were performed after first polar body formation, the rate of first cleavages and subsequent development apparently decreased. Only a small percentage of oocytes underwent normal cleavage when inseminated after second polar body formation, although fertilization membrane was formed normally in all oocytes. Figure 5 shows the increased rate of abnormal cleavage accompanying increased sperm concentrations in oocytes with second polar body: with moderate sperm concentration (8×10^4 per ml) the rate of normal cleavage was 53%, but with high sperm concentration (8×10^6 per ml), the rate of normal cleavage was only one per cent. However, even at the same high sperm concentration, normal cleavage occurred in 87% of the oocytes which completed germinal vesicle breakdown. Thus, these results suggested that the increased rate of abnormal cleavage was due to the occurrence of polyspermy. The occurrence of polyspermy was confirmed by cytological observation.

Chambers and Chambers (1949) have suggested that there is a decline in proper functional interactions between sperm and egg cytoplasm when insemination is performed after the optimum ripening period. In the present study, however, some fertilized oocytes, which were inseminated after second polar body formation, showed normal cleavage and developed to early bipinnaria if the sperm concentration was light. It is suggested that as long as polyspermy was prevented, the oocyte remained viable, even after second polar body formation.

Earlier investigators reported that starfish oocytes become susceptible to polyspermic fertilization after standing in sea water less than two hrs (Chambers, 1923; Clark, 1936). In their studies, the occurrence of polyspermy and abnormal development were not considered in relation to various periods of oocyte maturation. Our study confirms that the number of starfish oocytes showing polyspermy gradually increases with increasing time intervals between first polar body formation and sperm addition, and that almost all the oocytes show polyspermy when the insemination is performed after second polar body formation. Even in the latter case, however, fertilization membrane is normally formed. It is postulated that polyspermy occurs before fertilization membrane formation and that there is some mechanism(s) of protection against polyspermy between the first sperm entry and fertilization membrane formation. The mechanism(s) may be gradually lost after first polar body formation and disappear completely after second polar body formation.

On the other hand, polyspermy occurs regardless of the fertilization membrane formation in immature oocytes which have intact germinal vesicles (Cayer, Kishimoto and Kanatani, 1975; Hirai, 1976). Thus, the protective mechanism(s) against polyspermy may be acquired during the maturation process.

In sea urchin eggs, it was recently demonstrated by Jaffe (1976) that polyspermy was quickly inhibited as a result of electrical depolarization brought about

by the entry of the first spermatozoon. In starfish oocytes, it was reported that potassium conductance of oocyte membrane changed during maturation, thus altering the form of action potential subsequently generated (Miyazaki, Ohmori and Sasaki, 1975). Potassium conductance was small before first polar body formation, and larger after it (Miyazaki, Ohmori and Sasaki, 1975). It is suggested that the change of potassium conductance during oocyte maturation may affect electrical depolarization brought about by the entry of the first spermatozoon. Further examination would be desirable.

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Summary

During the course of starfish oocyte maturation, the optimum period for insemination is between germinal vesicle breakdown and the first polar body formation. The rate of polyspermy increases with increasing time intervals between the first polar body formation and sperm addition, although the fertilization membrane is formed normally in all oocytes. As long as polyspermy is prevented, however, the oocytes remain viable even after the formation of second polar bodies. Thus, it is postulated that there is some mechanism(s) of protection against polyspermy between the first sperm entry and the fertilization membrane formation. The mechanism(s) may be gradually lost after the first polar body formation and disappear completely after the second polar body formation.

LITERATURE CITED

Cayer, M. L., T. Kishimoto, and H. Kanatani, 1975. Formation of the fertilization membrane by insemination of immature starfish oocytes pretreated with calcium-free sea water. *Dev., Growth Differ.*, **17**: 119–125.

Chambers, R., 1923. The mechanism of the entrance of sperm into the starfish egg. *J. Gen. Physiol.*, **5**: 821–829.

Chambers, R., and E. L. Chambers, 1949. Nuclear and cytoplasmic interrelations in the fertilization of the *Asterias* egg. *Biol. Bull.*, **96**: 270–282.

Clark, J. M., 1936. An experimental study of polyspermy. *Biol. Bull.*, **70**: 361–384.

Fol, H., 1879. Recherches sur la fecondation et le commencement de l'henogenie chez divers animaux. *Geneve Soc. Phys. Mem.*, **26**: 89–397.

Delage, Y., 1901. Etudes experimentales sur la maturation cytologigue et sur la parthenogenese artificielle ches les echinodermes. *Arch. Zool. Exp. 3 ser.*, **9**: 285–326.

Goldforb, A. J., 1935. Viscosity changes in aging unfertilized eggs of *Arbacia punctulata*. *Biol. Bull.*, **68**: 191–206.

Hirai, S., 1976. The role of germinal vesicle material on the swelling of sperm nuclei penetrated in immature oocytes of the starfish. *Bull. Mar. Biol. Stn., Asamushi*, **15**: 165–171.

Jaffe, L. A., 1976. Fast block to polyspermy in sea urchin eggs is electrically mediated. *Nature*, **261**: 68–71.

Lillie, R. S., 1915. Momentary elevation of temperature as a means of producing artificial parthenogenesis and the conditions of its action. *J. Exp. Zool.*, **5**: 375–428.

Miyazaki, S., H. Ohmori, and S. Sasaki, 1975. Potassium rectifications of the starfish oocyte membrane and their changes during oocyte maturation. *J. Physiol.*, **246**: 55–78.

Miyazaki, S., H. Ohmori, and S. Sasaki, 1975. Action potential and non-linear current-voltage relation in starfish oocytes. *J. Physiol.*, **246**: 37–54.

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REPRODUCTION AND DEVELOPMENT OF THE HERMAPHRODITIC SEA-STAR, *ASTERINA MINOR* HAYASHI

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A number of papers have been published on the development of sea-stars belonging to the genus *Asterina,* but the entire process of the development from eggs to juveniles is known only in four species: *A. gibbosa* (Ludwig, 1882; Goto, 1898; MacBride, 1896), *A. burtoni* (James, 1972), *A. coronata japonica* (Komatsu, 1975) and *A. batheri* (Kano and Komatsu, 1978). Among these, description of *A. burtoni is* quite brief and information on the development of other asterinids is fragmental.

Sea-stars generally are gonochoric, but a few hermaphroditic asterinid species, namely *A. batheri, A. gibbosa, A. scobinata* and *A. pancerii* (Ohshima, 1929; Delavault, 1966; Dartnall, 1970) have been known. The details of breeding in these hermaphroditic species, however, are not well documented, although the gonadal structure and development of some species like *A. gibbosa* (MacBride, 1896) and *A. batheri* (Ohshima, 1929; Kano and Komatsu, 1978) have been reported in detail.

The present study was initiated to determine the breeding and development of *A. minor,* which was recently described as a new species (Hayashi, 1974). The preliminary observations revealed that the present species had a distinct breeding behavior. Moreover, it is found that *A. minor* is hermaphroditic and is able to self-fertilize. This feature being unique among sea-stars, it was felt worthwhile to have a thorough understanding of the breeding and development of this species.

The present paper describes the structure of the gonad, breeding behavior, and development throughout metamorphosis in *A. minor.* It also incorporates the observations on isolated cultures studied during the breeding season.

Materials and Methods

Specimens of *A. minor* were collected from the undersurface of stones at the intertidal zone of Kushimoto, Wakayama Pref., Japan, on several occasions during May of 1972 to June of 1977. They were brought to the laboratory of Toyama University and kept either in groups or individually.

The development of the species from fertilization to the completion of metamorphosis was observed with the help of a dissecting microscope and an inverted microscope. Measurements of living embryos were executed with an ocular micrometer. Examination of the skeletal system was performed on alcohol-fixed specimens treated with potassium hydroxide solution.

For microscopic observation of the reproductive organs, fresh specimens were measured and weighed, and then fixed in Bouin's solution. The fixed material was

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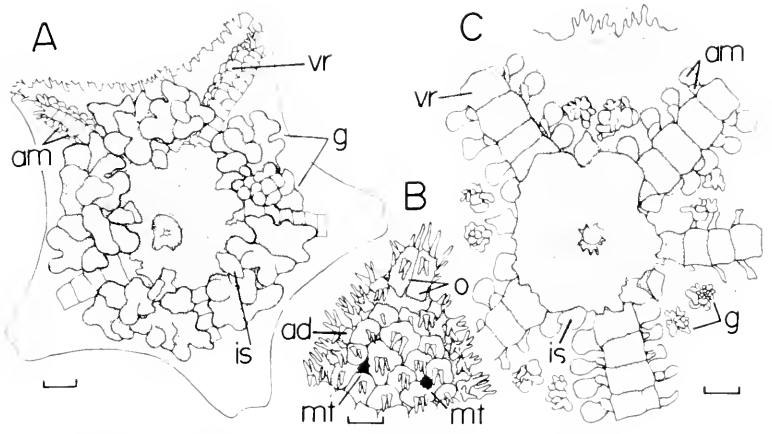


FIGURE 1. A) Aboral view of the arrangement of mature gonads in the specimen, collected on April 25, 1975, just before spawning. Aboral body wall and viscera were removed. am, ampulla; g, gonad; is, interradial septum; vr, vertebral ridge; Scale = 1 mm. B) Remnants of germinal substance, mostly egg fragments, attached to the gonopores in the specimen, collected on June 13, 1974, which was after spawning. ad, adambulacral plate; mt, remnants of germinal substance; o, oral plate. Scale = 500 μm. C) Aboral view of the gonads in the specimen collected on June 13, 1974, which was after spawning. am, ampulla; g, gonad; is, interradial septum; vr, vertebral ridge. Scale = 500 μm.

serially sectioned at 7 μm by routine paraffin method and stained with Delafield's hematoxylin and eosin.

OBSERVATIONS AND RESULTS

Breeding season

In the present study, no observations were made to confirm spawning in the field. However, it is felt that a possible breeding season of *A. minor* is during May at Kushimoto. This prediction is based on the following circumstantial evidence. In the specimens examined, the gonads were largest in size and nearly mature in late April (Figs. 1A, 2A–F). In adults collected in June, some particles, possibly fragments of ova, were often observed near the gonopores (Fig. 1B). Similar particles were usually observed in the specimens spawned under laboratory conditions. The gonads of the individuals collected in June showed an atrophy and contained degenerating ova (Figs. 1C, 3A, 3B). Spawning in the laboratory as observed for 3 years occurs in the month of May (Table I). The juveniles of this species, each bearing two pairs of tube-feet in each arm, were collected in the month of June. They were estimated to be 20 to 30 days old since their fertilization. In the later months (July onward), the juveniles found in the field were larger than those collected in June (Table II).

Structure of the gonad

A pair of gonads lies at each interradial portion and each gonad is composed of cluster of tubules (Figs. 1A, 1C). Histological observations on the specimens

TABLE I

Occurrence of spawning of specimens of Asterina minor *in the laboratory.*

Number of animals kept together	Date of collection in the field	Time and date of the commencement of spawning in the laboratory
40	April 25, 26, 1974	21:20 May 8, 1974
2	April 25, 1975	17:05 May 2, 1975
10	April 25, 1975	13:00 May 11, 1975
4	April 25, 1975	9:20 May 24, 1975
2	Feb. 16, 1976	20:25 May 12, 1976
2	April 28, 1976	17:30 May 19, 1976
2	April 28, 1976	20:55 May 22, 1976
2	April 28, 1976	0:00 May 25, 1976
2	April 28, 1976	20:00 May 25, 1976

measuring 2.5 to 6.0 mm in R show that this species is a spatial hermaphrodite. Each gonad consists of both the ovarian tubules and the testicular ones (Figs. 2A, 2B) and has a common gonoduct which opens on the oral side of the disk (Figs. 3A, 3B). In the breeding season, the ovarian tubules show pale yellow color and the testicular tubules are whitish and semi-transparent. In general, the testicular tubules lie near the gonoduct and are smaller than the ovarian tubules located in the peripheral region (Fig. 2C). The distribution of the ovarian and testicular tubules, however, varies in different gonads and also in different individuals. In some cases, both sex elements exist in a single tubule (Figs. 2D, 2F). After spawning, all gonads become shrunken and transparent and show a green tint (Figs. 1C, 3A, 3B).

Breeding behavior

Breeding assemblage was observed in the laboratory every year. Although spawning was not observed in the field, assemblage of this species was often found

TABLE II

Number and size distribution of juveniles (under 17 pairs of tube-feet) collected in the field.

Number of tube-feet in the longest arm (in pairs)	June 10–12, 1975	July 3–6, 1974	Sept. 11–13, 1973	Feb. 16, 1976
2	10	4		
3		31	1	
4		27	5	
5		6	26	
6			32	
7			42	
8			29	
9		1	7	1
10		1	3	
11		4	5	
12		22	19	
13		36	9	
14		18	3	
15		6	2	
16		3	1	
17		2	1	

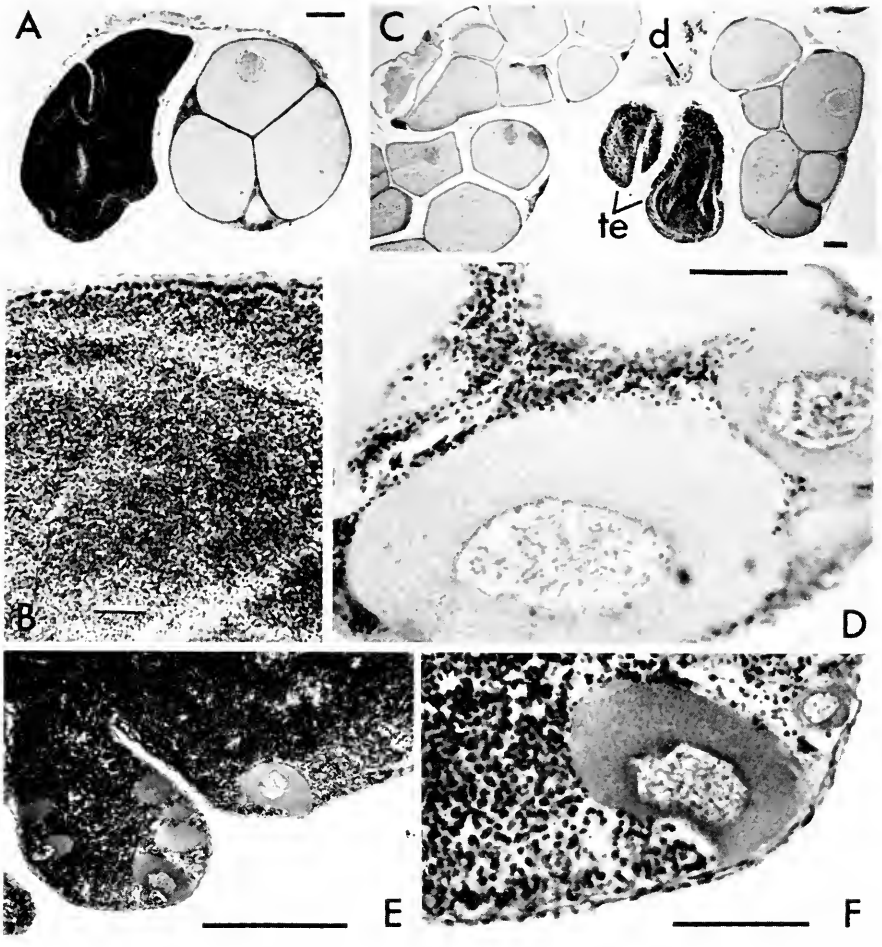


FIGURE 2. Section of the gonad of a specimen of *Asterina minor*, which was fixed just before spawning. Hematoxylin-eosin stain. A) A testicular and an ovarian tubule, containing mature sperms and ova, respectively. B) Magnified picture of the testicular tubule in Figure 2A. C) Section of gonad showing that testicular tubules (te) are situated close to the gonoduct (d). D) A hermaphroditic gonad, in which ovarian part is dominating. E) A hermaphroditic gonad, in which testicular part is dominating. F) Magnified picture of a part of Figure 2E. Scale = 100 μm in A, C, E. Scale = 50 μm in B, D, F.

in the field in May. The following is a description of the typical process of breeding assemblage.

Forty animals which were collected on April 25 and 26, 1974, began to organize into two groups in a large glass container in the laboratory several days after their collection. These groups were not very stable and animals moved frequently from one group to the other. Generally after about 10 days the assemblages became stable, and no animals seemed to move from their respective group. At this time animals in either group clung to one another along the margins of their bodies, or they were imbricated with others to some extent.

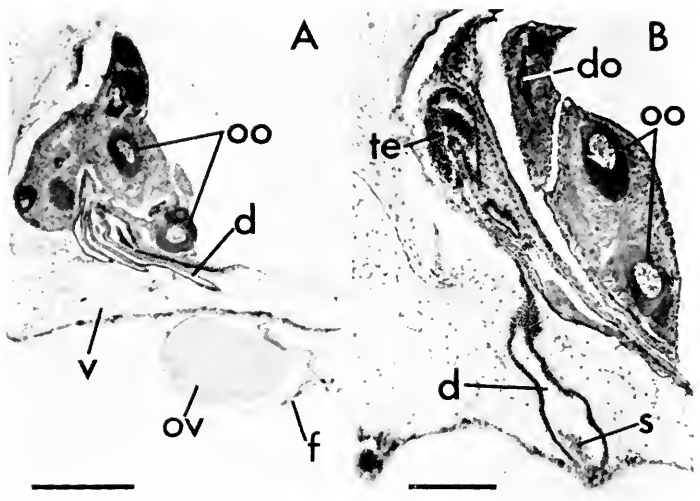


FIGURE 3. Sagittal section of the gonad of a specimen of *Asterina minor*, showing gonoduct and gonopore. A) This specimen was fixed during spawning. Note a spawned egg (ov) close to the body surface of the adult and fertilization membrane (f). d, gonoduct; oo, ovarian ova; v, ventral body wall of the adult. Scale = 50 μm. B) This specimen was fixed just after spawning was finished. Note a remnant of sperms (s) in the gonoduct (d) and degenerating ova (do). oo, ovarian ova; te, testis. Scale = 25 μm.

At 21:20 May 8, one animal in an assemblage comprised of 25 individuals began to spawn (a, in Fig. 4). As will be described later the eggs got attached to the substratum after their release. About 15 min thereafter, about 80 eggs were laid by this animal. At 22:50, three other animals (b, c, d, in Fig. 4) began to spawn and several minutes later each animal had delivered about 20 eggs. About 30 min thereafter, four more animals (e, f, g, h, in Fig. 4) began to spawn. Two

TABLE III

Spawning of specimens of Asterina minor *in isolated culture.*

Size of animal (mm) R	r	Fresh weight of newly collected animal (mg)	Fresh weight of animal after spawning (mg)	Decrease of weight by spawning (%)	Number of eggs spawned	Term in isolated culture (day)
7.0	4.2	188.3	106.0	44	297	27
6.5	4.5	149.4	100.4	33	233	87
5.6	3.3	148.3	—	—	180	85
5.5	4.1	94.1	77.7	17	243	21
5.4	4.0	196.4	92.8	53	261	21
5.1	3.7	79.0	69.7	12	127	19
5.1	3.3	62.3	45.2	27	132	29
5.0	3.8	89.3	69.8	22	137	39
5.0	3.2	67.6	56.3	17	116	28
4.7	3.5	37.8	29.5	22	87	21
4.2	3.8	45.8	34.9	24	44	100
4.1	2.9	16.4	10.5	36	6	33

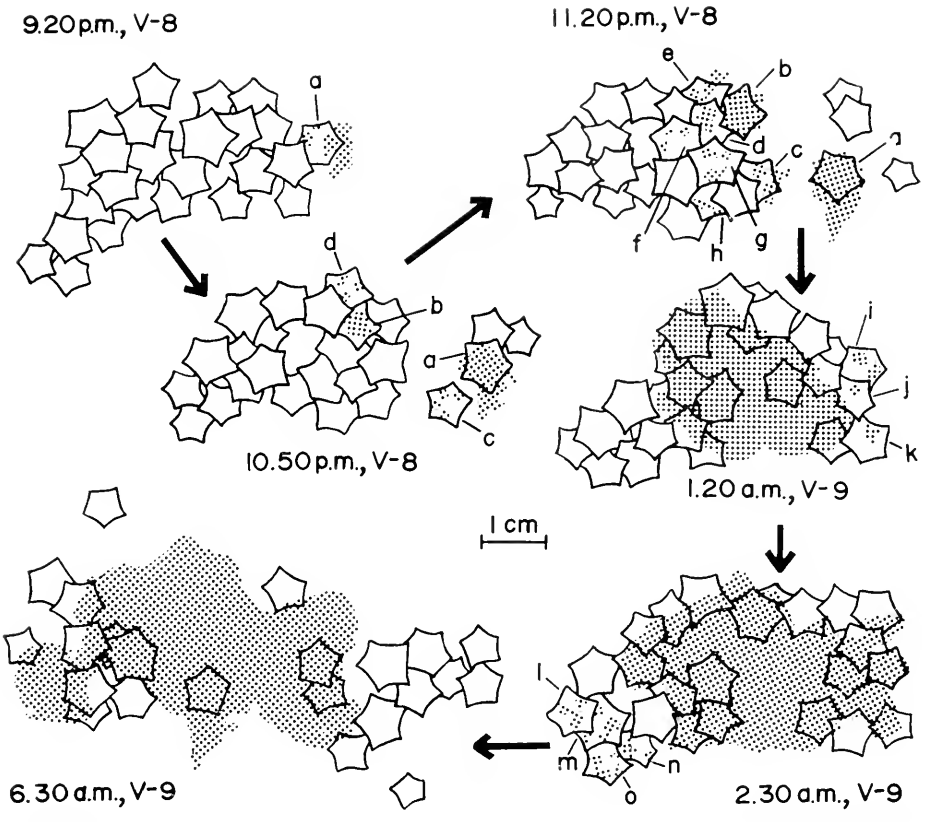


FIGURE 4. Sequential change of the distribution of individuals in a breeding assemblage. Dotted areas show the deposition of eggs. See text for detail.

hours later, three more animals (i, j, k, in Fig. 4) were found to be spawning, and soon four other animals (l, m, n, o, in Fig. 4) followed. After that, the majority of animals in this assemblage were found to be spawning. About 6 hr after the first spawning, all animals seemed to have completed spawning. At 6:30 the following morning (May 9), the assemblage was almost disordered. In the other assemblage, spawning commenced 3 hr after the onset of the spawning in the first one. The process of spawning in the other assemblage was very similar to that in the first. In the evening of May 9, the assemblages were completely disorganized and constituent individuals in either assemblage got mixed. After the disorganization of the assemblages, two egg masses were found, and these were not protected by the adults.

Isolated culture, a proof of self-fertilization

From the characteristic breeding assemblage observed, it seems that this species engages in cross-fertilization. However, there is a possibility that the species also indulges in self-fertilization, since histological observations show

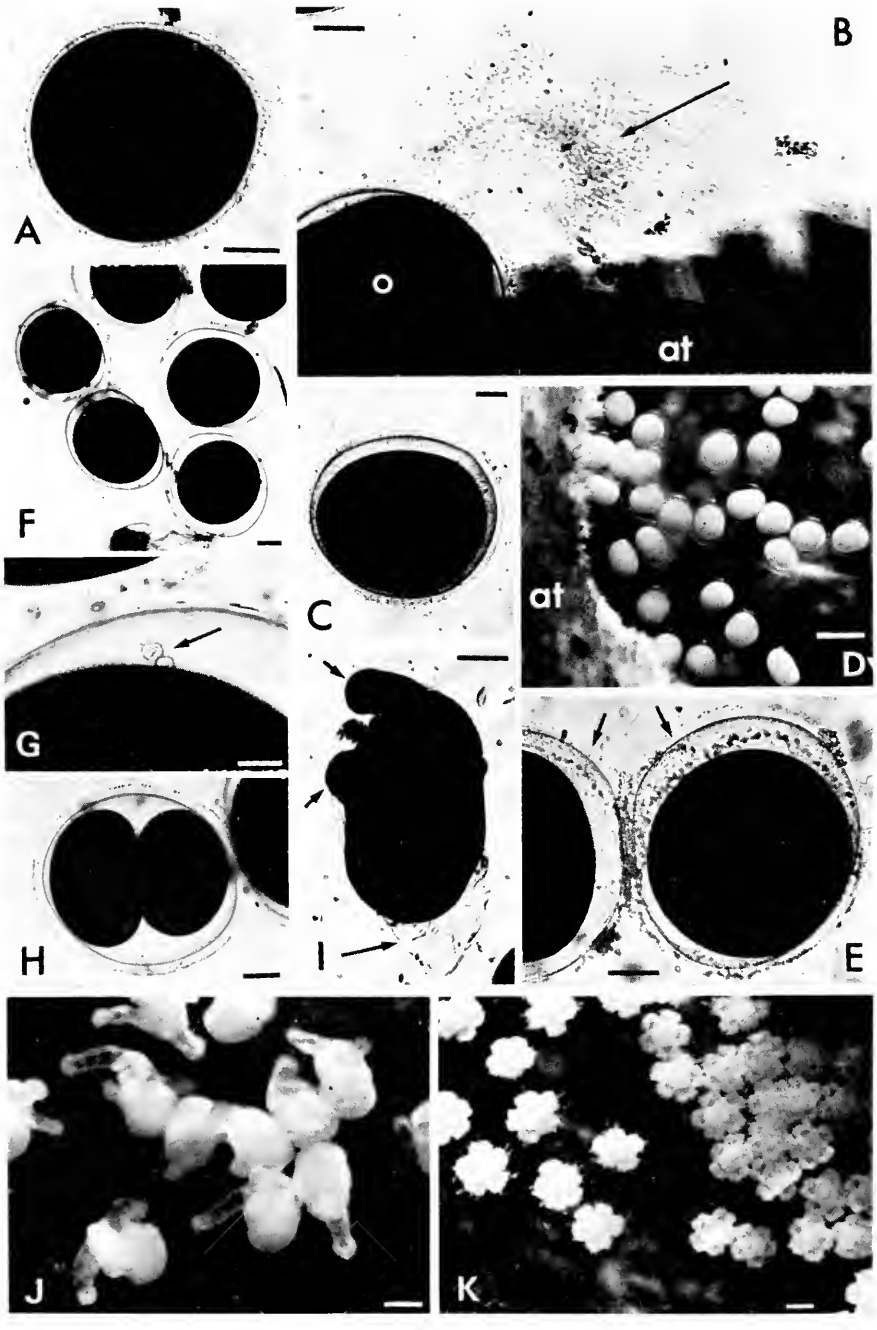


FIGURE 5. Development of specimens of *Asterina minor*. All pictures show living specimens. A) Egg just after spawning. Fertilization membrane was not yet formed. Sperms are crowding around the jelly layer. B) A mass of sperms (arrow) is seen near the spawning

both male and female elements simutlaneously maturing in one individual. In order to know whether the species was able to self-fertilize, observations were made on individuals in isolated culture. Specimens, immediately after their collection in the field, were kept individually in small glass jars. Temperature during the culturing was maintained similar to that of the natural habitat, 15 to 25° C. The majority of animals in the isolated culture deposited eggs and these eggs were fertilized with the sperms ejected from the same animal in the absence of any artificial treatment. The details in respect of isolated culture and self-fertilization are given in Table III.

The eggs possibly became mature during the travel through the gonoduct or else as soon as they were released. The number of eggs spawned by one adult is related to the size of the adult. The self-fertilized eggs developed into normal embryos and became normal juveniles through metamorphosis (Fig. 5K). No differences were observed in the fertilization rates or developmental processes in the specimens held in mass culture and those kept in an isolated culture. Adults were collected in different months of the year and kept separately, but they spawned within a limited span of time in the laboratory.

The above observations demonstrate that *A. minor* has an ability to self-fertilize when kept singly. However, it is not clear whether or not self-fertilization occurs when the animal is spawning in the breeding assemblage.

Development

In this species no fertilization membrane was observed in the freshly laid eggs (Fig. 5A).

The egg is spherical with a diameter of 437 ± 5 μm (mean $\pm$ s.e., $n = 37$). They are transparent yellow and have a jelly layer of about 20 μm thick. A few minutes after spawning, sperm masses are released from the same adult (Fig. 5B) and the sperms are dispersed. The head of the sperm is spherical, about 3 μm in diameter, and the tail is about 30 μm in length. The eggs a few minutes after spawning, attach to the substratum and the neighbouring eggs by their sticky jelly layer. Ten minutes after spawning, elevation of the fertilization membrane is recognized. Figure 5C shows fertilization membrane in an egg 30 min after spawning. The spawned eggs are laid on the bottom in one layer (Figs. 5D, 5E). About 2 hr after spawning, polar bodies are seen in the perivitelline space, which is about 50 μm in height (Figs. 5F, 5G). Three hours after spawning at 20 to 23° C, the first cleavage occurs through the animal-vegetal axis (Figs. 5H, 6A), and 40 min thereafter it is followed by the second cleavage which is perpendicular

adult (at). Fertilization membrane is being formed in an egg (o), about 10 min after spawning. C) Egg about 30 min after spawning. D) Adult (at) and eggs spawned from it, 1 hr after the commencement of spawning. E) Magnified picture of a part of Figure 5D. Two eggs are attached to each other by their jelly layers (arrows), which lie outside of the fertilization membrane. F) Eggs 2 hr after spawning. G) Magnified view of F. Note polar bodies (arrow). H) First cleavage, 3 hr after spawning. I) Hatching brachiolaria, view from the ventro-lateral (left) side. Long and short arrows indicate fertilization membrane and brachiolar arms, respectively. J) Developed brachiolariae, creeping on the substratum. K) Metamorphosed juveniles, 15 days after spawning. Scale = 50 μm in D, G. Scale = 100 μm in A, B, C, E, F, H, I. Scale = 200 μm in J. Scale = 100 μm in K.

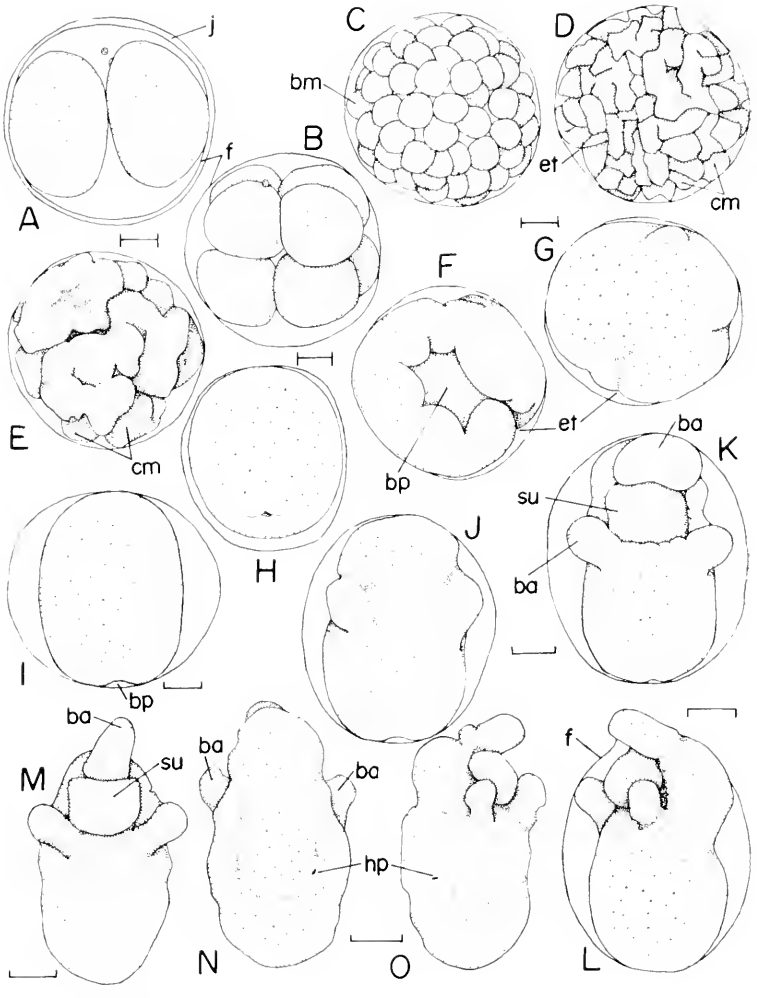


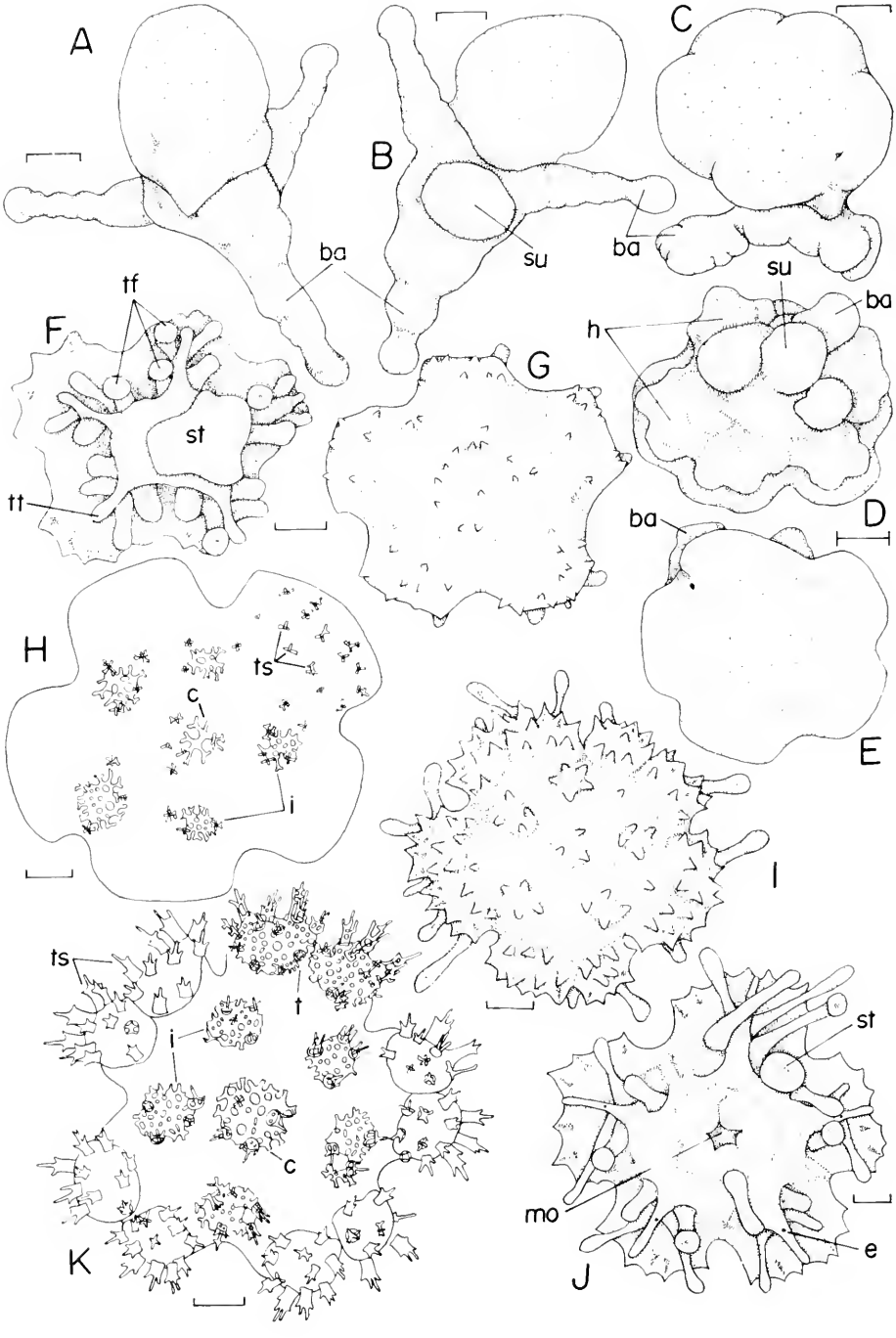
FIGURE 6. Development of specimens of *Asterina minor*. All drawings are from a living specimen, jelly layer is not shown except in A. All scales show 100 μm. A) Two-cell stage. f, fertilization membrane; j, jelly layer. B) Eight-cell stage, 4.5 hr after spawning. f, fertilization membrane. C) 64-cell stage, 6.5 hr after spawning. bm, blastomere. D) Early wrinkled blastula, 10 hr after spawning. cm, cell mass; et, egression tract. E) The most wrinkled blastula, 12.5 hr after spawning. F) Early gastrula, 18 hr after spawning. Egression tracts (et) are still recognized on the surface of the embryo, view from the vegetal pole. bp, blastopore. G) Early gastrula, 18 hr after spawning, view from the animal pole. et, egression tract. H) Gastrula, 36 hr after spawning. I) Gastrula, 40 hr after spawning. bp, blastopore. J) Brachiolaria, 2 and a half days after spawning, view from the ventro-lateral (right) side. K) Brachiolaria, 3 days after spawning, ventral view. ba, brachiolar arm; su, sucker. L) Brachiolaria at the commencement of hatching, 4 days after spawning, view from the ventro-lateral (left) side. f, fertilization membrane. M) Brachiolaria, just after hatching, ventral view. ba, brachiolar arm; su, sucker. D) Same as M, dorsal view. ba, brachiolar arm; hp, hydropore. O) Same as M, ventro-lateral (right) view. hp, hydropore.

to the first. The cleavage is total, equal, and radial. The embryo reaches the eight-cell stage (Fig. 6B) and the 64-cell stage (Fig. 6C), 4.5 and 6.5 hr respectively after spawning. Eight hours after spawning, the embryos enter into the wrinkled blastula stage. The process of wrinkling has been earlier reported in detail (Komatsu, 1976). Figures 6D and 6E illustrate an early wrinkled blastula and most wrinkled blastula, respectively. Twenty-four hours after spawning, the wrinkled blastula stage is completed.

At the end of the wrinkled blastula stage, gastrulation by invagination takes place (Figs. 6F, 6G). Thirty-six hours after spawning, the gastrula begins to rotate within the fertilization membrane (Fig. 6H) and becomes enlarged along the archenteric axis. Figure 6I shows a gastrula 40 hr after spawning. It measures about 500 μm in length and 350 μm in width. Two days after spawning, the ventral side of the embryo becomes flattened and the rudiments of the brachiolar arms (lateral arms) appear at the ventro-lateral side of the embryo. These arms grow gradually and the third arm (anterior arm) emerges from the ventral side of the anterior portion of the embryo. Two and a half days after spawning, the embryo becomes pear shaped, with three distinct brachiolar arms and a rudimentary sucker which appears in the area surrounded by the brachiolar arms (Fig. 6J). Three days after spawning, the brachiolaria measures 550 μm in total length (Fig. 6K). The anterior half of the embryo, which bears three brachiolar arms and a sucker, corresponds to the stalk of the larva and the posterior half is the disk of the larva. Lateral arms are longer, 150 μm in length, than the anterior arm, which is about 100 μm. A blastopore and a hydropore are observed at the posterior tip and at the dorsolateral (right) side of the larva, respectively. Three and a half days after spawning, the blastopore is closed.

About four days after spawning, the brachiolaria is hatched from the fertilization membrane (Figs. 5I, 5L), usually at the anterior portion. Figures 6M, 6N and 6O show a freshly hatched larva. The present species has no pelagic life and the brachiolariae creep on the substratum with their developed brachiolar arms throughout the brachiolaria stage (Fig. 5J). One day after hatching, the brachiolariae attach to the substratum with their brachiolar arms and not with the sucker (Figs. 7A, 7B). This attachment is a sign of the commencement of metamorphosis. At this stage, the brachiolar arms are markedly long and appear semitransparent excepting at the tip ends. The anterior arm measures 250 μm, and the lateral arms are 350 μm in length.

About 12 hr later, the disk begins to transform into a subpentagonal form (Fig. 7C), and the stalk begins to shrink rapidly. The rudiments of the tube-feet are seen on the hydrolobes, these are clearly visible in the future oral side of the adult disk. Figures 7D and 7E show a larva one day after the one shown in Fig. 7C. This larva is about 500 μm in diameter and has a hydropore in an interradius of the future aboral side of the disk. A degeneration of the stalk, including the brachiolar arms and sucker, progresses gradually. About 7 days after spawning, the larvae are freed from the substratum due to the extreme degeneration of the brachiolar arms. They are able to move by means of their tube-feet (Figs. 7F, 7G). On the aboral side of the larva at this stage, one central and five interradial plates are recognized (Fig. 7H). Metamorphosis is completed with the opening of the mouth about 10 days after spawning (Figs. 7I, 7J). At this stage,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the juvenile is 700 μm in diameter, and bears a red eye-spot at the basal portion of each terminal tentacle. On the oral side, a rudiment of the stalk is still observed. In each interradius, the formation of a pair of oral plates is noticed. Skeletal plates on the aboral side are shown in Fig. 7K. It is of interest to note that a slit is seen at the midline of each terminal plate, making it appear as if one terminal plate is composed of two pieces. Fifteen days after spawning, that is about 5 days after the completion of metamorphosis, the juveniles start moving from the place where they were laid (Figs. 5K, 8A). At this stage, each oral plate bears a spine which is pointed at the center of the mouth (Figs. 8B, 8C). On the aboral side of disk, small radial plates are being formed (Fig. 8D). After the spawning the juveniles grow to the size of 1000 and 1200 μm in diameter after 20 and 30 days, respectively. In specimens 30 days after spawning, each radial plate bears one spine at the center. In each interradius of the aboral side, one pair of the secondary plates is formed. On the oral side, a pair of adambulacral plates is formed in each arm and each adambulacral plate is furnished with one spine.

The juveniles were kept in the laboratory several months after the completion of metamorphosis, but no further development was observed beyond the stage 30 days after spawning (20 days after the completion of metamorphosis). Field surveys were then undertaken to obtain supplementary material for understanding post-metamorphic development. As shown in Table II, a number of juveniles were collected. Among these, two animals are described here in detail. One animal collected on July 3, 1974, was 1500 μm in diameter and had three pairs of the tube-feet in each arm (Figs. 9, 10, 11A). At each interradius, one pair of superomarginal plates was present, each plate having two prominent spines. On the oral side, five pairs of oral plates encircle the mouth. In each arm, two pairs of adambulacral plates and one pair of inferomarginal plates were present. The first adambulacral plate had two small spines. The second adambulacral plate was smaller than the first, and each of them had one spine. The other specimen collected on Sept. 7, 1973, was 3000 μm in diameter and possessed seven pairs of tube-feet in each arm (Figs. 11B, 11C). This specimen had well-developed aboral skeletal plates, some of which were imbricated with each other. Between the neighboring interradial plates, there was papular area, each of which had one papula. In one of the interradial plates there was a concave portion and this might be a rudiment of the madreporite. Each arm had several pairs of supero-

FIGURE 7. Development of specimens of *Asterina minor*. All drawings are made from living specimens, except H and K, which were treated with KOH solution before examination. All scales show 100 μm. A) Brachiolaria, 5 days after spawning, strongly attached to substratum with brachiolar arms (ba), dorsal view. B) Same as A, ventral view. ba, brachiolar arm; su, sucker. C) Metamorphosing larva, 12 hr after that shown in Figures 7A and B, view from the future aboral side of the juvenile. ba, brachiolar arm D) Metamorphosing larva, 1 day after that shown in Figure 7C, future oral view. ba, brachiolar arm; h, hydrolobe; su, sucker. E) Same as D, future aboral view. ba, brachiolar arm. F) Metamorphosing larva, 7 days after spawning, future oral view. st, stalk; tf, tube-foot; tt, terminal tentacle. G) Same as F, future aboral view. H) Aboral skeletal plates, in the same stage shown in Figures 7F and G. c, central plate; i, interradial plate; ts, spines on the terminal plate. I) Juvenile just after the completion of metamorphosis, 10 days after spawning, aboral view. J) Same as I, oral view. e, eye-spot; mo, mouth; st, markedly degenerated stalk. K) Aboral skeletal plates, in the same stage shown in Figures 7I and J. c, central plate; i, interradial plate; t, terminal plate; ts, spines on the terminal plate.

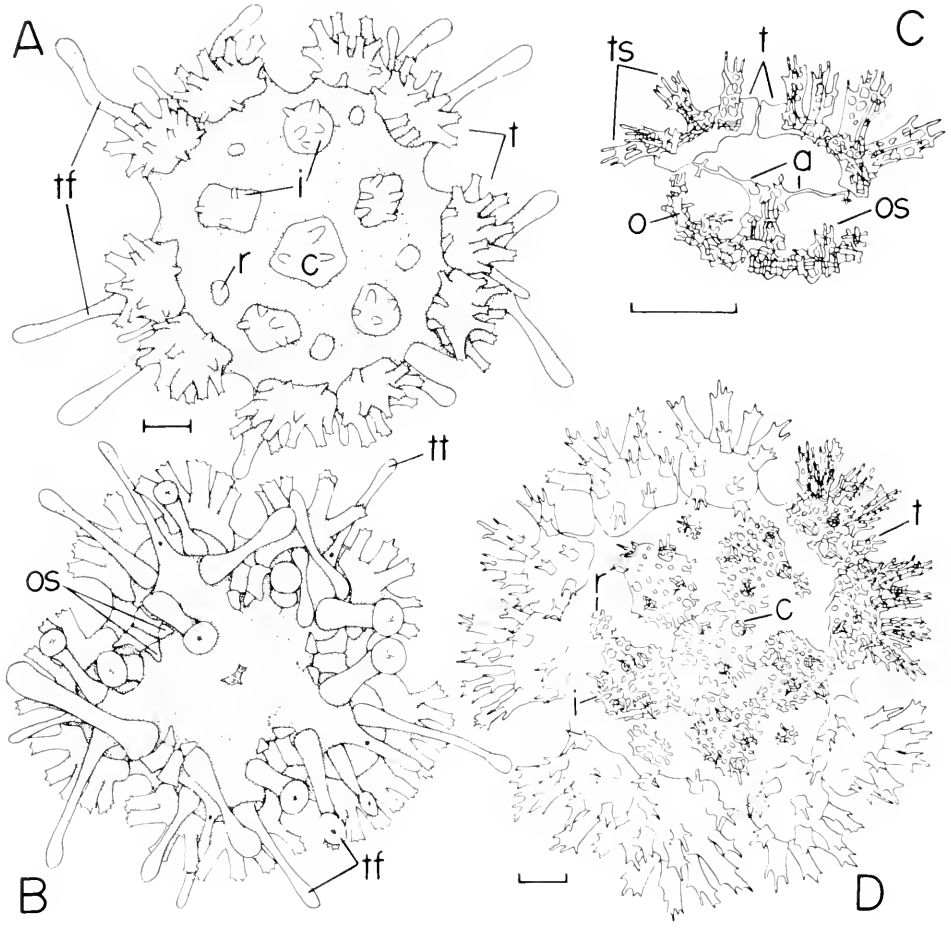

FIGURE 8. Juvenile of specimens of *Asterina minor,* five days after the completion of metamorphosis, having two pairs of tube-feet. A and B are drawn on living specimens and C and D are on KOH treated specimens. All scales show 100 μm. A) Aboral view. c, central plate; i, interradial plate; r, radial plate; t, terminal plate; tf, tube-foot. B) Oral view. os, oral spine; tf, tube-foot; tt, terminal tentacle. C) Skeletal plates of an arm, oral view. a, ambulacral plate; o, oral plate; os, oral spine; t, terminal plate; ts, spine on the terminal plate. D) Aboral skeletal plates. c, central plate; i, interradial plate; r, radial plate; t, terminal plate.

marginal plates and inferomarginal plates, the latter both in size and number were larger than the former. Six pairs of adambulacral plates were present in each arm, and each of the three proximal plates (1st, 2nd, and 3rd) possessed three spines. In the interradial area of the oral side, there were several ventro-lateral plates in addition to adambulacral plates and inferomarginal plates. One slit was always recognizable along the midline of the terminal plate.

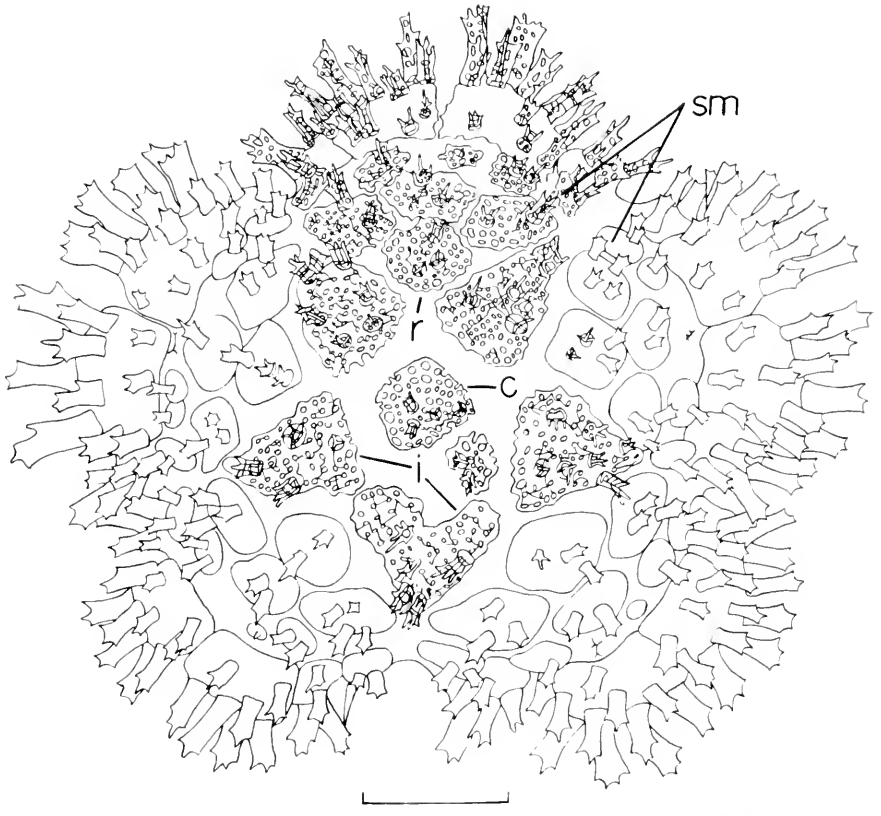

FIGURE 9. Aboral skeletal plates of a juvenile specimen of *Asterina minor,* with three pairs of tube-feet in each arm, KOH treated. c, central plate; i, interradial plate; r, radial plate; sm, superomarginal plate. Scale = 250 μm.

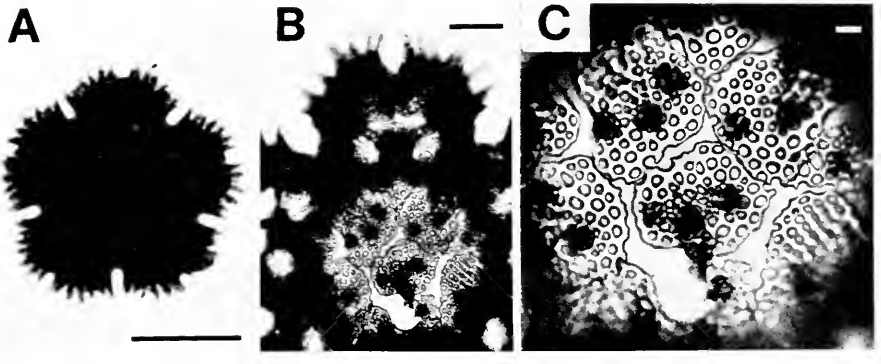

FIGURE 10. Skeletal plates of a juvenile specimen of *Asterina minor,* in the same stage shown in Figure 9, KOH treated. A) Shadow picture showing spines on terminal plates and contour of the body. Scale = 500 μm. B) Picture of plates in one arm and disk, in the same specimen shown in Figure A. Shaded area corresponds to plates in the oral side. Scale = 150 μm. C) Magnified picture of the central portion of Figure B. Scale = 150 μm.

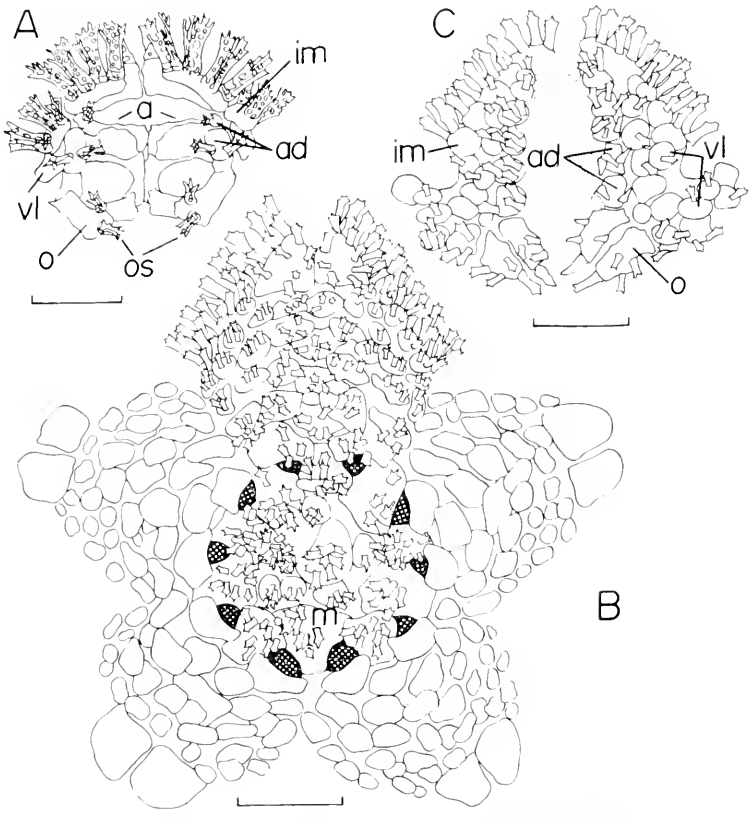

FIGURE 11. Skeletal plates of juvenile specimens of *Asterina minor*, treated with KOH. A) Oral skeletal plates in one arm in the specimen shown in Figure 9. a, ambulacral plate; ad, adambulacral plate; im, inferomarginal plate; o, oral plate; os, oral spine; vl, ventro-lateral plate. Scale = 200 μm. B) Aboral skeletal plates of a specimen having seven pairs of tube-feet in each arm, hatched portions show papular area. m, interradial plate with a rudiment of madreporite. Scale = 450 μm. C) Skeletal plates in the oral side of one arm in the specimen shown in Figure B. ad, adambulacral plate; im, inferomarginal plate; o, oral plate; vl, ventro-lateral plate. Scale 500 = μm.

DISCUSSION

A. minor lacks the bipinnaria stage and develops without pelagic life as known in *A. gibbosa* and *A. exigua* (Ludwig, 1882; MacBride, 1896; Mortensen, 1921). In general, asteroids whose development is direct have pear-shaped brachiolaria with three brachiolar arms. In species belonging to *Asterina*, the brachiolar arms of *A. batheri* and *A. coronata japonica* are short and blunt (Komatsu, 1975; Kano and Komatsu, 1978). The brachiolariae of these species have pelagic life. On the other hand, the brachiolar arms of *A. minor* are well developed and resemble those of *A. gibbosa* and *A. exigua*, and yet these brachiolariae spend benthonic life creeping on the substratum before metamorphosis. It is likely, therefore, that well-developed brachiolar arms are one of the adaptive characters of the benthonic life.

As described before, asteroids, barring a few hermaphroditic species, are gonochoric (Delavault, 1966). Sexuality of asterinids has been fairly well studied since Cuénot (1887) reported the occurrence of protandric hermaphroditism in *A. gibbosa*. Among asterinid species, in addition to *A. gibbosa*, hermaphroditism is known in *A. pancerii*, *A. batheri* and *A. scobinata* (Ohshima, 1929; Cognetti, 1954; Dartnall, 1970; Kano and Komatsu, 1978). In *A. batheri* a few hermaphroditic individuals occur among gonochoric individuals (Ohshima, 1929). No difference seems to exist in the sexual status of this species from different geographical regions (Kano and Komatsu, 1978). In this study, it was found that *A. minor* is a spatial hermaphroditic and all individuals bear hermaphroditic gonads comprising both functional testes and ovaries. In the breeding season, both elements mature simultaneously and the eggs can be fertilized with the sperm released from the same individual. This study has presented for the first time evidence of definite self-fertilization in asteroids.

Chia (1968) reported that *Leptasterias hexactis* aggregates under rocks during breeding season. Such a gathering is usual behavior in brooding species (Kubo, 1951). It is interesting to note that *A. minor* shows a distinct breeding assemblage, although it is not a brooding species and is capable of self-fertilizing. These facts may imply a complex historical background through which *A. minor* has been speciated.

The data accumulated so far indicate that genus *Asterina*, or its closely related groups, despite intimate similarity in adult morphology in different species, shows a remarkable variety in the mode of development and the method of breeding (Ludwig, 1882; MacBride, 1896; Goto, 1898; Mortensen, 1921; James, 1972; Komatsu, 1975; Kano and Komatsu, 1978). This observation may bring out the importance of studies on the development of *Asterina* group which may not only clarify the ontogeny of a given species but may also help towards understanding the evolution of asteroid development as a typical group having divergent breeding methods and development.

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Summary

1. The breeding season of *Asterina minor* is estimated to be during the month of May in Kushimoto, Japan. *A. minor* shows a characteristic breeding assemblage and the eggs are laid on the substratum in a mass spawning. The eggs are not protected by the adults.

2. *A. minor* is a spatial hermaphrodite, where ovaries and testes in an individual become mature simultaneously. Isolated individuals are capable of self-fertilizing and the self-fertilized eggs develop normally.

3. The spawned eggs are spherical, yellow, and 437 μm in average diameter. They attach to the substratum with a sticky jelly layer. Cleavage is total and radial.

4. Eggs through the wrinkled blastula stage develop into a pear-shaped brachiolaria bearing three brachiolar arms within the fertilization membrane.

5. About four days after spawning, the brachiolariae hatch from the fertilization membrane and creep on the substratum with well-developed brachiolar arms. There is no evidence of pelagic life in the present species.

6. One day after hatching, brachiolariae attach firmly to the substratum with the brachiolar arm and undergo a rapid transformation of the body (metamorphic climax). Metamorphosis is completed with the opening of the mouth about 10 days after spawning. The newly metamorphosed juvenile is about 700 μm in diameter and each arm bears two pairs of the tube-feet and one red eye-spot at the base of the terminal tentacle.

7. The reproduction and larval development of *A. minor* are unique, and the study may prove a good guideline for understanding the evolution of reproduction and development in Asteroidea.

LITERATURE CITED

Chia, F-S., 1968. The embryology of a brooding starfish, *Leptasterias hexactis* (Stimpson). *Acta Zool.,* **49**: 321–364.

Cognetti, G., 1954. La proteroginia in una popolazione di *Asterina pancerii* Gasco del Golfo di Napoli. *Boll. Zool.,* **21**: 77–80.

Cuénot, L., 1887. Contributions á l'étude anatomique des Asterides. *Arch. Zool. Exp. Gén.,* **2(5)**: 1–144.

Dartnall, A., 1970. Some species of *Asterina* from Flinders, Victoria. *Victorian Nat.,* **87**: 1–4.

Delavault, R., 1966. Determinism of sex. Pages 615–638 in R. A. Boolootian, Ed., *Physiology of Echinodermata.* Interscience, N. Y.

Goto, S., 1898. Some points in the metamorphosis of *Asterina gibbosa. J. Sci. Coll. Imp. Univ. Tokyo,* **12**: 227–242.

Hayashi, R., 1974. A new sea-star from Japan, *Asterina minor* sp. nov. *Proc. Jap. Soc. Syst. Zool.,* **10**: 41–44.

James, D. B., 1972. Note on the development of the asteroid *Asterina burtoni* Gray. *J. Mar. Biol. Assoc. India,* **14**: 1–2.

Kano, Y. T., and M. Komatsu, 1978. Development of the sea-star, *Asterina batheri* Goto. *Dev. Growth Differ.,* **20**: 107–114.

Komatsu, M., 1975. Development of the sea-star, *Asterina coronata japonica* Hayashi. *Proc. Jap. Soc. Syst. Zool.,* **11**: 42–48.

Komatsu, M., 1976. Wrinkled blastula of the sea-star, *Asterina minor* Hayashi. *Dev. Growth Differ.,* **18**: 437–440.

Kubo, K., 1951. Some observations on the development of the sea-star, *Leptasterias ochotensis similispinis* (Clark). *J. Fac. Sci., Hokkaido Univ. Ser. VI, Zool.,* **10**: 97–105.

Ludwig, H., 1882. Entwicklungsgeschichte der *Asterina gibbosa* Forbes. *Z. Wiss. Zool.,* **37**: 1–98.

MacBride, E. W., 1896. The development of *Asterina gibbosa. Q. J. Microsc. Sci.,* **38**: 339–411.

Mortensen, Th., 1921. *Studies of the development and larval forms of echinoderms.* G. E. C. Gad, Copenhagen, 216 pp.

Ohshima, H., 1929. Hermafrodita marsetelo *Asterina batheri* Goto. *Annot. Zool. Jap.,* **12**: 333–349.

Reference: *Biol. Bull.*, **157**: 275–287. (October, 1979)

THE EFFECT OF SIZE, TEMPERATURE, OXYGEN LEVEL AND NUTRITIONAL CONDITION ON OXYGEN UPTAKE IN THE SAND DOLLAR, *MELLITA QUINQUIESPERFORATA* (LESKE)

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Because metabolism often represents a large source of expended energy, accurate measurement of metabolism is essential for construction of energy budgets. Metabolic processes, as measured indirectly by oxygen uptake, however, may vary with physical condition and physiological state. It is often difficult to assess the results of all these variables on metabolism, and some effects are either discounted or estimated.

Such estimations may be misleading, especially in echinoids, where relatively few studies have been made and where different species of echinoids may respond differently to certain variables. The purpose of this study is to obtain a relatively accurate estimation of metabolic energy expenditure in the sand dollar, *Mellita quinquiesperforata*, by measuring oxygen consumption seasonally at ambient water temperatures, under various degrees of starvation, during the day and night, and at different oxygen concentrations on various size animals.

Materials and Methods

For monthly measurement of oxygen consumption, five or six animals of various sizes were collected from Mullet Key, a sub-tidal, semi-protected sandy beach area along the Gulf of Mexico, adjacent to the mouth of Tampa Bay, Florida (27° 38′ N; 82° 44′ W). After allowing the guts to clear overnight, animals were placed in individual dishes containing either 200 ml (for small animals), 500 ml (for medium-sized animals) or 1000 ml (for large animals) of freshly aerated sea water. After 0.5 hr, a 1-cm layer of mineral oil (which blocks oxygen diffusion) was poured over the surface of the water. At this time, an initial water sample was taken from a dish with no animal (control). A known volume of water (approx. 30 ml) was removed from each dish at 1 and 2 hr after sealing; the water was gently stirred just before sampling. Oxygen in water samples was measured using the micro-Winkler method (Hoar and Hickman, 1967). Sea water used for all experiments was collected from the field and filtered through 0.22 μ Millepore filters before use. A constant temperature chamber (dimly lit) stimulated monthly environmental water temperatures and fluctuated less than 0.2° C during each experiment. Salinity varied little between experiments (33.0–34.9‰). After 2 hr, each animal was removed, weighed, and placed in a drying oven at 80° C. Dried animals were weighed and ground to powder in a Wylie mill. A small sample of this material was used for measurement of total nitrogen by the micro-Kjeldahl (Holland, 1964). Monthly rates of oxygen consumption are expressed on a wet, dry and total body nitrogen weight basis. Analysis of the

data was done on values obtained after 1 hour as tests showed *M. quinquiesperforata* to be an oxygen conformer.

To ascertain how rate of oxygen consumption is affected by reduced oxygen tensions in sea water, animals of similar weight (7 g dry weight) were placed in dishes containing a known volume of sea water (100 ml) in ten experiments. The dishes were sealed with a lid through which an oxygen electrode was mounted. The rate of respiration in these closed dishes was measured every 10 minutes. The water was stirred during each reading by a magnetic stirbar located just beneath the oxygen electrode. The water temperature in all these experiments was 25° C.

In addition to monthly measurements using a closed system, the rate of oxygen consumption was measured in June in an "open" or "flow-through" system, similar to the one described in Hoar and Hickman (1967). Six animals were placed in individual dishes which were closed to general water circulation except for entrance and exit holes. Water entered the dishes and was continuously siphoned into stoppered Erlenmeyer flasks. The rate of flow through the dishes was measured each day and adjusted so that oxygen tension never fell below 80% saturation. Animals used in this experiment were acclimatized to summer water temperatures (29–33° C). Experiments were run at 15, 20, 25, 30, and 35° C. For each experimental temperature, the water in the flow-through apparatus was changed and different animals were used. Animals were not placed into the dishes until all feces had been expelled (usually overnight); oxygen readings were begun after animals had been held at experimental temperatures in the dishes for one day. Oxygen concentration was measured in the Erlenmeyer flasks using a Beckmann Field-Lab oxygen meter fitted with a platinum electrode. Readings were taken at 4-hr intervals for 48 continuous hours. The experimental temperature was maintained by placing the whole flow-through apparatus in a constant temperature chamber. A dim light switched on at 0700 hr and off at 2200 hr simulating day-night conditions. After each experiment, animals were removed from the dishes, weighed and dried as described above. Results from the flow-through system were compared with results from a closed respiratory system. One-hour closed system measurements were run at the same temperature at the beginning of each flow-through experiment.

The effect of starvation upon the rate of oxygen consumption was measured at different times of the year by starving animals in filtered sea water for periods up to 1 month. Water in the holding tanks was renewed every 2 days with freshly collected and filtered sea water. During starvation, water temperature in tanks simulated ambient temperatures. The oxygen consumption of animals which were starved for various times (5–30 days), was measured in a closed system.

Results of all oxygen uptake experiments are expressed as log-log linear regression equations with $\log_{10}$ rate of oxygen uptake and $\log_{10}$ body weight (wet, dry or body nitrogen weight) being the two variables. Slopes and intercepts from different seasons or experiments were compared using analysis of covariance (Snedecor and Cochran, 1971). Variances were checked with Bartlett's Chi-square (Snedecor and Cochran, 1971). For ease in comparing rates of oxygen consumption at different temperatures, pooled slopes were used when statistically valid and intercepts were adjusted as follows; new intercept equals average of Y values minus pooled slope times average of X values.

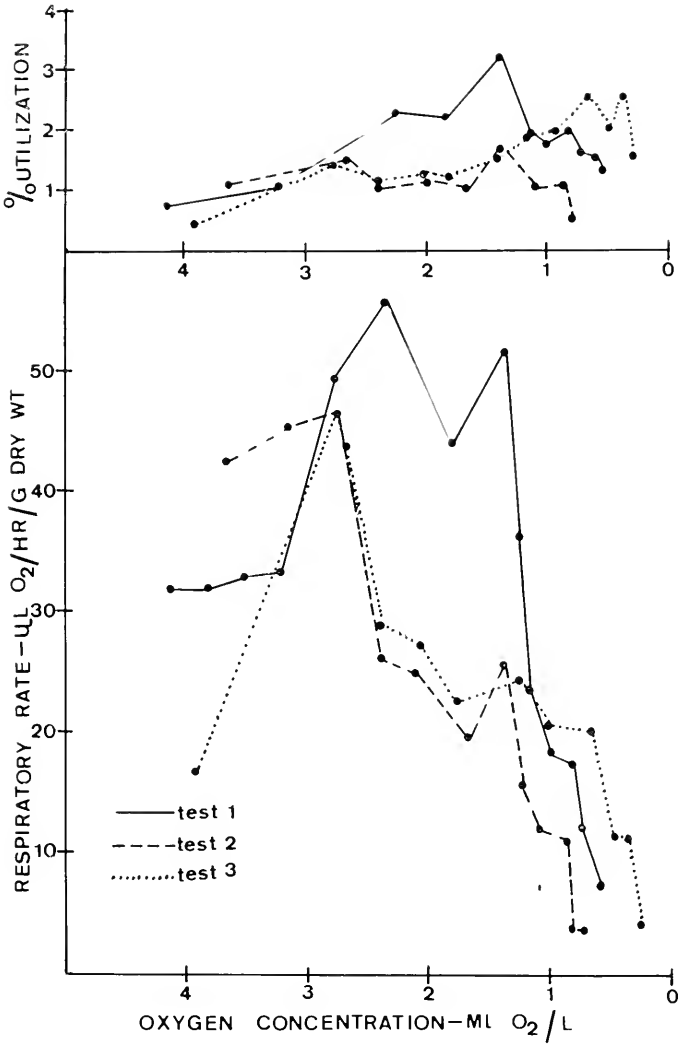

FIGURE 1. Rate of oxygen uptake (μl O_2/hr/g dry weight) and percent utilization of available oxygen by *Mellita quinquiesperforata* in decreasing oxygen concentrations in three representative experiments. A closed system was used for measurement.

RESULTS

Rates of respiration of *Mellita quinquiesperforata* conform to oxygen concentrations in water (Fig. 1). Oxygen consumption decreases with decreasing oxygen pressure in a nearly linear manner. At very low levels of external oxygen (< 1 ml O_2/liter), a low level of respiration is maintained.

In monthly oxygen measurements at ambient temperatures, rates of respiration are dependent on both body weight (wet, dry or body nitrogen) and water

TABLE 1

Summary of linear regression equations for ml O_2 respired/hr per animal vs. *dry body weight in non-starved* Mellita quinquiesperforata *at monthly ambient water temperatures and in starved sand dollars for various periods of starvation at ambient water temperatures. The form of the linear regression equations is $\log_{10}$ ml O_2 respired/hr per animal (Y) equals $\log_{10}$ g dry body weight (X) times slope plus intercept. A closed system was used for measurement.*

Date	Dry weight of animals (g)	Ambient water temperature (° C)	# days starved	N	Slope	"F" for slope	Intercept	Intercept (adjusted)
			Non-starved animals					
VII/4/72	1.5–26.5	31	—	5	0.5448	180.81**	−0.7508	−0.7541
VII/25/72	0.3–17.6	30	—	6	0.5754	61.46**	−0.9960	−0.9707
VIII/30/72	2.2–16.0	33	—	6	0.5799	41.44**	−0.8954	−0.8655
IX/30/72	1.0–21.9	27	—	5	0.5174	49.88**	−0.9671	−0.9908
X/24/72	0.4–26.8	25	—	5	0.6221	308.72**	−1.0426	−1.0027
XI/15/72	1.0–15.4	22	—	5	0.5136	1167.25**	−0.9850	−1.0119
XII/15/72	2.4–15.9	20	—	6	0.5285	18.55**	−1.0551	−1.0717
I/16/73	2.0–13.8	20	—	6	0.5116	30.17**	−0.9577	−0.9876
II/27/73	4.1–12.0	15	—	6	0.6093	10.59*	−1.1714	−1.1174
V/14/73	1.7–35.8	28	—	6	0.6704	21.60**	−1.0208	−0.8706
			Starved animals					
VI/30/72	0.6–54.6	30	5	5	0.8410	56.02**	−1.1018	
VII/21/72	3.1–23.4	31	18	4	0.7011	14.75**	−1.2526	
X/16/72	0.5–29.0	25	20	5	0.9297	106.48**	−1.3979	
VIII/14/73	2.4–14.2	29	24	5	0.9600	17.29*	−1.3739	
XI/15/73	2.0–28.2	21.5	12	5	0.2978	1.32	−0.8849	

* Significant at 95% level.
** Significant at 99% level.

TABLE II

Summary of analysis of covariance for testing significance of differences between slopes and intercepts for regression equations of oxygen uptake in Mellita quinquiesperforata *at different temperatures, in different nutritional states, on a dry (DW) and nitrogen (NW) weight basis, and in closed and open respirometers. The form of the linear regression equation is* $\log_{10}$ *ml* O_2 *respired/hr per animal (Y) equals* $\log_{10}$ *g dry body weight or nitrogen weight (X) times slope plus intercept. For comparing slopes, summer months are considered as VI, VII, and VIII; fall months are IX, X, and XI; winter months are XII, I, and II; and spring months are III, IV, and V.*

Dates or experiments being tested	Pooled slope	χ^2 variance	"F" for slope	df	"F" for intercept	df
Tests for heterogeneity among experimental dates						
All non-starved dates (DW)	0.5485	13.61	0.3027	9, 36		
Summer non-starved dates (DW)	0.5374	5.10	0.1323	2, 11	21.7575**	2, 27
Fall non-starved dates (DW)	0.5614	4.20	2.2056	2, 9	0.3768	2, 23
Winter non-starved dates (DW)	0.5327	0.90	0.0827	2, 12	7.8714**	2, 29
All non-starved dates (NW)	0.5670	14.55	1.1036	8, 32	1154.6722**	8, 327
All starved dates (DW)	0.8216	2.37	1.5747	4, 14		
All starved dates (NW)	0.8132	7.77	2.5699	4, 14	15.9071**	4, 75
Tests for heterogeneity among grouped regressions						
Starved *vs* non-starved summer dates (DW)	0.5789	0.83	7.7766**	1, 24	2.3639	1, 25
Starved *vs* non-starved fall dates (DW)	0.6611	15.43**	6.2446**	1, 21	1.8405	1, 27
Starved *vs* non-starved summer dates (NW)	0.6359	0.01	2.1658	1, 21	0.0364	1, 22
Starved *vs* non-starved fall dates (NW)	0.6572	10.42**	2.9027	1, 21	0.2687	1, 22
Closed *vs* open at 25° C	0.6772	1.09	0.0750	1, 8	62.4133**	1, 9
Closed *vs* open at 20° C	0.5496	0.90	0.0436	1, 26	23.2153**	1, 27
Closed *vs* open at 30° C	0.4115	12.52**	0.0456	1, 20	11.8181**	1, 21

* Significant at 95% level.
** Significant at 99% level.

temperature (Table I; Fig. 2). Small animals have proportionally higher rates of respiration than do large animals when values are expressed on a dry weight (pooled yearly slope of 0.5485; Table II) or a nitrogen weight (pooled yearly slope of 0.5670; Table II) basis. Differences between slopes are not significant when slopes are compared together by year or by season (Table II). Degree of gonadal development had no effect on respiratory rates.

Although there was no significant difference between slopes, rates of respiration did change significantly with water temperature, as indicated by significant "F" values for intercepts (Table II). To simplify comparison at different temperatures, intercepts were adjusted using a pooled slope of 0.5485 (monthly slopes were not significantly different) for all experiments. Using these adjusted intercepts, a 5-g animal (dry weight) would have highest rates of respiration on VII/4/72 at 31° C (0.4259 ml O_2/hr) and lowest rates on II/27/73 at 15° C (0.1845 ml O_2/hr) (Fig. 2). Rates of respiration slowly decrease between 30 and 22° C (fall months) but decline greatly between 31 and 30° C (VII/4/72 and VII/25/72, respectively). The respiratory rate is lower at 33° C than at 31° C (Fig. 2). Q_{10} values for field acclimatized animals are 1.38 from the temperature range 15 to 33° C, 1.69 from 15 to 31° C, 1.93 from 22 to 31° C, 1.13 from 22 to 30° C and 1.42 from 15 to 22° C.

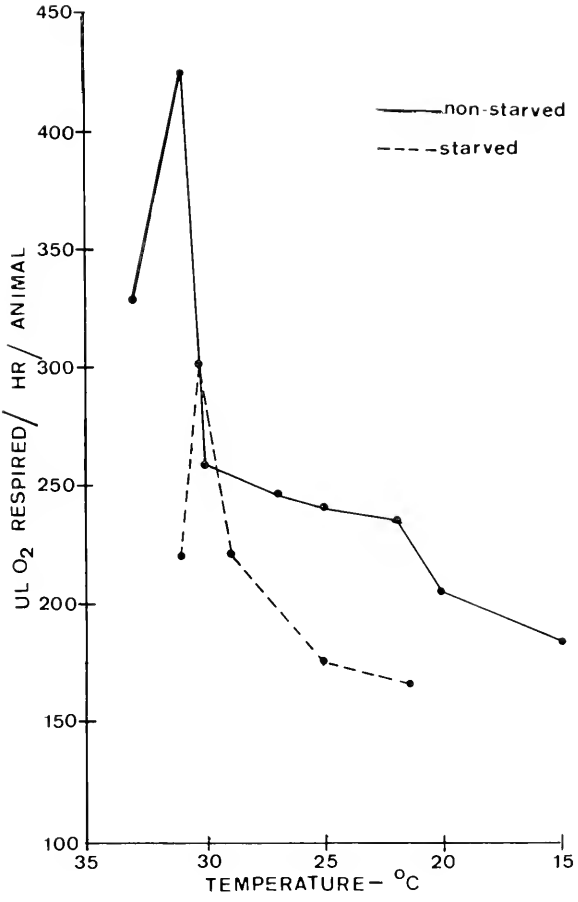

FIGURE 2. Relationship between oxygen consumption (μl O_2 respired/hr per animal) and water temperature for a starved and a non-starved 5-g (dry) specimen of *Mellita quinquiesperforata*. Rates were calculated from regression equations given in Table I.

Respiratory rates decline with starvation; the degree to which the rate declined depended on length of time animals were starved, water temperatures during starvation, and weight of the animal. Because animals were starved for different lengths of time at different water temperatures, it is difficult to assess the effect of these variables independently. Respiratory rates became more depressed as the period of starvation increased (Table I). A 1-g dry animal which was starved for 18 days at 31° C (VII/21/72) would use 53.63 μl O_2/hr, whereas a 1-g animal starved for 5 days at 30° C (VII/30/72) would use 76.24 μl O_2/hr (calculated using a pooled slope of 0.8670 and adjusted intercepts, Table I).

Effects of starvation depend on weight of the animals. On the basis of body dry weight, starvation has a proportionally greater effect on the respiratory rate of small starved animals as indicated by higher values for "starved" slopes (Table I). A 20-g (dry weight) starved animal has slightly higher rates and a

5-g (dry weight) starved animal has lower rates when compared with non-starved animals of similar size (Table I; Fig. 2). On a seasonal basis, the slopes for starved and non-starved animals are significantly different (Table II).

When respiratory rates are expressed on a nitrogen basis, O_2 uptake of small, starved animals is not as depressed when compared to non-starved animals (Fig. 3) and the regression slopes and intercepts of the two groups are not significantly different (Table II). Starved animals of all sizes have significantly less nitrogen/g dry weight than non-starved animals (Lane, 1977; Fig. 4) and small animals lose (metabolize) slightly more nitrogen during starvation than do larger animals (Lane, 1977). Small animals have lower rates of respiration when starved, but they have less body nitrogen as well when compared with larger animals.

Rates of respiration depend on the type of system used for measuring respiration. Comparison of respiration in closed and open systems at the same temperature shows intercepts, hence rates, of O_2 consumption are significantly higher in the open system (Table II). Using pooled slopes and adjusted intercepts for each

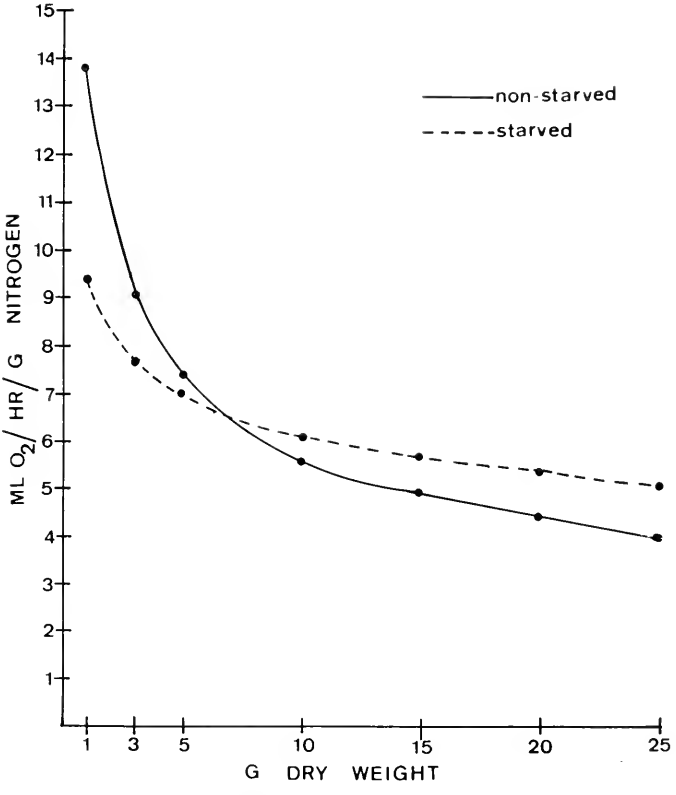

FIGURE 3. Relationship between oxygen consumption (ml O_2 respired/hr/g nitrogen) and dry body weight for starved and non-starved specimens of *Mellita quinquiesperforata* at fall water temperatures. Rates were calculated as follows; non-starved animals-$\log_{10}$ ml O_2/hr/g nitrogen equals 1.1414 minus 0.3823 times $\log_{10}$ g dry body weight; starved animals-$\log_{10}$ ml O_2/hr/g nitrogen equals 0.9740 minus 0.1835 times $\log_{10}$ g dry body weight.

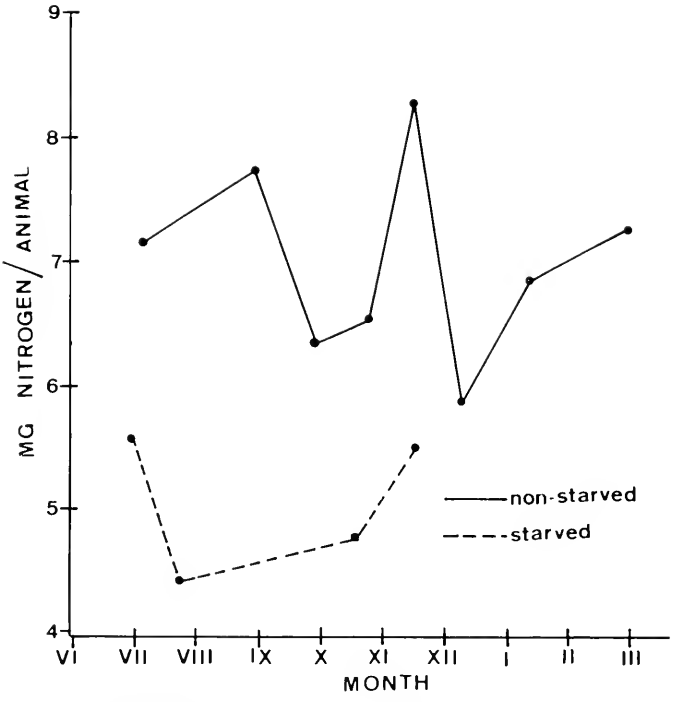

FIGURE 4. Seasonal changes in the amounts of total nitrogen in a starved and non-starved 1-g specimen of *Mellita quinquiesperforata*. Values were calculated using pooled seasonal slopes and adjusted intercepts. Slopes used for non-starved animals are: summer, 0.8989; fall, 0.9462; winter, 1.0301. Slopes used for starved animals are: summer, 0.9561; fall, 1.0499.

temperature, a 10-g animal (dry weight) would respire 0.3003 ml O_2/hr per animal and 1.0598 ml O_2/hr per animal at 25° C; 0.2252 ml O_2/hr per animal and 0.5564 ml O_2/hr per animal at 20° C; and 0.4990 and 0.8158 ml O_2/hr per animal at 30° C in closed and open systems respectively. Comparison of respiration during the day and night in an open system showed no trends.

DISCUSSION

As found with many other echinoderms and invertebrates in general, the respiratory rates of *Mellita quinquiesperforata* are modified by a variety of factors. Body weight or age is one such factor. Smaller (younger) animals have proportionally higher rates of respiration than larger (older) animals when rates are based on both body weight or body nitrogen. Similarly, this effect of size (age) on rate of O_2 uptake has been found in all other echinoids studied and regression slopes ($\log_{10}$ ml O_2 respired/hr per animal versus $\log_{10}$ body weight) for echinoids generally fall between 0.500 and 0.700 (Lewis, 1967, 1968; McPherson, 1968; Percy, 1971; Webster, 1972; Miller and Mann, 1973). An adequate or complete explanation for this phenomenon is lacking. The decreasing surface area with increasing weight explanation does not appear to apply in the case of *M. quinquies-*

perforata. Surface area (from shape) is approximately body weight 0.66, while respiration is body weight 0.5485. In addition, the body wall of echinoids has been shown to be the principal consumer (90%) of oxygen in whole respiring animals (Giese, Farmanfarmaian, Hilden, and Doezema, 1966; Webster, 1972; Belman and Giese, 1974; Webster and Giese, 1975). Stephans, Volk, Wright, and Backlund (1978) similarly concluded that the epidermis of the test of the sand dollar is responsible for a large portion of the total oxygen consumption by the sand dollar, *Dendraster excentricus*. Since the outer surface of the body wall is directly exposed to oxygenated water, the surface to volume argument should hardly apply to these echinoids.

In *M. quinquiesperforata*, it would appear that smaller (younger) animals may simply have 'faster' metabolic systems, *i.e.*, shorter half-lives of enzymes, faster turnover of proteins. Evidence for this comes from similar decreases in rate with increasing animal weight which were found for the processes of feeding, carbon excretion, ammonia excretion and radioactive uptake in this sand dollar (Lane, 1977). With starvation, smaller sand dollars metabolize proportionally greater amounts of nitrogen than larger animals. Fuji (1962, 1967) and Miller and Mann (1973) found similar allometry in different functions of other echinoids. Stephans *et al.* (1978), however, found that differences in rates of amino acid uptake between small and large sand dollars disappeared when surface area was used as the basis of expression.

As with *M. quinquiesperforata*, other echinoids have depressed respiratory rates with starvation. Farmanfarmaian (1966) observed that V_{O_2}'s were reduced by as much as 50% after a month of starvation. Giese (1967) noted a general

TABLE III

Summer and fall comparisons for calories expended in respiration/month of starved and non-starved Mellita quinquiesperforata. *For starved animals, values were calculated from VIII 14 72 (summer) with a slope of 0.867 and an adjusted intercept of −1.3353 and XI 15 73 (fall) with a slope of 0.7720 and an adjusted intercept of −1.3099. For non-starved animals, a slope of 0.5485 and an adjusted intercept of −0.7541 (VII 4 72) was used for calculating summer values and a slope of 0.5485 and an adjusted intercept of −1.0119 (XI 15 72) was used for fall values. 4.8 cal/ml O_2 respired was used to convert ml O_2 into calories.*

Dry weight of animals	Cal respired/ month/starved animal	Cal respired/ month/non-starved animal	Starved values/ non-starved values × 100
	Summer comparison		
1 g	159.67	608.80	26.22%
10 g	1175.71	2152.65	54.61%
20 g	2144.11	3148.39	68.10%
40 g	3910.80	4604.72	83.93%
	Fall comparison		
1 g	169.31	336.26	50.35%
10 g	1001.55	1188.98	84.23%
20 g	1710.05	1738.95	98.33%
40 g	2919.97	2543.33	114.80%

decline in rate of oxygen uptake over a month of starvation. Webster (1972) found that the greatest decrease in V_{O_2} occurred in the first week of starvation with little change thereafter. Boolootian and Cantor (1965) reported that the respiratory rate fell to a low level after three weeks of starvation and remained constant thereafter. Differences in response of respiratory rate among echinoids with starvation may perhaps be explained by differences in nutritional history at the beginning of the experiment and consequent differences in substrate being metabolized during starvation. Wallace (1973) found that the V_{O_2} of starving crabs fell in steps corresponding to the type of nutrient reserve being metabolized. Carbohydrate reserves were first utilized, with lipid next and protein last. Differences in response of small and large specimens of *M. quinquiesperforata* to starvation may also be due to differences in substrate being metabolized. If so, large animals may have had proportionally greater reserves of either carbohydrate or lipid (in the gonad). Consequently, small animals may have been metabolizing protein while larger animals were metabolizing carbohydrate or lipid, resulting in more depressed rates of respiration in small animals. The proportionally greater decrease in amount of body nitrogen in small, starved animals seems to support this conclusion.

Higher rates of respiration in small, non-starved specimens of *Mellita* may also have been due to effects of SDA or greater activity in non-starved sand dollars. Effects of both factors, however, appear to be minimal. Activity of starved and non-starved animals was similar in closed containers. As reported by Lilly (1979) for two species of sea urchins, the effect of SDA was most pronounced just after feeding and declined to pre-feeding levels after 3 hr. Rates of O_2 uptake in the sand dollar were measured approximately 24 hr after feeding. Hence SDA should be of slight consequence.

Differences between respiratory rates in starved and non-starved animals may represent energy used in growing (Table III). If so, 26% of respiration energy is used for summer maintenance and the remainder (74%) is energy expended for growth in a 1-g, non-starved specimen of *M. quinquiesperforata*. In an actively growing 20-g animal (dry weight), 68% of the respiration energy is used for summer maintenance and 32% respresents energy for growth (Table III). In fall, more non-starved respiration energy is used in maintenance and less in growth for all size sand dollars. Both the greater amounts of energy expended in growth by small sand dollars and the seasonal difference in amounts of energy for growth by all size animals are consistent with information from growth studies on this animal (Lane, 1977). From growth studies, a 1-g animal would expend 136 calories for growth/month in the summer (Lane, 1977). Difference between respiratory rates of starved and non-starved 1-g animals indicate 449 calories/month would be spent in growth. Likewise in fall, a 1-g animal would add 91 calories/month in growth (Lane, 1977) and expend 167 calories/month in growing (from difference between starved and non-starved respiration). For larger animals in fall, starved respiration was greater than non-starved. This anomaly may have resulted from differences in the nutritional condition of "freshly collected" animals. Negative growth reported for large animals in fall (Lane, 1977) could indicate that "freshly collected" specimens of *M. quinquiesperforata* were

already starved when taken from the field, and starvation in the laboratory produced no further change in respiratory rate.

Although laboratory experiments were not specifically designed to characterize type of acclimation response in *M. quinquiesperforata,* monthly oxygen measurements made at ambient water temperatures did demonstrate partial acclimatization in sand dollars. V_{O_2}'s were higher in January at 20° C than in December at the same temperature. Likewise, acclimatization to summer water conditions may have resulted in lower rates of respiration on VII/25/72 at 30° C as compared to the much higher rates three weeks earlier on VII/4/72 at 31° C. As with many other echinoids (Farmanfarmaian and Giese, 1963; Moore and McPherson, 1965; McPherson, 1968; Percy, 1971; Webster, 1972), this compensatory response was only partial in *Mellita* since respiration was still higher in summer than in winter.

The sensitivity of the respiratory rate in *M. quinquiesperforata* (Q_{10}) was relatively low between 22 and 30° C. In the Tampa Bay area, the change in water temperature over the 22 to 30° C range is rapid during the spring warming and fall cooling. The respiratory insensitivity over this range would, therefore, appear to have some adaptive value. The decline in V_{O_2} at 33° C may indicate metabolic malfunction as this temperature is close to the lethal limits (~ 38° C) of this sand dollar.

Oxygen consumption may be modified by type of system used for measuring respiration. Miller and Mann (1973) for *S. drocbachiensis,* Webster and Giese (1975) for *Strongylocentrotus purpuratus* and this study found that rates of respiration were higher in flow-through systems when compared with closed systems. Although this increased rate may be caused by increased availability of oxygen, activity of animals may also be increased in flowing water. Activity of *M. quinquiesperforata* was much greater in the flow-through system than intermittent activity of sand dollars in closed systems. Although sand dollars are exposed to a continual exchange of water in their environment, much of the time they are partially buried and do not or can not continually wave their spines back and forth as was the case in the open system. Therefore, the monthly oxygen measurements made in closed dishes are considered more representative of routine metabolism.

Due to various factors which modify metabolic rate, and the different units used to express respiratory rate, it is difficult to compare absolute rates of respiration among various echinoids. When compared to the respiratory rates of tropical and temperate echinoids as given by Webster (1975), V_{O_2}'s of *M. quinquiesperforata* (as found in this study) are slightly higher than rates for tropical echinoids of similar weight.

Summary

1. Rates of respiration in a closed vessel conformed to oxygen concentrations in surrounding water until a low level (1 ml O_2/liter) of oxygen was attained.

2. Respiratory rates of small animals were proportionally higher on both a dry weight and nitrogen weight basis than rates of large animals (slope for ml O_2 respired/hr per animal was 0.5485 for dry weight and 0.5670 for nitrogen) in a closed vessel.

3. Respiratory rates of field acclimatized animals were slightly higher at summer temperatures (30–33° C) than at winter water temperatures (15–20° C) with evidence for partial acclimatization presented.

4. Respiratory rates of starved animals were lower than rates of non-starved animals with starvation depressing the rates of smaller animals more than rates of larger animals.

5. Respiratory rates in an open system were approximately twice as high as rates in a closed system due to greater activity of animals in the open system.

6. Comparison of day versus night respiration showed no obvious trends.

7. Respiratory rates of *M. quinquiesperforata* are slightly higher than rates of a similar weight, tropical, regular urchin as reported in the literature.

LITERATURE CITED

Belman, B. W., and A. C. Giese, 1974. Oxygen consumption of an asteriod and an echinoid from the Antarctic. *Biol. Bull.*, **146**: 157–164.

Boolootian, R. A., and M. A. Cantor. 1965. Preliminary report on respiration, nutrition and behavior of *Arbacia punctulata*. *Life Sci.* **4**: 1567–1571.

Farmanfarmaian, A. A., 1966. The respiratory physiology of echinoderms. Pages 245–265 in R. A. Boolootian, Ed., *Physiology of Echinodermata*. Interscience Publishers, New York.

Farmanfarmaian, A. A., and A. C. Giese, 1963. Thermal tolerance and acclimation in the western purple sea urchin *Strongylocentrotus purpuratus*. *Physiol. Zool.*, **36**: 237–243.

Fuji, A., 1962. Studies on the biology of the sea urchin V. Food consumption of *Strongylocentrotus intermedius*. *Jpn. J. Ecol.*, **12**: 181–186.

Fuji, A., 1967. Ecological studies on the growth and food consumption of Japanese common littoral sea urchin, *Strongylocentrotus intermedius* (A. Agassiz). *Mem. Fac. Fish., Hokkaido Univ.*, **15**: 83–160.

Giese, A. C., 1967. Changes in the body-component indexes and respiration with size in the purple sea urchin *Strongylocentrotus purpuratus*. *Physiol. Zool.*, **40**: 194–200.

Giese, A. C., A. Farmanfarmaian, S. Hilden, and P. Doezema, 1966. Respiration during the reproductive cycle in the sea urchin *Strongylocentrotus purpuratus*. *Biol. Bull.*, **130**: 192–201.

Hoar, W. S., and C. P. Hickman, Jr., 1967. *A Laboratory Companion for General and Comparative Physiology*. Prentice-Hall, New Jersey, 296 pp.

Holland, L. Z., 1964. Variation of pervisceral fluid protein content with reproduction and nutritional state in the purple sea urchin. *Master's Thesis. Stanford University*, 66 pp.

Lane, J. M., 1977. Bioenergetics of the sand dollar, *Mellita quinquiesperforata* (Leske, 1778). *Ph.D. dissertation. University of South Florida*, 363 pp.

Lewis, J. B., 1967. Comparative respiration of tropical echinoids. *Comp. Biochem. Physiol.*, **41**: 649–652.

Lewis, J. B., 1968. Respiration in the tropical sea urchin *Diadema antillarum* Philippi. *Physiol. Zool.*, **41**: 476–480.

Lilly, G. R., 1979. The influence of diet on the oxygen uptake of the sea urchins, *Tripneustes ventricosus* and *Strongylocentrotus drobachiensis*. *Comp. Biochem. Physiol.*, **62A**: 463–470.

McPherson, B. F., 1968. Feeding and oxygen uptake of the tropical sea urchin *Eucidaris tribuloides* (Lamarck). *Biol. Bull.*, **135**: 308–321.

Miller, R. J., and K. H. Mann, 1973. Ecological energetics of the seaweed zone in a marine bay on the Atlantic coast of Canada. III. Energy transformations by a sea urchin. *Mar. Biol.*, **18**: 99–114.

Moore, H. B., and B. F. McPherson, 1965. A contribution to the study of the productivity of the sea urchins *Tripneustes esculentus* and *Lytechinus variegatus*. *Bull. Mar. Sci. Gulf Caribb.*, **15**: 855–871.

Percy, J. A., 1971. Thermal acclimitization and acclimation in the echinoid *Strongylocentrotus*

droebachiensis (O. F. Muller) 1776. *Ph.D. dissertation. Memorial University*, Newfoundland, 251 pp.

SNEDECOR, G. W., AND W. G. COCHRAN, 1971. *Statistical Methods*. 6th Ed. The Iowa State University Press, Ames, 593 pp.

STEPHENS, G. C., M. J. VOLK, S. H. WRIGHT, AND P. S. BACKLUND, 1978. Transepidermal accumulation of naturally occurring amino acids in the sand dollar, *Dendraster excentricus*. *Biol. Bull.*, **154**: 335–347.

WALLACE, J. C., 1973. Feeding, starvation and metabolic rate in the shorecrab, *Carcinus maenas*. *Mar. Biol.*, **20**: 277–281.

WEBSTER, S. K., 1972. The respiratory physiology of *Strongylocentrotus purpuratus* Stimpson with particular reference to the reproductive cycle. *Ph.D. dissertation, Stanford University*, 120 pp.

WEBSTER, S. K., 1975. Oxygen consumption in echinoderms from several geographical locations, with particular reference to the Echinoidea. *Biol. Bull.*, **148**: 157–164.

WEBSTER, S. K., AND A. C. GIESE, 1975. Oxygen consumption of the purple sea urchin with special reference to the reproductive cycle. *Biol. Bull.*, **148**: 165–180.

Reference: *Biol. Bull.*, **157**: 288–296. (October, 1979)

DEVELOPMENT AND BEHAVIOR OF AN INTERGENERIC CHIMERA OF HYDRA (*PELMATOHYDRA OLIGACTIS* INTERSTITIAL CELLS: *HYDRA ATTENUATA* EPITHELIAL CELLS)

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Hydra has so few cell types that it should be possible to map out the developmental and behavioral functions of each. By combining cell types of different species, one might trace roles by identifying species characters in the resulting chimeric animals.

Many of hydra's highly specialized cell types (nerve cells, nematocytes, gametes) are part of a single lineage of cells that is continually being renewed by proliferation and differentiation of a stem cell called the interstitial cell or "I cell." The entire interstitial cell lineage can be removed from a hydra by various means (Marcum and Campbell, 1978a; Campbell, 1979). The resulting animal is termed an "epithelial hydra," and is composed only of ectodermal and endodermal epithelial cells. This viable epithelial shell can then be repopulated by I cells, since they will migrate throughout the depleted animal from a small, temporary graft of normal tissue. A number of chimeric strains in hydra have been made in this fashion (Saffitz, Burnett and Lesh, 1972; Sugiyama and Fujisawa, 1978; Marcum and Campbell, 1978b). In order to assign roles to the different cell lineages, one would use the most dissimilar species as parents. However, grafting success (and hence presumably tissue compatibility) decreases as species diversity increases (Campbell and Bibb, 1970), so that most hydra chimeras have been constructed of cells from the same or closely related species.

One pair of dissimilar species, *Hydra attenuata* and *Pelmatohydra oligactis*, will partially tolerate intergrafting and a considerable literature suggests that it may be possible to make a stable chimera between their cells (Evlakowa, 1946; Brien and Reniers-Decoen, 1955; Kolenkine and Bonnefoy, 1976). Since it is possible to remove the interstitial cell lineage from *H. attenuata*, we repopulated epithelial *H. attenuata* with *P. oligactis* interstitial cells. The reciprocal graft is not possible since a technique for removing I cells from *P. oligactis* has not been found. This report describes some developmental and behavioral similarities and differences between the chimeras and the two parental species.

Material and Methods

Specimens of *Hydra attenuata* from Lake Zurich, specimens of *Pelmatohydra oligactis* collected in Grant Lake, Mono County, California, and the chimeras were all grown in "M" solution lacking bicarbonate (Muscatine and Lenhoff, 1965) by standard methods (Lenhoff and Brown, 1970). Epithelial specimens of *H. attenuata* were produced by a double colchicine treatment (Marcum and Campbell,

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1978a) to eliminate I cells. Distal halves of epithelial specimens of *H. attenuata* were repopulated with I cells by axially grafting them to proximal halves of normal *P. oligactis* polyps. The graft junction was marked by a permanent constriction in the ectoderm (Kolenkine and Bonnefoy, 1976) and by different coloration of the endoderm in these two species. Grafts were left intact for 3 to 5 days and then the *P. oligactis* epithelial tissue was cut away. The resulting repopulated hydra were maintained for up to 6 months by methods appropriate for epithelial hydra (Marcum and Campbell, 1978a).

Cell compositions were determined using David's (1973) maceration procedure. Heat shocks were applied by immersing 10-ml test tubes containing individual hydra in 2 ml of medium into a preheated waterbath for 30 min. Afterwards the tubes were left at room temperature for 12 hr, and then the hydra were cultured normally. Time-lapse films were made using a 16-mm Bolex camera outfitted with extension tubes behind the lens, with illumination through heat filters.

Feeding response was measured using the methods of Lenhoff (1969). Inhibition of nematocyst discharge (Smith, Oshida and Bode, 1974) was carried out by feeding hydra to repletion and then releasing single shrimp, successively, onto tentacles at measured times later. The number of shrimp contacts which occurred before two became trapped was recorded. Only 20 trials were offered each polyp; a score of 20 indicated that the hydra did not catch the *Artemia*.

Results

Genetic composition of chimeras

Although the methods used for producing chimeras seem straightforward, we considered it important to demonstrate that the chimeras were, in fact, composed of *H. attenuata* epithelial and *P. oligactis* interstitial cells.

The genetic origin of interstitial cells was verified by analysis of chimera nematocysts. Table I shows the dimensions and Figure 1 shows the morphologies

Table I

Nematocyst sizes. Length and width (standard deviations in parenthesis) are all in μm. Each measurement represents about 20 nematocysts. Chimera polyps a, b, c, and d are progeny from different grafts.

Hydra strain	Stenotele	Atrichous isorhiza	Desmoneme
H. attenuata	14.8 × 11.4 (2.2) (1.9)	9.7 × 4.0 (1.0) (0.2)	8.1 × 5.6 (0.6) (0.4)
P. oligactis	12.9 × 9.7 (0.5) (0.5)	7.8 × 4.3 (0.5) (0.5)	6.7 × 4.8 (0.4) (0.3)
Chimera polyp a	12.1 × 9.5 (0.7) (0.7)	8.2 × 4.0 (0.7) (0.2)	5.7 × 4.2 (0.5) (0.4)
polyp b	11.9 × 9.1 (0.6) (0.8)	8.8 × 4.4 (0.5) (0.6)	6.2 × 4.6 (0.4) (0.4)
polyp c	11.0 × 8.3 (1.0) (0.7)	8.8 × 3.8 (0.6) (0.3)	6.1 × 4.0 (0.5) (0.1)
polyp d	11.7 × 8.9 (0.6) (0.5)	9.1 × 4.0 (0.8) (0)	6.1 × 4.2 (0.2) (0.2)

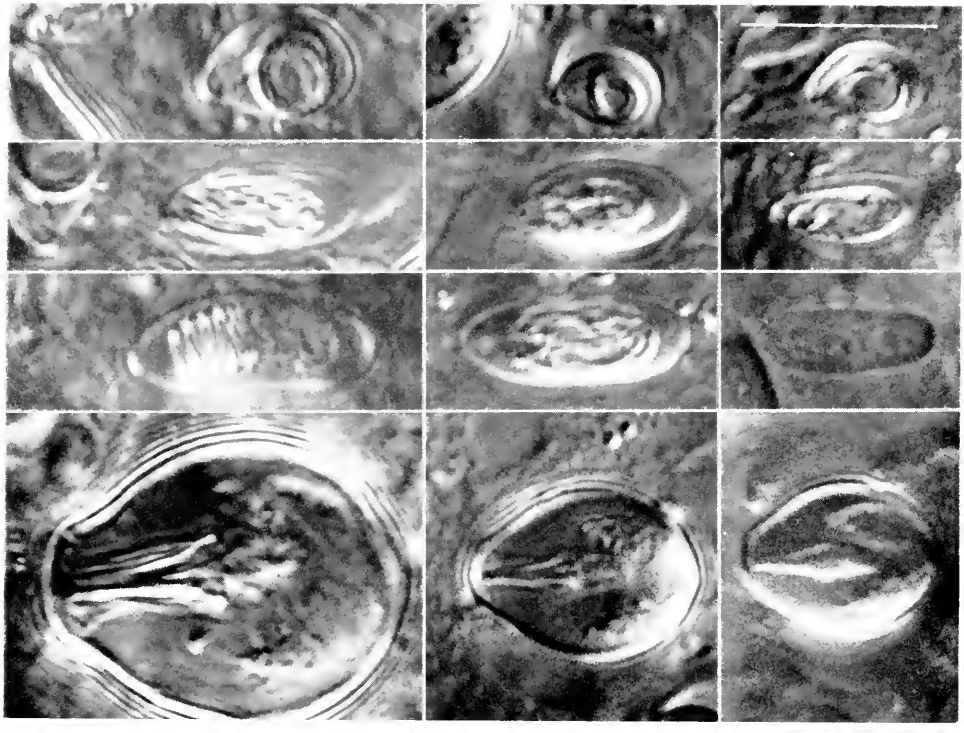

FIGURE 1. Nematocyst structures of *H. attenuata* (left), *P. oligactis* (middle), and chimera (right). Nematocyst types, bottom to top, are: stenotele, holotrichous isorhiza, atrichous isorhiza, and desmoneme. All figures except the chimera holotrichous isorhiza represent mature, mounted nematocysts. Chimeras rarely have mounted holotrichous isorhizas, but the species specific tubule pattern is visible in complete but immature capsules in gastric region nests, as shown here. Scale indicates 10 μm.

of nematocysts of the chimeras and both parents. These data are taken from animals more than 4 months after chimeras were made. The small sizes of the chimera nematocysts are similar to those of *P. oligactis* and not *H. attenuata*. The morphologies of the chimera nematocysts (Fig. 1) are also unambiguously those of *P. oligactis*. The absence of transverse coils in the holotrichous isorhiza is the

TABLE II

Relative proportions of nematocyst types in tentacles. Numbers express percentages of total nematocysts. (Between 500 and 1000 nematocysts were counted in the distal parts of tentacles, with the number of polyps indicated at left).

Species	Nematocyst type		
	Stenotele	Isorhizas	Desmoneme
H. attenuata (n = 2)	3.5 (±0.7)	12.5 (±4.9)	84 (±4)
P. oligactis (n = 3)	34 (±9)	5.7 (±1.5)	60 (±9)
Chimera (n = 6)	37 (±8)	6.7 (±1.0)	57 (±7)

most notable character of *P. oligactis* stingers (Ewer, 1948), and the chimera also lacks these. In addition, the slender shape of the stenotele, the bluntly oval shape of the holotrichous isorhiza, and the reniform atrichous isorhiza are all characteristically *P. oligactis*

The two parental species differ in the relative abundances of the different nematocyst types. Table II shows that in this character the chimeras closely resemble *P. oligactis* and not *H. attenuata*. However, one abnormality of the chimeras was the nearly complete absence of mature holotrichous isorhizas. None were found on animals whose nematocytes were measured (Table I), and few were seen in this study. The photograph in Figure 1 is of an immature holotrichous isorhiza.

The genetic origin of epithelial cells was ascertained in two ways. First, in color both the ectoderm and endoderm were found to resemble *H. attenuata* rather than *P. oligactis*. The chimeric ectoderm was colorless, and the endoderm pink, as in *H. attenuata*. *P. oligactis* has yellow granules in the ectoderm and an orange endoderm. Second, it is known that epithelial tissue controls graft tolerance (Campbell and Bibb, 1970). Therefore, two chimeras that had been established for 6 months were bisected and halves were grafted back to the two parental strains. In the chimera/*H. attenuata* grafts, the graft junctions became imperceptible within a day and no incompatibility was detected during the next 8 days of culture. In the chimera/*P. oligactis* grafts, the graft junctions were still constricted after 1 day and by the sixth day the two halves had separated.

We conclude that the chimeras had *H. attenuata* epithelial cells and *P. oligactis* interstitial cell lineage, and that this composition remained stable throughout the study.

Morphology of chimeras

Chimeras were always smaller than either parent (Fig. 2). Measurements of ten chimeras and parents grown on a regime of six shrimps/day averaged 2.0 mm (chimera), 7.0 mm (*H. attenuata*) and 9.0 mm (*P. oligactis*) in extended length. Tentacle number of budding individuals averaged 5.8 (chimeras), 6.5 (*H. attenuata*) and 6.4 (*P. oligactis*) per polyp. The body column and tentacles of chimeras never seemed to elongate as much as those of either parental species, and this was a contributing factor to their short lengths. The chimeras were slightly more stalked than the *H. attenuata* parent, but not as pronouncedly so as the *P. oligactis* parent.

Budding

The most clear-cut difference between buds of the parental species is the arrangement of tentacle rudiments. Buds of *P. oligactis* first acquire two lateral tentacles, and after these have grown long, two more intercalated rudiments arise (Fig. 3a). In *H. attenuata* the tentacle rudiments arise nearly synchronously and are all about the same length (Fig. 3c). The chimeric pattern (Fig. 3b) is clearly of the *H. attenuata* type.

The budding rates of chimeras were about normal. In one experiment three polyps each of *H. attenuata*, *P. oligactis* and chimera were fed six shrimps/day,

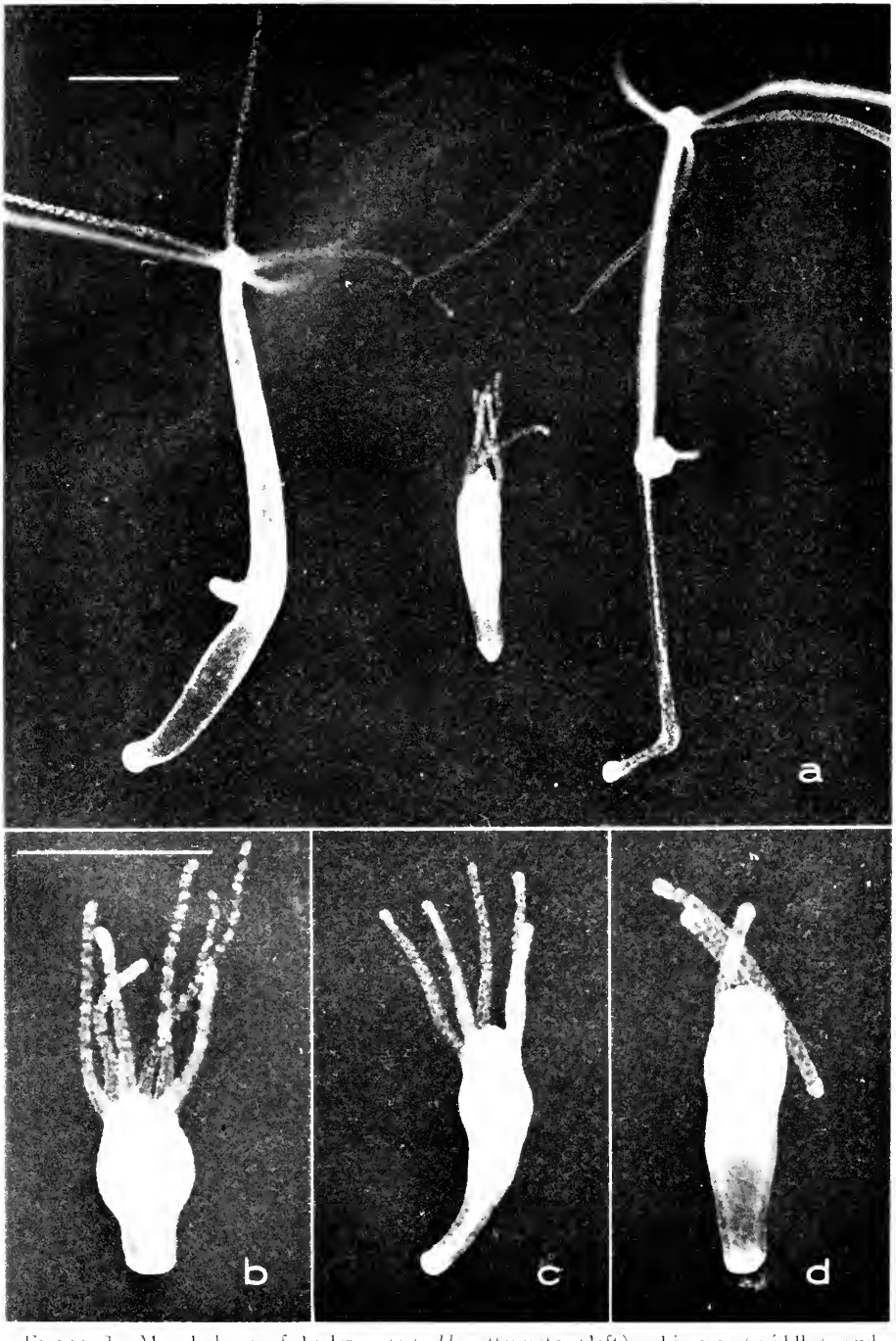

FIGURE 2. Morphology of hydra. (a) *H. attenuata* (left), chimera (middle) and *P. oligactis* (right). All three hydras are of the same age, growing under the same conditions. (b)–(d) different chimera individuals showing typical poses. Scale indicates 1 mm.

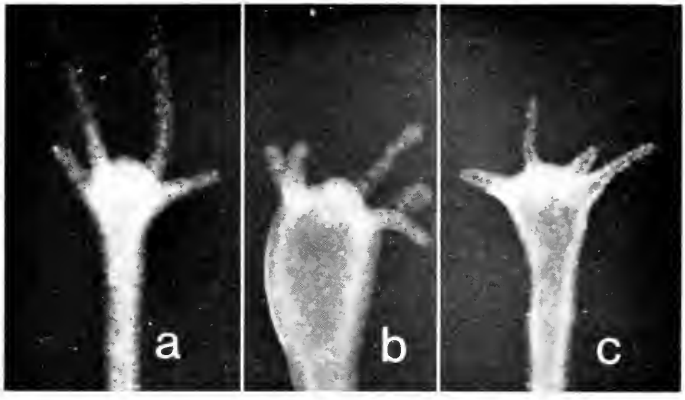

FIGURE 3. Pattern of tentacle development on buds. (a) *P. oligactis* (b) chimera, (c) *H. attenuata.*

long enough for each to produce five buds. Budding rates for these three strains were, respectively, 0.26, 0.26, and 0.20 buds/day. Early morphogenesis, until basal disk formation, of the developing buds occurred at normal rates. However, chimera buds remained attached to their parents for an average of 8 days, while both parental buds detached after 3 days. Mature chimera buds were very small, averaging 1.2 mm in length (extended), whereas *H. attenuata* buds average 5.1 mm and *P. oligactis* buds averaging 5.4 mm in length. Chimera buds did not themselves begin to bud for 19 days, while parental buds budded after 10 (*H. attenuata*) or 11 (*P. oligactis*) days.

Regeneration

Polyps that had been fed six shrimps/day for 6 days were cut in half transversely and both parts allowed to regenerate, without feeding. Tentacle regeneration was assayed by the presence of tentacle rudiments. Basal disk regeneration was assayed by adherence to the dish.

The chimeras ($n = 3$) and both parental species (*H. attenuata,* $n = 8$; *P. oligactis,* $n = 7$) regenerated tentacles from the proximal half in 2 days. The distal halves of chimeras and of *H. attenuata* regenerated basal disks in 2 days. However, the *P. oligactis* proximal halves had not regenerated basal disks in 26 days. Thus, in basal disk regeneration the chimera resembled the epithelial cell parent.

Feeding behavior

The chimeras (as well as the two parental strains) showed typical (Lenhoff, 1969) feeding responses to *Artemia.* When shrimp touched the tentacles they adhered, indicating desmoneme nematocyte discharge, and were paralyzed, indicating stenotele nematocyte discharge. Tentacles holding shrimp underwent considerable writhing, and shrimp were brought to the mouth repeatedly. However, in the chimera the shrimp were never swallowed. The chimeras were thus unable to feed themselves.

To determine if the swallowing behavior itself was deficient in the chimeras,

TABLE III

Inhibition of nematocyst discharge following satiation. Numbers represent average number of trials before hydra caught and paralyzed two Artemia. *Twenty was the maximum number of trials allowed for any polyp. These data represent about 100 sets of trials.*

Time after feeding (min.)	Hydra strain		
	H. attenuata	*P. oligactis*	Chimera
10–30	5.8	7.5	5.4
30–60	12	16	8.4
60–120	10	20	6.7

we strung isolated hydranths on nylon fishing line in "M" solution and measured the creeping movement along the line. In normal hydra the hydranth will move rapidly along the line in the direction of the mouth, as the hydranth attempts to swallow the line. After the first 20 min, *H. attenuata* ($n = 1$) had moved 2.4 mm and *P. oligactis* ($n = 1$) had moved 0.9 mm. Two chimera hydranths failed to move during 3.5 hr of observation.

Normal hydra exhibit a mouth opening response to glutathione. The duration of opening reflects the intensity of the response (Lenhoff, 1969). The durations of the feeding responses to 10^{-5} M glutathione (26° C, pH 7.0) were measured with the following results: *H. attenuata* ($n = 10$), 35.2 ± 18.6 min; *P. oligactis* ($n = 11$), 41.9 ± 13.5 min; chimera ($n = 26$), 21.7 ± 6.8 min.

We also tested the satiation response, as manifested by the failure of nematocysts to discharge, and consequently the failure to trap *Artemia*, after the hydra is fed (Smith, Oshida and Bode, 1974). Table III shows data from these experiments. The chimeras showed a reduced satiation response up to two hours after feeding.

Body motility

Chimeras were much less active than were the two parents. Chimeras never somersaulted nor extended fully, while parental polyps frequently did. We analyzed body contractions and pulsations using time-lapse motion pictures showing all three polyp types in the same dish. By "contractions" we refer to a marked shortening of the body column followed by a reextension. "Pulsation" refers to peristaltic waves traveling proximally down the column.

In contraction frequency, chimeras (0.25/min) were intermediate between *H. attenuata* (0.08/min) and *P. oligactis* (0.34/min). In pulsation frequency chimeras (1.07/min) resembled *H. attenuata* (1.03/min). *P. oligactis* did not exhibit pulsations.

DISCUSSION

The major objective of this research was to produce a chimeric strain of hydra in order to distinguish developmental and behavioral contributions of the epithelial and interstitial cell lineages. Other studies of this nature (Sugiyama and Fujisawa, 1978; Marcum and Campbell, 1978b) showed the feasibility of this approach using closely related strains or species. In the present study we sought to examine the

feasibility of using distantly related species, in this case species of separate genera.

In several traits it was possible to attribute chimeric development and behavior to particular cell types. The pattern of tentacle origin on buds, the rate of basal disk regeneration, heterospecific grafting specificity, color, and prominence of columnar peristaltic waves were all clearly characteristic of *H. attenuata*. Thus, these traits are determined by epithelial cells. On the other hand, nematocyst morphology, nematocyst concentration, and interstitial cell temperature sensitivity (Fradkin, Lee, and Campbell, unpublished) were distinctively those of the *P. oligactis*, indicating that these characteristics are due to the interstitial cell lineage. These results fit the pattern so far uncovered (Campbell, 1979) that morphological and morphogenetic traits derive principally from the epithelial cell genotype.

In several respects the evolutionary divergence between *H. attenuata* and *P. oligactis* was apparently too great to allow normal chimera functioning. These chimeras were very delicate, and had such altered behavior that they could neither eat nor sommersault by themselves. Repopulation of epithelial hydra by interstitial cells of the same species yields hydra of normal behavior (Marcum and Campbell, 1978b) suggesting that the behavioral defects observed here are due to cell incompatibilities rather than due to the repopulation procedure. Therefore, in constructing chimeras for purposes of deducing cellular roles one must work with more closely related species. However, chimeras containing cells as divergent as *H. attenuata* and *P. oligactis* certainly may be useful in unravelling cellular mechanisms. It would be interesting, for example, to see if abnormal neuromuscular contacts might be responsible for the inadequacy of the feeding behavior or the absence of column elongation.

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Summary

An intergeneric chimera was produced by repopulating epithelial *Hydra attenuata* (lacking the interstitial cell lineage) with interstitial cells of *Pelmatohydra oligactis*. The chimera's morphology and morphogenesis generally resembled that of *H. attenuata*, for example in the pattern of bud tentacles, in basal disk regeneration rate, and in heterografting specificity. Nematocyst characters of the chimera were the *P. oligactis* type. In behavior the chimeras were intermediate in some respects but deficient in others. For example, chimeras were unable to feed by themselves or to extend the column. This study illustrates the value of chimeras in deducing which cell types control the various aspects of development and behavior.

Note added in proof

Data from cell type composition studies show that the percentage of nerves among the total cells counted for *H. attenuata* is 5.5%, for *P. oligactis*, 5.7%, and for the Chimera, 6.4%. A total of 5000 to 9000 cells were counted for 6 to 9 individuals (6 individuals of *H. attenuata*, 6 of *P. oligactis*, and 9 of Chimera).

LITERATURE CITED

BRIEN, P., AND M. RENIERS-DECOEN, 1955. La signification des cellules interstitielles des hydres d'eau douce et le problème de la résèrve embryonnaire. *Bull. Biol. Fr. Belg.*, **89**: 285–325.

CAMPBELL, R. D., 1979. Development of hydra lacking interstitial and nerve cells ("epithelial hydra") *Symp. Soc. Dev. Biol.*, **37**: 267–293.

CAMPBELL, R. D., AND C. BIBB, 1970. Transplantation in coelenterates. *Transplant. Proc.*, **2**: 201–211.

DAVID, C. N., 1973. A quantitative method of maceration of hydra tissue. *Wilhelm Roux' Arch. Entwicklungsmech. Org.*, **171**: 259–268.

EVLAKOWA, V. F., 1946. Form-building migration of regenerative material in the hydra. *C.R. Academy of Science, Moscow*, **53**: 373–376.

EWER, R. F., 1948. A review of the Hydridae and two new species of *Hydra* from Natal. *Proc. Zool. Soc. Lond.* **118**: 226–244.

KOLENKINE, X., AND A. M. BONNEFOY, 1976. Etude structurele des jonctions et "contacts" myoepitheliaux dans l'affrontment heterospecifique *Pelmatohydra oligactis* et *Hydra attenuata*. *J. Microscop. Biol. Cell.*, **27**: 59–68.

LENHOFF, H. M., 1969. pH profile of a peptide receptor. *Comp. Biochem. Physiol.*, **28**: 571–586.

LENHOFF, H. M., AND R. D. BROWN, 1970. Mass culture of *Hydra:* an improved method and its application to other aquatic invertebrates. *Lab. Anim.*, **4**: 139–154.

MARCUM, B. A., AND R. D. CAMPBELL, 1978a. Development of Hydra lacking nerve and interstitial cells. *J. Cell. Sci.*, **29**: 17–33.

MARCUM, B. A., AND R. D. CAMPBELL, 1978b. Developmental roles of epithelial and interstitial cell lineages in hydra: analysis of chimeras. *J. Cell Sci.*, **32**: 233–247.

MUSCATINE, L., AND H. M. LENHOFF, 1965. Symbiosis of *Hydra* and algae. I. Effects of some environmental cations on growth of symbiotic and aposymbiotic *Hydra*. *Biol. Bull.*, **128**: 415–425.

SAFFITZ, J. E., A. L. BURNETT, AND G. E. LESH, 1972. Nervous system transplantation in hydra. *J. Exp. Zool.*, **179**: 215–223.

SMITH, S., J. OSHIDA, AND H. BODE, 1974. Inhibition of nematocyst discharge in hydra fed to repletion. *Biol. Bull.*, **147**: 186–202.

SUGIYAMA, T., AND T. FUJISAWA, 1978. Genetic analysis of developmental mechanisms in hydra. V. Cell lineage and development of chimera hydra. *J. Cell Sci.*, **32**: 215–232.

Reference: *Biol. Bull.*, **157**: 297–305. (October, 1979)

ON THE POPULATION BIOLOGY AND NATURE OF DIAPAUSE OF *LABIDOCERA AESTIVA* (COPEPODA: CALANOIDA) [1]

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Knowledge of the seasonal distribution patterns of many marine copepods led Fish and Johnson (1937) to postulate that these species might produce resting eggs as an adaptive mechanism to insure survival at times of the year unfavorable to a planktonic existence. It was not until 1972 (Zillioux and Gonzalez, 1972) that conclusive results demonstrating the production of resting eggs by a marine copepod were obtained. During the last decade, it has become increasingly evident that the production of resting eggs by temperate, inshore marine copepods is a widespread event in this group (Zillioux and Gonzalez, 1972; Kasahara, Onbe and Kamigaki, 1975; Landry, 1975a; Grice and Gibson, 1975, 1977; Grice and Lawson, 1976; Uye and Fleminger, 1976). These studies have documented the existence of resting eggs in bottom sediments (Kasahara, Uye and Onbe, 1974, 1975; Kasahara, Onbe, and Kamigaki, 1975; Grice and Gibson, 1975, 1977; Uye and Fleminger, 1976) and demonstrated their production by females collected from the plankton (Grice and Gibson, 1975, 1977; Grice and Lawson, 1976; Uye and Fleminger, 1976; Zillioux and Gonzalez, 1972). These investigations have shown that physical factors such as temperature, light, salinity, and oxygen concentration affect the maintenance and termination of the resting egg. The events which actually trigger the induction of resting egg production are still poorly understood. The elucidation of factors which influence the induction, maintenance, and termination of copepod resting eggs is necessary to achieve a better understanding of the biology, evolution and distribution patterns, both in time and space, of neretic temperate-boreal copepods which are key elements in the food web.

Labidocera aestiva is a large calanoid copepod reported to occur in coastal waters from the Gulf of St. Lawrence to the Gulf of Mexico (Wheeler, 1901; Grice, 1956; Fleminger, 1957; Cronin, Daiber and Hulbert, 1962; Anraku, 1964; Van Engel and Tan, 1965; Bowman, 1971; Fleminger, 1975). The populations occurring north of Cape Hatteras are seasonally abundant in the plankton, with maximum numbers of nauplii, copepodites, and adults occurring in the summer and fall, and disappearing by mid-December. Grice and Gibson (1975) and Grice and Lawson (1976) have demonstrated the production of resting eggs by females of *L. aestiva* collected in the field and their presence in bottom sediments.

During the summer-fall period in 1977, a preliminary investigation of subitaneous and resting egg production by *L. aestiva* from Vineyard Sound, Massachusetts was conducted. This work indicated that the production of resting eggs commenced in early September. The surface water temperature was 19.5° C. The data presented in this paper document the seasonal variation in subitaneous and resting

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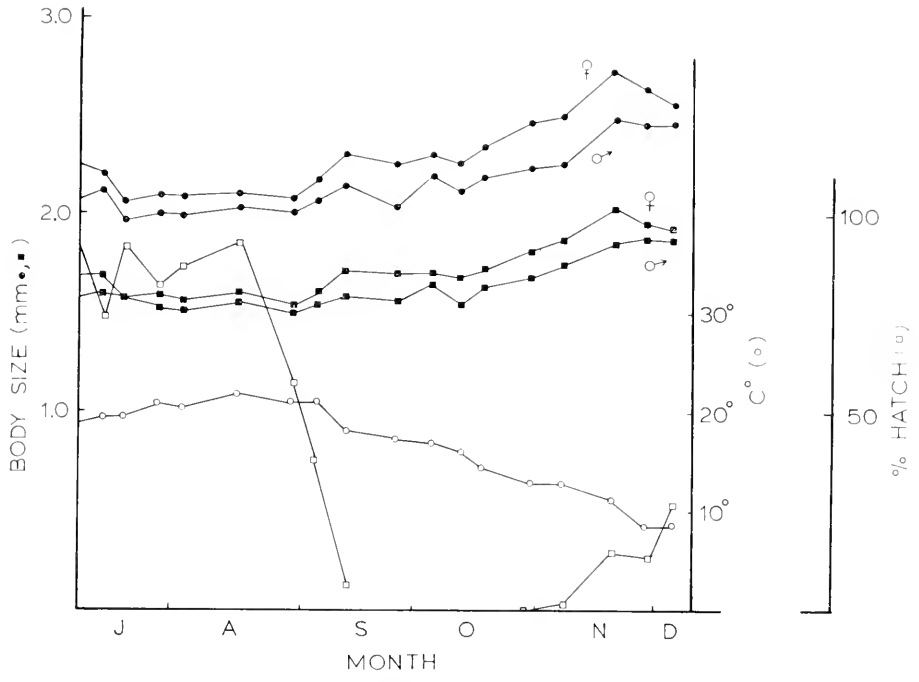

FIGURE 1. Trends in body size (cephalothorax, solid square, total length solid circle), percent hatch (open square), and surface water temperature (open circle) for the sampling period July 7, 1978 to December 6, 1978.

egg production during the summer-fall period in 1978. It is shown that the proportion of subitaneous eggs produced is greater during the summer, decreases markedly in September, and persists as a small percent throughout the fall. Moreover, the major change from subitaneous to resting egg production precedes the initiation of increasing adult body size and decreasing surface water temperatures by 2 weeks. It is demonstrated that resting eggs which are chilled at 5° C for a minimum period of 30 days, will hatch synchronously within 2 days when warmed to 21° to 23° C. Comparable eggs kept continuously at 19° to 23° C will hatch over a longer period of time and not synchronously. This paper discusses the significance of these findings as they relate to diapause induction, maintenance, and termination, as well as the population dynamics of *L. aestiva*.

Materials and Methods

Specimens of *Labidocera aestiva* were collected from July through December at weekly intervals from a 1 to 2 m depth in Vineyard Sound, Massachusetts by towing a 1-m, 243-μ mesh net for a 10 min period. Adult males and females were removed from these two samples by pipette. Twenty individuals of each sex were preserved in 5% buffered formalin each week, except for collections in late fall when very few adults were present. Two measurements of body size were obtained for each

preserved specimen (*i.e.*, cephalothorax, and total length) using a stereomicroscope and ocular micrometer. During the summer months, eggs were collected by placing 10 females together in separate 100-ml dishes. *Gymnodinium nelsoni* was added as food, and the dishes were placed overnight in an incubator at 19° C, with a 12 to 12-hr photoperiod. The following morning eggs were removed by pipette and counted. The percent hatch was determined for an aliquot (100–120) of these eggs. During the summer, eggs usually hatched within 2 days. The same procedure of collection, counting, and determination of percent hatch was followed for fall eggs. Fall eggs which did not hatch within 4 days were placed in filtered sea water (glass fiber) in 75-ml screw-top glass jars. The jars were incubated at 19° C or refrigerated at 5° C. At the appropriate time, the refrigerated jars were warmed to 21° to 23° C and percent hatch determined. At the same time, the percent hatch in the jars kept at 19° C was determined.

Results

The trends in body size, percent immediate hatch, and surface water temperature for the six-month period between July and December 1978 are shown in Figure 1. The standard deviations for body size ranged between 0.01 and 0.17. For each sex, changes in cephalothorax length paralleled total length. At each census throughout the sampling period, female body size was larger than male body size. An inverse relationship was observed for body size and surface water temperature for both females and males, and is depicted for adut females in Figure 2a. The smallest adults were collected during July and August when water temperatures ranged between 20° to 22° C. A marked increase in size began in September reaching a maximum in late November.

The percent immediate hatch (*i.e.*, within 2–4 days) of eggs collected from grouped females was greatest from July to mid-August, ranging between 74 and 93%. Subsequently the percent immediate hatch began to decrease and was observed to vary between 6 and 27% from mid-September to early December. The marked decrease in the portion of eggs hatching immediately began in late August, and preceded the initial drop of surface water temperatures to below 20° C (Figs. 1, 2b) and the time at which body size began to increase. No obvious difference in total egg production was observed, although differences may exist.

The percent hatch of eggs incubated at 19° or 5° C and then later warmed to room temperature (21°–23° C) is shown in Table I. The data presented are for eggs laid within 1 day of collection for five samplings between early September and late November. In general the percent hatch of eggs kept at 5° C and then warmed to 21° to 23° C was higher than those incubated at 19° C. Moreover, the hatch of the 5° C eggs occurred synchronously within two days of being warmed to 21° to 23° C. It was determined from periodic examination of the jars held at 19° C that non-synchronous hatching occurred throughout the incubation interval. Some of the unhatched eggs at 19° C appeared dead (brown, granulated interior), whereas others appeared viable (greenish hue).

Discussion

An inverse relationship between body size and temperature has been demonstrated for a number of marine and freshwater copepods (Coker, 1933; Marshall,

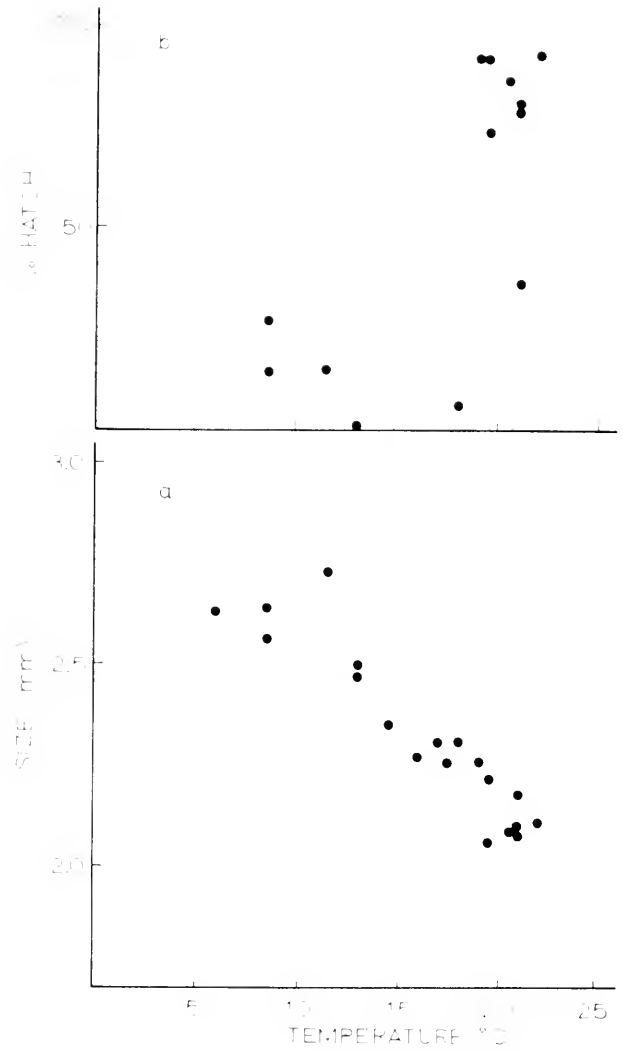

FIGURE 2. Scatter diagrams of female total body length (a), and percent hatch (b) versus surface water temperature at the time of collection.

Nicholls, and Orr, 1934; Aycock, 1942; Deevey, 1960a, b; McLaren, 1965; Deevey, 1966; see review in Hutchinson, 1967; Durbin and Durbin, 1978). The seasonal pattern of size variation reported herein for *Labidocera aestiva* from Vineyard Sound is similar to that reported by Deevey (1960a) for another population of the species from Delaware Bay studied in 1930. However, the patterns are shifted in time. The smallest individuals are found during August in Vineyard Sound and September in Delaware Bay. Deevey (1960a) reports that the average surface water temperature in Delaware Bay was 22° C during September. This value

TABLE I

Percent hatch of incubated (19°–23° C) and refrigerated Clutch 1 eggs after warming to 21°–23° C, for five samplings between early September and late November.

Date collected	Date incubated		Period of incubation or refrigeration (days)	% Hatch
	5°	19–23°		
Sep 27	Oct 2		75	96
Sep 27		Oct 2	75	61
Oct 5	Oct 10		111	95
Oct 5		Oct 10	111	42
Sep 7	Sep 11		58	83
Sep 7		Sep 11	58	2
Oct 19	Oct 23		126	95
Oct 19		Oct 23	126	55
Nov 21	Nov 27		87	95
Nov 21		Nov 27	87	95

corresponds to temperatures recorded for Vineyard Sound in August (Fig. 1). The pattern, however, varies from year to year within an area, inasmuch as the smallest individuals during 1932 in Delaware Bay were collected in early August, when temperatures were then highest. It is obvious that body size is correlated more with the surface water temperature than with the time of collection.

In the present study, there was no evidence of discrete generations for *L. aestiva* in Vineyard Sound. Breeding appeared to be continuous throughout the summer and fall. Deevey (1960a) suggested that spawning was continuous during the summer for the Delaware Bay populaton, but that a break occurred between early August and September, resulting in a burst of larger individuals late in October. Moreover, it was suggested that the offspring of the large-sized fall individuals would not mature until the following year. More recent studies of *L. aestiva* from Vineyard Sound (Grice and Gibson, 1975; Grice and Lawson, 1976) have demonstrated that the offspring of the fall individuals survive the winter as resting eggs, and hatch the following spring. It is probable that the same life history pattern is common to the Delaware Bay population.

In Vineyard Sound, the population continues to breed at temperatures as low as 8.5° C (Fig. 1), producing both subitaneous and resting eggs. On the other hand, Deevey (1960a) reports that 12° C is the lower limit of breeding in Delaware Bay. This difference could reflect acclimatization or genetic adaptation of individuals to the slightly differing thermal regimes of Vineyard Sound and Delaware Bay (see Bumpus, 1957). It has been shown for a wide variety of marine invertebrates that the tolerance limits for a number of biological functions differ between populations exposed to different environmental regimes (see Battaglia and Beardmore, 1978).

Whatever the factor(s) inducing the production of resting eggs by *L. aestiva* from Vineyard Sound, it is shown that the response (*i.e.* initiation of dormancy) is first expressed in late August or early September. The intensity of the response increases as time progresses and the surface water temperatures decrease. This intensification is expressed as an increase in the percent production of resting

eggs and a decrease in subitaneous eggs. There is a strong correlation between temperature and the type of egg produced (Fig. 2b). However, temperature alone is not sufficient to account for the observed switch in egg production in early September (Fig. 1). The same temperatures (*i.e.* 18°–20° C) which are coincident with the production of resting eggs in September are observed in early summer when production is restricted to subitaneous eggs. Temperature may influence the type of egg produced, but it would appear that other factors are also involved.

The data show that subitaneous eggs are produced throughout the summer-fall period, and that resting eggs are produced only during the fall. There are clearly two egg types being produced in the fall. Subitaneous eggs produced at this time take longer to hatch (2–4 days) at 21° to 23° C than those produced in the summer (1–2 days).

The rate of embryonic development of copepod eggs is influenced by egg size, temperature, and environmental conditions experienced by the parents (McLaren, 1965; 1966; McLaren, Corkett, and Zillioux, 1969; Landry, 1975b; Hart and McLaren, 1978). For *L. aestiva* the observed difference in rate of development most probably reflects a maternal effect relating to the physiological condition of the female at the time of collection, as was shown for *Pseudocalanus* by Hart and McLaren (1978). No obvious size difference was observed between summer and fall subitaneous eggs of *L. aestiva* in the present study.

The fall subitaneous eggs of *L. aestiva* were detected by placing females collected at that time in an incubator at 19° C. If the subitaneous eggs produced by fall females were collected and held at temperatures equivalent to the ambient values in Vineyard Sound, their development would be retarded and they could be mistaken for resting eggs. On the other hand, fall eggs which do not hatch within 2 to 4 days at 21° to 23° C are to be regarded as resting eggs. As first demonstrated by Grice and Gibson (1975) resting eggs of *L. aestiva* remain viable for as long as 120 days at 5° C. It was suggested that a minimum chilling period of of 2 to 4 weeks at 5° C was required to break the dormant condition. The results of the present study show that if resting eggs are placed at 19° C without chilling, hatching will take place, but it takes longer and occurs sporadically. Chilling of resting eggs results in the reduction of time in actual diapause and synchronization of hatching. The diapause and hatching response of resting eggs of *L. aestiva* to different temperatures corresponds to several examples of insect and freshwater copepod diapause (Church and Salt, 1952; see Hutchinson, 1967; Stross, 1969b; Dean and Hartley, 1977a, b). The dormant state in these animals consists of a period of diapause followed by post-diapause development and then hatching (see Mansingh, 1971). During the diapause period, development is arrested and cannot resume even if conditions are favorable. Once the diapause is broken the individual is competent to resume development as soon as adverse conditions are terminated. For *L. aestiva* the actual breaking of diapause (reactivation) can take place at 19° C, but the process is faster at colder temperatures. Post-diapause development, however, is slower at reduced temperatures than at high temperatures. Therefore, individual chilled eggs terminate diapause at different times, and further development is retarded as long as the eggs remain chilled. The chilled eggs accumulate at a stage of readiness, and when exposed to higher tempera-

tures are competent to proceed with development, resulting in synchronous hatching. On the other hand, individual resting eggs held continuously at 19° C terminate the diapause condition at different times (as the chilled eggs), but since the higher temperature is favorable for post-diapause development to proceed there is no accumulation of individuals at the boundary between diapause and post-diapause so that hatching is asynchronous.

Two attributes expressed by resting eggs are cold-hardiness and synchronous hatching. Resting eggs remain viable at 2° C for as long as 6 months (Grice and Gibson, 1975) whereas summer subitaneous eggs remain viable for no longer than 15 days (Grice, unpublished). The tolerance of fall subitaneous eggs has not been investigated. The cold resistance of resting eggs enables survival during the winter. Synchronization of development promotes the reproductive success of the first generation appearing in the early summer by ensuring that individuals will attain reproductive maturity at the same time. If hatching was sporadic, the number of mature individuals in the population at any one time might not attain a size sufficient for successful mating encounters to occur.

The effects of temperature on the maintenance and termination of resting eggs of *L. aestiva* are similar to those observed for other plants and animals which overwinter. In the majority of cases for which diapause induction has also been investigated, photoperiod and temperature have been shown to be the two most important factors affecting the initiation of dormancy (Harvey, 1957; Stross and Hill, 1965; see Hutchinson, 1967; Stross, 1969a, b; Watson and Smallman, 1971; see Mansingh, 1971; de March, 1977; see Clutter, 1978). The pattern of egg production observed in this study would occur if a developmental stage perceived a cue which then triggered an irreversible sequence of events leading to one type of egg or another. The fact that resting egg production precedes the decline of surface water temperatures suggests that such a stimulus may exist. The successful species will evolve a dormancy response to a factor which closely parallels the stress (in this case temperature) but which itself is extremely stable (such as photoperiod). That two egg types are produced during the fall could be due to the extended survival of summer females which lay subitaneous eggs and the newly developed fall females programmed to produce resting eggs. Moreover, because no two individuals are alike, some females may respond to a weak stimulus, whereas others require a more intense exposure. In the spruce budworm (Harvey, 1957), diapause-free development occurs under a long day regime (*i.e.,* greater than 15 hr of light) in a portion of the population. As day length is increased the number of non-diapausing insects increases, reaching 100% in continuous light. Universal diapause occurs when day length is less than 15 hr. Current progress in our laboratory indicates that short photoperiods (less than 12 hr light) are effective in inducing the production of resting eggs by laboratory-reared *L. aestiva.* This will be reported on at length in a subsequent paper.

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Summary

The calanoid copepod, *Labidocera aestiva* was collected from Vineyard Sound, Massachusetts between July and December 1978. Adult size (cephalothorax and total body length) was inversely proportional to surface water temperature at the time of collection. The major switch from subitaneous to resting egg production occurred during late August to early September, but a small percent of subitaneous eggs continued to be produced throughout the fall. Resting eggs were cold-resistant and when chilled at 5° C hatched synchronously when warmed to 21° to 23° C. Individual resting eggs kept continuously at 19° C took longer to hatch and emergence was asynchronous. The resting eggs of *L. aestiva* appear to be in a state of diapause similar to many insects, and it is suggested that photoperiod is the primary cue inducing the production of resting eggs.

LITERATURE CITED

Anraku, M., 1964. Influence of the Cape Cod canal on the hydrography and on the copepods in Buzzards Bay and Cape Cod Bay, Massachusetts. I. Hydrography and distribution of copepods. *Limnol. Oceanogr.*, **9**: 46–60.

Aycock, D., 1942. Influence of temperature on size and form of *Cyclops vernalis* Fischer. *Journal Elisha Mitchell Scientific Society*, **58**: 84–93.

Battaglia, B., and J. Beardmore, Ed., 1978. *Marine Organisms: Genetics, ecology and Evolution*. Plenum Press: New York, 757 pp.

Bowman, T., 1971. The distribution of calanoid copepods off the southeastern United States between Cape Hatteras and southern Florida. *Smithson. Contrib. Zool.* No. 96, 58 pp.

Bumpus, D., 1957. Surface water temperatures along Atlantic and Gulf coasts of the United States. *U. S. Fish. Wildl. Serv.-Special Scientific Report* #214, 153 pp.

Church, N., and R. Salt, 1952. Some effects of temperature on development and diapause in eggs of *Melanoplus bivattatus* (Say) (Orthoptera: Acridae). *Can. J. Zool.*, **30**: 90–171.

Clutter, M., 1978 (ed.). *Dormancy and Developmental Arrest*. Academic Press: New York, 316 pp.

Coker, R. E., 1933. Influence of temperature on size of freshwater copepods (Cyclops). *Internationale Revue der gesamten Hydrobiologie and Hydrographie*, **29**: 406–436.

Cronin, L., J. Daiber and E. Hulbert, 1962. Quantitative seasonal aspects of zooplankton in the Delaware River estuary. *Chesapeake Sci.*, **3**: 63–93.

Dean, R. and J. Hartley, 1977a. Egg diapause in *Ephippiger cruciger* (Orthoptera: Tettigoniidae). I. The incidence, variable duration and elimination of the initial diapause. *J. Exp. Biol.*, **66**: 173–183.

Dean, R., and J. Hartley, 1977b. Egg diapause in *Ephippiger cruciger*, (Orthoptera: Tettigonidae). II. The intensity and elimination of the final egg diapause. *J. Exp. Biol.* **66**: 185–195.

Deevey, G., 1960a. The zooplankton of the Delaware Bay Region. *Bull. Bingham Oceanog. Collect* Yale Univ., **17**: 5–53.

Deevey, G., 1960b. Relative effects of temperature and food on seasonal variations in length of marine copepods in some American and western European waters. *Bull. Bingham Oceanog. Collect* Yale Univ., **17**: 54–86.

Deevey, G., 1966. Seasonal variations in length of copepods in South Pacific New Zealand waters. *Aust. J. Mar. Freshwater Res.*, **17**: 155–168.

de March, B. G., 1977. The effects of photoperiod and temperature on the induction and termination of reproductive resting stage in the freshwater amphipod *Hyallela azteca* (Sanssure). *Can. J. Zool.*, **55**: 1595–1600.

Durbin, E., and A. Durbin, 1978. Length and weight relationships of *Acartia clausi* from Narragansett Bay, R. I. *Limnol. Oceanogr.*, **23**: 958–969.

Fish, C., and M. Johnson, 1937. The biology of zooplankton populations in the Bay of Fundy and Gulf of Maine with special reference to production and distribution. *J. Fish. Res. Bd. Can.*, **3**: 189–322.

Fleminger, A., 1957. New calanoid copepods of *Pontella* Dana and *Labidocera* Lubbock with notes on the distribution of genera in the Gulf of Mexico. *Tulane Stud. Zool.*, **5**: 19–34.

Fleminger, A., 1975. Geographic distribution and morphological divergence in American coastal zone planktonic copepods of the genus *Labidocera*. *Estuarine Res.*, **1**: 392–419.

Grice, G., 1956. A qualitative and quantitative seasonal study of the copepods of *Alligator* Harbor. Florida State University Studies, No. 22. *Papers from The Oceanographic Institution, No. 2*, pp. 37–76.

Grice, G., and V. Gibson, 1975. Occurrence, variability, and significance of resting eggs of the calanoid copepod, *Labidocera aestiva*. *Mar. Biol.*, **31**: 335–357.

Grice, G., and V. Gibson, 1977. Resting eggs in *Pontella meadi* (Copepoda: Calanoida). *J. Fish. Res. Bd. Can.*, **34**: 410–412.

Grice, G. and T. Lawson, 1976. Resting eggs in the marine calanoid copepod, *Labidocera aestiva* Wheeler. *Crustaccana*, **30**: 9–12.

Hart, R., and I. McLaren, 1978. Temperature acclimation and other influence on embryonic development in the copepod *Pseudocalanus*. *Mar. Biol.*, **45**: 23–30.

Harvey, G., 1957. The occurrence and nature of diapause-free development in the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortrichidae). *Can. J. Zool.*, **35**: 549–572.

Hutchinson, G. E., 1967. *A Treatise on Limnology. Vol. II.* John Wiley and Sons, Inc.: New York, 1115 pp.

Kasahara, S., T. Onbe, and M. Kamiyaki, 1975. Calanoid copepod eggs in sea-bottom buds. III. Effects of temperature, salinity, and other factors on the hatching of resting eggs of *Tortanus forcipatus*. *Mar. Biol.*, **31**: 31–35.

Kasahara, S., S. Uye, and T. Onbe, 1974. Calanoid copepod eggs in sea-bottom muds. *Mar. Biol.*, **26**: 167–171.

Kasahara, S., S. Uye, and T. Onbe, 1975. Calanoid copepod eggs in sea-bottom muds. II. Seasonal cycles of abundance in the populations of several species of copepod and their eggs in the Inland Sea of Japan. *Mar. Biol.*, **31**: 25–29.

Landry, M., 1975a. Dark inhibition of egg hatching of the marine copepod *Acartia clausi* Giesbr. *J. Exp. Mar. Biol. Ecol.*, **20**: 43–47.

Landry, M., 1975b. Seasonal temperature effects and predicting development rates of copepods. *Limnol. Oceanogr.*, **20**: 434–440.

Mansingh, A., 1971. Physiological classification of dormancies in insects. *Can. Entomol.* **103**: 983–1009.

Marshall, S., A. Nicholls, and A. Orr, 1934. On the biology of *Calanus finmarchicus*. V. Seasonal distribution, size, weight and chemical composition in Loch Striven in 1933, and their relation to the phytoplankton. *J. Mar. Biol. Ass.*, **19**: 793–827.

McLaren, I., 1965. Some relationships between temperature and egg size, body size, development rate, and fecundity of the copepod, *Pseudocalanus*. *Limnol. Oceanogr.*, **10**: 528–538.

McLaren, I., 1966. Predicting development rate of copepod eggs. *Biol. Bull.*, **131**: 457–469.

McLaren, I., C. J. Corkett, and E. J. Zillious, 1969. Temperature adaptations of copepod eggs from the Arctic to the tropics. *Biol. Bull.*, **137**: 486–493.

Stross, R., 1969a. Photoperiod control of diapause in *Daphnia*. II. Induction of winter diapause in the Arctic. *Biol. Bull.*, **136**: 264–273.

Stross, R., 1969b. Photoperiod control of diapause in *Daphnia*. III. Two stimulus control of long-day, short-day induction. *Biol. Bull.*, **137**: 359–374.

Uye, S., and A. Fleminger, 1976. Effects of various environmental factors on egg development of several species of *Acartia* in southern California. *Mar. Biol.*, **38**: 253–262.

Van Engel, W., and E. Tan, 1965. Investigations of inner continental shelf waters of lower Chesapeake Bay. Part VI. The copepods. *Chesapeake Sci.*, **6**: 183–189.

Watson, N., and B. Smallman, 1971. The role of photoperiod and temperature in the induction and termination of an arrested development in two species of freshwater cyclopoid copepods. *Can. J. Zool.*, **49**: 855–862.

Wheeler, W., 1901. The free-swimming copepods of the Woods Hole region. *Bull. U. S. Fish Comm.* (1899): 157–192.

Zillioux, E., and J. Gonzalez, 1972. Egg dormancy in the neretic calanoid copepod and its implications to overwintering in boreal waters. *Arch. Oceanogr. Limnol., European Marine Biology Symposium*, **5**: 217–230.

Reference: *Biol. Bull.*, 157: 306–319. (October, 1979)

LOCOMOTION IN THE PRIMITIVE PULMONATE SNAIL *MELAMPUS BIDENTATUS*: FOOT STRUCTURE AND FUNCTION

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Most land pulmonates crawl by means of muscular waves that travel along the foot from posterior to anterior. These waves, classified as direct waves by Vlés (1907), have been analyzed by Jones (1973) for the land slug *Agriolimax*. A great variety of wave types has been described for prosobranch gastropods; the most primitive type is thought to be the retrograde type, in which the waves travel in a direction opposite to the gastropod's progress (Miller, 1974; Trueman, 1975). Jones and Trueman (1970) have analyzed the mechanism of retrograde waves in the limpet *Patella* and have reviewed gastropod locomotory waves (Jones, 1975; Trueman, 1975).

The purpose of the work reported here has been to analyze the unusual locomotion of the snail *Melampus bidentatus*. This basommatophoran snail is a member of one of the most primitive families of pulmonates, the Ellobiidae. Information on its mode of locomotion may cast light on the origin of the locomotory behavior exhibited by the more advanced land pulmonates.

Although Morton (1955) indicated that land-inhabiting ellobiids, including *Melampus,* crawl in a way that others have classified as retrograde waves (Jones, 1975), my examination of events in *Melampus* locomotion has not supported that classification. Rather, *Melampus* exhibits a form of locomotion that fits neither the direct nor retrograde wave category nor any of the other locomotor categories of Miller (1974). *Melampus* locomotion consists of a repeated sequence of events, the crawl-step, in which the posterior of the foot slides along but the anterior is lifted and placed. Hydraulic forces, described by Chapman (1958; 1975) for a variety of soft-bodied animals, are an important component in the locomotion of *Melampus*. The blood is acted on by the columellar muscles as well as the intrinsic pedal musculature, and the transverse subdivision of the foot allows the posterior-to-anterior transfer of blood to be coordinated with the muscular events.

Materials and Methods

Animal. Adult specimens of *Melampus bidentatus* Say with shell lengths of 7 to 11 mm were collected in salt marshes of Woods Hole, Massachusetts. They were maintained in large, covered fingerbowls lined with filter paper moistened with 75% sea water (Instant Ocean) and provided with napa (Chinese) cabbage and crushed eggshells. A photoperiod of 8L: 16D and constant temperature of 16° C suppressed reproductive activity (Apley, 1968).

Photographic Techniques. Crawling snails were filmed with a Bolex Macro-

zoom 160 super 8 mm camera at 18 frames per second. Side and bottom views were obtained simultaneously by the use of mirrors.

Marking the Foot. Snails were anaesthetized according to the method of Price (1977). Three or more spots of India ink were injected just beneath the surface of the sole. The animals were allowed to recover overnight in fresh 75% sea water.

Anatomy. Pedal and columellar muscles were studied in live and narcotized snails and specimens fixed in alcoholic Bouins. Serial sections of snails quick-frozen in liquid nitrogen while crawling (Jones, 1973) provided information on the muscle contraction patterns during locomotion. The 10 μm sections were stained with Mallory's triple stain (Pantin, 1948).

Force Recordings. Snails were allowed to crawl over a plexiglass platform having a 2-mm gap into which a 1-mm bar was inserted. The bar was attached to a force transducer (Statham Micro-Scale Accessory Model UL5) connected to an amplifier (Gilson IC-MP module). The resulting forces were recorded on a chart recorder (Gilson ICT-SH). The transducer was connected so as to measure either upward and downward forces or backward and forward forces as the snail's foot passed across it. The translucent platform on which the snail crawled was positioned just above the writing surface of the recorder so that the crawling snail was filmed with the chart recorder's activity in the background. The absolute magnitude of the forces was not relevant to this analysis.

Results

Stages in the crawl-step

As illustrated in Figure 1, the foot of *Melampus bidentatus* is divided anatomically into an anterior region (the propodium) and a posterior region (the metapodium) by a permanent transverse groove. The mouth region surrounded by the oral veil or lappets forms an important part of the locomotory surface.

In *Melampus* locomotion a single series of muscular events propagates along the foot at one time. I have called this mode of locomotion a crawl-step because the anterior part of the foot is lifted free of the substratum. Characteristic postures assumed by the foot during the crawl-step have been identified in frame-by-frame analysis of motion picture films of crawling snails. Postures identified with three easily recognized stages of the crawl-step are shown in Figure 1. Each stage is characterized by the following events:

Stage I. Metapodial shortening/propodial extension. The posterior third of the metapodium shortens as a single movement and the propodium simultaneously extends to its longest dimension. The head and oral veil are lifted off the substratum.

Stage II. Metapodial extension. A series of local muscular contractions within the metapodium produce one or more waves that ripple forward along the sides of the metapodium from the medial region of the metapodium toward the transverse groove. The region of the metapodium ahead of the elevations bulges outward and when the bulge reaches the transverse groove the posterior edge of the propodium is displaced upward and forward by the expanded metapodium. Mean-

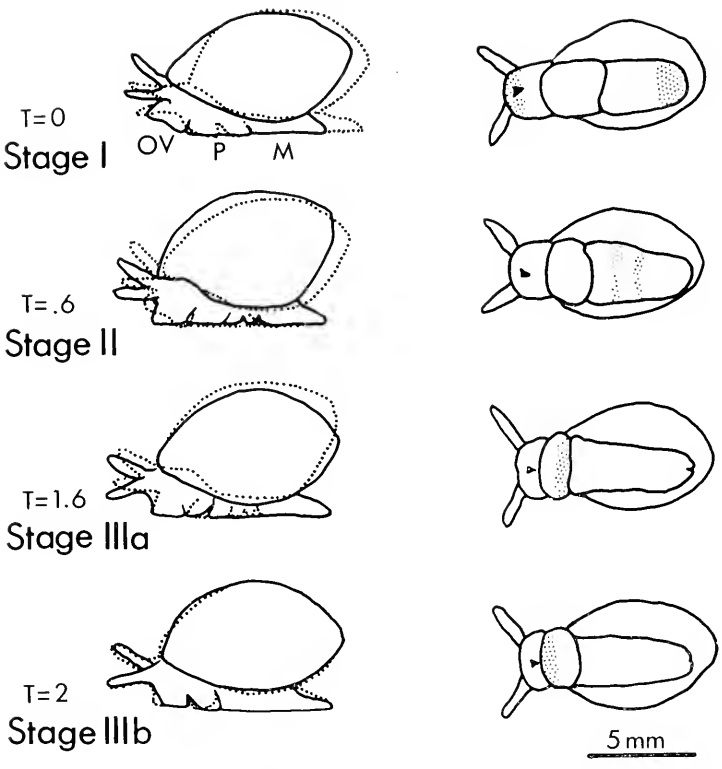

FIGURE 1. The crawl-step of *Melampus bidentatus*. Side and bottom views traced from motion picture frames for Stage I: metapodial shortening/propodial extension, Stage II: metapodial extension, and Stage III: propodial elevation, posterior (a) and then anterior (b) phase. Cumulative elapsed time in seconds is given to the left. Dotted lines (side views) give the snail's posture at onset of the movement characteristic of each stage and the unbroken lines represent the posture upon completion of the movement. Stippled regions (bottom view) indicate portions that were lifted during each stage. Subdivisions of the locomotory surface are labeled in the bottom view of Stage I: OV, oral veil-mouth region; P, propodium of the foot; M, metapodium of the foot.

while the posterior region of the metapodium narrows and flattens dorsoventrally and the shell tilts forward as the oral veil is lowered to the substratum.

Stage III. Propodial elevation. The posterior region of the propodium is elevated first (IIIa, Fig. 1) and then the anterior region is elevated (IIIb, Fig. 1). The oral veil–mouth region forms an area of contact with the substratum throughout this stage. The shell tilts backwards.

In the motion picture from which the tracings shown in Figure 1 were made, the snail progressed 2.2 mm in 2.3 sec. Snails with shell lengths of 10 to 11 mm typically crawled 2 to 4 mm/step at a rate of 10 to 20 steps/min at 20° C.

Foot morphology

Figure 2 shows the relationship of the columellar muscles to the foot. In *Melampus* the columella itself is largely resorbed during development, leaving

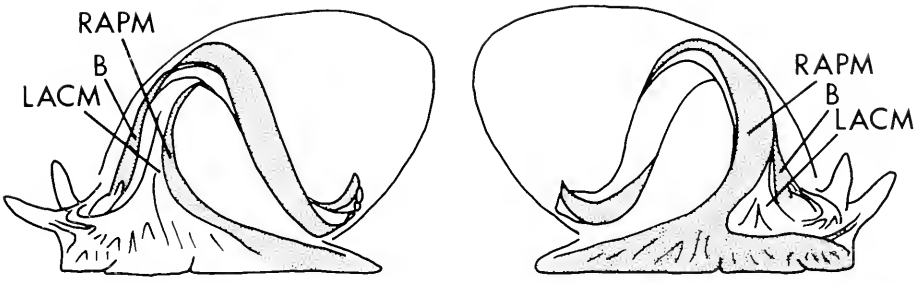

FIGURE 2. Relationship between columellar muscles and the foot in *Melampus*. A, view of animal's left side; B, right side. Left anterior cephalic muscle (LACM) is unshaded, the right anterior and right and left posterior muscle (RAPM) stippled, and buccal muscle (B) crosshatched.

the columellar muscles attached to the inner wall of the shell (Morton, 1955). The muscles follow the inner surface of the shell within the body whorl in approximately a 360° turn from origin to insertion in the extended foot. In addition to the buccal retractor muscle, two major subdivisions of the columellar muscle are apparent in *Melampus*. The left anterior and cephalic muscle (LACM, Fig. 2) originates in several adjacent bundles and further subdivides into muscle bands that insert around the head, into the left tentacle and oral veil, the left side of the propodium and approximately the anterior third of the left side of the metapodium. The right anterior and right and left posterior muscle (RAPM, Fig. 2) has a single origin on the shell and divides into muscle bands that insert on the right tentacle, the right oral veil, the right propodium, the anterior portion of the metapodium and bilaterally in the posterior region of the metapodium. Thus the muscles enter the foot in a quite asymmetrical pattern with those muscles on the left side that support the shell being the more massive. The columellar muscle system of *Melampus* probably includes both the longitudinal and columellar muscle groups described for *Lymnaea* by Plesch, Janse and Boer (1975).

In serial sections of snails quick-frozen in the act of crawling, there is no evidence that intrinsic bands of muscle fibers form discrete layers above the sole of the foot. Rather, the fibers that could be traced appear to be derived from columellar muscles. This is apparent in the parasagittal section which cuts through the left anterior and cephalic columellar muscle (Fig. 3C). The muscle fibers form a meshwork enclosing small, spherical blood spaces, shown in a region of the metapodium (Fig. 3E). These are similar to those in the foot of the limpet, *Patella* (Jones and Trueman, 1970). These small spaces are contrasted with the large blood sinuses that are present in the anterior region of the foot (Fig. 3A, D).

When sections of snails frozen in different stages of the step cycle are compared, one of the most obvious differences is in the orientation of the transverse groove. In Stage I, the metapodial shortening/propodial extension results in a propodium that is long and a transverse groove that is shallow and anteriorly-slanting (Fig. 3A). In Stage IIIa elevation of the transverse goove and posterior region of the propodium pulls the groove into a deep backward-slanting indentation (Fig. 3C). As the propodial elevation moves into the anterior propodium (Stage

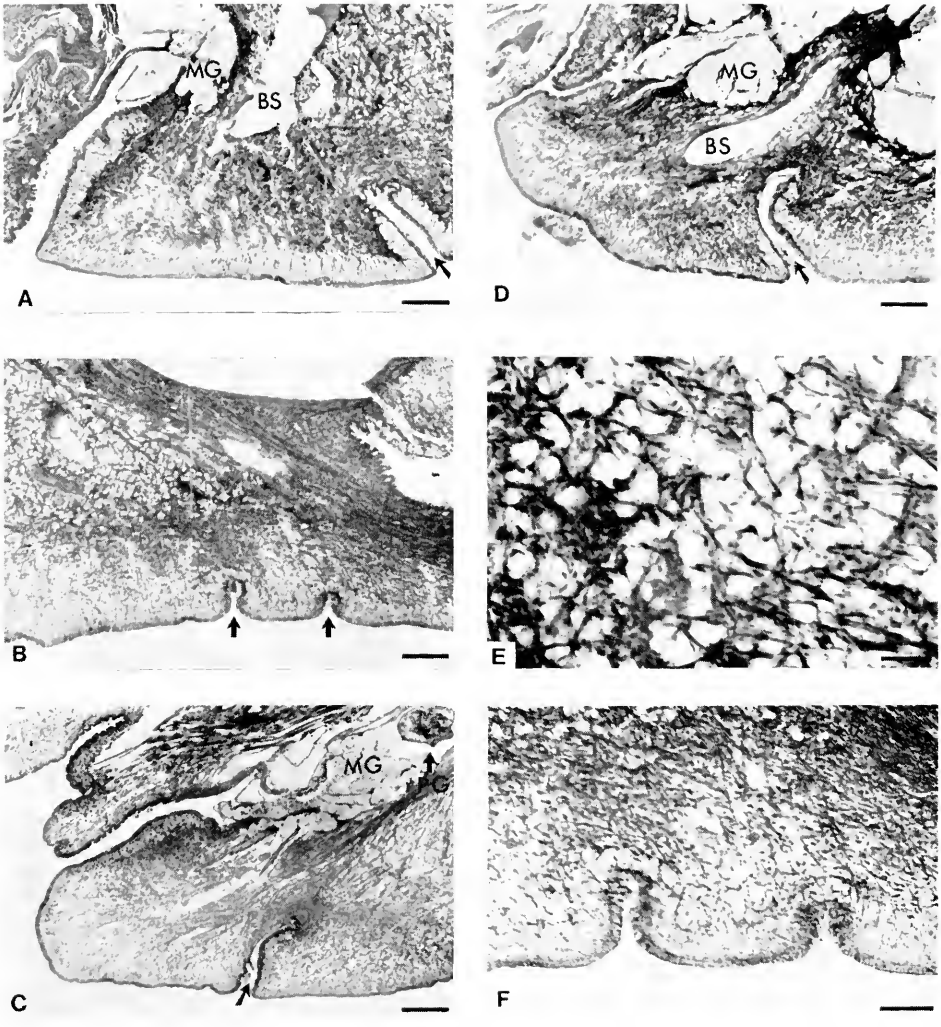

FIGURE 3. Sections prepared from specimens frozen in liquid nitrogen while crawling. In each case, anterior is to the left. A. Stage I posture, mid-sagittal section showing propodial region of foot and tranverse groove (arrow). Scale bar 250 μ. B. Stage II posture, mid-sagittal section showing metapodial region with two metapodial waves (arrows). Scale bar 250 μ. C. Stage IIIa posture, left para-sagittal section through left anterior and cephalic muscle bands in the propodial region of the foot. Arrow indicates transverse groove. Scale bar 250 μ. D. Stage IIIb posture, mid-sagittal section showing propodial region of the foot and transverse groove (arrow). Scale bar 250 μ. E. Detail of metapodial tissue showing small blood spaces. Scale bar 25 μ. F. Detail of muscle contraction pattern in the metapodial waves in Figure 3B. Scale bar 75 μ. Abbreviations used: BS, large blood sinus; MG, suprapedal mucus gland; PG, pedal ganglion.

IIIb) the transverse groove region is relaxed and assumes a condition intermediate between that of Stage IIIa and Stage I (Fig. 3D).

Metapodial contractile waves are shown in Figure 3B and the muscle pattern

contributing to them is apparent at higher magnification in Figure 3F. The sole of the foot is lifted straight up rather than pulled in a slanting angle forward. It appears that the elevations are caused by a local tightening of the mesh-work of fibers within the foot. Anterior to the elevations, the blood spaces are larger than in the contracted regions behind the waves. A consequence of anterior movement of such a pattern of waves would be the forward displacement of blood.

Marked-foot experiments

In this and the following sections an attempt was made to characterize more precisely the sequence of events and the forces underlying forward progression during the crawl-step. The aim of marked-foot experiments was to analyze temporally the changes in the relative size of the propodium and metapodium and the movement of points within those regions. This was achieved by filming the foot of snails that had spots of ink injected into the sole. In every fourth frame of the film the position of the ink spots was determined, relative to a fixed point behind the snail's foot. Sample data from five crawl-steps are illustrated in Figure 4. The beginning and end of one crawl-step is included between the vertical dashed lines.

The following features are apparent from such an analysis: First, shortening of the posterior metapodium, measured as advancement of the end of the foot, is mirrored in time and magnitude by advancement of the anterior edge of the propodium. Thus during posterior shortening the length of the snail's foot does not change. The remaining events in the crawl-step are all forward shifts of intermediate points along the foot, relative to the fixed posterior and anterior

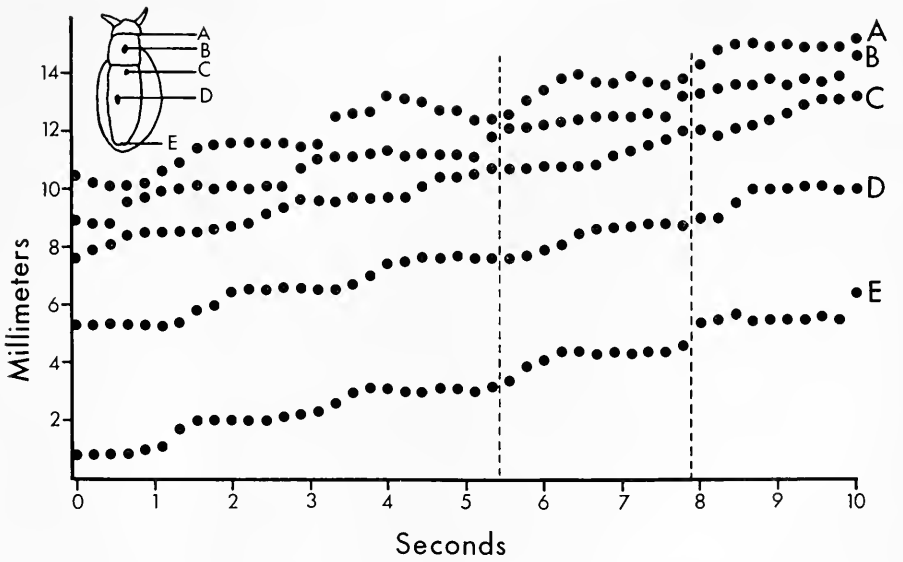

FIGURE 4. Progress of the anterior (A) and posterior (E) edges of the foot and three spots of injected ink (B-D) was charted for every fourth frame of an 18 frames/sec motion picture film. The events of one complete crawl step occur between the vertical dashed lines.

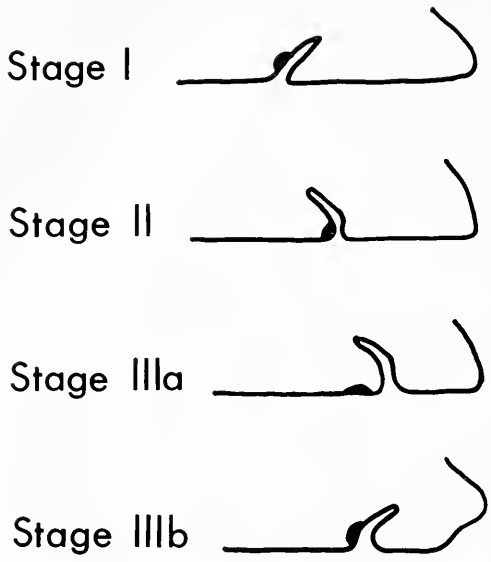

FIGURE 5. Diagrammatic illustration of the relationship of an ink spot to the transverse groove. Motion picture films indicated that during locomotion the spot of ink disappeared within the transverse groove from Stage IIIb through Stage I and was visible during Stages II and IIIa.

edges of the foot. These forward shifts proceed from posterior to anterior: the metapodial waves carry point D and then point C forward. Finally, point B moves forward as the anterior propodium is lifted off the substratum.

Films of a snail with a differently placed ink spot shed light on events occurring in the region of the transverse groove during locomotion. The ink spot was in the anterior metapodium almost within the transverse groove. During locomotion, the spot appeared and disappeared with each crawl-step. The position of the spot during the crawl-step is illustrated diagrammatically in Figure 5. It became visible during metapodial extension in Stage II and was eclipsed by expansion of the posterior propodium as the anterior propodium was elevated (Stage IIIb). Both this and the histological evidence indicate that although the transverse groove is a constant feature of the foot, a greater or lesser region of the pedal sole anterior or posterior to this region can be drawn up into the groove. Both changes in blood volume in the propodium and metapodium adjacent to the groove, and contraction of columellar muscles which insert close to the groove, alter its configuration.

Upward and downward forces

Direct observations and motion picture films indicate that portions of the *Melampus* foot are elevated during locomotion. Forward sliding movements along the mucus-covered substratum might also be expected to exhibit an upward component. Transducer recordings of upward and downward forces were obtained

to determine which portions of the sole are experiencing upward or downward (weight-bearing) forces during each stage in the crawl-step cycle.

The forces exerted during locomotion were measured as snails crawled across a moveable bar positioned in a slit in a plexiglass platform. Five to six step cycles were required for snails to crawl across the bar and therefore at each successive step the transducer measured forces from a more posterior region of the foot. The pattern of upward and downward deflections recorded in this manner was fairly constant from crossing to crossing and animal to animal. Figure 6A is typical of the recordings that were produced. These data were interpreted as described in the methods. Postures characteristic of Stages I–III for each step were identified with particular points on the force recording as illustrated in Figure 6A.

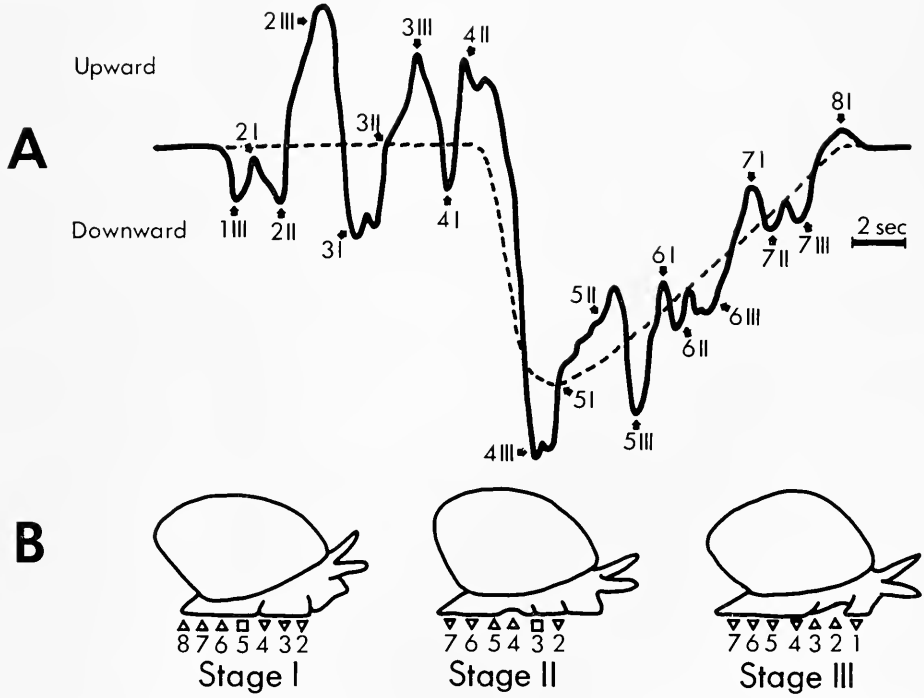

FIGURE 6. A is the force transducer recording of upward and downward forces exerted on a bar as a snail crawled across it. A portion of the snail's foot was in contact with the bar for six complete steps and the end and beginning of two other steps, for a total of 24 sec. Each point at which the simultaneously-recorded motion picture film indicated that the snail assumed one of the postures characteristic of Stage I, II or III of the crawl step cycle was marked on the transducer recording. The dotted line represents a baseline adjustment that was inserted to compensate for the overwhelming effect of passage of the shell mass onto the bar. In B the information obtained from the force recording is represented as a pattern of upward and downward-pointing triangles showing the distribution of vertical forces exerted by the foot on the substratum at Stages I, II and III of the step cycle. Regions exerting no vertical force are indicated by squares. The position of the triangles or squares under the foot marks the position of the recording bar each time the posture characteristics of a particular stage was assumed.

Note that in the force recording in Figure 6A there is a dramatic downward registration following stage II of the fourth step. At that time the shell weight shifts forward onto the bar. From then until the foot is pulled off the bar, all the upward deflections fail to rise above the original baseline. The dotted line in Figure 6A was inserted as a relative baseline in an attempt to correct for the overwhelming effect of shell weight on the force recordings. Using that correction, the results of these experiments were summarized in Figure 6B. The following conclusions can be drawn: In metapodial shortening/propodial extension the propodium is weight-bearing while the posterior region of the metapodium exerts an upward force on the substratum. In metapodial lengthening the anterior region of the propodium and the posterior region of the metapodium are weight-bearing while the waves moving forward in the anterior region of the metapodium exert an upward force. In propodial elevation the oral veil and the entire metapodium are weight-bearing during elevation of the posterior and anterior propodium.

Forward and backward forces

Lissmann (1946) defined the sliding progression of a snail's foot along a substratum as being the product of forces acting parallel to the ground, including internal and external forces acting to change the shape of the snail's body and the reactions from the ground. The concept of the internal forces has been advanced and direct measurements of fluid pressure made for a variety of animals since that time (see Chapman, 1975). The reactions of the ground to the passage of the snail's foot were measured in this study to indicate what was happening along the length of the foot at each stage in the crawl-step cycle. Lissmann's (1946) reactions from the ground were defined as the static reaction, which is the force exerted on the substratum in a backward direction by stationary areas while other regions of the foot are advanced, and sliding friction, which is the forward force that moving portions of the foot exert. These backward and forward forces were recorded and interpreted in a manner similar to that described for upward and downward forces, but with the transducer measuring forces in the horizontal plane.

In general the sole of the foot did not noticeably protrude into the space in the plexiglass slit adjacent to the recording bar, but rather moved smoothly onto the bar. There were two notable exceptions to this generalization: if the spacing of the animal's approach to the bar was such that anterior metapodial advancement or propodial advancement began at the edge of the slit, then the foot region bulged downward into the gap and thereby displaced the bar forward by pushing it from behind. A sample recording of the forward and backward forces is shown in Figure 7A. The labels on the recording show the points at which the snail's posture matched the characteristic postures for stages I, II, and III in each of the six crawl steps. The distribution of forces beneath the foot at each stage is illustrated in Figure 7B. During metapodial shortening/propodial extension, the posterior metapodium and the anterior propodium register forward sliding friction while the anterior metapodium and posterior propodium show the static reaction. In metapodial extension the entire metapodium registers a forward force and the propodium registers a backward force. In propodial elevation the propodium is the only region showing forward sliding friction.

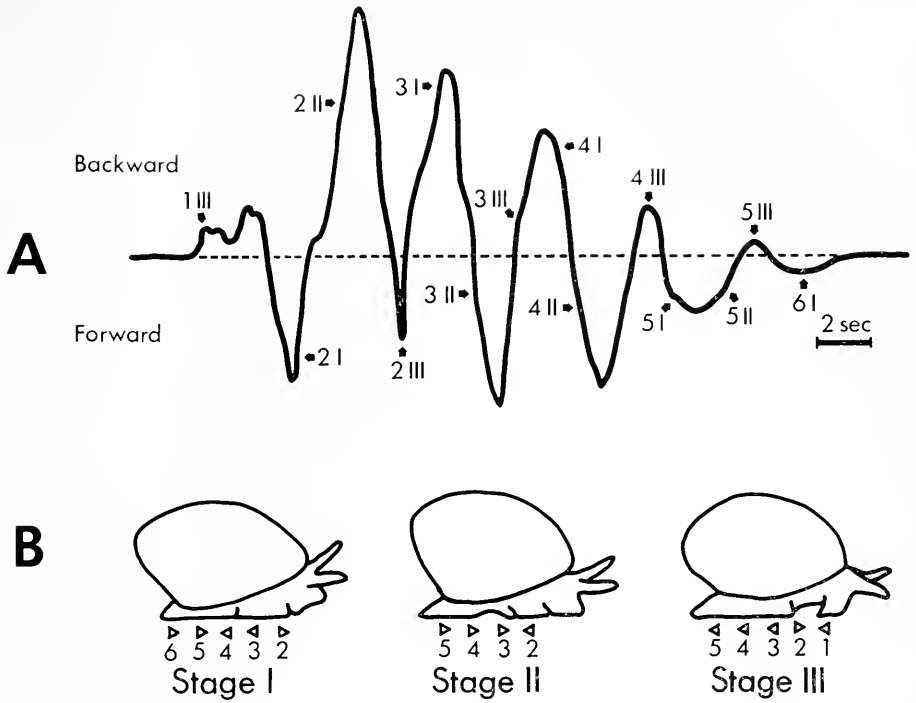

FIGURE 7. A is the force transducer recording of forward and backward forces exerted by a snail's foot as it crawled across a bar. The snail required four complete crawl-step cycles and portions of two other cycles to complete the crossing. Information was obtained in a manner similar to that described for Figure 6. B is the pattern of forward and backward forces exerted by the foot on the substratum in Stages I, II and III of the crawl-step.

DISCUSSION

The crawl-step of *Melampus* is a complex sequence of events compared with the direct pedal waves of most land pulmonates. Figure 8 gives a model for *Melampus* locomotion that was constructed from the histology, cinematography and force transducer recordings presented in the results.

According to this model the Stage I metapodial shortening/propodial extension result from a single muscular event: contraction of columellar muscles in the posterior half of the metapodium. This action not only draws the end of the foot upward and forward, as indicated by the transducer recordings, but it causes some blood to leave the posterior region of the foot. This blood could flow across the low transverse groove into the propodium and into the anterior region of the body. The pattern of forces recorded from the foot at this stage indicates that the weight is primarily borne by the posterior propodium and that it and the anterior metapodium experience a backward drag (the static reaction). The combination of downward and forward forces recorded from the anterior propodium are what would be expected in a region experiencing invasion of blood from the posterior. The observation that the propodium bulged downward into the crack

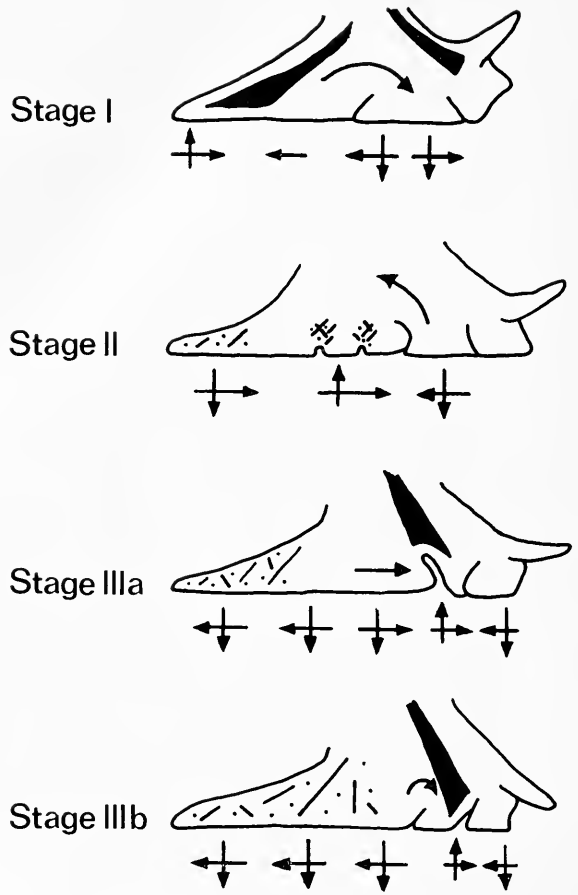

FIGURE 8. Model of mechanical events producing locomotion in *Melampus*. Arrows below the foot show forces exerted on the substratum; arrows within the body show fluid dynamics; dark regions in the body indicate muscle contraction patterns. See text for discussion.

adjacent to the recording bar indicates that relaxed tonus in the propodium may contribute to the hydraulic expansion.

In Stage II, metapodial extension is produced by low-amplitude waves travelling from the middle toward the anterior metapodium. Their action is depicted as squeezing blood forward within the ventral portion of the foot until it accumulates behind the transverse groove. The resulting change in orientation of the groove causes blood in the posterior propodium to move up into the large blood sinuses in the dorsal propodium and anterior metapodium. The snail's weight rests on the anterior propodium and the posterior metapodium while the middle of the foot shifts forward. The observed narrowing of the metapodium that begins in this stage and continues through Stage III probably exerts a tonic force on the blood and favors forward movement of the foot. This tonic force may be produced

by body wall musculature rather than by pedal muscle fibers derived from the columellar muscles.

In Stage III, propodial elevation results from contraction of columellar muscle bands, chiefly those of the left anterior and cephalic columellar muscle. The transverse groove region is elevated in Stage IIIa. This allows the anterior edge of the metapodium to expand into the space formerly occupied by the posterior propodium. Again, the combination of downward and forward forces indicates that metapodial extension results from an hydraulic event. By Stage IIIb the more anterior region of the propodium is elevated by columellar muscles and the posterior region returns to the substratum in advance of its former position. This forward displacement is due, at least in part, to the extension of the metapodium in Stage IIIa. Throughout Stage III the oral veil and the posterior metapodium are static, weight-bearing regions that support the forward progression of the anterior region of the foot.

The hydraulic expansion of the anterior region of the foot of *Melampus* is similar to hydraulic events described for burrowing in the naticid snail *Polinices josephinus* (Trueman, 1968). The work of Schiemenz (1884) and, more recently, Russell-Hunter and Russell-Hunter (1968) and Russell-Hunter and Apley (1968) revealed that in naticids water is taken up into special channels to aid in the expansion of the foot. Both the burrowing *Polinices* and the crawling *Melampus* advance in a similar stepwise fashion.

The deeply cleft transverse groove and elevation of the propodium in a stepping locomotion are features that have arisen independently in several lines of Ellobiidae that are adapted to a hard substratum (Morton, 1955). Morton (1955) suggests that it may be especially adaptive in progression over broken or irregular surfaces. The model presented above indicates that the transverse groove has a valve-like function, rendering the metapodium relatively independent of the propodium during metapodial extension. This enhanced control over hydraulic events may have been important in the transition from the marine habitat to land in the Ellobiidae.

Morton (1955) describes the locomotion of ellobiids with a divided foot as ". . . fixing down the anterior third of the foot well in advance of the animal and drawing the remainder forwards upon it" (p. 151). This brief description neglects the hydraulic expansion of the propodium and thereby exaggerates the superficial similarity between *Melampus* locomotion and the "loping" locomotion of another primitive pulmonate, *Otina otis*. According to Vlés' (1913) account of *Otina* locomotion, the transversely subdivided foot is used in a stepping fashion in which the anterior is lifted and placed forward, rendering the foot long, and then the posterior is pulled forward, rendering the foot short. The hydraulic component that is responsible for maintaining the *Melampus* foot at a relatively constant length is apparently absent in *Otina* locomotion. Furthermore, no matter in what order the events of *Melampus* locomotion are considered, the contraction pattern within the propodium clearly passes from posterior to anterior and therefore the crawl-step locomotion exhibited by this snail cannot be considered a retrograde wave. Thus the locomotion of *Melampus* cannot be cited to support the supposition that the retrograde wave locomotion seen in stylommatophoran gastropods during escape behavior is a primitive type of locomotor behavior within the pulmonates.

The only feature of *Melampus* locomotion that is similar to the locomotion of

higher land snails and slugs is the anteriorly-travelling ripple that produces metapodial lengthening. This wave of contraction is apparently produced by intrinsic pedal musculature and, although the *Melampus* foot appears to lack the specialized musculature that produces the waves in *Agriolimax* (Jones 1973), observation of the side view clearly shows that the elevated region of the wave is compressed and its forward movement forces a bolus of fluid forward. The fact that these metapodial waves do not originate at the end of the foot is very significant in an attempt to relate them to the direct waves of stylommatophorans. This is the case because, as Jones (1975) has pointed out, the multiple direct wave pattern of higher pulmonates could not be derived by adding waves to the single direct wave type of locomotion such as that exhibited by *Onchidella,* in which the wave begins by shortening of the posterior region of the foot (Vlés, 1907). Such an action renders the foot shorter during locomotion than when it is at rest and the propagation of many waves at the posterior of the foot before the first wave passes off the anterior would cause the foot to become prohibitively short. The wave pattern in higher pulmonates, in contrast, is initiated at the onset of locomotion at a site near the anterior part of the foot, where constriction of the elevated region results in a compensatory stretching of a more posterior part of the foot and the foot length remains constant (Lissmann, 1945; Jones, 1975). Thus the metapodial waves of *Melampus* could represent the survival of the motor pattern that gave rise to multiple direct wave locomotion in stylommatophorans. Morton's (1955) description of locomotion in ellobiids that lack a transverse groove suggests that this wave pattern may be more pronounced in less specialized members of the family.

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Summary

The foot of *Melampus* is subdivided into an anterior propodium and a posterior metapodium by a permanent transverse groove. Locomotion in *Melampus* consists of repetition of a cycle of events that pass from posterior to anterior; this cycle has been named a crawl-step Three stages in the crawl-step have been identified: Metapodial shortening is produced by the action of columellar muscles and this action forces blood anteriorly to extend the propodium. Metapodial lengthening is produced by muscle action within the metapodium and extends the metapodial region forward at the expense of the propodium. Propodial elevation is produced by columellar muscles and prepares the propodium to "step" forward while fluid invasion occurs in the first stage.

LITERATURE CITED

Apley, M. L., 1968. Field and experimental studies on pattern and control of reproduction in *Melampus bidentatus* (Say). *Ph.D. dissertation, Syracuse University,* 154 pp. *Diss. abstr.* V29, p. 1527B (#68–13,810).

CHAPMAN, G., 1958. The hydrostatic skeleton in the invertebrates. *Biol. Rev.* **33**: 338–71.
CHAPMAN, G., 1975. Versatility of hydraulic systems. *J. Exp. Zool.*, **194**: 249–270.
JONES, H. D., 1973. The mechanism of locomotion of *Agriolimax reticulatus* (Mollusca: Gastropoda). *J. Zool., (Lond.)*, **171**: 489–98.
JONES, H. D., 1975. Locomotion. Pages 1–32. in V. Fretter & J. Peake, Eds., *Pulmonates*, Academic Press, London.
JONES, H. D., AND E. R. TRUEMAN, 1970. Locomotion of the limpet, *Patella vulgata* L. *J. Exp. Biol.*, **52**: 201–16.
LISSMAN, H. W., 1946. The mechanism of locomotion of gastropod molluscs. II. Kinetics. *J. Exp. Biol.*, **22**: 37–50.
MILLER, S. L., 1974. The classification, taxonomic distribution, and evolution of locomotor types among prosobranch gastropods. *Proc. Malacol. Soc. Lond.*, **41**: 233–72.
MORTON, J. E., 1955. The evolution of the Ellobiidae with a discussion on the origin of the Pulmonata. *Proc. Zool. Soc. Lond.*, **125**: 127–68.
PANTIN, C. F. A., 1948. *Notes on Microscopical Technique for Zoologists.* Cambridge, Cambridge University, 79 pp.
PLESCH, B., C. JANSE, AND H. H. BOER, 1975. Gross morphology and histology of the musculature of the freshwater pulmonate *Lymnaea stagnalis* (L.). *Neth. J. Zool.*, **25**: 332–52.
PRICE, C. H., 1977. Regeneration in the central nervous system of a pulmonate mollusc, *Melampus*. *Cell Tissue Res.*, **180**: 529–36.
RUSSELL-HUNTER, W. D. AND M. RUSSELL-HUNTER, 1968. Pedal expansion in the naticid snails. I. Introduction and weighing experiments. *Biol. Bull.*, **135**: 548–62.
RUSSELL-HUNTER, W. D. AND M. L. APLEY, 1968. Pedal expansion in the naticid snails. II. Labelling experiments using inulin. *Biol. Bull.*, **135**: 563–73.
SCHIEMENZ, P., 1884. Uber die Wasseraufnahme bei Lamellibranchiaten und Gastropoden. *Mitt. Zool. Stn. Neapel*, **5**: 509–43.
TRUEMAN, E. R., 1968. The mechanism of burrowing in some naticid gastropods in comparison with that of other molluscs. *J. Exp. Biol.*, **48**: 663–78.
TRUEMAN, E. R., 1975. *The Locomotion of Soft-Bodied Animals.* American Elsevier, New York, 200 pp.
VLÉS, F., 1907. Sur les ondes pedieuses des mollusques reptateurs. *Comptes Rendu Acad. Sci.*, **145**: 276–8.
VLÉS, F., 1913. Observations sur la locomotion d'*Otina otis* Turt. Remarques sur la progression des gasteropodes. *Bull. Soc. Zool. Fr.*, **38**: 242–50.

Reference: *Biol. Bull.*, **157**: 320–333. (October, 1979)

THE ECTOPARASITISM OF *BOONEA* AND *FARGOA* (GASTROPODA: PYRAMIDELLIDAE) [1]

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Pyramidellids are now well known to be ectoparasites feeding on the body fluids of invertebrates (Ankel, 1938, 1949a, 1949b, 1959; Fretter and Graham, 1949, 1962; Fretter, 1951). Most of the verified hosts are polychaetes, gastropods and bivalves, but there also appear to be various minor host groups such as polyplacophorans and echinoderms (Robertson and Orr, 1961). A defect of many of the reports of hosts and even of "feeding" experiments is that no definite observations on feeding were made. It is unsatisfactory to report a "host" when a pyramidellid is merely found "on" or "with" an invertebrate, whether it be in the field or in the laboratory. Pyramidellids, being foraging animals, sometimes assume positions on living substrates other than their hosts. Also, there can be behavioral preludes to feeding that do not culminate in feeding. A dilemma is that feeding cannot be verified *in situ* in the field, while in the laboratory some pyramidellids will feed on "hosts" they would never naturally encounter; the feeding of other pyramidellids is observed with great difficulty in the laboratory, even when the probable natural host is offered. Under the circumstances, what should be done is to observe for consistent associations with "hosts" in the field and then to determine whether these "hosts" are fed on in the laboratory.

The degree to which pyramidellids are host-specific remains unresolved. Fretter and Graham (1949, 1962), even while recording the European species *Odostomia ambigua* (*O. "eulimoides"*) on *Pecten maximus, Chlamys opercularis* and *Ostrea edulis,* emphasized that pyramidellids are host-specific. Berry (1955) went so far as to suggest that host-specificity accounts for the large number of pyramidellid species. There may be pyramidellids that are specific to one host species, but there are a few suggestive data that others are not. Ankel and Christensen (1963) observed *Odostomia rissoides* (*O. "scalaris"*) in Denmark feeding on five species in the laboratory: *Lacuna vincta* (*L. "divaricata"*), *Littorina "saxatilis", Hydrobia ulvae, Rissoa membranacea* and *Chlamys opercularis.* All but the last species live in the same habitat. There are, too, literature records of *O. rissoides* with *Mytilus edulis.* Ankel and Christensen (1963) also assembled literature data showing that *O. ambigua* had been found with four pectinid species, *Mytilus edulis, Ostrea edulis, Hiatella* (*"Saxicava"*) *rugosa* and *Turritella communis.* Minichev (1971) found *Odostomia fujitanii* on various mollusks in the Sea of Japan, and under experimental conditions it fed readily on *Littorina brevicula, Tegula rustica, Umbonium costatum, Turritella fortilirata, Arca boucardi,* etc. According to LaFollette (1979), the southern California species *Chrysallida cincta* has at least six hosts: *Haliotis corrugata, H. fulgens, Tegula eiseni, Norrisia norrisi, Astraea undosa* and *A. gibberosa* (all archaeogastropods).

[1] Woods Hole Oceanographic Institution Contribution No. 4313.

The purposes of the present paper are: to tabulate literature records of the "hosts" of all eastern North American odostomioids (*Boonea* and *Fargoa* species), to present (with emphasis on *Boonea seminuda* and *B. bisuturalis*) original field data on their occurrences and frequencies with "hosts", to present laboratory data on the "hosts" that were fed upon, and to present laboratory data on the preferences that *B. seminuda* and *B. bisuturalis* had for two hosts. Of prime interest was the sympatric occurrence and abundance of these two species, and how they divide resources. Two species in the related genus *Fargoa* were also of interest by way of comparison, but fewer data are available about them.

Justification for separating the genera *Boonea* and *Fargoa* from *Odostomia* has recently been presented elsewhere (Robertson, 1978). The systematics of the species reported on here was also treated in the same paper. *Boonea impressa* is considered one species, although the populations in North Carolina and northwest Florida have planktotrophic larval development while a Texas population is lecithotrophic. The nomenclature and systematic sequences of the non-pyramidellid mollusks in the tables follow Abbott (1974) except that *Bittium* is used instead of *Diastoma* (Houbrick, 1977).

Materials and Methods

Odostomioids were collected in the vicinities of Plymouth and Woods Hole, Massachusetts, and Beaufort and Wilmington, North Carolina. Correspondents sent living specimens and habitat data from Sapelo Island, Georgia, and Galveston, Texas. In all, seven species were collected alive, and careful note was taken on whether they were consistently on a particular living substrate—the presumptive host. "Hosts" were obtained near Woods Hole, on the New Jersey coast, near Beaufort, and from Texas.

Quadrats were used to determine the abundance of *Boonea* relative to its hosts and to other mollusks in the same habitats. The quadrats were placed at random in habitats suitable for two *Boonea* species (for *B. seminuda,* the muddy sand shallow subtidal zone where *Crepidula fornicata* is common at the N end of Quissett Harbor, 3 km NE of Woods Hole, mid-August 1978; for *B. bisuturalis,* the stony low intertidal zone, underlain by sand and peat, where *Littorina littorea* is common at the shore opposite Flume Pond, S of Gunning Point, 4 km NNE of Woods Hole, early and late August, 1978). There was time only for two quadrats for each species. Three of the quadrats were 1.0×1.0 m; the fourth, because of a rising tide, had to be 0.5×0.5 m. All mollusks (except *Bittium alternatum* in the *Boonea seminuda* habitat) in each quadrat were collected, identified, counted and weighed (the weights include the shells). The five species of *Boonea* and *Fargoa* whose presumptive hosts were determined in the field were kept with these hosts in bowls of sea water in the laboratory. After it was learned that *Boonea* would feed on mollusks other than the presumptive hosts, all mollusks found in *Boonea* habitats plus a selection of species from other habitats were offered. Two polychaete species found in the same habitat as *B. bisuturalis* were also offered, as was the ascidian *Molgula.* The two *Fargoa* species were offered the serpulid *Hydroides dianthus,* a few other (unidentified) polychaetes, and various mollusks (*Littorina littorea, Crepidula fornicata* and *Anachis avara*).

TABLE I

Literature records of eastern North American species of Boonea *and* Fargoa *with "hosts" (nomenclature brought up to date).*

Species	"Host"	Area	Association observed in field (F) or lab. (L).	True feeding observed	Author
B. seminuda	*Argopecten irradians*	Beaufort, NC	F	no	Hackney, 1944, p. 60
B. seminuda	*Crepidula fornicata*	Woods Hole, MA	F, L	yes	Robertson, 1957
B. seminuda	*Argopecten gibbus*	off Ocracoke and Core Banks, NC	F, L	yes	Wells and Wells, 1961; Wells, Wells and Gray, 1964, p. 577
B. seminuda	*Crepidula fornicata*	West Dennis, MA	F, L	yes	Merrill and Boss, 1964; Boss and Merrill, 1965
B. seminuda	*Crucibulum striatum*	Cape Cod, MA	L	yes	Merrill and Boss, 1964; Boss and Merrill, 1965
B. seminuda	*Crassostrea virginica*	West Dennis, MA	L	no	Boss and Merrill, 1965
B. seminuda	*Mercenaria mercenaria*	Cape Cod, MA	L	no	Boss and Merrill, 1965
B. seminuda	*Argopecten irradians*	Cape Cod, MA	L	no	Boss and Merrill, 1965
B. seminuda	*Modiolus modiolus*	Cape Cod, MA	L	no	Boss and Merrill, 1965
B. seminuda	*Placopecten magellanicus*	Cape Cod, MA	L	no	Boss and Merrill, 1965
B. seminuda	*Argopecten irradians*	North Carolina	F	no	Porter, 1974, 1976
B. bisuturalis	*Geukensia demissa*	Long Island, NY	F	no	Pelseneer, 1928, p. 172
B. bisuturalis	*Mytilus edulis*	Long Island, NY	F	no	Pelseneer, 1928, p. 172
B. bisuturalis	*Mya arenaria*	Gulf of St. Lawrence	F	no	Medcof, 1948
B. bisuturalis	*Crassostrea virginica*	New England	F, L	yes	Loosanoff, 1956
B. bisuturalis	*Crassostrea virginica*	West Dennis, MA	F, L	yes	Merrill and Boss, 1964; Boss and Merrill, 1965
B. bisuturalis	*Littorina littorea*	Woods Hole, MA	F, L	yes	Scheltema, 1965
B. bisuturalis	*Bittium alternatum*	Woods Hole, MA	L	yes	Scheltema, 1965
B. bisuturalis	*Crepidula fornicata*	West Dennis, MA	L	no	Boss and Merrill, 1965
B. bisuturalis	*Mercenaria mercenaria*	Cape Cod, MA	L	no	Boss and Merrill, 1965
B. bisuturalis	*Argopecten irradians*	Cape Cod, MA	L	no	Boss and Merrill, 1965
B. bisuturalis	*Crucibulum striatum*	Cape Cod, MA	L	no	Boss and Merrill, 1965
B. bisuturalis	*Modiolus modiolus*	Cape Cod, MA	L	no	Boss and Merrill, 1965
B. bisuturalis	*Crepidula plana*	Cape Cod, MA	L	no	Boss and Merrill, 1965

TABLE I—*Continued*

Species	"Host"	Area	Association observed in field (F) or lab. (L).	True feeding observed	Author
B. bisuturalis	*Placopecten magellanicus*	Cape Cod, MA	L	no	Boss and Merrill, 1965
B. bisuturalis	*Littorina littorea*	Woods Hole, MA	F, L	yes	Robertson, 1967
B. bisuturalis	*Ilyanassa obsoleta*	Duxbury Beach, MA	F	no	Bullock and Boss, 1971
B. bisuturalis	*Mytilus edulis*	Duxbury Beach, MA	F	yes	Bullock and Boss, 1971
B. bisuturalis	*Littorina littorea*	Duxbury Beach, MA	F	no	Bullock and Boss, 1971
B. bisuturalis	*Urosalpinx cinerea*	Duxbury Beach, MA	F	no	Bullock and Boss, 1971
B. bisuturalis	*Crepidula convexa*	Duxbury Beach, MA	F	no	Bullock and Boss, 1971
B. bisuturalis	*Crepidula fornicata*	Duxbury Beach, MA	F	no	Bullock and Boss, 1971
B. bisuturalis	*Nassarius trivittatus*	Duxbury Beach, MA	F	no	Bullock and Boss, 1971
B. bisuturalis	*Ilyanassa obsoleta*	[Woods Hole, MA]	L	yes	Abbott, 1972, p. 45
B. impressa	*Crassostrea virginica*	Florida	F	no	Dall, 1883, p. 331
B. impressa	*Crassostrea virginica*	South Carolina	F, L	yes	Hopkins, 1956
B. impressa	*Crassostrea virginica*	Chesapeake Bay	L	yes	Allen, 1958
B. impressa	*Bittium varium*	Chesapeake Bay	L	yes	Allen, 1958
B. impressa	*Crepidula convexa*	Chesapeake Bay	L	yes	Allen, 1958
B. impressa	*Triphora nigrocincta*	Chesapeake Bay	L	yes	Allen, 1958
B. impressa	*Urosalpinx cinerea*	Chesapeake Bay	L	yes	Allen, 1958
B. impressa	"polychaete" worm	Chesapeake Bay	L	no?	Allen, 1958
B. impressa	*Molgula*	Chesapeake Bay	L	no?	Allen, 1958
B. impressa	cf. *Fargoa gibbosa*	Chesapeake Bay	L	yes	Allen, 1958
B. impressa	*Crassostrea virginica*	Beaufort, NC	L	yes	Wells, 1959
B. impressa	*Crassostrea virginica*	Mississippi Sound	F	no	Moore, 1961, p. 33
B. impressa	*Crassostrea virginica*	North Carolina	F	no	Porter, 1974, 1976
F. dianthophila	*Hydroides dianthus*	Beaufort, NC	L	no	Wells and Wells, 1961
F. dianthophila	*Hydroides dianthus*	Buzzard's Bay, MA	F, L	yes	Roberge, 1968
F. dianthophila	*Hydroides microtis*	off Core Bank, NC	L	no	Wells and Wells, 1969
F. dianthophila	*Hydroides floridanus*	N. E. & N. W. Florida	L	no	Wells and Wells, 1969
F. dianthophila	*Hydroides crucigera*	Wakulla and Franklin Co, FL	L	no	Wells and Wells, 1969
F. dianthophila	*Hydroides dianthus*	N. W. Florida	L	no	Wells and Wells, 1969
F. bartschi	*Hydroides dianthus*	[Woods Hole, MA]	[F, L]	[yes]	Robertson, 1978

To be considered in a *feeding position* odostomioids had to be stationary on a "host" for about two or more minutes, and less than a proboscis length away from an accessible portion of the potential "host's" soft tissues. Usually after assuming a feeding position, odostomioids *probing* their "hosts" partially everted and inverted their proboscises one or more times, near or onto the "host's" soft tissues (probing rarely occurs away from any host). *Feeding* was recorded only when the proboscis was fully everted, when the "host" was pierced, and when the buccal pump within the proboscis "vibrated" to and fro with peristaltic waves.

The *Boonea* and *Fargoa* animals and the "hosts" were kept together until feeding, death, or termination of the experiment occurred. The interactions were monitored with a binocular dissecting microscope. Differences in the number of "hosts" available and the time available for observations account for the differences in the "hosts" that were offered each species in Table V.

The movements of two *Boonea* species towards two hosts at a distance were quantified. Round, plastic washbasins (diameter 30 cm) were filled with sea water to a depth of 5 cm. Four *Crepidula fornicata* chains were placed at equal distances around the perimeter and four groups of four large specimens of *Littorina littorea* were placed between them. Sixty individuals of *Boonea seminuda* or *B. bisuturalis* (all about the same size) were placed in the center of each basin and allowed to move freely. The littorinas also moved freely and occasionally one had to be placed back in the water after it crawled up the side of the basin. Otherwise the basins were undisturbed for 3 hrs, after which time the littorinas and crepidulas were removed and the number of *Boonea* individuals on each was counted. All *Boonea* animals were then removed from their hosts and the basin. The basin was washed and new sea water was added. Each experiment was done twice each day. *Boonea* was not allowed to feed except when in the basin (6 hrs per day). There were ten trials for *B. seminuda*, and eight trials for *B. bisuturalis*. The data for each species were tested for homogeneity to determine whether the two sets of data could be lumped and treated as two experiments (Sokal and Rohlf, 1969). Chi-squares were then computed.

Results

There are literature records of three species of *Boonea* with 22 species of mollusks, one species of polychaete and one species of ascidian (Table I). Only 18 of the 44 records were based on actual feeding observations. Table I also shows that two species of *Fargoa* are recorded with four species of *Hydroides*. Only two of the seven records were based on feeding observations.

In the present study, three species of *Boonea* were found alive and with hosts (all of them molluscan; Table II). Four species of *Fargoa* were found alive, but only two of them were with hosts (both *Hydroides dianthus*; Table II).

Four *Boonea seminuda* animals were found in the winter with *Littorina littorea* in the habitat occupied at other seasons by *B. bisuturalis* (shore opposite Flume Pond, near Woods Hole). *B. bisuturalis* was occasionally found in the summer with *B. seminuda* on *Crepidula fornicata* (N end of Quissett Harbor, near Woods Hole).

The quadrat data (Tables III and IV show that *Boonea seminuda* and *B.*

TABLE II

Field observations on Boonea *and* Fargoa *species found alive in the present study: localities and presumptive hosts.*

Species	Area	In feeding position on	Association
B. seminuda	Woods Hole	*Crepidula fornicata*	common
B. seminuda	Woods Hole	*Littorina littorea*	rare
B. seminuda	Woods Hole	*Argopecten irradians*	rare
B. seminuda	Beaufort	*Crepidula fornicata*	common
B. seminuda	Beaufort	*Argopecten irradians*	common
B. bisuturalis	Plymouth	*Ilyanassa obsoleta*	common
B. bisuturalis	Woods Hole	*Littorina littorea*	common
B. bisuturalis	Woods Hole	*Crepidula fornicata*	fairly rare
B. bisuturalis	Woods Hole	*Argopecten irradians*	fairly rare
*B. impressa**	Beaufort	*Crassostrea virginica*	common
*B. impressa**	Wilmington	*Crassostrea virginica*	common
*B. impressa**	Sapelo I.	*Crassostrea virginica*	common
B. "impressa"†	Galveston	*Crassostrea virginica*	common
B. "impressa"†	Galveston	*Ischadium recurvum*	rare
B. "impressa"†	Galveston	*Geukensia demissa*	rare
F. dianthophila	Woods Hole	*Hydroides dianthus*	common
F. dianthophila	Beaufort	*Hydroides dianthus*	common
F. bushiana	Beaufort	not found	
F. bartschi	Woods Hole	*Hydroides dianthus*	common
F. bartschi	Beaufort	not found	
F. bartschi	Wilmington	*Hydroides dianthus*	common
F. bartschi	Texas	not found	
F. gibbosa	Beaufort	not found‡	

* Planktotrophic.
† Lecithotrophic.
‡ Muddy sand *Zostera* substrate.

bisuturalis occur at low densities relative to the biomass and numbers of their hosts. The percentages derived from the weights in Tables III and IV are all similar, ranging from 0.035 to 0.165 with only the predominant hosts considered and 0.029 to 0.079 when all potential hosts are pooled.

When starved to some degree, *Boonea* species fed on many of the gastropods and bivalves that were offered them (Table V), including not only their probable predominant hosts and other species in the same habitats (Tables III–IV) but even species from other habitats. *B. bisuturalis* fed on a record 37 mollusk species (Table V). It did not feed on the polychaetes *Nereis* (*Neanthes*) *succinea* and *Hydroides dianthus,* the chiton *Chaetopleura apiculata* or the ascidian *Molgula manhattensis.*

Aside from a few interactions that probably were not monitored long enough, the only proffered gastropod and bivalve "hosts" that were not observed even to elicit feeding positions or probing were a large gastropod with a thick integument (*Busycon*), and small species with tight closure (*Caecum, Mysella* and *Gemma*). The bivalves *Anadara, Anomia, Tellina* and *Cumingia* were fed on reluctantly or not at all.

Starved Texas *Boonea "impressa"* was frequently observed to feed cannibalisti-

TABLE III

Weights and numbers of individual mollusks from two 1.0 × 1.0 m quadrats in a habitat where Boonea seminuda *is relatively abundant on* Crepidula fornicata, *showing the abundance of the* Boonea.

Species	Quadrat 1 Weight (g) / [no. individuals]	Quadrat 2 Weight (g) / [no. individuals]
1. *Crepidula fornicata* (adults)	256.484 [74]	159.472 [49]
2. *Littorina littorea* (mainly large adults)	29.874 [15]	6.526 [4]
3. *Crassostrea virginica* (juvenile and adults)	25.143 [5]	0.531 [1]
4. *Bittium alternatum* (juveniles)	not weighed or counted*	
5. *Anomia simplex* (juvenile to medium-sized)	1.221 [21]	3.972 [42]
6. *Crepidula convexa* (mainly juveniles)	1.187† [78‡]	3.228† [140‡]
7. *Urosalpinx cinerea* (adults)	3.707 [2]	0 [0]
8. *Crepidula plana* (juvenile and adults)	0.477 [2]	0.006 [1]
9. *Boonea seminuda* (mainly juveniles)	0.090 [24]	0.063 [25]
Totals	318.183 [221]	173.798 [262]
(excluding *Bittium*)		
Weight of *Boonea:*		
percent of weight of *Crepidula fornicata*	0.035	0.040
percent of weight of all "hosts"	0.029	0.037

* Very numerous mainly above the substratum on the red alga *Polysiphonia;* rank estimated.
† Including egg sacs.
‡ Excluding egg sacs.

cally (Table V), unlike the other species. *B. bisuturalis* was once seen to feed on another odostomioid, *Fargoa bartschi* (Table V).

The degree of unselectiveness in feeding differs among the *Boonea* species. *B. "impressa"* (from Texas) fed on 36 out of the 37 gastropod and bivalve species offered to it (97%) (Table V). *B. bisuturalis* fed on 37 out of the 46 gastropod and bivalve species offered to it (80%) (Table V). *B. seminuda* fed on 22 out of the 36 gastropod and bivalve species offered to it (61%) (Table V). *B. seminuda* is more selective than either of the other species, feeding reluctantly or not at all on most neogastropods (*Urosalpinx* through *Ilyanassa* in Table V) and many bivalves.

The "hosts" were pierced in all accessible places (Table V), including tentacles which might be thought too sensitive for this (Ankel and Christensen, 1963). *Melampus* and *Littorina "saxatilis"*, kept submerged with difficulty, were fed on only under water.

Boonea was never seen to feed on individual hosts much smaller than itself. (In the case of *Odostomia,* Ankel and Christensen, 1963, often observed that the parasite was larger than the host.) Only tiny juvenile *B. bisuturalis* were seen to feed on tiny juvenile *Littorina littorea.* The many juveniles of the smaller mollusks encountered in the quadrats (Tables III–IV) no doubt would be immune from parasitism by adult *Boonea.*

In the laboratory, *Fargoa dianthophila* and *F. bartschi* probed and fed only on *Hydroides dianthus. F. dianthophila* fed readily both on the collar and on the branchiae. *F. bartschi* was seen to feed (on the collar) only once.

In the experiments on the presumably chemosensory responses of *Boonea* to

two hosts at a distance, *Boonea seminuda* had a total of 234 individuals (77%) on *Crepidula fornicata* and 72 individuals (23%) on *Littorina littorea* in ten trials. The data for *B. seminuda* were found to be sufficiently homogeneous ($0.70 < P < 0.80$) to be treated as a single sample. A Chi-square with one degree of freedom, corrected for continuity, was then performed, resulting in a probability much less than 5 in 10,000 ($P << 0.0005$) that *B. seminuda* was randomly distributed between *C. fornicata* and *L. littorea*. *B. seminuda* showed a clear preference for *C. fornicata*. Boss and Merrill (1965) also demonstrated the same preference.

In the 8 trials of *Boonea bisuturalis* a total of 102 individuals (29%) were on *Crepidula fornicata*, while 246 individuals (71%) were on *Littorina littorea* after 3 hr. The data for *B. bisuturalis* were also tested for homogeneity. These data were found not to be uniform in magnitude, although they all differed in the same direction. The pooled Chi-square was highly significant, but the heterogeneity Chi-square was significant as well. Chi-squares were then computed for each of the eight trials. The Chi-squares for the first three trials are not significant. In the final five trials, after acclimating to laboratory conditions, *B. bisuturalis* showed a clear preference for *L. littorea* ($P << 0.0005$ in trials 4–6; $0.005 > P > 0.001$ in trials 7–8).

Discussion

Boonea seminuda and *B. bisuturalis* occupy different (but slightly overlapping) habitats. Judging by the Woods Hole field data (Tables II–IV), the feeding

Table IV

Weights and numbers of individual mollusks (juveniles as well as adults) from one 1.0 × 1.0 m quadrat and one 0.5 × 0.5 m quadrat in a habitat where Boonea bisuturalis *is relatively abundant near and on* Littorina littorea, *showing the abundance of the* Boonea.

Species	Quadrat 1 (1.0 × 1.0 m) Weight (g)/ [no. individuals]	Quadrat 2 (0.5 × 0.5 m) Weight (g)/ [no. individuals]
1. *Littorina littorea*	132.005 [2,156]	68.647 [708]
2. *Mercenaria mercenaria*	0 [0]	33.997 [2]
3. *Mya arenaria*	0.219 [7]	25.828 [14]
4. *Petricola pholadiformis*	0.901 [3]	11.051 [13]
5. *Urosalpinx cinerea*	20.418 [48]	2.288 [5]
6. *Littorina "saxatilis"*	1.511 [63]	0.530 [17]
7. *Boonea bisuturalis*	0.069 [10]	0.113 [24]
8. *Crepidula convexa*	0.147* [18†]	0 [0]
9. *Mitrella lunata*	0.021 [3]	0.029 [7]
10. *Bittium alternatum*	0.046 [5]	0.013 [5]
11. *Littorina obtusata*	0.001 [1]	0.019 [1]
12. *Argopecten irradians*	0.017 [2]	0 [0]
13. *Anomia simplex*	0.006 [1]	0.001 [1]
Totals	155.361 [2,317]	142.516 (797]
Weight of *Boonea:*		
percent of weight of *Littorina littorea*	0.052	0.165
percent of weight of all "hosts"	0.044	0.079

* Including one cluster of egg sacs.
† Excluding one cluster of egg sacs.

TABLE V

Systematic list of mollusks offered to Boonea seminuda (*from Woods Hole*), B. bisuturalis (*from Woods Hole*), *and* B. "impressa" (*from Galveston*) *in bowls in the laboratory, and the feeding interactions observed. Occurrence (habitat coincidence) is considered in terms of the likelihood of contact. Abbreviations: A—absent; AS—anterior siphon; BES—base of excurrent siphon; C—common; CNO—cannibalism not observed; DIMC—deep in mantle cavity; EDIFE—edge and deep in from edge; F—foot; FEF-front end of foot; FFRD—fed on fairly readily; FFRL—fed on fairly reluctantly; FRD—fed on readily; FRL—fed on reluctantly; IC—inaccessible; IFE—in from edge; IP—impenetrable; IS—incurrent siphon; LIT—large and integument thick; M—mantle; MBTS—mantle between two siphons; ME—mantle edge; MES—mantle through excurrent siphon; MF—mantle fusion; NMILE—not monitored long enough; OL—operculigerous lobe; PH—predominant host; R—rare; RC—reluctant cannibalism; RF—rear end of foot; RH—rear of head; SA—siphonal area; SBF—side and base of foot; SF—side of foot; SH—side of head; STC—small and tight closing; T—tentacle; TIES—through incurrent and excurrent siphon; U—unpalatable; USF—under side of foot.*

Taxa	*Boonea seminuda*						*Boonea bisuturalis*						*Boonea "impressa"*					
	Occurrence	Feeding position	Probing	Feeding	Puncture site	Remarks	Occurrence	Feeding position	Probing	Feeding	Puncture site	Remarks	Occurrence	Feeding position	Probing	Feeding	Puncture site	Remarks
Polyplacophora																		
Chaetopleura apiculata							R	–	+	–	—	U or IC						
Gastropoda																		
Lacuna vincta	A	+	+	+	F	FFRD	R	+	+	+	DIMC	FFRD	A	+	+	+	SF	FFRD
Littorina littorea	C	+	+	+	M (EDIFE)	FFRD	C	+	+	+	SF; ME; OL	PH; FRD	A	+	+	+	ME; OL	FRD
Littorina obtusata	A	+	+	+	ME; SBF	FFRD	R	+	+	+	DIMC	FFRD	A	+	+	+	T	FFRD
Littorina "saxatilis"	A	+	+	+	ME	FFRL	C	+	+	+	SF	FFRL	A	+	+	+	ME	FFRD
Littorina irrorata							A	+	+	+	SF	FFRD						
Hydrobia cf. *H. totteni*	A	+	+	+	T	FRD	A	+	+	+	USF	FRD	A	+	+	+	SF	FRD
Caecum pulchellum							A	–	–	–	—	STC						
Bittium alternatum	C	+	+	+	DIMC	FFRL	C	+	+	+	DIMC	FFRD	A	+	+	+	DIMC	FFRD
Cerithiopsis emersoni							R	+	–	–	—	U						
Seila adamsi	R	+	+	–	—	U	R	+	+	–	—	U						
Crepidula fornicata	C	+	+	+	ME; SF	PH; FRD	R	+	+	+	ME	FFRD	A	+	+	+	ME or SF	FFRD
Crepidula convexa	C	+	+	+	RH	FFRD	C	+	+	+	SF	FFRD	A	+	+	+	ME	FFRL
Crepidula plana	R	+	+	+	ME	FFRD	R	+	+	+	ME	FFRD	R	+	+	+	ME	FFRL
Urosalpinx cinerea	R	+	+	–	—	U	C	+	+	+	ME	FFRD	A	+	+	+	ME	FFRD
Nucella lapillus	A	+	+	+	ME	FFRL	A	+	+	+	ME	FFRL	A	+	+	+	USF	FRD
Eupleura caudata	A	–	–	–	—	NMILE	A	+	+	+	ME	FFRD	A	+	+	+	ME	FFRD
Anachis lafresnayi	A	+	+	+	DIMC	FRL	R	+	+	+	ME; AS	FFRD	A	+	+	+	ME	FFRD
Anachis avara	A	+	+	+	F	FRL	R	+	+	+	AS	FFRD	A	+	+	+	SF	FFRD
Mitrella lunata	A	+	+	+	F	FFRD	C	+	+	+	SF	FFRD	A	+	+	+	SH	FFRD
Busycon canaliculatum	A	–	–	–	—	U; LIT												
Nassarius vibex	A	+	+	–	—	FRL?	A	+	+	+	RF	FFRD	A	+	+	+	SF	FFRD
Nassarius trivittatus							A	+	+	+	ME	FFRD						
Ilyanassa obsoleta	C	+	+	+	SF	FRL	A*	+	+	+	RF	FFRD	A	+	+	+	ME	FFRD
Boonea seminuda	C	–	–	–	—	CNO												
Boonea bisuturalis							C	–	–	–	—	CNO						

TABLE V—*Continued*

Taxa	*Boonea seminuda*						*Boonea bisuturalis*						*Boonea "impressa"*					
	Occurrence	Feeding position	Probing	Feeding	Puncture site	Remarks	Occurrence	Feeding position	Probing	Feeding	Puncture site	Remarks	Occurrence	Feeding position	Probing	Feeding	Puncture site	Remarks
Boonea "impressa"													C	+	+	+	FEF	RC
Fargoa bartschi							R	+	+	+	SF	FRL						
Melampus bidentatus	A	+	+	+	ME?; DIMC	FFRL	A	+	+	+	ME	FFRL	A	+	+	+	T	FFRD
Bivalvia																		
Solemya velum	A	+	+	+	ME	FFRL	A	+	+	+	ME	FFRD	A	+	+	+	ME	FFRD
Anadara transversa	A	+	+	−	—	U	R	+	+	+	ME	FRL	A	+	+	+	ME	FRL
Anadara ovalis	A	+	+	−	—	U	R	+	+	+	ME	FRL	A	+	+	+	ME	FRL
Mytilus edulis	A	+	+	+	M (IFE)	FFRD	R†	+	+	+	ME	FFRD	A†	+	+	+	ME	FFRL
Brachidontes exustus													C	+	+	+	ME	FRD
Ischadium recurvum													C	+	+	+	ME	FRD
Geukensia demissa	A	+	+	+	MES	FFRD	A	+	+	+	ME	FFRD	C	+	+	+	ME	FFRD
◦ *Argopecten irradians*	R	+	+	+	ME	FFRD	R	+	+	+	ME	FFRL	A	+	+	+	ME	FRD
Anomia simplex	C	+	−	−	—	U	R	+	+	+	ME	FRL	A†	+	−	−	—	U
Crassostrea virginica	R	+	+	+	ME	FFRD	A	+	+	+	ME	FFRD	C	+	+	+	ME	PII; FRD
Mysella planulata							A	−	−	−	—	STC						
Laevicardium mortoni	A	+	−	−	—	U	A	+	+	+	ME	FFRD	A	+	+	+	ME	FFRL
Spisula solidissima	A	−	−	−	—	U	A†	+	+	+	SA	FFRD	A†	+	+	+	SA	FFRD
Mulinia lateralis							A	+	+	+	MBTS	FFRD						
Ensis directus							A	+	+	−	—	NMLE						
Tellina agilis	A	+	−	−	—	U	A	+	+	−	—	U	A	+	+	+	IS	FRL
Cumingia tellinoides	A	+	−	−	—	U	A	+	+	+	IS	FRL	A	+	+	+	MBTS	FRL
Mytilopsis leucophaeata													C	+	+	+	DIMC (TIES)	FRD
Mercenaria mercenaria	A	+	+	+	ME	FFRL	R	+	+	+	IS	FFRL	C	+	+	+	MBTS	FFRL
Gemma gemma							A	−	−	−	—	STC						
Petricola pholadiformis	A	+	−	−	—	U	C	+	+	+	MF	FFRL	A	+	+	+	ME	FFRD
Mya arenaria	A	+	+	+	IS	FFRL	C	+	+	+	MBTS	FFRD	A	+	+	+	BES	FFRL
Lyonsia hyalina	A	+	+	−	—	U	A	+	+	+	SA	FFRD						
Totals		32	27	22				40	40	37				37	36	36		

* At Woods Hole; association common at Plymouth, MA.
† Juvenile host used.

experiments (Table V) and the experiments on the attractiveness of different hosts, *B. seminuda* clearly feeds primarily on *Crepidula fornicata* and *B. bisuturalis* primarily on *Littorina littorea*. Thus the two *Boonea* niches differ in respect to preferred habitat and predominant host, and competition for space and food is largely avoided.

Assuming that what happens in the laboratory also happens in nature and that preferences for the predominant hosts are not overriding, it seems probable that some of the other mollusk species living in the same habitats serve as natural secondary hosts for *Boonea*. Leaving aside *Crepidula fornicata*, *B. seminuda* occurs with five other common mollusk species; four of these were fed on in the laboratory (Table V). Four rare species co-occur, and three of these were also fed on in the laboratory (Table V). Leaving aside *Littorina littorea*, *B. bisuturalis* occurs with seven other common species; all of these were fed on in the laboratory (Table V). Fifteen rare species co-occur, and all but two of these were also fed on in the laboratory (Table V). Rarely, *B. seminuda* is associated with *Littorina littorea* in the field (Table II) and hence rather definitely feeds on it in nature. The same holds for *B. bisuturalis* with *Crepidula fornicata* (Table II). Predominant hosts are thus reversed occasionally. Leaving aside *Crassostrea virginica*, *B. "impressa"* occurs with six other common species; all of these were fed on in the laboratory (Table V). Of these, *Geukensia demissa* and the *Ischadium* appear in Table II and hence are particularly likely natural secondary hosts.

Molgula and an unidentified polychaete are the only non-molluscan hosts reported to be fed on by a *Boonea* (Allen, 1958; *B. impressa*). *Molgula* was not fed on by *B. bisuturalis* despite intermittent monitoring for three weeks. *B. bisuturalis* sometimes assumed a feeding position on either of the siphons, just as it does on bivalves. Probing into a siphon was seen once. Allen (1958) may also have seen probing. The tunic of *Molgula* is probably too thick and tough for penetration by a *Boonea* proboscis. The cuticle of *Nereis* is probably also impenetrable.

Boonea clearly is a generalist molluscivore, by no means being host-specific. If some selective catastrophe befell any of the predominant hosts, the *Boonea* populations no doubt would be greatly reduced but would survive on other molluscan hosts.

Boonea bisuturalis is an indigenous species at Woods Hole, having been described (as *Turritella bisuturalis*) by Say (1822) with "Boston harbour" as the type-locality. *Littorina littorea*, on the other hand, is introduced and has been at Woods Hole only since 1875 (Wells, 1965). (This in itself indicates that *B. bisuturalis* is not host-specific.) It would be interesting to know the host or hosts of *B. bisuturalis* before this introduction. Assuming that there has been no habitat change, *Mya arenaria* (see Medcof, 1948), *Petricola pholadiformis*, *Urosalpinx cinerea* and *Mercenaria mercenaria* are possibilities. Note also that at Plymouth, *Ilyanassa obsoleta* was a common substrate (Table II) in a mud habitat. *Crassostrea virginica*, the predominant host of *B. impressa* (which hybridizes or intergrades clinally with *B. bisuturalis* in the New York–New Jersey area), was never found to be a living substrate for *B. bisuturalis* at Woods Hole. *Crassostrea* was, however, reported as a natural host elsewhere in New England by Loosanoff (1956) and Boss and Merrill (1965).

In terms of biomass, *Crepidula fornicata* and *Littorina littorea,* the predominant hosts of *Boonea seminuda* and *B. bisuturalis,* are dominant in their respective habitats (Tables III and IV). *L. littorea* is also dominant numerically. The low densities of the two *Boonea* species relative to the biomass and numbers of their hosts (Tables III and IV) make it likely that these odostomioids do little harm to their hosts—injuries such as reported by Cole and Hancock (1955), Loosanoff (1956), and Allen (1958) were not observed. Judging by the four quadrats and the two species, a given biomass of host may support a relatively stable percentage biomass of *Boonea.* Note that the percentages derived from the weights in Tables III and IV are all similar.

The anomalous results of the experiments with *B. bisuturalis* are probably due to the individuals having just been collected. The trials with *B. seminuda* started after these animals had been in the laboratory for two weeks feeding on *Crepidula fornicata* and *Littorina littorea.* Something caused the *B. bisuturalis* to move randomly in their first 48 hr in the laboratory. Possibly they were disoriented or hungry.

In contrast to the pair of *Boonea* species at Woods Hole, the pair of *Fargoa* species is in direct competition with each other for space and food (*Hydroides dianthus*). They occasionally exist even on the same individual worm. *F. dianthophila* is the commoner and smaller of the two species. Sometimes it rides in and out of the worm's tube while sitting on the worm's operculum. At other times it sits on the tube. *F. bartschi* never enters the tube since (at least when adult) it is too long to turn around.

In the Woods Hole area *Hydroides dianthus* is sparsely and patchily dispersed, and spatfalls vary in success at each locality from year to year (personal observations). Not surprisingly, the *Fargoa* populations are commensurately sparse, patchy and sporadic. *Hydroides dianthus* is sympatric with other species of *Hydroides* (*Eupomatus* is a synonym) off North Carolina, northeast and northwest Florida. *Fargoa dianthophila* occurs with three of these other *Hydroides* species (Wells and Wells, 1969). The same is probably true of *F. bartschi,* which ranges from Massachusetts to Texas. Thus the two *Fargoa* species seem both to be specialists host-specific to the genus *Hydroides.* This occurrence of more than one odostomioid on the same serpulid is reminiscent of *Odostomia plicata, O. lukisi, O. unidentata* and "*Chrysallida*" (*Partulida*) *spiralis* of Europe, all of which parasitize *Pomatoceros triqueter* (Ankel, 1959; Fretter and Graham, 1962).

Boonea is a generalist and *Fargoa* a specialist. It seems likely that the former condition is the more primitive, and that pyramidellids were not originally host-specific.

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Summary

1. Three *Boonea* species (occurring in sympatric species pairs) occupy different habitats and have different molluscan host preferences. In the field, *B. seminuda* is preferentially with *Crepidula fornicata* or *Argopecten irradians*, *B. bisuturalis* with *Littorina littorea* (introduced), *Ilyanassa obsoleta* or *Crassostrea virginica*, and *B. impressa* with *C. virginica*. Weights of the first two species are about 0.03 to 0.17% those of their hosts. In the laboratory, *B. seminuda* was attracted much more to *Crepidula fornicata* than to *Littorina littorea*. With *B. bisuturalis* it was vice versa.

2. In the laboratory, *B. seminuda* fed on 22 out of the 36 gastropod and bivalve "hosts" offered; *B. bisuturalis* fed on 37 out of 45, and *B. impressa* fed on 36 out of 37. Some of these mollusks probably serve as secondary hosts in nature. *Boonea* definitely is not host-specific. Polychaetes, *Chaetopleura* and *Molgula* were not fed on.

3. *Fargoa dianthophila* and *F. bartschi*, two much rarer species, compete with each other for space and food by both obligately parasitizing species in the genus *Hydroides*, sometimes co-occurring on the same individual. *Fargoa* species do seem to be host-specific.

LITERATURE CITED

Abbott, R. T., 1972. *Kingdom of the seashell.* Crown Publishers, New York, 256 pp.

Abbott, R. T., 1974. *American seashells; the marine Mollusca of the Atlantic and Pacific coasts of North America.* Ed. 2. Van Nostrand Reinhold Co., New York, 663 pp. 24 pls.

Allen, J. F., 1958. Feeding habits of two species of *Odostomia*. *Nautilus*, **72**: 11–15.

Ankel, F., and A. M. Christensen, 1963. Non-specificity in host selection by *Odostomia scalaris* Macgillivray. *Vidensk. Medd. Dan. Naturhist. Foren.*, **125**: 321–325.

Ankel, W. E., 1938. Beobachtungen an Prosobranchiern der schwedischen Westküste. *Arkiv för Zoologi*, **30A**(9): 1–27, 2 pls.

Ankel, W. E., 1949a. Die Mundbewaffnung der Pyramidelliden. *Archiv für Molluskenkunde*, **77**: 79–82.

Ankel, W. E., 1949b. Die Nahrungsaufnahme der Pyramidelliden. *Verhandlungen der Deutschen Zoologen, Kiel*, **1948**: 478–484.

Ankel, W. E., 1959. Beobachtungen an Pyramidelliden des Gullmar-Fjordes. *Zool. Anz.*, **162**: 1–21.

Berry, S. S., 1955. Importance of the large pyramidellid elements in the West American fauna. *Am. Malacol. Union Annu. Rept.*, **1954**: 25.

Boss, K. J., and A. S. Merrill, 1965. Degree of host specificity in two species of *Odostomia* (Pyramidellidae: Gastropoda). *Proc. Malacol. Soc. Lond.*, **36**: 349–355, pl. 15.

Bullock, R. C., and K. J. Boss, 1971. Non-specificity of host-selection in the ectoparasitic snail *Odostomia* (*Menestho*) *bisuturalis* (Say) (Gastropoda: Pyramidellidae). *Breviora*, **363**: 1–7.

Cole, H. A., and D. A. Hancock, 1955. *Odostomia* as a pest of oysters and mussels. *J. Mar. Biol. Assoc. U.K.*, **34**: 25–31, 2 pls.

Dall, W. H., 1883. On a collection of shells sent from Florida by Mr. Henry Hemphill. *Proc. U. S. Natl. Mus.*, **6**: 318–342, pl. 10.

Fretter, V., 1951. *Turbonilla elegantissima* (Montagu), a parasitic opisthobranch. *J. Mar. Biol. Assoc. U.K.*, **30**: 37–47.

Fretter, V., and A. Graham, 1949. The structure and mode of life of the Pyramidellidae, parasitic opisthobranchs. *J. Mar. Biol. Assoc. U.K.*, **28**: 493–532.

Fretter, V., and A. Graham, 1962. *British prosobranch molluscs; their functional anatomy and ecology.* Ray Society, London, xvi + 755 pp.

HACKNEY, A. G., 1944. List of Mollusca from around Beaufort, N. Carolina, with notes on *Tethys*. *Nautilus*, **58**: 56–64.

HOPKINS, S. H., 1956. *Odostomia impressa* parasitizing southern oysters. *Science*, **124**: 628–629.

HOUBRICK, R. S., 1977. Reevaluation and new description of the genus *Bittium* (Cerithiidae). *Veliger*, **20**: 101–106, 1 pl.

LAFOLLETTE, P. I., 1979. Observations on the larval development and behavior of *Chrysallida cincta* Carpenter, 1864 (Gastropoda: Pyramidellidae). *West. Soc. Malacol. Annu. Rept.*, **11**: 31–34.

LOOSANOFF, V. L., 1956. Two obscure oyster enemies in New England waters. *Science*, **123**: 1119–1120.

MEDCOF, J. C., 1948. A snail commensal with the soft-shell clam. *J. Fish. Res. Board Can.*, **7**: 219–220.

MERRILL, A. S., AND K. J. BOSS, 1964. Reactions of hosts to proboscis penetration by *Odostomia seminuda* (Pyramidellidae). *Nautilus*, **78**: 42–45, pls. 4–5.

MINICHEV, Y. S., 1971. Contribution to the biology of some Pyramidellidae (Gastropoda, Pyramidellidae) of the Pos'yet Bay of the Sea of Japan (in Russian). *Acad. Sci. U.S.S.R., Zool. Inst. Explorations of the fauna of the seas*, **8**(16): 221–229.

MOORE, D. R., 1961. The marine and brackish water Mollusca of the state of Mississippi. *Gulf. Res. Rep.* **1**(1): 1–58.

PELSENEER, P., 1928. Les parasites des mollusques et les mollusques parasites. *Bull. Soc. Zool. Fr.*, **53**: 158–189.

PORTER, H. J., 1974. *The North Carolina marine and estuarine Mollusca—an atlas of occurrence*. University of North Carolina Institute of Marine Sciences, Morehead City, vi + 351 pp.

PORTER, H. J., 1976. Spiral cord variation of *Odostomia impressa* (Say) and *O. seminuda* (C. B. Adams); family Pyramidellidae. *Bull. American Malacol. Union Inc.* **1976**: 38–41.

ROBERGE, A. G., 1968. *Odostomia dianthophila* (Gastropoda, Pyramidellidae) from Buzzard's Bay, Mass., a northern range extension. *Nautilus*, **81**(4): iii.

ROBERTSON, R., 1957. Gastropod host of an *Odostomia*. *Nautilus*, **70**: 96–97.

ROBERTSON, R., 1967. The life history of *Odostomia bisuturalis*, and *Odostomia* spermatophores (Gastropoda: Pyramidellidae). *YearB. Am. Philos. Soc.*, **1966**: 368–370.

ROBERTSON, R., 1978. Spermatophores of six eastern North American pyramidellid gastropods and their systematic significance (with the new genus *Boonea*). *Biol. Bull.*, **155**: 360–382.

ROBERTSON, R., AND V. ORR, 1961. Review of pyramidellid hosts, with notes on an *Odostomia* parasitic on a chiton. *Nautilus*, **74**: 85–91.

SAY, T., 1822. An account of some of the marine shells of the United States. *J. Acad. Nat. Sci. Phila.*, **2**: 221–248, 257–276, 302–325.

SCHELTEMA, A. H., 1965. Two gastropod hosts of the pyramidellid gastropod *Odostomia bisuturalis*. *Nautilus*, **79**: 7–10.

SOKAL, R. R., AND F. J. ROHLF, 1969. *Biometry; the principles and practice of statistics in biological research*. Freeman, San Francisco, xxi + 776 pp.

WELLS, H. W., 1959. Notes on *Odostomia impressa* (Say). *Nautilus*, **72**: 140–144.

WELLS, H. W., 1965. Maryland records of the gastropod, *Littorina littorea*, with a discussion of factors controlling its southern distribution. *Chesapeake Sci.*, **6**: 38–42.

WELLS, H. W., AND M. J. WELLS, 1961. Three species of *Odostomia* from North Carolina, with description of new species. *Nautilus*, **74**: 149–157.

WELLS, H. W., AND M. J. WELLS, 1969. New host and distribution records of *Odostomia dianthophila*. *Nautilus*, **82**: 109–110.

WELLS, H. W., M. J. WELLS AND I. E. GRAY, 1964. The calico scallop community in North Carolina. *Bull. Mar. Sci. Gulf Caribb.*, **14**: 561–593.

Reference: *Biol. Bull.*, 157: 334–343. (October, 1979)

FIELD AND LABORATORY STUDIES OF *GLUGEA HERTWIGI* (MICROSPORIDA) IN THE RAINBOW SMELT *OSMERUS MORDAX* [1]

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Glugea hertwigi-induced microsporidosis is a disease of the smelt, *Osmerus mordax*. *O. mordax* is an anadromous species that has been successfully introduced to temperate freshwater areas. Haley (1957) has provided some evidence which indicates *G. hertwigi* was in part responsible for the decline of the smelt fishery in the Atlantic. The incidence of *Glugea* infection reaches a seasonal peak of nearly 90% in juvenile Lake Erie smelt each summer and fall; Nepszy, Budd, and Dechtiar (1978) estimate vast economic losses in the smelt fishery in the Great Lakes due to mortality of infected juveniles.

G. hertwigi infections typically localize as parasite colonies in the submucosal layer of the intestine. Mortality of the smelt host is believed to occur by starvation; in addition, infected fish have navigation problems, are more susceptible to predation, and less able to recover from environmental stress (Legault and Delisle, 1967; Nepszy and Dechtiar, 1972). Spawning female smelt in Lake Erie characteristically have numerous *G. hertwigi* cysts in the ovaries as well as along the intestine. Chen and Power (1972) reported a 42% decrease in fecundity of *Glugea* infected females. The microsporidan *Plistophora ovariae* infecting the golden shiner *Notemigonens crysoleucas* is an example of transovarian parasite transmission (Summerfelt and Warner, 1970).

G. hertwigi is an obligate intracellular parasite completing its life cycle within a single host cell. After ingestion by a smelt, the infective spore is believed to discharge a polar filament with a velocity suitable for penetrating the intestinal mucosal cell layer (Ishihara, 1968; Weidner, 1972, 1976). The vegetative stages of the parasite do not cause host cell degeneration but stimulate hypertrophy and abnormal development into a "xenoma" (Weissenberg, 1968, 1976; Weidner, 1974). "Xenoma" refers to the unique association between an hypertrophied host cell and developing intracellular parasites (Weissenberg, 1968, 1976). The host-cell component is induced to undergo extensive growth during vegetative development (schizogony) by the parasite. Eventually the parasite differentiates into mature spores (sporogony) which fill the central region of the xenoma. By this stage, a combination of host animal response and parasite growth transform the xenoma into a thick-walled "Glugea-cyst" filled with innumerable spores (Sprague and Vernick, 1968; Weissenberg, 1968, 1976; Weidner, 1973, 1976). *G. hertwigi* cysts range from 0.4 to 5.0 mm in diameter; heavy smelt infections

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number over 200 xenomas per host (Legault and Delisle, 1967; Nepszy and Dechtiar, 1972).

McVicar (1975), Olson (1976) and Weissenberg (1968) transmitted *Glugea* species to fish held in the laboratory. Weidner (1973) and Stunkard and Lux (1965) suggested that invertebrate filter-feeders may serve as natural vectors or transport hosts for fish microsporidans. Olson (1976) determined a low level of *Glugea stephani* infection occurred by ingestion of spores directly. McVicar (1975) transmitted *G. stephani* through spore-carrying vectors and by injection of spores into the peritoneal cavity of adult fish. Weissenberg (1968) did not determine whether *G. anomala* was inititated by a spore-carrying vector or by direct ingestion of the spores.

We thought it would be of interest to examine *G. hertwigi* growth and multiplication in ovaries of spawning female smelt; follow transmission of infections; and examine the microstructure of early xenoma growth.

Materials and Methods

Adult smelt

Spawning female smelt were collected from Wheatley, Ontario, on Lake Erie and from the Jones River, near Plymouth, Cape Cod, Massachusetts. A total of 150 fish from each location were examined internally for the presence of *Glugea hertwigi* cysts. The intestine and ovaries from infected and non-infected fish were excised, cut into small pieces and fixed in pH 7.4 phosphate buffered glutaraldehyde overnight at 4° C. After several buffer rinses, the material was post-fixed in phosphate buffered 2% osmium tetroxide for 2 hr at 4° C, dehydrated in ethanol and embedded in Epon. One micron sections were cut on a Dupont-Sorvall MT-2B microtome and stained with 1% toluidine blue. Parasite cysts were removed from adult smelt, homogenized, pelleted in pH 7.4 phosphate buffer, stored at 4° C and used for the transmission experiments.

Transmission experiments

Eggs and milt were stripped from spawning smelt, mixed 1:5 respectively and kept in a well-aerated nylon mesh cone. Naturally fertilized eggs were also collected from the river-bed. Anticipating large mortalities, a non-infected smelt population was located in Long Pond, Cape Cod, Massachusetts. Several years' examination of smelt from this pond had indicated they were completely free of *G. hertwigi* infection. Young smelt (20–25 mm) in Long Pond were attracted at night to a strong light at the surface and collected by hand-net. All fish were maintained at the National Marine Fisheries Service Aquarium, Woods Hole, in 20° C filtered fresh water taken from the Jones River well above the spawning sites. All fish were maintained on a diet of phyto- and zooplankton seined from Long Pond. The following methods of parasite transmission were attempted.

Experiment 1. Laboratory reared and collected smelt were exposed to a suspension of *G. hertwigi* spores placed in the tanks. The water was well aerated but not filtered for the following 48 hr.

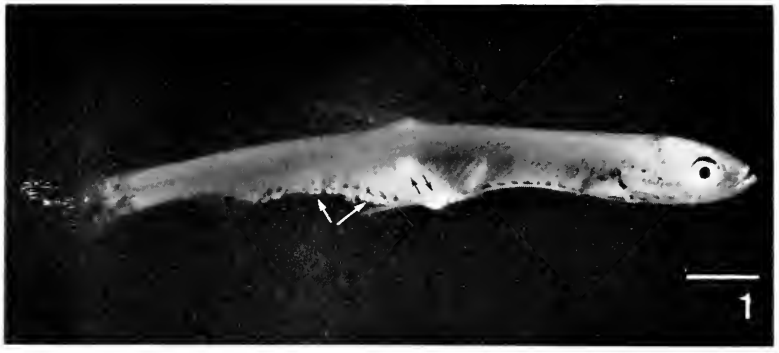

FIGURE 1. Young specimen of the smelt, *Osmerus mordax*, experimentally infected with *Glugea hertwigi*. The yearly incidence of natural infections nears 90% in juveniles with consequent vast mortalities. Seven days post infection, xenomas (white arrows) develop proximate to larger 4-week-old "Glugea cysts" (black arrows). Bar represents 2.5 mm.

Experiment 2. Laboratory reared smelt (10 mm, 6 weeks post spawning) were fed spore-carrying zooplankton (cladocerans and copepods) on 2 consecutive days. The plankton was first exposed to a spore suspension for 30 min, washed once with water, examined to ensure the presence of spores in their digestive tracts and then fed to the smelt.

Experiment 3. Collected smelt (15 mm, approximately 8 weeks post spawning) were fed spore-carrying plankton as above.

Experiment 4. Smelt from Experiment 1, 4 weeks after exposure to a spore suspension, were fed spore-carrying plankton as above. As a control, a number of laboratory reared and collected smelt were maintained unexposed to spores.

Development of xenomas

Young smelt from all tanks were observed and photographed with a Wild M–4 Makroskope 5 days, 1, 2 and 3 weeks after spore feeding. Intestinal tissue from experimental and control smelt were prepared for microscopy as outlined above.

TABLE I

Experiment number	Source of smelt	Sample size	Method of exposure	Period of incubation	Number of smelt infected	
					Light < 5 (per host)	Heavy > 50 (per host)
1	mixture of lab reared and collected	20	spore suspension	28 days	8	0
2	lab reared	5	spore-carrying plankton	7 days	0	5
3	collected	14	spore-carrying plankton	7 days	0	14
4*	mixture	8	spore-carrying plankton	7 days	0	8

* Smelt from Experiment 1, carrying light infections 28 days after exposure to the spore suspension.

Results

Adult smelt

In spawning females *Gluga hertwigi* cysts were found in the ovaries of more than 50% of the infected fish in Lake Erie and in 25% of infected fish in the Jones River. The ovaries of Lake Erie fish were heavily infected whereas those of the Jones River were lightly infected. Sections of infected ovaries showed the parasite was isolated from the scattered ova by the cyst wall. No free spores or developing stages of *G. hertwigi* were observed outside the cysts, in ova or in ovarian tissue.

Parasite transmission

The results of the experiments are summarized in Table I. Positive results were obtained from both methods of spore transmission; however, the intensity of infection and its effect differed.

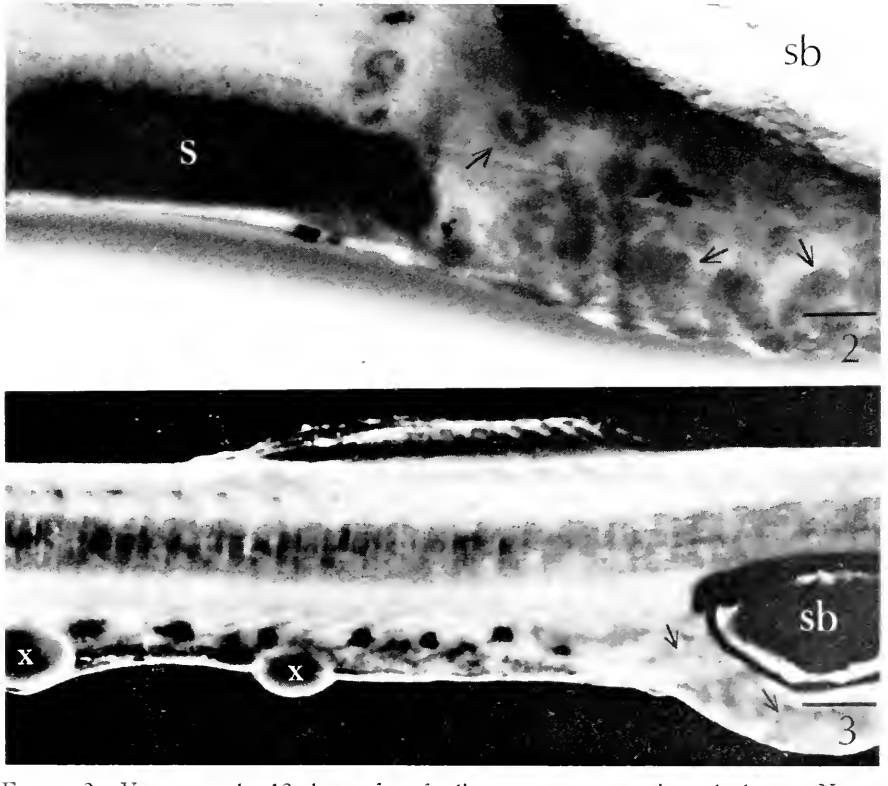

FIGURE 2. Young smelt, 12 days after feeding on spore-carrying plankton. Numerous xenomas (arrows) begin just behind the stomach and continue along the gut to the vent. S, stomach; sb, swimbladder. Bar represents 180 μm.

FIGURE 3. Young smelt previously infected after exposure to a spore suspension developed numerous new xenomas (arrows) along the intestine when fed spore-carrying plankton. X, xenomas from exposure to spores directly. Bar represents 750 μm.

Experiment 1. Laboratory-reared and collected fish were exposed to a suspension of *G. hertwigi* spores. Four weeks after exposure, 40% of the smelt exhibited one or two *Glugea* cysts in the posterior region of the intestine. These cysts were uniformly dense and protruded from the submucosa distending the peritoneal cavity. The fish fed continually throughout the experimental period. Several fish died during the period, were examined and one was found infected with a single *Glugea* cyst.

Experiment 2. Laboratory-reared fish were exposed to spore-carrying plankton. One week after exposure all fish exhibited numerous small xenomas beginning just behind the stomach and continuing along the entire length of the intestine to the vent (Fig. 2). Gradually the fish stopped feeding, had difficulty swimming and all died by 16 days after exposure to the spores.

Experiment 3. Collected smelt were exposed to spore-carrying plankton. The results were similar to those in Experiment 2; however, these fish stopped feeding and died at about 25 days after exposure to the parasite.

Experiment 4. Smelt from Experiment 1, 4 weeks after being exposed to a spore suspension, were fed spore-carrying plankton. One week after feeding, all fish exhibited numerous small xenomas along the intestine as well as the large posterior xenomas (Fig. 3). All fish died within 2 weeks after exposure to the spore-carrying vectors. Controls were examined periodically and found free of infection.

Parasite development

Parasite growth was rapid at 20° C. Examination of the intestine from a heavy infection (Experiments 2, 3, and 4) showed the extensive tissue involvement. Infections protruded to the serosa and were easily dissected away intact (Fig. 4). Extensive host cell hypertrophy was the obvious feature of sectioned material (Fig. 5). Xenomas ranged from 20 to 50 μm in diameter. Smaller xenomas contained one or two greatly enlarged host cell nuclei; whereas the larger xenomas

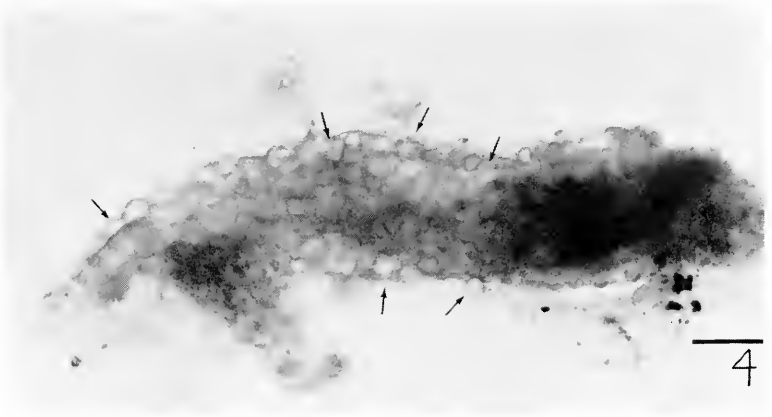

FIGURE 4. Whole intestine 1 week after exposure to spore-carrying plankton. Xenomas (arrows) protrude to the serosa and are easily dissected away intact. Bar represents 80 μm.

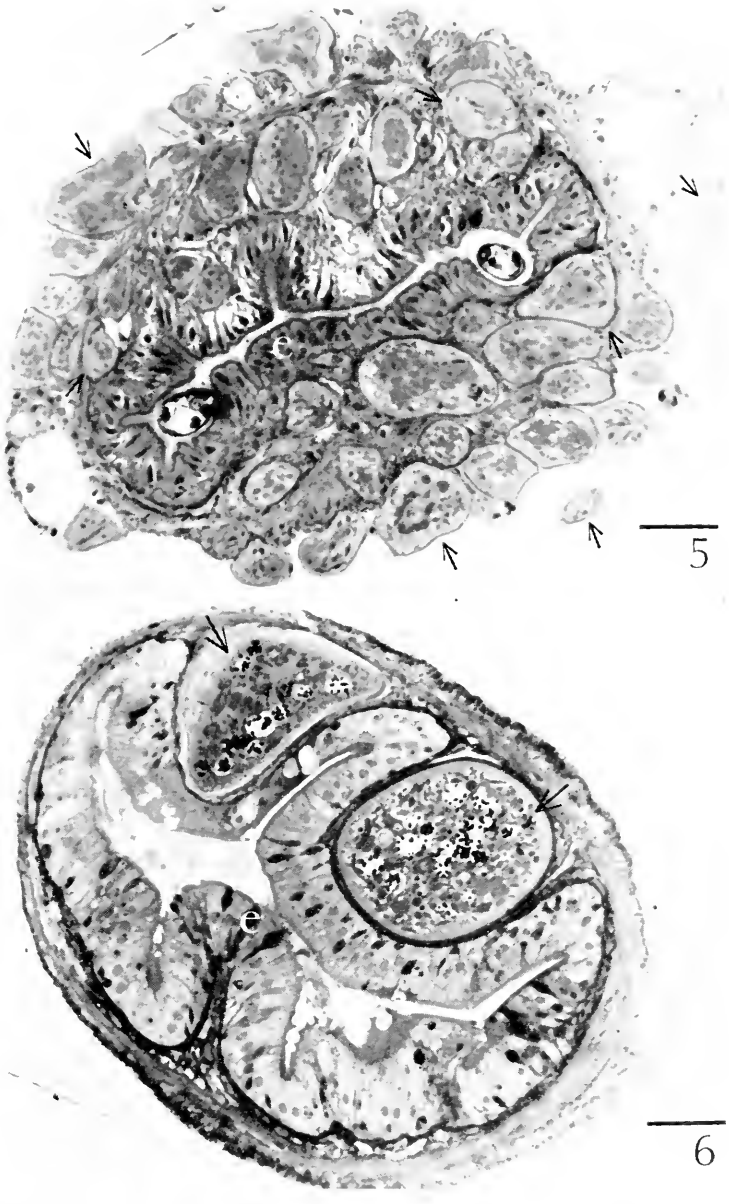

FIGURE 5. Cross section of intestine similar to that shown in Figure 4. At least 40 xenomas (arrows) 20 to 50 μm in diameter develop within the submucosa and protrude from the mucosa. Infected host cells hypertrophy and contain early schizont stages of the parasite. e, epithelium. One micron Epon section; 1% toluidine blue stain; bar represents 50 μm.

FIGURE 6. Cross section of intestine from lightly infected smelt 2 weeks after feeding on a spore suspension. Progressive hypertrophy increased the xenomas to 100 to 125 μm in diameter. Xenomas develop in the submucosal layer causing mechanical distension of the epithelium (e). Sporogony stages and free dense spores fill the central region of the xenoma. One micron Epon section; 1% toluidine blue stain; bar represents 50 μm.

were multinucleated with many nuclei lobed or branched, indicating nuclear division. At 1 week the parasite formed schizont colonies peripheral to the host cell nuclei. After 2 weeks of growth there was considerable increase in host cell hypertrophy and *Glugea* maturation to spores. Xenomas ranged from 100 to 125 μm in diameter with host cell nuclei, cytoplasmic components and *G. hertwigi* schizonts particularly obvious in the peripheral region of the xenomas; sporoblastic stages of the parasite were common in the central region (Fig. 5).

Discussion

Transovarian parasite transmission is known from a number of microsporidan species (Kudo, 1966). Recently, Summerfelt and Warner (1970) demonstrated a *Plistophora ovariae* infection in viable eggs of the golden shiner, *Notemigonus crysoleucas*. Although the ovaries of spawning female smelt were often loaded with *G. hertwigi* parasites, our thorough examinations indicate *G. hertwigi* are not present in germinative or egg cells. Previously, there has been limited success in experimental peroral transmission of fish microsporidans (Delisle, 1969; McVicar, 1975; Summerfelt and Warner, 1970; Stunkard and Lux, 1965). However, Olson (1976) successfully transmitted *G. stephani* to the English sole, *Parophyrys vetulus* in water temperatures above 15° C. Several authors are convinced that transport vectors are necessary to concentrate *Glugea* spores for natural transmission of certain fish microsporidosis (Haley, 1957; Putz and McLaughlin, 1970; Stunkard and Lux, 1965). Small filter-feeding animals may serve as transport hosts and in addition, may stimulate the spores to hatch and infect the fish. Weidner (unpublished observations) has observed such a phenomenon with *G. stephani* in the winter flounder, *Pseudopleuronectes americanus*. In this study, transmission of *G. hertwigi* to both laboratory-reared and collected smelt was successful at 20° C, either by direct spore consumption or ingestion of spore-carrying vectors; however, a major magnitude of difference exists in the intensity between direct spore and vector transmitted infections. Vector transmission produced massive infections along the entire intestine in all test subjects.

Smelt are selective predators, taking cyclopoid and calanoid copepods and several species of cladocerans as their first food (Reif and Tappa, 1966; Siefert, 1972). Presumably, the natural *G. hertwigi* infection occurs through the ingestion of spore-carrying filter feeders by very young smelt. Release of spores from infected adult smelt occurs via two routes. Scarborough (unpublished observations) has observed the expulsion of parasite xenomas from ovaries during spawning. In this manner, female adult smelt may concentrate *G. hertwigi* spores in the immediate vicinity of developing young. Further, infected smelt carrion were seen being preyed upon by small crustaceans in the nursery areas after spawning. Nepszy and Dechtiar (1972) found that heavily infected adult smelt were unable to recover from spawning stress; mass mortalities in the spawning grounds consisted of infected adults.

Massive infections of *G. hertwigi* consequent to ingestion of spore vectors are fatal to both collected and laboratory reared smelt. Mechanical distention of the intestinal tissue and starvation are thought to be the cause of death. *Osmerus mordax* is a difficult species to raise in the laboratory and the minimal condition

of the reared fish probably precluded a greater susceptibility to the effects of multiple infections. Young smelt tolerate light infections for at least several weeks and likely carry them into adulthood. Nepszy and Dechtiar (1972) found that *G. hertwigi* colonies can remain in smelt for much of the host's life, and stress will significantly increase the mortality rate in these fish over uninfected smelt.

It is well documented that the life cycle of microsporidans begins with injection of the sporoplasm through a spore tube into the host (Vavra, 1976; Weidner, 1972). It is assumed that the discharging tube of *G. hertwigi* spores penetrates through the gut basal lamina delivering the parasite into submucosa cells.

Whether transmitted directly by spores or via spore-carrying vectors, development of *G. hertwigi* was identical, and paralleled that described for other *Glugea* species (Sprague and Vernick, 1968; Weissenberg, 1968, 1976). Changes in macroscopic appearance of xenomas correlated with microscopic examinations of the sectioned material. While the host cell component of the xenoma remains viable, the parasite develops numerous schizonts within the peripheral cytoplasm. The host cytoplasm and nucleus hypertrophy in apparent response to the parasite's presence; subsequently, the nucleus undergoes an amitotic budding. As the xenoma size increases, a cellular capsule delimits the xenoma from the surrounding tissue; this capsule becomes enveloped by host connective tissue layers. While maturation of the parasite progresses through sporogony, host cell components begin to degenerate and spores fill the interior. The cyst stage consists of spores and scattered vestiges of host cell components surrounded by a wall.

Although Weissenberg (1976) believed the host cell to be a presumptive macrophage, the cell type which can support *G. hertwigi* remains undetermined. Our observations indicate massive infections will produce xenomas easily separated from the intestine. Xenomas were observed associated with various visceral organs, including cells below the peritoneal lining. It is not known how *G. hertwigi* enters ovarian tissue; presumably, initially infected host cells enter the blood stream and are delivered to favorable environs for growth, such as the highly vascularized ovaries. *Nosema michaelis,* a microsporidan infecting the blue crab, *Callenectes sapidus,* undergoes vegetative growth in the gut wall and these cells subsequently circulate to muscle tissue for continued development (Weidner, 1972).

This particular host-parasite association likely will be of some use in the study of xenomas. The transparency of young smelt lends itself to *in vivo* study of drug effects on parasite infection and xenoma development. Tissue culture techniques may now be carried out on isolated xenomas since these larger tumor cells are readily detectable in young smelt and easily removed aseptically from the gut serosa.

Thanks are recorded to Mr. Charlie Wheeler of the National Marine Fisheries Service Aquarium, Woods Hole, Massachusetts; to Mr. Stephen Nepszy of the Canadian Ministry of Natural Resources, Lake Erie Fisheries Research Station, Wheatley, Ontario, and special appreciation to Mr. James Kennedy of the Massachusetts Fish and Wildlife Service, Buzzards Bay, Massachusetts.

Summary

Glugea hertwigi-induced microsporidosis is a disease of the smelt *Osmerus mordax*. The yearly incidence of infection reaches over 50% in adult smelt and as

high as 90% in juveniles. Primary infections localize as large intracellular colonies in submucosal cells of the digestive tract. Field observations indicate the ovaries of spawning females are the secondary site of infection. *G. hertwigi* was successfully transmitted to both laboratory-reared and collected young smelt at 20° C by small filter-feeding vectors and by direct ingestion of spores. Infections transmitted by spore-carrying vectors numbered hundreds per animal, and were visible along the intestine one week after feeding. Large parasitized host-cells (xenomas) extended from the intestinal serosa and were easily recovered. *G. hertwigi* infections acquired by direct spore feeding numbered one or two per animal; these fish have the capacity to develop many new infections by feeding on spore-carrying vectors. Microscopic study revealed that *G. hertwigi* development was indeed within a single greatly hypertrophied host cell. After 1 week of growth, 20 to 50-μm xenomas contained a few enlarged host nuclei and vegetative *G. hertwigi;* after 2 weeks, the xenomas measured 100 to 125 μm, exhibited multiple host nuclei and numerous *G. hertwigi* sporoblasts and spores.

LITERATURE CITED

Chen, M., and G. Power, 1972. Infection of American Smelt in Lake Ontario and Lake Erie with the microsporidian parasite *Glugea hertwigi* (Weissenberg). *Can. J. Zool.,* **50**: 1183–1188.

Delisle, C., 1969. Bimonthly progress of a non-lethal infection of *Glugea hertwigi* in young-of-the-year smelt, *Osmerus eperlanus mordax*. *Can. J. Zool.,* **47**: 871–876.

Haley, A. J., 1957. Microsporidian parasite, *Glugea hertwigi* in American Smelt from Great Bay region, New Hampshire. *Trans. Am. Fish. Soc.,* **83**: 84–90.

Ishihara, R., 1968. Some observations on the fine structure of sporoplasm discharged from spores of a microsporidian, *Nosema bombycis*. *J. Invertebr. Pathol.,* **12**: 245–257.

Kudo, R. R., 1966. *Protozoology*. 5th ed. Charles C Thomas, Publ., Springfield. 1135 pp.

Legault, R. O., and C. Delisle, 1967. Acute infection by *Glugea hertwigi* (Weissenberg) in young-of-the-year rainbow smelt, *Osmerus eperlanus mordax* (Mitchill). *Can. J. Zool.,* **45**: 1291–2.

McVicar, A. H., 1975. Infection of plaice *Pleuronectes platessa* L. with *Glugea* (*Nosema*) *stephani* (Hagenmuller, 1899) (Protozoa: Microsporidia) in a fish farm and under experimental conditions. *J. Fish. Biol.,* **7**: 611–619.

Nepszy, S. J., J. Budd, and A. O. Dechtiar, 1978. Mortality of young-of-the-year rainbow smelt (*Osmerus mordax*) in Lake Erie associated with the occurrence of *Glugea hertwigi*. *J. Wildl. Dis.,* **14**(2): 233–239.

Nepszy, S. J., and A. O. Dechtiar, 1972. Occurrence of *Glugea hertwigi* in Lake Erie rainbow smelt (*Osmerus mordax*) and associated mortality of adult smelt. *J. Fish. Res. Bd. Can.,* **29**: 1639–1641.

Olson, R., 1976. Laboratory and Field Studies on *Glugea stephani* (Hagenmuller), a microsporidian parasite of pleuronectid flat-fishes. *J. Protozool.,* **23**(1): 158–164.

Putz, R. E., and J. A. McLaughlin, 1970. Biology of Nosematidae (Microsporidia) from freshwater and euryhaline fishes. Pages 124–132 in S. F. Snieszko, Ed., *A Symposium on Diseases of Fishes and Shellfishes.*. Special Publication No. 5, Am. Fish. Soc., Washington, D. C.

Rief, C. B., and D. W. Tappa, 1966. Selective predation; smelts and cladocerans in Harveys Lake. *Limnol. Oceanog.,* **11**: 437.

Siefert, R. E., 1972. First food of Larval Yellow Perch, White Sucker, Bluegill, Emerald Shiner, and Rainbow Smelt. *Trans. Am. Fish. Soc.,* **101**(2): 219–225.

Sprague, B., and S. H. Vernick, 1968. Light and electron microscope study of a new species of *Glugea* (Microsporidia, Nosematidae) in the 4-spined stickleback *Apeltes quadranus*. *J. Protozool.,* **15**(3): 547–571.

Stunkard, H., and F. E. Lux, 1965. A microsporidian infection of the digestive tract of the winter flounder, *Pseudopleuronectes americanus*. *Biol. Bull.,* **129**: 371–385.

SUMMERFELT, R. C., AND M. C. WARNER, 1970. Incidence and intensity of *Plistophora ovariae*, a microsporidian parasite of the Golden Shiner, *Notemigonus crysoleucas*. Pages 142–160 in S. F. Snieszko, Ed., *A Symopsium on Diseases of Fishes and Shellfishes*. Special Publication No. 5, Am. Fish. Soc., Washington, D. C.

VAVRA, J., 1976. Development of Microsporidia. Pages 87–110 in T. C. Cheng and L. A. Bula, Jr., Eds., *Comparative Pathology Vol. 1. Biology of the Microsporidia*. Plenum Publ., New York.

WEIDNER, E., 1972. Ultrastructural study of microsporidian invasion into cells. *Z. Parasitenk.*, **40**: 227–242.

WEIDNER, E., 1973. Studies of microsporidian disease transmission in winter flounder and smelt. *Biol. Bull.*, **145**(2): 459.

WEIDNER, E., 1974. Microsporidiosis in aquatic animals. Pages 77–82 in R. L. Amborski, Ed., *Proceedings of Gulf Coast Regional Symopsium on Diseases of Aquatic Animals*. Center Wetland Resources, LSU Press, Baton Rouge.

WEIDNER, E., 1976. Some aspects of microsporidian physiology. Pages 110–126 in T. C. Cheng and L. A. Bulla, Jr. Eds., *Comparative Pathobiology Vol. 1, Biology of the Microsporidia*. Plenum Publ., New York.

WEISSENBERG, R., 1968. Intracellular development of the microsporidia *Glugea anomala* (Moniez) in hypertrophying migratory cells of the fish *Gasterosteus aculeatus* L., an example of the formation of "Xenoma" tumors. *J. Protozool.*, **14**: 44–56.

WEISSENBERG, R., 1976. Microsporidian interactions with host cells. Pages 203–207 in T. C. Cheng and L. A. Bulla, Jr. Eds., *Comparative Pathobiology, Vol. 1, Biology of the Microsporidia*. Plenum Publ., New York.

Reference: *Biol. Bull.*, **157**: 344–355. (October, 1979)

DEVELOPMENT OF TAIL MUSCLE ACETYLCHOLINESTERASE IN ASCIDIAN EMBRYOS LACKING MITOCHONDRIAL LOCALIZATION AND SEGREGATION

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Ascidians (subphylum Urochordata; class Ascidiacea) develop a large number of mitochondria during oogenesis. At fertilization many of these become localized into a myoplasmic crescent and are subsequently segregated by the determinate cleavage mechanism into the muscle lineage cells of the developing larva. In some species colored yolk granules are associated with the mitochondria; they serve as visible markers of the mitochondrial localization and its segregation (Conklin, 1905; Berrill, 1929; Berg and Humphreys, 1960). Many different staining techniques have been used to establish that oviparous species have mitochondrial segregation even in the absence of a visible crescent (*e.g.*, Meves, 1913; Duesberg, 1915; Mancuso, 1952; Reverberi, 1956).

This obvious association of large numbers of mitochondria with the ascidian larval muscle cell lineage has raised questions about whether the crescent mitochondria are (a) permissive, (b) selective, or even (c) instructive of muscle differentiation. An equally important question is whether segregation of the cytoplasmic determinants responsible for muscle differentiation (Whittaker, 1973) is linked directly to the mechanisms that localize and segregate mitochondria into the muscle cells. The results of various observations and experiments that have attempted to answer these questions prove to be contradictory. The suggestion has therefore persisted in some of the review literature that mitochondrial localizations are causally related to muscle cell determination and development (Brachet, 1960, 1974; Minganti, 1961; Reverberi, 1961, 1971).

Conklin (1931) displaced mitochondria by centrifugation of unfertilized ascidian eggs and concluded from his results that localizations of mitochondria were not the cause of muscle determination. Mitochondria could be driven out of the finely granular plasm in which they were found without preventing the formation of muscle during subsequent development. When the plasm itself was displaced, the larval muscles were also displaced. Tung, Ku, and Tung (1941) noted that mitochondrial masses moved by centrifugation to neural and ectodermal regions did not cause these cells to develop myofibrillae.

The first contradictory evidence came from centrifugation experiments by Ries (1939) who found that displacement of the indophenol oxidase-containing plasm of the ascidian egg resulted in a change in muscle development; he did not know at the time that this enzyme was a mitochondrial oxidase. La Spina (1958) also showed that mitochondrial displacement resulted in some abnormalities of muscle development. Direct interference with mitochondrial function, using inhibitors of the mitochondrial enzymes, resulted in the development of embryos markedly

deficient in muscle structures (de Vincentiis, 1956; Reverberi, 1957). Recently, Bell and Holland (1974) have found by microsurgically dividing centrifugally stratified eggs in various orientations and fertilizing the fragments that a certain limited number of mitochondria appear to be necessary for muscle differentiation. Their data also confirm the possibility that mitochondrial localizations might be causally related to muscle differentiation.

One of the difficulties with these various traumatic and disruptive experimental interventions is that they cause severe abnormalities in the embryos. Results are subject to considerable selection and interpretation by the investigator. In nature, however, a situation occurs in which the question can be clearly resolved in a qualitative way. Certain ascidian species have secondarily evolved anural larvae which no longer develop the larval tail (Berrill, 1931). One of these species, *Molgula arenata,* differentiates larval tail muscle up to the point of producing histospecific muscle acetylcholinesterase (Whittaker, 1979). This species does not localize mitochondria or segregate them into the muscle lineage cells.

Materials and Methods

Materials

The specimens of *Molgula arenata* Stimpson used in this study were dredged from sand flats near Senator Shoal in northern Nantucket Sound at Cape Cod, June through November. Control observations were made on embryos of two urodele species: *Ciona intestinalis* (L.), obtained in the vicinity of Woods Hole, Massachusetts, and *Molgula occidentalis* Traustedt of the Florida Gulf, purchased from the Gulf Specimen Company, Panacea, Florida. These three species do not have a visibly colored mitochondrial crescent.

Gametes were obtained by techniques described previously (Whittaker, 1979). Embryos were cultured in filtered sea water at $18 \pm 0.1°$ C using a refrigerated constant temperature water bath. Since the time of first division is variable in *M. arenata* (Whittaker, 1979), development times are expressed as time after the 2-cell stage.

Histochemistry

Acetylcholinesterase was localized in embryos by the Karnovsky and Roots (1964) procedure after 2 to 3 min fixation in cold (5° C) 80% ethanol (Durante, 1956). Incubation was for 12 hr at 18° C. Various substrate and inhibitor controls for the identity of the enzyme are presented elsewhere (Whittaker, 1979).

Succinic dehydrogenase activity was detected by the standard technique for bound mitochondrial enzymes described by Pearse (1972) using sodium succinate as substrate and nitro blue tetrazolium as the electron acceptor. Cytochrome oxidase was localized by the diaminobenzidine reaction of Seligman, Karnovsky, Wasserkrug, and Hanker (1968) including 20 μg/ml Sigma C-40 catalase in the reaction medium to prevent peroxidase activity. The fixative used for both enzymes was Karnovsky's (1965) fixative, but with formaldehyde and glutaraldehyde each reduced to 1.5%, half of the originally recommended strength. Fixation was for 5 to 15 min at 5° C. Incubation time for each of the mitochondrial enzymes

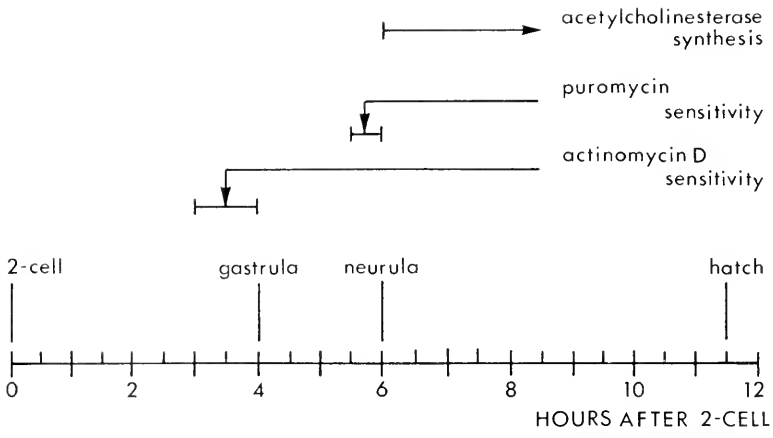


FIGURE 1. Time of various developmental stages and other events in specimens of *Molgula arenata* at 18° C.

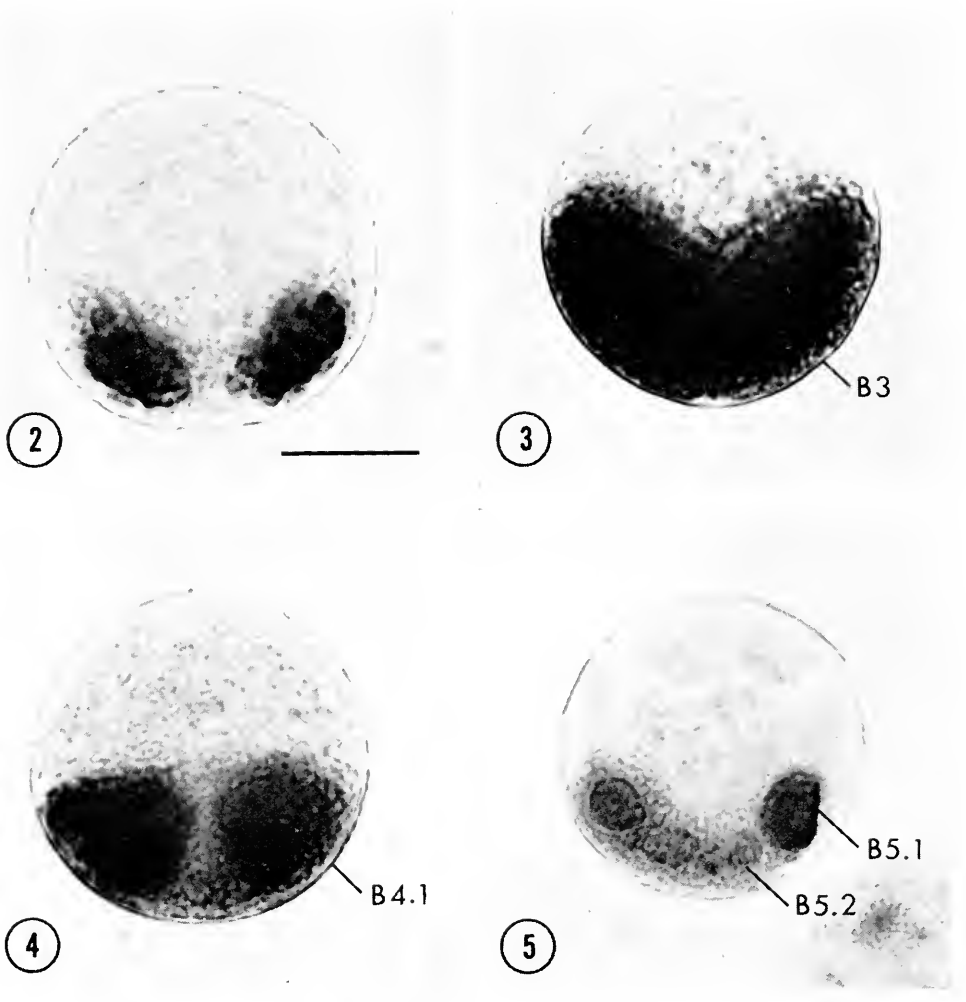
was 90 to 120 min at 22° C. Succinic dehydrogenase staining did not occur when sodium succinate was omitted from the incubation medium; the enzyme activity was not affected significantly by 10 mM sodium azide. Cytochrome oxidase activity did not occur in the presence of either 10 mM sodium azide or 10 mM potassium cyanide. Cytochrome c greatly enhances the sensitivity of the reaction but cytochrome oxidase gives a modest reaction with 3,3'-diaminobenzidine in the absence of substrate. Concentrations of the reaction products in both of these histochemical reactions appear to follow the Beer-Lambert law; color density is proportional to enzyme activity and therefore proportional to the numbers of mitochondria (Cabrini, Vinuales, and Itoiz, 1969; Marinos, 1978).

After the respective histochemical reactions the embryos were dehydrated in ethanol, cleared in xylene, and mounted in damar resin. The three enzyme procedures produced essentially permanent color reactions.

RESULTS

Development of acetylcholinesterase

Development of larval tail muscle acetylcholinesterase in *Molgula arenata* initially followed the same pattern found in urodele ascidian species: the enzyme became histochemically detectable at neurulation. Color first appeared at 6 hr after the 2-cell stage (Fig. 1) and enzyme activity accumulated gradually over the next few hours until a modest level of activity was attained (Fig. 2). In some larvae this activity reached a level as high as 20% of that found in the larvae of comparable urodele species of *Molgula*, but the mean activity found was 5 to 6% of the urodele level (Whittaker, 1979). Tail development was completely suppressed in *M. arenata* and except for acetylcholinesterase development presumptive muscle tissues did not otherwise develop beyond the early neurula stage; there was no obvious myofibrillar synthesis.



FIGURES 2–5. Embryos of *Molgula arenata* stained histochemically for acetylcholinesterase.

FIGURE 2. Hatched larva.

FIGURE 3. Embryo cleavage-arrested in cytochalasin B at the 4-cell stage and reacted for enzyme at 8 hr after the time of the 2-cell stage.

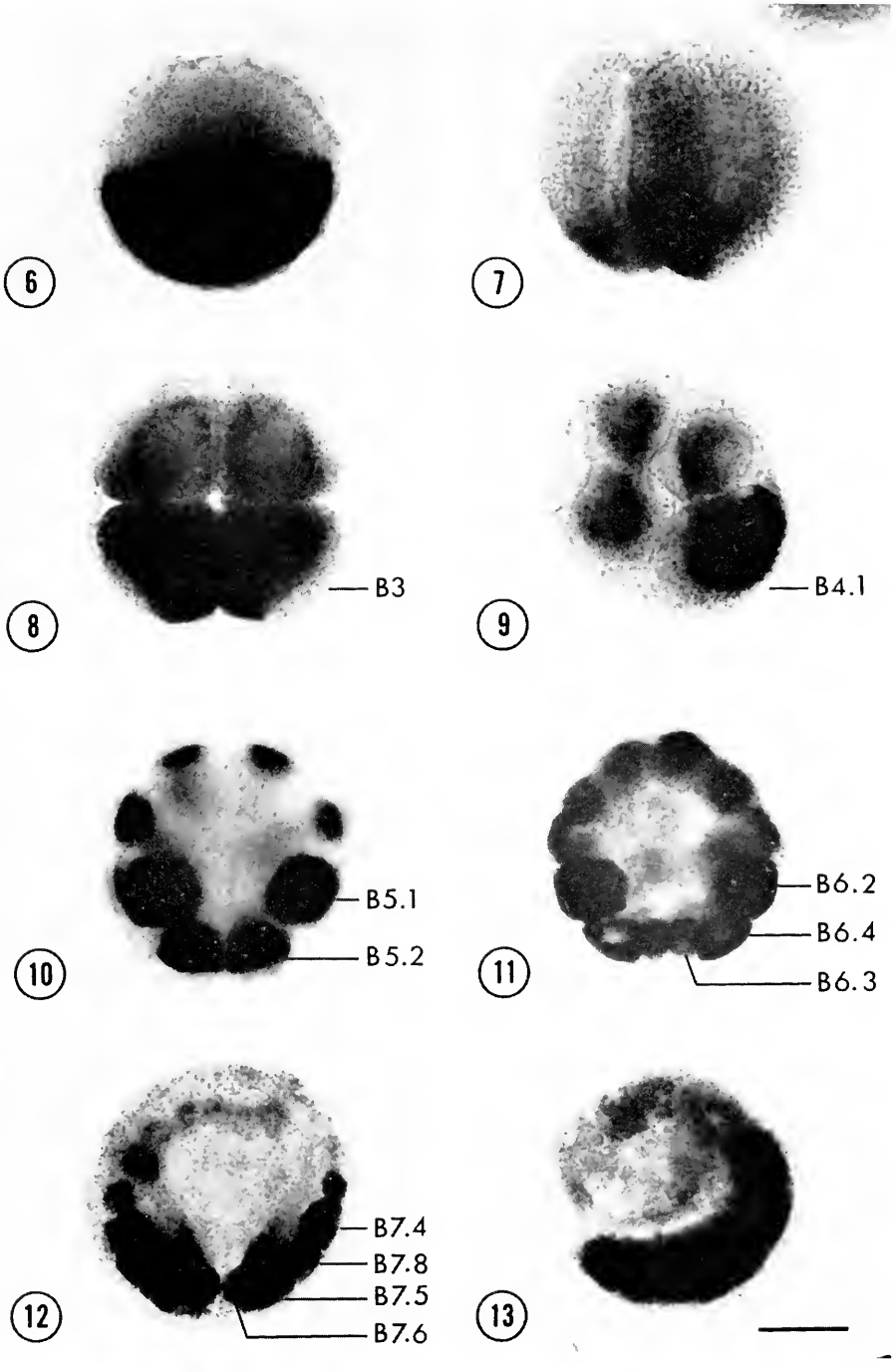
FIGURE 4. Embryo cleavage-arrested at the 8-cell stage and reacted for enzyme at 8 hr after the 2-cell stage.

FIGURE 5. Embryo cleavage-arrested at the 16-cell stage and reacted for enzyme at 8 hr after the 2-cell stage.

The muscle cell lineage designations are according to Ortolani (1955). All figures are the same magnification; the bar in Figure 2 is 40 μm long.

Segregation of a muscle determinant

Previous studies with cleavage-arrested embryos of *C. intestinalis* have shown that blastomeres of the muscle lineage in arrested embryos will eventually develop acetylcholinesterase (Whittaker, 1973). Similar experiments with *M. arenata*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13

produced the same results, at least in the earlier stages. Such observations support the theory that an autonomously acting cytoplasmic determinant of acetylcholinestrase development is being segregated in the muscle lineage cells.

Embryos of *M. arenata* were placed in 2 μg/ml cytochalasin B (Sigma) at various cleavage stages after fertilization. These were then reacted for acetylcholinesterase 8 to 9 hr after the 2-cell stage. The maximum cell numbers producing acetylcholinesterase and the relative positions of these cells in the embryo matched those of the known ascidian muscle cell lineage: one blastomere at 1-cell, two at 2-cell, two at 4-cell, two at 8-cell, and four at 16-cell. Figures 3–5 depict the last three of these stages.

A majority of the embryos at all of the various cleavage-arrested stages developed acetylcholinesterase in one or more of the muscle lineage blastomeres and a modest number of embryos at 2-, 4- and 8-cell stages produced the maximum lineage numbers of reacting cells (two). Interestingly, few embryos at the cleavage-arrested 16-cell stage produced enzyme in more than two cells, one on each bilateral side. Figure 5 illustrates one of the embryos in which all four of the muscle lineage cells produced acetylcholinesterase. Usually only the B5.1 cells synthesize acetylcholinesterase. This restriction to two rather than four cells is conceivably related to the lesion that results in a limited expression of acetylcholinesterase in *M. arenata*.

A limitation of expression seemed also to occur in later stages as well, but there the results were less certain. The chorion of *M. arenata* adheres closely to the embryo and appears to exert tension during development. Consequently, the acetylcholinesterase-producing muscle lineage cells in cleavage-arrested 32-cell and 64-cell stages tend to remain aggregated together in two bilateral groups. Aggregation combined with high yolk content of the cells and the large number of nuclei which accumulate make it essentially impossible to discern cell boundaries within groups of older myoblasts.

Acetylcholinesterase dependence on protein and RNA synthesis

The time at which enzyme was first detected histochemically (Fig. 1) is apparently the time of first acetylcholinesterase synthesis. When 200 μg/ml puromycin di-HCl (Sigma) was added to embryos of *M. arenata* 30 min before the time (at 6 hr) of first acetylcholinesterase staining no enzyme was detected histochemically at 8 to 9 hr time. Similarly, embryos placed in puromycin at

FIGURES 6–13. Embryos of *Ciona intestinalis* stained histochemically for succinic dehydrogenase.

FIGURE 6. Unfertilized egg.

FIGURE 7. 2-cell stage.

FIGURE 8. 4-cell stage.

FIGURE 9. 8-cell stage. Side view of the bilaterally symmetrical embryo.

FIGURE 10. 16-cell stage.

FIGURE 11. 32-cell stage.

FIGURE 12. 64-cell stage.

FIGURE 13. Middle tailbud stage at about the time of first melanocyte differentiation (12 hr development at 18° C).

The muscle lineage desginations are according to Ortolani (1955). All figures are the same magnification; the bar in Figure 13 is 40 μm long.

6 hr produced, even after many hours, only the slight amount of enzyme activity that would ordinarily be detected at 6 hr time. Puromycin at 200 μg/ml causes 95 to 99% inhibition of protein synthesis in ascidian embryos (Whittaker, 1973, 1977). Presumably the cytoplasmic factor being segregated is not a preformed inactive acetylcholinesterase since the occurrence of activity seems to depend directly on protein synthesis.

There was also an actinomycin D sensitivity period for acetylcholinesterase synthesis in this species. This occurred between 2 and 3 hr before the time of enzyme synthesis (Fig. 1). When embryos were treated continuously with 20 μg/ml actinomycin D (Sigma, Grade III) beginning at 3 hr after the 2-cell stage, no acetylcholinesterase developed subsequently. If treatment was started at 4 hr some slight amount of enzyme activity was found at hatching time. Progressively more enzyme activity was found the later after 4 hr that actinomycin D treatment was started. Since actinomycin D at this concentration produces a maximal inhibition of RNA synthesis in ascidian embryos (Smith, 1967; Mansueto-Bonaccorso, 1971), occurrence of acetylcholinesterase probably requires a specific embryonic period of RNA synthesis. Enzyme messenger RNA (mRNA) synthesis most likely occurs during this time. On the basis of other studies (Whittaker, 1977), one can assume that enzyme synthesis would be resistant to actinomycin D if a performed mRNA for the enzyme were present.

Mitochondrial segregation in urodele embryos

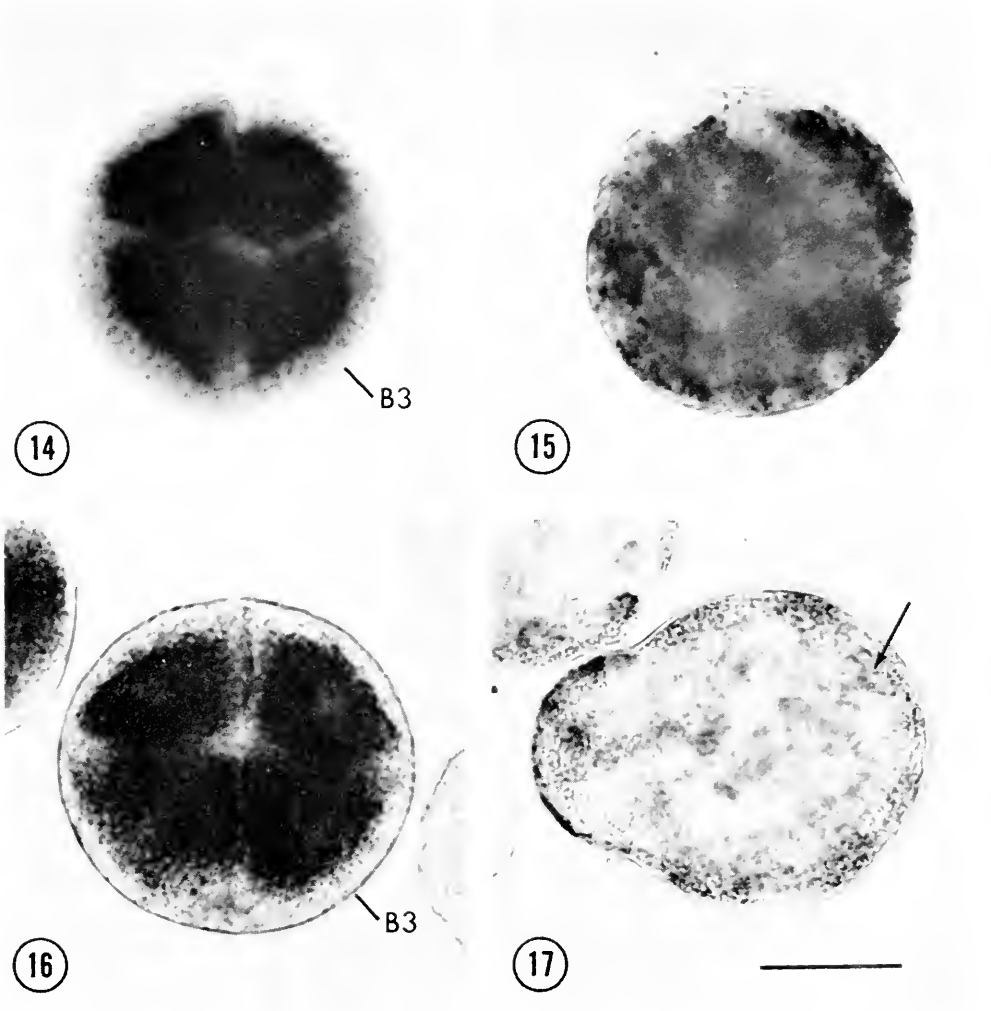
Succinic dehydrogenase reactions in early embryonic stages of *C. intestinalis* showed clearly the pattern of mitochondrial distribution in the embryos (Figs. 6–13). Before germinal vesicle breakdown, which occurs in the oviduct, mitochondria are distributed uniformly in the subcortical cytoplasm of the egg, as seen by the distribution of succinic dehydrogenase in cryostat sections (Patricolo, 1964). After germinal vesicle breakdown and before fertilization there is a migration of mitochondria to the vegetal half of the egg (Fig. 6). After fertilization they migrate further into the vegetal half of the egg and many of them eventually form a mitochondrial crescent, which is seen most clearly at the 2-cell stage (Fig. 7).

Although mitochondria were distributed elsewhere in the egg and embryos, a major concentration occurred in the muscle lineage cells, according to the lineage patterns established by Conklin (1905) and Ortolani (1955). One should note particularly the distribution of activity at the 4-cell stage (Fig. 8) where there is obviously segregation of much more activity into the B3 pair of blastomeres. At the tailbud stages one sees the strong localization of enzyme in the differentiating muscle cells of the tail (Fig. 13). Identical results were obtained with embryos of another species, *M. occidentalis*.

Similar enzyme distributions can be shown with a second mitochondrial enzyme, cytochrome oxidase. Histochemical localizations of this enzyme were identical in both *C. intestinalis* and *M. occidentalis* to those shown in Figs. 6–13 for succinic dehydrogenase. This duplication lends additional confidence to the likelihood that these staining reactions reveal the mitochondrial distribution rather than simply reactive peculiarities of a particular enzyme.

Mitochondrial enzymes in anural embryos

There was no evidence at any embryonic stage that mitochondria were differentially segregated into muscle lineage blastomers of *M. arenata*. Embryos were examined at cleavage stages and at various later developmental stages. At second cleavage the two posterior (B3) blastomeres are slightly larger than the



FIGURES 14–17. Embryos of *Molgula arenata* stained histochemically for the mitochondrial enzymes succinic dehydrogenase and cytochrome oxidase.

FIGURE 14. 4-cell stage embryo stained for succinic dehydrogenase.

FIGURE 15. Embryo 6 hr after the 2-cell stage stained for succinic dehydrogenase.

FIGURE 16. 4-cell stage embryo stained for cytochrome oxidase.

FIGURE 17. Hatching larva stained for cytochrome oxidase. The arrow indicates the position of one arm of the muscle cell crescent.

All figures are the same magnification; the bar in Figure 17 is 40 μm long.

anterior pair. Figure 14 shows that distribution of succinic dehydrogenase occurs in proportion to the size of the blastomeres. There is no obvious differential segregation of activity into the posterior pair. At the neurula stage (Fig. 15) the developing larva had no enzyme localization in those presumptive muscle rudiments that so clearly showed the presence of acetylcholinesterase in other experiments (Fig. 2). Cytochrome oxidase staining revealed the same result: no segregation of enzyme at either the 4-cell stage (Fig. 16) or in the hatching larva (Fig. 17). As described in detail elsewhere (Whittaker, 1979), the hatching larva emerges "head" end first from the chorion; in Fig. 17 the smaller bulge at the left is the emerging anterior end of the larva. The crescent of myoblasts is located at the position of the arrow. There is no differential accumulation of cytochrome oxidase staining in this region of the embryo, or elsewhere.

Discussion

Preformation of mitochondria in the ascidian oocyte is an apparent adaptation to the needs of rapid development in oviparous embryos. Presumably the embryo is unable to synthesize sufficient mitochondria during a brief development time to meet the high energy requirements of larval tail muscle, and must prepare some of these in advance of embryonic development. Ascidian embryos synthesize additional mitochondria during development. Mancuso (1962) noted bilobed mitochondrial structures especially in myoplasmic regions of the *Ciona* embryo, and concluded that muscle mitochondria multiply during embryogenesis. Measurements of mitochondrial enzyme activity indicate that only about half of the number of mitochondria found at larval hatching occur preformed in the fertilized egg (D'Anna and Metafora, 1965; D'Anna, 1966).

According to enzyme activity measurements by Berg (1956, 1957) on separated blastomeres at the 4-cell stage roughly two-thirds of the original mitochondria are segregated (in *Ciona*) into the two B3 muscle lineage blastomeres. A large proportion of these become actively segregated into the later cells of the muscle lineage. While mitochondrial preformation and segregation may be necessary for optimal physiological function of the larva at hatching, the important question is whether this initial concentration directly provides information in the developing muscle system.

Larvae of most ascidian species have a histospecific acetylcholinesterase that occurs in the tail muscle cells (Durante, 1956, 1959). One anural species, *M. arenata*, also developed enzyme in the presumptive muscle cells of the aborted tail (Whittaker, 1979), which indicates that these cells are unquestionably programmed to become muscle. Although myofibrils were not obvious, and probably did not occur, the cells nevertheless differentiated part of their histospecific acetylcholinesterase.

Evidence from experiments with cleavage-arrested embryos of *Ciona* (Whittaker, 1973) and microsurgically isolated partial embryos (Whittaker, Ortolani, and Farinella-Ferruzza, 1977) suggests that ascidian embryos localize and segregate a cytoplasmic determinant concerned with synthesis of acetylcholinesterase by the developing muscle cells. This factor appears to be neither the enzyme itself nor a preformed mRNA for acetylcholinesterase, and is probably an agent responsible

for activating particular genetic programs in the embryo (Whittaker, 1973). A similar determinant apparently occurs in *M. arenata*. Segregation of a determinant was demonstrated by cleavage-arresting embryos of *M. arenata* with cytochalasin B, and finding the eventual development of acetylcholinesterase in blastomeres of the ascidian muscle lineage pattern. Also, normal occurrence of acetylcholinesterase activity was blocked by puromycin and actinomycin D, suggesting thereby that there is neither preformed enzyme nor preformed mRNA for acetylcholinesterase.

Histochemical staining of the mitochondrial enzymes succinic dehydrogenase and cytochrome oxidase in embryos and larvae of *M. arenata* showed unequivocally that no "crescent" localization of mitochondria occurred after fertilization and no subsequent differential segregation of mitochondria took place. The conclusion that anural embryos lack a differential localization and segregation of mitochondria is thought to be valid for two reasons. Results with the same techniques in a normal urodele embryo series (Figs. 6–13) unambiguously show differential mitochondrial distribution. Secondly, concentrations of the histochemical reaction products follow the Beer-Lambert law, and the human eye is sufficiently sensitive to distinguish even minor concentration differences in the products.

In *M. arenata* there is an uncoupling of mitochondrial behavior and muscle cell differentiation. Mitochondria are not localized and not differentially segregated; yet there is determination of larval muscle and its partial differentiation. This shows without question that the mitochondrial accumulation in muscle cells of ascidian embryos has no direct informational relationship to the cellular differentiation. At the same time it is likely that the cytoplasmic determinants of muscle are not physically associated with the myoplasmic mitochondria. One cannot, however, rule out the possibility that the same physical mechanism is involved in both mitochondrial segregation and segregation of the determinants responsible for muscle differentiation. If so, the processes operate independently of one another.

The demonstration of disjunction between mitochondrial segregation and muscle differentiation is a potent example of the intrinsic superiority of nature's experiment. Theodore Boveri urged us long ago to seek the natural experiment where possible: "the investigator of living processes will make it his special concern to find out abnormalities, in which he has not intervened with his crude methods, where he can penetrate into the nature of the alteration" (Boveri, 1908, p. 216).

I thank Dr. Arthur Humes, Director of the Boston University Marine Program at the Marine Biological Laboratory, for the use of dredging facilities, and Mr. Charles H. Henry Jr. of the Case Western Reserve University Dental School for his patient preliminary study of succinic dehydrogenase staining in embryos of *Ciona*. The work was supported by Grant HD 09201, awarded by the National Institute of Child Health and Human Development, DHEW.

Summary

The ascidian *Molgula arenata* produces an anural larva in which development of the tail and other urodele features has been suppressed. The embryos never-

theless developed part of the histospecific tail muscle acetylcholinesterase; the presumptive myoblasts have obviously acquired the muscle differentiation program. When cleavage-stage embryos were prevented from undergoing further division by treatment with cytochalasin B, acetylcholinesterase eventually developed in blastomeres of the muscle lineage. These anural embryos apparently segregate a cytoplasmic determinant concerned with acetylcholinesterase development into cells of the muscle lineage. In this species there was no localization and segregation of mitochondrial succinic dehydrogenase and cytochrome oxidase in the muscle lineage, as found in embryos of two urodele ascidians, *Ciona intestinalis* and *Molgula occidentalis*. The causal determinant of histospecific acetylcholinesterase expression is not, therefore, a differential localization of mitochondria nor is segregation of the muscle determinants linked directly to mitochondrial segregation.

LITERATURE CITED

Bell, W. A., and N. D. Holland, 1974. Cholinesterase in larvae of the ascidian, *Ciona intestinalis*, developing from fragments cut from centrifuged eggs. *Wilhelm Roux' Arch. Entwicklungsmech. Org.*, **175**: 91–102.

Berg, W. E., 1956. Cytochrome oxidase in anterior and posterior blastomeres of *Ciona intestinalis*. *Biol. Bull.*, **110**: 1–7.

Berg, W. E., 1957. Chemical analyses of anterior and posterior blastomeres of *Ciona intestinalis*. *Biol. Bull.*, **113**: 365–375.

Berg, W. E., and W. J. Humphreys, 1960. Electron microscopy of four-cell stages of the ascidians *Ciona* and *Styela*. *Dev. Biol.*, **2**: 42–60.

Berrill, N. J., 1929. Studies in tunicate development. I. General physiology of development of simple ascidians. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, **218B**: 37–78.

Berrill, N. J., 1931. Studies in tunicate development. II. Abbreviation of development in the Molgulidae. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, **219B**: 281–346.

Boveri, T, 1908 Zellen-Studien VI. Die Entwicklung dispermer Seeigel-Eier. Ein Beitrag zur Befruchtungslehre und zur Theorie des Kernes. *Jenaische Zeitschrift für Naturwissenschaft*, **43**: 1–292.

Brachet, J., 1960. *The Biochemistry of Development*, Pergamon Press, London, 320 pp.

Brachet, J., 1974. *Introduction to Molecular Embryology*, Springer-Verlag, New York, 176 pp.

Cabrini, R. L., E. J. Vinuales, and M. E. Itoiz, 1969. A microspectrophotometric method for histochemical quantification of succinic dehydrogenase. *Acta Histochem.*, **34**: 287–291.

Conklin, E. G., 1905. The organization and cell lineage of the ascidian egg. *J. Acad. Natl. Sci. (Phila.)*, **13**: 1–119.

Conklin, E. G., 1931. The development of centrifuged eggs of ascidians. *J. Exp. Zool.*, **60**: 1–120.

D'Anna, T., 1966. Attività della citocromo-ossidasi durante lo sviluppo embrionale dell'uovo di Ascidie. *Boll. Zool.*, **33**: 351–360.

D'Anna, T., and S. Metafora, 1965. Adenosinetriphosphatase activity in the embryonic development of ascidian eggs. *Acta Embryol. Morphol. Exp.*, **8**: 267–277.

de Vincentiis, M., 1956. Sullo sviluppo delle uovo di *Phallusia mamillata* in condizioni di anaerobiosi. *Experientia*, **12**: 381–382.

Duesberg, J., 1915. Recherches cytologiques sur la fécondation des Ascidiens et sur leur développement. *Carnegie Inst. Wash. Contrib. Embryol.*, **3**: 33–70.

Durante, M., 1956. Cholinesterase in the development of *Ciona intestinalis* (Ascidia). *Experientia*, **12**: 307–308.

Durante, M., 1959. Sulla localizzazione istochimica della acetilcolinesterasi lungo lo sviluppo di alcune ascidie e in appendicularie. *Acta Embryol. Morphol. Exp.* **2**: 234–243.

Karnovsky, M. J., 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Biol.*, **27**: 137A–138A.

Karnovsky, M. J., and L. Roots, 1964. A "direct-coloring" thiocholine method for cholinesterases. *J. Histochem. Cytochem.*, **12**: 219–221.

La Spina, R., 1958. Lo spostamento dei plasmi mediante centrifugazione nell'uovo vergine di ascidie e il conseguente sviluppo. *Acta Embryol. Morphol. Exp.*, **2**: 66–78.

Mancuso, V., 1952. Ricerche istochimiche nell'uovo di Ascidie. II. Distribuzione delle ossidasi, perossidasi e della succino-deidrogenasi. *Rend. Ist. Super. Sanità*, **15**: 265–269.

Mancuso, V., 1962. L'uovo di *Ciona intestinalis* (Ascidia) allo stadio di 8 blastomeri osservato al microscopio elettronico. *Acta Embryol. Morphol. Exp.*, **5**: 32–50.

Mansueto-Bonaccorso, C., 1971. Incorporazione di precursori marcati in presenza di actinomicina nello sviluppo delle uova di Ascidie. Penetrazione dell'actinomicina D-H^3 nei diversi stadi. *Atti Accad. Naz. Lincei Rend.*, **50**: 164–166.

Marinos, E., 1978. Cytochrome oxidase activity of mitochondria in *Xenopus laevis* previtellogenic oocytes. *J. Histochem. Cytochem.*, **26**: 658–662.

Meves, F., 1913. Ueber des Verhalten des plastosomatischen Bestandteiles des Spermiums bei der Befruchtung des Eies von *Phallusia mamillata*. *Arch. Mikrosk. Anat. Entwicklungsmech.*, **82**: 215–260.

Minganti, A., 1961. Recent investigations on the development of ascidians. Pages 255–276 in S. Ranzi, Ed., *Symposium on the Germ Cells and Earliest Stages of Development*, Institut Intern. d'Embryologie and Fondazione A. Beselli, Milan.

Ortolani, G., 1955. The presumptive territory of the mesoderm in the ascidian germ. *Experientia*, **11**: 445–446.

Patricolo, E., 1964. Ricerche istochimiche sull'uovo di Ascidie in sviluppo. *Ric. Sci.*, **34** (**II-B**): 71–76.

Pearse, A. G. E., 1972. *Histochemistry, Theoretical and Applied*, Third Edition. Vol. 2 [1342–1343], Churchill Livingstone, London.

Reverberi, G., 1956. The mitochondrial pattern in the development of the ascidian egg. *Experientia*, **12**: 55–56.

Reverberi, G., 1957. The role of some enzymes in the development of ascidians. Pages 319–340 in A. Tyler, R. C. Borstel, and C. B. Metz, Eds., *The Beginnings of Embryonic Development*, American Association for the Advancement of Science, Washington, D. C.

Reverberi, G., 1961. The embryology of ascidians. *Adv. Morphog.*, **1**: 55–101.

Reverberi, G., 1971. Ascidians. Pages 507–550 in G. Reverberi, Ed., *Experimental Embryology of Marine and Fresh-water Invertebrates*, North-Holland/American Elsevier, New York.

Ries, E., 1939. Versuche über die Bedeutung des Substanzmosaiks für die embryonale Gewebedifferenzierung bei Ascidien. *Archiv für Experimentelle Zellforschung*, **23**: 95–121.

Seligman, A. M., M. J. Karnovsky, H. L. Wasserkrug, and J. S. Hanker, 1968. Nondroplet ultrastructural demonstration of cytochrome oxidase activity with a polymerizing osmiophilic reagent, diaminobenzidine (DAB). *J. Cell Biol.*, **38**: 1–14.

Smith, K. D., 1967. Genetic control of macromolecular synthesis during development of an ascidian: *Ascidia nigra*. *J. Exp. Zool.*, **164**: 393–405.

Tung, T. C., S. H. Ku, and Y. F. Y. Tung, 1941. The development of the ascidian egg centrifuged before fertilization. *Biol. Bull.*, **80**: 153–168.

Whittaker, J. R., 1973. Segregation during ascidian embryogenesis of egg cytoplasmic information for tissue-specific enzyme development. *Proc. Natl. Acad. Sci. U.S.A.*, **70**: 2096–2100.

Whittaker, J. R., 1977. Segregation during cleavage of a factor determining endodermal alkaline phosphatase development in ascidian embryos. *J. Exp. Zool.*, **202**: 139–153.

Whittaker, J. R., 1979. Development of vestigial tail muscle acetylcholinesterase in embryos of an anural ascidian species. *Biol. Bull.*, **156**: 393–407.

Whittaker, J. R., G. Ortolani, and N. Farinella-Ferruzza, 1977. Autonomy of acetylcholinesterase differentiation in muscle lineage cells of ascidian embryos. *Dev. Biol.*, **55**: 196–200.

ABSTRACTS OF PAPERS PRESENTED AT THE MARINE BIOLOGICAL LABORATORY

Abstracts are arranged alphabetically by first author. Author and subject references will be found in the regular volume index, appearing in the December issue.

GENERAL SCIENTIFIC MEETINGS

AUGUST 20–22, 1979

Morphogenesis in grafted Hydra attenuata: *positive dominance, negative dominance, and pattern regulation.* JAMES ALFRED ADAMS.

Positive dominance (induction) and negative dominance (inhibition) have been studied in grafted *Hydra attenuata*. Grafted animals are prepared having three gastric regions, tandemly arranged, with original disto-proximal orientation of the graft pieces being maintained. Grafted animals prepared in this fashion (3g hydras) have the ability to regenerate heads and feet in the vicinities of the graft borders. These regenerates are referred to as a secondary head (2°h) and secondary feet (2°f). Gradients in the frequencies of both 2°h and 2°f regeneration are observed in these animals. Using Chi-square methods and a 95% level of confidence, one finds a significantly greater frequency of 2°h regeneration at the graft border farthest from the terminal head ($0.050 > P > 0.010$). Likwise, a higher frequency of 2°f regeneration occurs at the graft border farthest from the terminal foot ($0.005 > P$). These data suggest that the terminal head or foot, by its presence, either inhibits or causes inhibition of a like structure occurring (negative dominance). This inhibitory effect decreases in a linear fashion with increasing distance from the terminal head or foot as the case may be.

However, when a tentacle and a small piece of attached hypostomal tissue is left on the middle and proximal graft pieces there is no inhibition of head formation at either graft border. This result shows that the inductive capacity (positive dominance) of the tentacle-hypostome region can overcome the negative dominance of the terminal head. This technique also affects 2°f formation on the 3g animal. The frequency of 2°f regeneration is enhanced significantly at the proximal graft border and decreased significantly at the distal border, with the effect that the gradient in foot formation observed in control 3g animals is abolished. Thus, a direct relationship, and perhaps an interdependency between the gradients of 2°h and 2°f regeneration in 3g *H. attenuata* is demonstrated.

Alternate pathways in the biosynthesis of testosterone by Rana pipiens. CHRISTOPHER A. ADEJUWON, SHELDON J. SEGAL, AND S. S. KOIDE.

The absence of immunoreactive progesterone, despite the presence of appreciable amounts of immunoreactive testosterone in unstimulated testes taken from specimens of *Rana pipiens*, led us to investigate the precursor role of progesterone in testicular testosterone biosynthesis in this amphibian species. Three sets of experiments were performed. The first was chromatographic analysis of testicular steroids. Testis slices (60–80 mg) were placed in 2.0 ml ringer's solution and were agitated with 10.0 ml petroleum ether and then with choloroform. After evaporating the solvent, the residue was dissolved in chloroform : methanol and applied to flourescent-coated silica gel thin-layer chromatography (TLC) plates. The chromatogram was developed in ethyl acetate : water : n-hexane : ethanol. The resulting spot was visualized with ultraviolet light using testosterone as a marker. The steroid present in the testicular extract had an R_f value of 0.63 compared to the R_f values of 0.67 and 0.72 for testosterone and progesterone, respectively. Radiolabeled pregnenolone co-migrated with progesterone in this system.

In the second experiment, *in vitro* stimulation of testosterone production by human chorionic gonadotropin (hCG) was demonstrated by incubating *Rana* testis slices with ringer's solution containing varying concentrations of hCG. Aliquots (100 μl) of the incubation medium were removed between 0.5 and 2.0 hr of incubation for testosterone determination

by radioimmunossay (RIA). Testosterone production was stimulated by hCG in a dose-related manner; the lower limit of sensitivity was 2 ng. Thirdly, *in vitro* conversion of precursor to ^{3}H-testosterone was demonstrated by incubations of ^{3}H-progesterone and ^{3}H-pregnenolone with testicular slices in ringer's solution containing a nicotinamide adenine dinucleotide phosphate-generating system. Steroids were extracted and analyzed on TLC. About 8.8% of ^{3}H-progesterone and 8.7% of ^{3}H-pregnenolone were transformed to ^{3}H-testosterone in 4 hr of incubation The present results, based on RIA, TLC, and hCG-stimulation show that testosterone is produced by testes taken from mature male frogs of the *R. pipiens* species. The Δ^5 and Δ^4 biosynthetic pathways appear to be equally important for *in vitro* testosterone formation. HCG concentrations as low as 2 ng stimulate testosterone production.

Supported by a grant from the George Hecht Fund. C. A. Adejumon is a recipient of a Rockefeller Foundation Fellowship.

Characterization of Spisula *sperm protamine.* Juan Ausio and K. E. Van Holde.

The major protein component of mature *Spisula* sperm nuclei appears to be a protamine. This material has been purified from ripe sperm by the following procedure: sperm were washed gently from excised gonads and homogenized in 5% acetic acid. After low-speed centrifugation, the nuclei were re-extracted with 25% acetic acid. These extractions remove most of the contaminating histones. The nuclei were next extracted with 0.25 N HCl, and the protamine so released was purified by chromatography on a CMC-25 column, using 1.2–1.8 M NaCl gradients.

The protamine so prepared migrated as a single band on urea gel electrophoresis, with the reservation that a small amount of material always remained at the top of the gel. Sedimentation equilibrium studies at pH 9.2, and treatment with 0.4 M NaCl revealed a nearly homogeneous material of molecular weight 29,400. The sedimentation coefficient was determined at a number of concentrations in 0.4 M NaCl, and the extrapolated value of $S_{20,w}^{0} = 2.0 \times 10^{-13}$ was obtained. This value, small for a protein of this mass, corresponds to a frictional ratio $f/f_o = 1.55$, indicating asymmetry or unfolding of the molecule. Upon increasing the ionic strength above 1 M, the sedimentation coefficient increases about 25%, probably from a condensation of this highly charged molecule.

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Phosphorylation of the 40s ribosomal subunit after fertilization of Arbacia punctulata *eggs.* Dennis Ballinger, Scott Peterson, and Tim Hunt.

A particularly prominent alteration in the pattern of protein phosphorylation after fertilization of *Arbacia punctulata* eggs occurs in a 31,000 MW protein whose labeling increases relative to other proteins. This increase can be detected 5 min after fertilization in eggs which have been labeled for 2 hr with $^{32}PO_4$.

The following criteria identify this protein as a component of the 40s ribosomal subunit: first, it co-sediments with 80s ribosomes on a low salt sucrose gradient; secondly, it co-sediments with the 40s ribosomal subunit on a high salt sucrose gradient; thirdly, its mobility in a two dimensional gel system designed to separate basic ribosomal proteins (acid urea/SDS) suggests that it may be the sea urchin analogue of protein S6 of yeast and mammalian ribosomes, but this identification is tentative at present. We hope to use this analytical system to determine whether the increased labeling represents *de novo* phosphorylation, or simply an increased rate of turnover of preexisting phosphate residues. Our data indicate, however, that at least one of these parameters must change after fertilization.

We have made one test of the hypothesis that the phosphorylation of this ribosomal protein may be connected in some way with the 20- to 30-fold increase in protein synthesis which occurs in the sea urchin after fertilization by asking whether the labeled subunits were preferentially associated with polysomes. We labeled embryos with $^{32}PO_4$ for 4 hr following fertilization, and analyzed extracts on sucrose gradients. The labeled 31,000 MW protein was found associated to the same extent with 80s ribosomes and polysomes in terms of cpm/A_{260}. These results are

indicative of little or no preferential association of phosphorylated subunits with mRNA in 4-hr embryos. Thus, the signficance of this phosphorylation, while suggestive, remains uncertain.

Difference between SDS-PAGE patterns of Labyrinthula *slimeways and vegetative cells.* Eugene Bell, Norio Nakatsuji, and Stephanie Sher.

The slimeways of *Labyrinthula* are synthesized by the cells and contain contractile proteins organized, in part, into filaments. To examine the relative protein complexities of cells and slimeways, colonies were labeled with ^{14}C reconstituted protein hydrolysate, homogenized, fractionated into cells and slimeways and then subjected to SDS PAGE. Labeled homogenates of whole colonies gave a minimum of 55 radioactive bands. Bands comigrating with chick skeletal muscle myosin and chicken gizzard were identified. The slimeway fraction showed five times fewer bands than the whole colony homogenate or the cell fraction homogenate, but the film densities of actin bands of the cell and slimeway fractions was the same, while the myosin band of the slimeway fraction was even more dense.

To try to understand the segregation of cell and slimeway proteins in the three-dimensional colony, we have reviewed the fine structure of *Labyrinthula* and propose the following scheme: In the extrusion of "extracellular" membranes and slimeway proteins from a cell bounded only by its plasma membrane (cell membrane I), the first event important for slimeway formation may be the ballooning of membrane vesicles from bothrosome apertures. The vesicles with associated proteins flatten into two layers and open where the layers make hairpin turns. The layer adjacent to the cell, cell membrane II, which is attached to the cell through the bothrosome, fuses with comparable layers from other bothrosomes of the same cell to form a second membrane around the cell. The cell is now invested with a second membrane of reverse polarity with respect to the plasma membrane. Thus, through vesicle formation and rupture, selected proteins and formed membrane pieces are brought to the exterior for slimeway construction.

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Conduction and dielectric studies of protein-methylglyoxal complexes. Stephen Bone.

Dielectric and conduction studies of bovine serum albumin (BSA), casein, collagen, and lysozyme treated with methylglyoxal have been made in an attempt to provide evidence for the existence of the charge-transfer mechanism proposed by Albert Szent-Györgyi (1979, *Biol. Bull.*, **157**: 398). The dry treated protein samples show a dielectric dispersion at sub-Hertz frequencies whereas no such trend is observed for the pure proteins. A similar low frequency dispersion has been shown to exist in the dielectric studies of the conventional charge-transfer complex (perylene + chloranil). These dispersions have been found to be associated with a bulk rather than an electrode interfacial effect. The observation of an increased DC conductivity and lowered conductivity activation energy for the protein-methylglyoxal complexes as compared with the untreated proteins is consistent with the formation of a charge-transfer complex. Analysis of the dielectric dispersions indicate that this dielectric loss cannot be interpreted using conventional dipolar relaxation theories, but they may be understood in terms of the hopping of mobile charge carriers. These carriers are thought to hop over potential energy barriers associated with intra- and inter-protein boundaries, and their mean free path length has been determined to be of the order of 1.5–1.8 nm for the BSA, casein, and lysozyme complexes and 16 nm for the collagen complex. These studies provide strong evidence for the existence of a charge-transfer interaction between proteins and methylglyoxal as originally proposed by Szent-Györgyi.

Pressure-flow relations in the perfused systemic circulation of squid. George B. Bourne.

An artificially perfused preparation of the posterior systemic circulation of squid was developed to permit examination of some physical and cholinergic factors affecting pressure-

flow relations. The posterior aorta was cannulated via the systemic heart, after which pressure was monitored by a strain-gauge manometer connected to the cannula at a T-junction by polyethylene tubing. Flow was measured by an electromagnetic flowmeter and also was monitored by sensing loss of fluid from the perfusion reservoir via an isometric muscle lever. The signals from the transducers were read out on a Brush recorder.

The preparation took approximately 15 min to stabilize when perfused by vigorously oxygenated, filtered sea water. Stability was maintained for 1 to 2 hr, after which the preparation decayed rapidly. The decay was characterized by increasing vascular resistance and severe oedema.

When flow was plotted against pressure, a curve which obeyed a power function was obtained. Acetylcholine caused a marked vasodilation. The threshold of response to acetylcholine was 10^{-11} M with 75% of maximal response occurring at 10^{-9} M. The vasodilatory action of acetylcholine was blocked by *d*-tubocurarine.

This work was supported by a Steps Toward Independence Program Fellowship and by an operating grant from the National Scientific and Engineering Research Council of Canada.

The numbers and sizes of cells in molluscan ganglia; simultaneous optical recording of activity from many neurons. [*Demonstration.*] M. B. Boyle, L. B. Cohen, and E. R. Macagno.

We have compared ganglia from the central nervous systems of a number of gastropod molluscs with the object of finding an animal having relatively large and relatively few nerve cells. We hope to use optical methods for monitoring activity to help us unravel the neuronal basis of behaviors. Freshly dissected ganglia were left in a 0.1 to 0.3 mg/ml solution of methylene blue in saline for 12 to 18 hr at room temperature, fixed in 4% formaldehyde in saline for about 30 min, dehydrated in 30, 60, 90, and 100% acetone, and cleared in methyl salicylate. The ganglia were then whole-mounted between cover slips using Permount. This procedure is designed to selectively stain cell bodies. We showed examples of buccal ganglia from *Aplysia californica*, *Dendronotus iris*, and *Tritonia diomedia*, and of circumesophageal ganglia from *Hermissenda crassicornis*, *Dendronotus iris*, and *Tritonia diomedia*. As viewed under the dissecting scope, the buccal ganglia of *Tritonia* and *Aplysia* appear to contain a relatively large number of small cells as compared with *Dendronotus*. The circumesophageal ganglia of *Hermissenda* seem to consist of cells whose average size is significantly smaller than that in either *Tritonia* or *Dendronotus*. *Dendronotus* appears to have relatively few small cells in either the buccal or the circumesophageal ganglia.

We also showed an example of a pre-recorded experiment done at Friday Harbor Laboratories on *Dendroronotus iris*. We detected spontaneous activity and activity in response to suction electrode stimulation of nerves from a circumesophageal ganglion using the merocyanine-rhodanine dye WW433.

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Optimal prey selection of Littorina littorea *by green crabs.* John E. Boynton.

Optimum foraging theory predicts that predators will be adapted to select prey of a size that returns maximal food energy for time invested. *Carcinus maenas* (green crab) has a varied diet which includes *Littorina littorea* (periwinkle). Crabs attack snails by several methods: crushing, shell edge chipping, and shell boring. Predictions of optimal foraging theory and correlations of attack armature and defensive armor were tested using this predator–prey system. Snails of all sizes were fed to a size-spectrum of individual crabs, and prey size selected was determined. Snail shell-length : dry-weight relations were measured. Parameters of crab claws were examined as well as snail shell thickness and length relations. Only snails in the size range of 5 to 17 mm were selected by crabs. Dry weight results indicate that snail soft tissue increases exponentially. Crabs would benefit more from going after larger snails rather than smaller snails. Crabs mostly crushed their prey. Crabs chose snails that fell within the upper range of that permitted by the individuals' claw gape, and

ignored smaller snails. This corresponds with data on snail populations in the salt marsh. Claw size seems to determine the prey size that can be attacked.

Chemoreception in catfish: effects of diet on behavior and body odor. Bruce Bryant and Jelle Atema.

Whole body-odor complexes are important in maintaining social structure, but this maintenance is not based on a rigid stimulus-response relationship as found in typical pheromone-mediated social behavior. In aquaria, the brown bullhead, *Ictalurus nebulosus,* forms territories and dominance hierarchies. The importance of body odor in maintaining these territories and hierarchies was investigated by observing the effects of a changing body odor of one member of a territorial pair. Body-odor change was induced by a behaviorally non-invasive manipulation—a change in diet.

Pairs of fish, fed identically for at least three weeks (Purina trout chow), were observed and both social and solitary behavioral units were recorded. A handling control preceded the diet manipulation and consisted of isolating and feeding the subdominant (β) fish on chow in a separate tank for 1 day. Two days after reintroduction into the main tank, (β) was isolated again but this time fed an identical volume of beef liver. In three of four tanks, a change from display behavior to aggressive nips and bites by α occurred when comparing control and experimental reintroductions of β. The fourth tank showed no change in aggressive behavior but a change in territorial status. β's territory suffered significantly more incursions following manipulation than during the control periods ($P < 0.05$, Friedman 2-way ANOVA). Inspection of β's solitary behavior units did not reveal effects of the diet change. The changes in behavior associated with a change in diet suggest that the maintenance of territorial hierarchy is dependent on chemical recognition of conspecifics which have become familiar; diet manipulation disrupts this recognition. The degree and time course of this disruption seems to suggest that it is not complete and that familiarity is regained within days.

New developments in the mariculture of Aplysia californica. Thomas R. Capo, Susan E. Perritt, and Carl J. Berg, Jr.

It recently has become possible to study the developmental neurobiology of *Aplysia californica* through the use of laboratory cultured animals. In order to optimize culture conditions we have emphasized three approaches. The first involved the evaluation of various antibiotics. The combination of Penicillin-G sodium salt (100.5 units/ml) and Streptomycin-sulfate (50 μg/ml) was routinely found to improve the survival of veliger cultures. The second approach was to find substrates other than *Laurencia pacifica,* for triggering the metamorphosis of *A. californica.* Two red alga available in the Woods Hole region, *Neoagardhcilla baileyi* and *Gracilaria* sp., have been successful. *Neoagardhcilla baileyi,* Strain A$_1$, has been cultured in the laboratory for six months (T. Capo, in preparation). With present techniques, more than adequate amounts of epiphyte-free *N. baileyi* have been grown for metamorphic and early juvenile stages. Finally, the technique for handling metamorphic stages has been simplified with the use of disposable 50-ml conical centrifuge tubes. By using sufficient algae to cover the base of the tube, sinking animals are brought into increased contact with the substrate on which metamorphosis takes place. The combined effects of these three approaches have simplified and improved the mass culture of *Aplysia californica.*

This work was funded by a grant from the Klingenstein Foundation.

Motor fields of pharyngeal motoneurons in Navanax, *an opisthobranch mollusc.* Mitchell S. Cappell, David C. Spray, David H. Hall, Abraham J. Susswein, and Michael V. L. Bennett.

Three orthogonal muscle groups comprise the *Navanax* pharynx: radials which run through the pharyngeal thickness and mediate expansion, and superficial circumferentials and longi-

tudinals which cause constriction and shortening. Stimulation of identified neurons in the buccal ganglion cause these pharyngeal movements. This work provides evidence that these neurons are true motoneurons, with specific and reproducible motor fields. Evidence indicating that these cells directly innervate muscle includes: constant latency EMGs are evoked by intrasomatic stimulation at moderately high frequencies, antidromic impulses are evoked by electrically stimulating the appropriate muscle area, and a peripheral nerve net is apparently absent.

Circumferential and longitudinal muscles consist of a superficial gridwork of a very consistent number of discrete bands. Localizability and reproducibility of motor fields was quantitatively determined with respect to these pharyngeal landmarks.

Motoneuron fields were identified by: movements following intrasomatic stimulation, area producing 1-1 EMGs with intrasomatic impulses, areas yielding antidromic impulses at low threshold, and axonal course through nerves innervating specific areas. Generally, these methods gave mutually consistent results.

To date fields of 10 motoneurons have been characterized. These fields show little variability. Four circumferential motoneurons, two per side, innervate fused circumferential bands located at the anterior of the pharynx. Of the two per side, one innervates ipsilaterally, the other bilaterally. Another left circumferential motoneuron bilaterally innervates the fused bands located posteriorly. Both giant (G) expansion motoneurons innervate radial muscles of the entire pharynx. The ventral left medium-sized (M) cell innervates the anterior dorsal pharynx bilaterally from about circumferential band 25 to 31. An adjacent left expansion motoneuron innervates the more posterior ventral and dorsal pharynx ipsilaterally. A small (S) left expansion motoneuron has a bilateral field comprising about the anterior half of the M cell. About another dozen observed fields are not yet adequately established. These include longitudinal motoneurons, none of which have been discovered previously. These data provide a further step towards understanding the neural control of feeding in *Navanax*.

Mitchell Cappell is supported in part by NIH training grant no. 5T 32 GM7288.

Ionophore induced sodium loading of nerve terminals: a model for long term facilitation of transmitter release. Milton P. Charlton, Charlie S. Thompson, and Harold L. Atwood.

Accumulation of sodium ions caused by sodium pump inhibitors, metabolic poisons, repetitive stimulation or direct injection increases spontaneous and evoked transmitter release at synapses. We induced sodium loading in nerve terminals by application of the sodium ionophore, Monensin. In the stretcher muscle of the spider crab (*Libinia*), application of 6 μM Monensin caused a 2- to 8-fold increase in the amplitude of postsynaptic potentials (psp) evoked when the excitor motorneuron was stimulated. Recovery following washout of Monensin took up to 50 min. A second dose gave a smaller response or none. The rate of increase of psp's following application of Monensin was greater in saline containing the normal concentration of sodium than in saline in which two-thirds of the sodium had been replaced by sucrose. This is consistent with a sodium ionophore role for Monensin in this system. Following application of Monensin in saline containing no calcium, normal saline (no Monensin) was reapplied and there was an immediate increase in psp amplitude as if Monensin had been working during its application in zero calcium saline. This and its miniscule calcium transporting ability militate against a calcium ionophore role of Monensin in our experiments. Extracellular focal recordings at synaptic sites showed that quantal content was increased by Monensin. Spontaneous transmitter release was increased by Monensin in crab, lobster and frog (cutaneus pectoralis) muscles; in the latter there was a 20-fold increase in spontaneous release. No changes in membrane potential or input resistance of muscle fibers were seen with Monensin treatment. The amount and duration of long lasting potentiation produced by tetanic stimulation were similar to the effects produced by Monensin. The data support a role for sodium in long-term facilitation and demonstrate the usefulness of Monensin for loading sodium in small cells.

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Some biochemical properties of an RNA-instructed DNA polymerase in developing sea urchin (Lytechinus pictus) *embryos.* Beverly S. Chilton, Marc R. Laufer, and Santo V. Nicosia.

The rate of protein synthesis in unfertilized (UF) sea urchin eggs increases dramatically during early cleavage. This has been attributed in increased oogenic mRNA availability and translational efficiency. The presence of an RNA-instructed DNA polymerase in UF eggs and early cleavage stage embryos suggests that the sea urchin may inherit a mechanism for the amplification of existing maternal mRNA's.

An RNA-instructed DNA polymerase was partially purified according to Slater and Slater (1972, *Nature New Biology* 237: 81–87) from UF eggs and early (2–16 cell) embryos. Using the synthetic template-primer $(rA)_n \cdot (dT)_{10}$, incorporation of (^{3}H)-dTTP (305 cpm/pM) by the polymerase from UF eggs and 16-cell embryos was maximal at 15° C. Incorporation kinetics were linear for 150 min. The effects of polymerase concentration on the rate of DNA synthesis resulted in similar sigmoidal curves for the enzyme from both UF eggs and 16-cell embryos. This suggests that the polymerase has a similar subunit structure and relationship to template-primer prior to and during embryogenesis.

Polymerase activity, expressed as pM (^{3}H)-dTTP/μg protein, was 0.17 and 0.53 for UF eggs ($n = 3$ animals/determination), and averaged 0.64 for two- and four-cell embryos. Specific activity (mean $\pm$ s.e.) was not significantly different for UF eggs (0.45 ± 0.08) compared to 16-cell embryos (0.55 ± 0.14). Thus RNA-dependent DNA polymerase is detectable throughout early development. However, when expressed on a per cell basis, specific activity decreases during this time. The partially purified polymerase from 16-cell embryos banded at a sucrose buoyant density of 1.15 to 1.16 g/ml, the same density as the particulate polymerase isolated from human tumors. This implies a viral association for the sea urchin RNA-dependent DNA polymerase which may play a role in early development.

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Hydrogen peroxide release from sea urchin eggs during fertilization: importance in the block to polyspermy. Michael Coburn, Herbert Schuel, and Walter Troll.

Polyspermy results in abnormal cleavage and halting of development by the blastula stage in sea urchins. For successful fertilization polyspermy must be avoided. We noted a novel block to polyspermy employed by *Arbacia punctulata:* the release of hydrogen peroxide after fertilization. We suggest that this hydrogen peroxide inactivated other sperm after entry of the first, ensuring proper development.

We have demonstrated the crucial role of hydrogen peroxide release in preventing polyspermy in *Arbacia* by causing 100% polyspermy through the addition of catalase to the sea water in which eggs were fertilized. Catalase is an enzyme of high specificity for the decomposition of H_2O_2 to water and oxygen. An early finding was confirmed by showing that sperm are inactivated by minute concentrations of H_2O_2, and that this action was blocked by catalase. We have further shown that catalase produces polyspermy within the first minute after fertilization, when present at the time of the addition of sperm to eggs.

Protease inhibitors such as soybean trypsin inhibitor (SBTI) have been shown to cause polyspermy in *Arbacia*. This and other protease inhibitors also act to prevent oxygen uptake and H_2O_2 production in human white blood cells. We now note that SBTI prevented the release of H_2O_2 from fertilized *Arbacia* eggs, similar to its action in white cells. Thus, the cause of polyspermy produced by protease inhibitors could be the inhibition of H_2O_2 release, rather than the prevention of normal raising of the protective fertilization envelope as was suggested previously. These observations reinforce the relationship between proteases and H_2O_2 production in biological systems.

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Association of centriole-like structures with the marginal band of a molluscan red cell. William D. Cohen and Iris Nemhauser.

Marginal bands (MBs) are continuous circumferential bundles of microtubules found classically in all non-mammalian vertebrate erythrocytes and in platelets. MBs are believed to function in cellular morphogenesis, but relatively little is known about their formation.

We have been engaged in a continuing survey of blood cells of local (MBL) invertebrates for possible presence of MBs, with the objectives of establishing the phylogenetic distribution of the MB system identifying useful cell types for experimental work on MB structure and physiology. Previously we have established that MBs are present in sipunculan red cells, *Limulus* amebocytes, and lobster and crab coelomycetes. We report here the presence of MBs in the hemoglobin-containing red cells of the arks *Anadara ovalis* and *Anadara transversa*. A striking feature of these molluscan red cell MBs is the obvious presence, on or adjacent to each MB, of a pair of centriole-like structures which we have tentatively named "perioles". The living red cells (*A. transversa*) are highly flattened and generally slightly elliptical (10–15 μm long axis). In cells lysed with Triton X-100 under microtubule-stabilizing conditions, the nucleus, cytoplasmic particles, and MB with associated perioles are directly observable in phase contrast (oil immersion). The MBs are usually elliptical, with the periole pair appearing at various points on the ellipse but most frequently near one end. The two perioles are normally adjacent; however, in some cases a gap (0.25–1.5 μm) occurs between them. In fixed, uranyl acetate-stained whole mounts of lysed cells (TEM) MB microtubules and the densely-staining perioles are readily visible. A trans-MB network of fine filaments is also present. The perioles appear to be within or on the MB, with extra-MB microtubules often 'focussing' upon them in pole-like fashion. Their centriole-like features as observed in whole mounts include (a) close pairing, sometimes with approximately right angle positioning, (b) cylindrical appearance with one closed end, and (c) size, approximately 0.25×0.17 μm. We suggest that the perioles may be MB organizing centers, functionally analogous to centrioles and basal bodies.

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Dependence of discrete wave frequency on pH in Limulus *ventral photoreceptors.* D. Wesley Corson and Alan Fein.

Discrete waves in the ventral photoreceptors of *Limulus* are thought to result from either direct or spontaneous activation of visual pigment molecules by the absorption of photon energy or thermal kinetic energy. We have found that the average frequency of occurrence of spontaneous discrete waves in darkness rises 15-fold over a 2 pH unit range (6.0–8.0) of artificial sea water that bathes the receptors. Over this same range, the increment of discrete wave frequency added by a steady dim light remains essentially constant.

Artificial sea water was buffered with 10 mM each of Pipes (pK = 6.8), Hepes (pK = 7.55) and Tris (pK = 8.30) and titrated to pH values in the range from 6.0 to 8.0 in half pH unit increments. The frequency of discrete waves was determined by counting the number of discrete waves in five 40-sec intervals at each experimental pH after a 10 min exposure. A dim steady light was adjusted to give an increment of approximately 1 discrete wave per second (0.99 ± 0.66 dw/sec; $\bar{x} \pm$ s.d.; $n = 9$) over the spontaneous frequency (0.89 ± 0.42 dw/sec, $n = 9$) at pH 7.0. Dim 20-msec test flashes showed that changes in the average discrete wave frequency were not the result of changes in the average discrete wave amplitude, and data were taken only from cells in which the spontaneous discrete wave frequency returned to its control level following each exposure.

In seven of nine cells, the spontaneous discrete wave frequency rose within the pH range of 6.0 (0.16 ± 0.17 dw/sec, $n = 4$) to 8.0 (2.41 ± 0.60 dw/sec, $n = 4$), while in the remaining two cells there was no appreciable rise in frequency from pH 7.0 (0.17 ± 0.06 dw/sec) to pH 8.0 (0.17 ± 0.10 dw/sec). No appreciable rise in the light-induced frequency increment occurred in either the seven responsive cells (1.30 ± 0.33 dw/sec at pH 6.0 *vs.* 0.79 ± 0.76 dw/sec at pH 8.0) or the two unresponsive ones (0.43 ± 0.14 dw/sec at pH 7.0 *vs.* 0.48 ± 0.26 dw/sec. at pH 8.0).

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Production and metabolism of steroids in Homarus americanus. Ernest F. Couch, C. A. Adejuwon, and S. S. Kohde.

We have reported that the mandibular gland of the lobster contains progesterone as determined by radioimmunoassay (Couch *et al. Biol. Bull.* **155**: 433, 1978). To learn the fate of the progesterone, tritium-labeled progesterone (1×10^6 cpm) was injected into the abdominal muscle of an adult female lobster. After 1.5 hr various tissues (blood, urine, heart, green gland, hind gut, gills, mandibular gland, hepatopancreas, ovary, nerve cord, and muscle) were excised, homogenized, and extracted with petroleum ether and ethyl ether. The radioactivity in the extracts was measured. The highest incorporation of progesterone occured in the mandibular gland (7500 cpm/mg wet wt.) and in the green gland (2750 cpm/mg wet wt.).

To determine the capability of lobster tissues to transform steroids, the mandibular gland, hepatopancreas, and ovary were incubated with ^{3}H-pregnenolone for 6 hr *in vitro* at room temperature. The reaction mixture was extracted with petroleum ether and ethyl ether. The extracts were pooled and dried. The residue was dissolved in chloroform : methanol (1 : 1) and spotted on silica gel TLC plates. The plates were developed in a mixture of petroleum ether : ethanol : water (100 : 80 : 20 by volume). The spots were visualized under UV light. By this method pregnenolone and progesterone were not separated. However, in the extract of the hepatopancreas, a unique radiolabeled metabolite ($R_f = 0.71$) was detected which was distinctly different from progesterone, testosterone, estrone, estradiol, and estriol. This UV-absorbing compound was also found in untreated hepatopancreas, but it was absent in ovary and mandibular gland. These results indicate that pregnenolone is transformed into a metabolite in the hepatopancreas under physiological conditions.

To establish a relationship between mandibular gland function and reproduction, ovaries from lobsters at varying stages of oocyte development were excised and fixed for morphological studies. Previous studies had shown the presence of small amounts of progesterone (about 0.2 ng/g of wet wt.) in immature ovaries but not in mature ovaries. In the present study, we observed that the size of the mandibular gland appears to vary during ovarian development; *i.e.*, the gland was small (about 12 mg/100 g of body wt.) during the dormant stage of egg production. It increased in size (about 25 mg/100 g of body wt.) during the time of early oocyte growth and development and receded to about 9 to 11 mg/100 g of body weight as maturation of oocytes occurred. These results suggest that mandibular gland function might be associated with ovarian development.

Supported by a grant from the George Hecht Fund.

Response specificity following behavioral training in the nudibranch mollusk Hermissenda crassicornis. Terry Crow and Nancy Offenbach.

Photopositive responses of the nudibranch mollusk *Hermissenda crassicornis* exhibit long-term behavioral plasticity that has been examined at the cellular level. The behavior is modified by an automated training procedure that stimulates the eyes and statocysts using light paired with rotation while the animals are confined to the ends of glass tubes filled with sea water. Random control procedures have demonstrated that the long-term behavioral modification is dependent on pairing light and rotation. Response latencies to move into an illuminated area are significantly increased for animals that received light paired with rotation. The increased response latencies following training are not the result of a decrease in general activity. When the same animals were tested in both light and dark after training we found that only response latencies to enter the illuminated area were increased ($P < 0.01$). Latencies to move to the end of the tubes in the dark wre not significantly changed. Significant light-dark differences were not found for random control or normal untrained animals. We next examined the response latencies of animals to initiate movement towards the illuminated area at the opposite end of the tube. We found a linear relationship between the start latencies (initiation of movement toward the light) and the latencies to enter light for normal untrained animals ($r = 0.93$, $P < 0.01$). Start latencies for trained animals were correlated with the latencies to enter light before ($r = 0.97$) and after training ($r = 0.99$). We found significant increases in start latencies following training for the light paired with rotation group ($P < 0.005$) while the start latencies did not change for random control groups.

The increase in the response latencies to enter light after training can be accounted for by the significant increase in start latencies for trained animals. This specific modified behavioral response to light following training will be examined at the cellular level in intact and semi-intact preparations.

In vitro *transcription in nuclei from* Arbacia punctulata. Lisa M. Davis.

A technique for isolating nuclei which are capable of incorporating ^{3}H-UTP into TCA precipitatable counts has been devised. This incorporation is inhibited by the presence of α-amanitin, it is dependent upon the presence of all four ribonucleotide triphosphates, and the reaction yields a product which is Ribonuclease A sensitive. The isolation procedure yields nuclei which are not aggregated and which are free of cytoplasmic contamination. Conditions for maximizing the reaction, with regards to the divalent metal ions and the salt concentrations, were also investigated. The procedure for isolating transcriptionally active nuclei called for the presence of $MgCl_2$, NP-40, and EGTA, in the homogenization buffer. The former two ingredients were important for obtaining clean, non-aggregated nuclei, and the latter for inhibiting nucleases. Nuclei from embryonic stages other than gastrula were not assayed for this transcriptional activity. However, the observation was made that nuclei isolated from embryos grown at 25° C were only 25% as active as those from embryos grown at 18° C. Following isolation, the nuclei were either assayed immediately, or stored at −20° C, with no loss of activity for at least 2 weeks.

The α-amanitin profile of this activity was determined; the reaction was inhibited 50% by both high and low levels of the toxin (100 and 5 μg/ml, respectively). Whether this result is due to the absence of any polymerase III activity, or to the possibility that the enzyme is sensitive to higher levels of amanitin, was not determined at the time of this writing. However, attempts were made to optimize for activity by polymerase III, by altering the Mg^{2+} concentrations and using Mn^{2+} and KCl concentrations which typically stimulate this activity. It was determined that the time course of the reaction, as well as the amanitin profiles, were identical with either 1 mM, 5 mM, or 10 mM Mg^{2+}. (The assay mix also contained 1 mM Mn^{2+} and 150 mM KCl).

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Chemical search image: prey exposure improves selective chemical detection by a predator (Homarus americanus). Charles Derby and Jelle Atema.

Considered as a species, lobsters (*Homarus americanus*) are omnivores; individuals, however, can be selective in prey preferences. Experience with prey can alter prey preferences by various methods: learning to search for prey in particular areas; learning to handle prey more efficiently; and learning to detect prey. The latter case, where encounters with prey improve the predator's detection of that prey, is called search image formation. This report describes chemical search image formation in lobsters.

Behavioral responses of ten lobsters to prey body odors were observed in 2.5-m-long troughs. Body-odor stimuli of two prey species, *Mytilus edulis* (blue mussel) and *Modiolus modiolus* (horse mussel), were prepared by placing five mussels in sea water for a fixed amount of time. For each stimulus, lobsters were observed for 10 min before and 10 min after stimulus introduction. Movements of appendages associated with alerting and searching (antennules, antennae, maxillipeds, claws, walking legs) were quantified, and differences between pre- and post-introduction periods were determined by Chi-square analyses. Detection thresholds for the two stimuli were determined by randomly presenting seawater controls and log-dilutions of both odors. Effects of feeding experience on thresholds were examined by initially feeding starfish and fish to all ten lobsters. After determining thresholds, lobsters were placed in a tank with either live *Mytilus* or live *Modiolus*. After 30 days of this exposure, thresholds were again determined. Changes in responses to experienced prey odors were compared relative to changes in response to nonexperienced prey odors. Seven lobsters became relatively more sensitive to odors of experienced prey; two showed no change; one became relatively less sensitive. Wilcoxon matched-pairs signed-rank test demonstrates significant at $P < 0.05$. This

experiment therefore demonstrates that chemical detection of prey improves as a function of experience with that prey; this supports the idea that lobsters can form chemical search images.

Effects of unilateral antennule ablations on food odor orientation in Homarus americanus. Dana Devine and Jelle Atema.

Recent electrophysiological investigations of the lobster antennule have suggested that the lobster can orient to odors by gradient discrimination through simultaneous comparison of bilateral receptor input from the two lateral flagella of the biramous antennule. Behavioral evidence supporting this hypothesis is anecdotal and scant, prompting the present study.

Lobsters were placed individually in 600-liter aquaria, where they occupied a shelter in the middle of the back wall. Two air life water flows were directed toward the center of the tank from the side walls, running continuously. For testing, a standard food odor was injected in either flow. The lobsters' behavior was observed and timed in response to this chemical stimulus. Three sets of data were obtained per lobster in the following order: untreated, after ablation of the right lateral flagellum, and after subsequent ablation of the ipsilateral medial flagellum. The following results were obtained. First, the initial direction choice during searching changed dramatically from 100% correct to roughly random upon ablation of one lateral flagellum. This illustrates the importance of lateral flagellar input for direction choice at the start of a search. Secondly, the paths followed by ablated animals when searching for the source of the stimulus were significantly more random than those of normal lobsters. Thus, lobsters missing one lateral flagellum could no longer efficiently follow the changing stimulus concentrations within the odor cloud. However, circus movements were never seen in ablated animals. Thirdly, ablation did not impair the lobsters' ability to detect the presence of a stimulus: the alert time did not increase. After ablation of the ipsilateral medial flagellum, there was a significant decrease in alert time which has no obvious explanation. These data give behavioral support to the hypothesis that the receptor input from both lateral flagella (perhaps from the aesthetasc hairs themselves) are used for efficient orientation to a food odor.

All the poly(A)(+)mRNA sequence complexity also occurs in poly(A)(−)-mRNA in sea urchin embryos. Roger Duncan and Tom Humphreys.

Polysomal RNA was purified from *Lytechinus pictus* hatched blastulae, and fractionated into poly(A)(+) and poly(A)(−)RNA by multiple passages through an oligo d(T)-cellulose column. A cDNA probe was synthesized using the poly(A)(+)mRNA as the template and AMV reverse transcriptase. The extent to which the poly(A)(+)mRNA sequence complexity also occurs in poly(A)(−)mRNA was determined by hybridizing the cDNA to both RNA populations. The complete hybridization reaction of the cDNA to its template poly-(A)(+)mRNA spans about 3 decades on a log $R_o t$ axis, indicating that a range of mRNA abundancies exists, and terminates with greater than 80% of the cDNA in hybrid form. The poly(A) (−)RNA also hybridizes greater than 80% of the cDNA, indicating that all the poly(A)(+)mRNA sequence complexity is also present in mRNA molecules containing little or no poly(A). The cDNA-poly(A) (−)RNA hydrization kinetics show that the poly-(A)(−)mRNA molecules which are driving the reaction are represented in the polysomes at about the same frequency as the poly(A)(+)mRNAs. The poly(A) and oligo(A) length and content of both RNA preparations was examined by digestion with RNase followed by gel electrophoresis. Comparison of the relative amount of poly(A) in the poly(A)(+) and poly-(A)(−)RNA preparations showed that greater than 90% of the mRNA molecules driving the cDNA-poly(A)(−)RNA hybridization reaction lack a poly(A) segment longer than 20 nucleotides. We conclude that a specific set of mRNA sequences which are exclusively polyadenylated does not exist in sea urchin embryo mRNA, confirming a recent *in vitro* translation comparison.

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Studies on actin mRNA-complimentary genomic DNA sequences in the sea urchin, S. purpuratus. David S. Durica, Jeffery A. Schloss, and William R. Crain.

An actin gene-containing plasmid recently isolated from *Drosophila melanogaster* (E. Fyrberg and N. Davidson, personal communication) was tested for interspecific sequence homology to sea urchin (*S. purpuratus*) DNA. The actin coding region of the cloned *Drosophila* DNA cross-reacted significantly in Southern transfer experiments using several restriction enzymes, hybridizing to a limited number of discrete bands (4–8 depending on the enzyme). Solution hybridization followed by HAP chromatography also indicated significant cross-reaction, with reassociation kinetics suggesting a homogeneous component repeated less than 10 copies per genome. Sequences which specifically cross-reacted with the *Drosophila* probe were cloned into the bacterial plasmid vector pBR322. These clones fall into two size classes which correspond to genomic Hind III fragments of 3.6 and 7.0 kb. Both clones tested positive when assayed for the ability to selectively capture actin mRNA from a total mRNA population. Both plasmids are presently being characterized by restriction and heteroduplex mapping to determine regions of homology between the two sea urchin plasmids and the *Drosophila* clone.

D.S.D. is a fellow of the Muscular Dystrophy Association. This research was also supported by NIH Training Grant #TG-HD07098.

Studies of the translational regulation of histone synthesis in Arbacia punctulata. Christopher Earl and Tim Hunt.

Histone synthesis in the somatic and embryonic cells of a variety of organisms is coupled to DNA replication. Control is exerted at the levels of transcription and translation, but the molecular basis of the regulatory mechanisms is unknown. We decided to test the hypothesis that histones bind specifically to histone mRNAs and block their translation. We prepared histones and histone mRNA from hatching blastulae of *A. punctulata* and added both to reticulocyte lysates. We found that histones did indeed inhibit protein synthesis, but that this inhibition was not specific for histone mRNA.

Incubation of *A. punctulata* embryos in 1 mM hydroxyurea inhibits both DNA synthesis and histone synthesis within 30 min, as measured by incorporation of radiolabeled thymidine and methionine or lysine, respectively. We therefore examined the stability of translatable histone mRNA *in vitro*. We prepared cytoplasmic extracts from four hour blastulae, untreated or treated for 90 min with 1 mM hydroxyurea. After homogenization and centrifugation, the postmitochondrial supernatants were equilibrated with reticulocyte salts on a Sephadex G–50 column. We mixed 3 volumes extract with 1 volume reticulocyte lysate to boost *in vitro* translation. Histone synthesis was significantly reduced in the hydroxyurea extracts after treatments longer than 1 hr, but it is not clear whether this result is due to active degradation of the histone messages or to a halt in their transcription and an unaltered rate of nonspecific turnover.

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Temperature-dependent timing of mitosis and cleavage in Lytechinus variegatus. Andrew Eisen and Shinya Inoué.

At a constant temperature individual embryos of the sea urchin *Lytechinus variegatus* divide at intervals with precision far greater than apparent in a batch of sibling embryos. A series of events recur with the exact same interval for the first four divisions. Taking the completion of cleavage as unity, the events occur at the following fractions of the cleavage interval: nuclear envelope breakdown (0.45), spindle formation (0.50), metaphase (0.06), anaphase-A onset (0.71), anaphase-B onset (0.79), cleavage onset (0.85), cleavage 50% completed (0.93). The fractions remain constant over the full range of temperatures which permit development (17–33° C). Thus a plot of division events *vs.* time yields a straight line for a single embryo at a given temperature. All lines intercept the abscissa just as the "nuclear streak" appears. Between 17° C and 27° C the slopes increase with temperature with a Q_{10}

of *ca.* 2. While the division schedule suggests a continuously operating temperature-dependent "clock", such is not the case. When the temperature is raised from 17 to 27° C, the schedule immediately shifts to the new rate. However, when the temperature is dropped during the second division from 27 to 17° C, the rate does not immediately shift. Rather, the shift is variously delayed. The delay can be as great as 23 min when the embryo is chilled very early after cleavage. Before metaphase the delay progressively decreases as though cells enter the new schedule at around metaphase regardless of the stage at chilling. Thus portions of the cell-division cycle (*ca.* 0.0–0.65) appear insensitive to temperature drops (from 27–17° C) and their rates are pre-determined by the temperature at an earlier time.

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Population density, size distribution and home range of the American Eel (Anguilla rostrata) *in the Great Sippewissett salt marsh.* Tim Ford and Evan Mercer.

The American Eel is increasing in commercial importance, yet little demographic or behavorial information is available for eels in coastal and inland habitats. Local eel populations were studied by trap-recapture methods along 600 m of tidal creek in Great Sippewissett marsh, Falmouth, Massachusetts, during summer 1979. The creek system varied in bottom topography and sediment type and encompassed both high and low marsh. Conical minnow traps (Gee-Gee Co.) were used which captured eels larger than 15 cm (standard length); the largest eel trapped was 62.5 cm. Eels were fin-clipped, using a site specific code. Additionally, animals were measured, and body blemishes noted. Intersection of body length, blemishes or marks, and fin notches allowed individual eels to be distinguished. A total of 300 eels were marked and 67 individuals recaptured; some of these several times. Capture-recapture data was analysed with Jolly's model, which estimated a population of 350 eels in the 600-m creek system. Of this population small eels (less than 30 cm) dominated captures in the narrow, soft-bottomed creeks of the high marsh, and larger eels (greater than 30 cm) in the broader, soft mud to sandy bottomed creeks of the low marsh. Distances travelled by recaptured eels gave an indication of home range. Two eels dispersed over 200 m; the majority dispersed over much less distance (average 38 ± 54 m), which suggests a small home range. Little movement was observed for eels greater than 40 cm and the largest eels were commonly recaptured several times at specific sites. The results indicate large eels may establish territories in low marsh, restricting small eels to high marsh creeks.

Studies by fluorescence of protochlorophyll(ide) and its phototransformation in dark-grown Euglena gracilis *var.* bacillaris. Marcia A. Frey, Randall S. Alberte, and Jerome A. Schiff.

Intact cells show a fluorescence emission peak at about 635 to 638 nm from excitation of protochlorophyll(ide) (Pchl(ide)) 634; no emission peak is seen in the 650 to 660 nm region. Emission peaks at 712 and 730 nm, also due to Pchl(ide) excitation, may represent aggregates. Emission at 642 nm appears to be due to protophenophytin/phorbide (Ppheo) based on excitation spectra. On exposure to light, the 638 nm peak decreases and chlorophyll (Chl) and, perhaps, pheophytin **a** (Pheo **a**) emission appears, but the 712 and 730 nm emissions do not change. Acetone extracts transferred to diethyl ether show emission bands at: 630 nm (Pchl(ide): excitation maxima at 435, 535, and 571 nm); 590 nm (Mg protoporphyrin IX (monomethyl ester?): excitation maxima at 417 and 551 nm); 512 nm (phytofluene: excitation maxima at 333, 348, and 368 nm); and in the 642 nm region (Ppheo: excitation maxima at 417, 525, 565, 585, and 638 nm). Acidification and washing of the ether yields more Ppheo and less Pchl(ide) and phytofluene. Extracts of light exposed cells show similar spectra but Pchl(ide) is diminished and an emission at 673 nm appears due to a mixture of Chl and Pheo **a** based on excitation spectra. On acidification, this emission decreases and appears to be due solely to Pheo **a**. As found previously by absorption, Pchl(ide) 634 is present and phototransformable to Chl but Pchl(ide) 650 is undetectable. Ppheo is detected in intact dark-grown cells and is probably not an extraction artifact; Ppheo to Pchl(ide) ratios of about 10:1 were found previously by absorption spectroscopy of extracts. Since Pheo **a** is

found after light exposure, one possibility of many is a photoconversion of Ppheo to Pheo **a**. The detection of Mg protoporphyrin IX suggests a control point just after this compound in the biosynthetic pathway of Chl. Fluorescence has permitted the detection of phytofluene, a precursor of carotenoids, in these cells.

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Electron spin resonance studies of protein-methylglyoxal complexes and model systems. Peter R. C. Gascoyne.

The interaction of methylglyoxal with bovine serum albumin, collagen, lysozyme and casein has been studied using electron spin resonance spectroscopy. A radical signal centered at $g = 2.005$ accompanied the reaction of methylglyoxal with the proteins studied and this radical was found to be stable to drying. If the lysine residues in the protein were first blocked by dimethylation, however, no such radicals could be induced by methylglyoxal treatment, indicating that the lysine sidechains of the protein were involved in the radical formation. This observation of the primary importance of lysine is consistent with the spectroscopic findings of Pethig and McLaughlin (1979, *Biol. Bull.*, **157**: 388).

In order to further understand the interaction between methylglyoxal and the protein lysine sidechains, a simple model, methylamine, has been investigated using a computer controlled stop-flow technique. In this way the development of the various radical species accompanying the mixing of molar methylglyoxal and methylamine solutions has been followed from 0.01–100 sec. Under a nitrogen atmosphere a singlet centered at $g = 2.005$ appeared in about 2 seconds followed by the development in a few tens of seconds of a septet with 8.5 Gauss splitting, and later by other radicals. The addition of sodium ascorbate greatly enhanced and accelerated the formation of the observed radicals and the presence of at least one additional radical species was detected after 20 sec. This observation of the importance of ascorbate is in agreement with the suggestion of Szent-Györgyi (1979, *Biol. Bull.*, **157**: 398) that the ascorbate molecule tends to facilitate the process of molecular charge transfer in methylglyoxal-amine systems.

Is there tubulin in spirochaetes? Sharon Greenberg.

The theory of the exogenous origin of microtubules in eucaryotic cells is based on the hypothesis that an early symbiotic relationship between primitive procaryotes, specifically spirochaetes, and eucaryotic cells, led to the evolution of motile organelles of existing eucaryotic organisms. This theory predicts the existence of tubulin-like proteins in spirochaetes.

The presence of tubulin in spirochaetes was investigated using the free-living, facultatively aerobic, halophilic spirochaete, *Spirochaeta halophila*. Microtubule-like cytoplasmic fibrils have previously been demonstrated in this bacterium by electron microscopy. Cultures of *S. halophila* were analysed for tubulin-like proteins by comparison of migration patterns on two polyacrylamide gel systems of whole-cell spirochaete protein fractions with an axoneme-derived tubulin standard prepared from sea urchin sperm. On 7.5% Laemmli slab and tube gels, there were protein bands in the spirochaete sample which apparently comigrated with α- and β-tubulin in the axoneme standard. On Fairbanks gels, the axoneme-derived tubulin standard produced the predicted single band at 55,000 MW. The putative spirochaete tubulin, however, ran as multiple bands; in some cases lower than the axoneme tubulin standard.

It would seem that on the basis of the Laemmli gel system there are proteins in the spirochaete sample with mobilities similar to that of authentic tubulins. When the same sample is run on Fairbanks gels, however, the apparent homology between the proteins breaks down. A conclusive statement cannot be made at this time, based on the results obtained from polyacrylamide gel electrophoresis, concerning the presence of tubulin-like proteins in the spirochaete *S. halophila*.

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Structural changes associated with increased coupling resistance in the septate axon of the crayfish. Robert B. Hanna, George D. Pappas, and Michael V. L. Bennett.

Increased coupling resistance was brought about by mechanical injury to identified segments of the lateral septate axon in the ventral nerve cord of the crayfish. The axons were fixed at specific time intervals following injury and prepared for electron microscopy. Both freeze-fracture replicas and thin sections were utilized. The intramembranous particles of the control electrotonic junctions are 12.5 nm in width, have a center-to-center spacing of 20 nm, and are organized in a loose hexagonal array. Following injury, the intramembranous particles of the identified high resistance electrotonic junctions were found to lose their organization and become more widely spaced. In some experiments the particle spacing and organization remained unchanged for as long as 45 min following injury. Thin sections corroborated the findings from freeze-fracture replicas.

Following injury there is an increased density of the axoplasm and an increased number of cellular organelles in the uninjured axon proximal to the junction. Concomitant with the increased cytoplasmic density, there is a mobilization of the Schwann cells which become interposed between the injured and uninjured segments (Pappas, Asada, and Bennett, 1971, *J. Cell Biol.*, **49**: 173–188). The mobilized Schwann cells develop increased extent of anastomosing tubular networks, which is continuous with their plasma membrane. Eventually the junctional complex is apparently internalized in the axoplasm of the uninjured axon.

Absorption properties of Platymonas *sp. Rey 2 containing a high proportion of chlorophyll* **b**. B. A. Hayhome, Jerome A. Schiff, Robert R. L. Guillard, and Randall S. Alberte.

Platymonas sp. Rey 2, a marine green prasinophyte alga, recently obtained in axenic culture (from Venezuela), has 55% of its total chlorophyll (**a** + **b**) in the form of chlorophyll **b** as determined by absorption spectra *in vivo* and *in vitro*, by chromatographic separation of the pigments followed by spectrophotometric determination and by fluorescence excitation spectra of the whole cells. Absorption spectra of the intact cells show a greatly increased absorption in the chlorophyll **b** region (653 nm) and a sharper drop on the long wavelength side of the chlorophyll **a** peak compared with organisms having the usual levels of chlorophyll **b** (*e.g., Chlorella pyrenoidosa* Emerson strain) or low levels (*Euglena gracilis* var. *bacillaris*). The total absorption cross section in the red region of the spectrum, however, is very similar for all three types of organism. This suggests that some antenna chlorophyll **a** of system 2 of photosynthesis is replaced by increased chlorophyll **b** which maintains the effective cross section for absorption but moves the absorption region to somewhat shorter wavelengths in the red in these organisms; this may be advantageous in competition for light with organisms having normal chlorophyll **b** levels. Plating efficiencies of 100% have been achieved for this organism and the conditions for treatment with chemical mutagens have been worked out; chlorophyll-**b**-deficient mutants would be of help in learning more about the biosynthesis of chlorophyll **b** and its role in photosynthesis.

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The major protein in Spisula *sperm nuclei is a protamine.* Louis Herlands and Juan Ausio.

When SDS gels are used to analyze the 0.25 N HCl extractable proteins isolated from *Spisula solidissima* sperm nuclei, a histone pattern similar to that of somatic cells is found. But in comparison with *Spisula* embryos there are few other proteins present, presumably because of the lack of contaminating cytoplasm and ribosomes in the sperm system. This

observation has led others to believe that *Spisula* is a typical histone-type sperm that would be a good model system for studying both nucleosome structure and the fate of sperm nuclear proteins after fertilization. However, when the same proteins are run on HOAc/urea gels, we see a new band which corresponds to an unusual protamine-like protein which is insoluble in SDS. More than 80% of its amino acid residues are Lys, Arg, Ser, or Ala. This new band is the major component; only small amounts of histones are present. These histones most likely come from immature sperm since first, there are several acetylated forms of H4; and secondly, gel scans indicate that the amount of histone in relation to protamine is variable. Digestion of *Spisula* sperm nuclei with micrococcal nuclease indicates that *Spisula* sperm chromatin is primarily *not* in the nucleosome configuration. Only small amounts of 140 BP protected DNA fragments are generated at early digestion times. The amount of this particle does *not* increase as digestion proceeds. The origin of this particle may either be immature sperm or perhaps a subset of chromatin in the mature sperm that is still organized with histones. Even though *Spisula* sperm contains predominantly protamine, an extremely stable PCA soluble limit, similar to somatic chromatins, is reached. But unlike chromatin, the DNA fragments are heterogeneous in size.

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Light-harvesting pigment-protein complexes from brown algae and diatoms: implications for the organization of the photosynthetic unit. D. L. Gustafson, A. L. Friedman, M. S. Rudnick, H. Lyman, and R. S. Alberte.

The pigment-protein complexes of three macrophytic brown algae, *Laminaria saccharina*, *Chorda filum* and *Pylaiella littoralis* and the diatom *Skeletonema costatum* were examined with respect to the organization of the photosynthetic unit (PSU) by fluorescence emission and excitation analysis and polyacrylamide gel electrophoresis of membrane detergent extracts. The fluorescence characterization of whole thalli, chloroplast membrane fragments, detergent solubilized thylakoids and gel electrophoretic zones were used to determine the paths of energy transfer among the light-harvesting pigment of the PSU. Pigment composition, pigment-protein biochemistry and physiological data support two paths of energy transfer. One path involves the coupled transfer of excitation energy from chlorophyll (Chl) **c** to Chl **a**, while the other involves energy transfer from the carotenoid fucoxanthin to Chl **a** and not to Chl **c**. Polyacrylamide gel electrophoresis of SDS solubilized thylakoid membranes of these algae results in two major pigment-protein zones. The slowest migrating zone shows fluorescence and absorption characteristics of a pigment-protein containing Chl **a** and Chl **c** with energy transfer from Chl **c** to Chl **a**. The other major zone shows spectral characteristics of a pigment-protein complex containing Chl **a** and fucoxanthin with energy transfer from fucoxanthin to Chl **a**. Physiological data obtained during nitrogen starvation of the diatom *Skeletonema* demonstrates a specific loss in fucoxanthin and Chl **a**. Accompanying these pigment losses are decreases in PSU size and a reduction in photosynthetic rates. In contrast, supraoptimal nitrogen levels lead to a cellular increase in these pigments and the pigment-protein complexes. The increase in pigment-protein may represent a nitrogen storage mechanism for these cells, and as such implies an opportuntistic strategy for their adaptation to fluctuating nutrient levels.

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Water relations and photosynthetic characteristics of the tall and short ecophenes of Spartina alterniflora. Arthur M. B. Hogan, David Mauzerall, and Randall S. Alberte.

The tall ecophene of *Spartina alterniflora* in Sippiwissett Marsh is inundated for the greatest part of the tidal cycle while the short ecophene is infrequently inundated. The present

investigation sought to account for the morphological differences by examining the water relations, morphological features, protein content and photosynthetic characteristics of the two ecophenes. All parameters were replicated at least ten times and data was significant to at least the 95% level. It was found that the short form was more succulent (41% greater) and heavier (18% greater) than the tall form. Both forms had similar leaf widths. The relative water content of the short form was lowest at midday during a high tide cycle and hence the short form was more water stressed. At pre-dawn the short form recovered to hydration levels similar to the tall form. Protein content per unit leaf area was 34% higher and 20% higher on a dry weight basis in the short form, strongly suggesting that nitrogen is not limiting growth of this ecophene. Leaf chlorophyll (Chl) content is similar ($2.9–3.2 \times 10^{-4}$ mg/mm^2) in the two forms when expressed per unit leaf area but 15% greater in the tall form when expressed on a dry weight basis. The Chl **a**:**b** ratios for both forms were 3.1 ± 0.1. Photosynthetic rates of the short form measured by oxygen evolution were 26% greater when expressed on either a Chl or an area basis than the tall form. Furthermore the photosynthetic unit (PSU) size in the short form was about 40% smaller than in the tall form. Based on the differences in leaf thickness, the short form possessed about 30% more PSUs per unit area as a result of the greater amount of tissue per unit surface area. The greater numbers of PSUs per unit area fully explains the higher photosynthetic rates per area in this form. The alterations in the packaging of the photosynthetic machinery probably represents an adaptive response to a more stressful environment of higher salinity and lower water availability. In addition, photosynthetically competent protoplasts were isolated in good yield from both ecophenes which will allow for further detailed examinations of these ecophenes.

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Brush border calmodulin: A structural protein of the microvillus core. Christine L. Howe and Mark S. Mooseker.

We are investigating the molecular basis for calcium regulation of microvillar motility in the brush border of intestinal epithelial cells. Results indicate that calmodulin is present in brush borders isolated from chicken, and that it is a major constituent of the microvillar filament bundle. Calmodulin is prepared by boiling suspensions of either Triton-treated brush borders or demembranated microvilli. No calmodulin is detected in the solubilized brush-border membrane fraction. The supernate derived from boiled microvilli contains one major polypeptide identified as calmodulin by first, comigration with brain calmodulin on SDS gels and on alkaline urea gels, where both proteins undergo a shift in mobility with Ca^{2+} present; and secondly, 4-fold activation of phosphodiesterase in the presence but not absence of calcium. The molar ratio of calmodulin to actin is approximately 1 : 10 in the intact brush border and increases to 1 : 3 in the isolated microvillus, indicating that calmodulin is predominantly localized in this structure. Calmodulin remains tightly associated with the microvillus core in the presence or absence of calcium. Furthermore, calmodulin does not bind to muscle F-actin *in vitro,* indicating that the binding of calmodulin to the microvillus core may involve protein(s) other than actin. These results suggest an intimate association between the microvillar actin filament bundle and the regulatory functions of this multifaceted protein in the brush border. Examples may include regulation of actomyosin interactions responsible for microvillar movement and calcium transport.

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Fairy rings: membrane particle arrays present during early stages of de novo *ciliogenesis in* Tetrahymena. L. A. Hufnagel.

During a study of membrane-cytoskeletal interactions related to morphogenesis in *Tetrahymena,* freeze-fracture EM was used to characterize membrane structural changes preceding formation of somatic cilia in log phase cells and oral cilia in cells synchronized for oral replacement. During early neogenesis of somatic cilia, a ring of irregularly spaced 100 to 130 Å particles was observed in the E-face of the plasma membrane. The diameter of the ring was

similar to that of cross-fractured ciliary axonemes, and a central group of 2–9 100 to 130 Å particles usually was seen within the ring. Ring particles numbered from 20 to 90, with an inverse relationship to the number of central particles. The rings resembled mushroom rings found in lawns after rainstorms; hence the name "fairy rings." Unlike the E-face, the P-face of the plasma membrane was evenly studded with small particles, with no apparent arrangement. Fairy rings were associated with specializations in membrane topography. Based on the observations, a tentative sequence for ring formation, origin of the ciliary necklace (a membrane specialization of the mature cilium) and concurrent topographical changes has been developed. According to this sequence, the central particles and parasomal sac (a basal body-associated plasma membrane invagination) appear earliest and may be intimately involved in positioning of basal bodies, while the fairy ring develops later, perhaps under the influence of the basal body. The fairy ring then migrates into the basal region of the nascent cilium, to become the ciliary necklace. These ideas are currently under further investigation by means of TEM. In contrast with these observations, fairy rings have not been observed during neogenesis of oral cilia. This supports the idea that the rings do not assist in the positioning of basal bodies and also suggests that they are not necessary for growth of cilia.

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Structure of aggregatoin factor on sponge cells and in gels. Susie Humphreys and T. S. Reese.

Aggregation factor has been visualized on aggregating *Microciona prolifera* cells and in factor gels by electronmicroscopy of specimens rapidly frozen and then either freeze-substituted and thin-sectioned, for freeze-fractured, deep-etched, and replicated without chemical fixation or cryoprotectant. Each factor macromolecule, a glycoprotein (MW 18×10^6 daltons), is a ring from which radiate several side chains which give it a "sunburst" shape which is well preserved by our freezing methods. Thus, the relationships of the factor molecules with each other and the cell surfaces of aggregating cells can be directly visualized. Preliminary examination of factor alone gelled in 10 mM $CaCl_2$ showed many images suggestive that factor retains its sunburst configuration in gels and that contact between side arms is the rule. Chemical dissociation of sponge tissue by divalent cation depletion extracts aggregation factor but cells aggregate rapidly when divalent cations and aggregation factor are restored (T. Humphreys). Replicas of cells aggregated in factor showed factor molecules over much of their surfaces, suggesting that factor "receptors" may be evenly distributed over the whole cell surface. In sections of reaggregating cells we could sometimes see the circular backbone sandwiched between two cells; some side chains contacted one cell and some from the same molecule contacted an adjacent cell. Our results suggest that cell-recognition sites are localized in the factor side chains and that the entire connection between cells could be made by single macromolecule bridges. Most significantly, our results demonstrate that factor could function by actually forming bridges between cells.

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Tubulin antibody induces microtubule depolymerization in vivo *and* in vitro. Shinya Inoué, Keigi Fujiwara, and Elaine D. Papafrangos.

Antibodies can be used for cytochemically localizing antigens or for functionally blocking reactions involving the antigen. We now find that a tubulin antibody (Anti-TL) can also depolymerize labile microtubules. The antibody was prepared against tubulin paracrystals induced in sea urchin *Strongyrocentrotus purpuratus* eggs by vinblastine. Upon microinjection of *ca.* 0.4 nanoliters of Anti-TL solution (containing *ca.* 150 mg/ml of immunoglobulin), the meiotic metaphase arrested spindle in the oocyte of *Chaetopterus pergamentaceous,* and the mitotic cleavage spindles and asters in *Lytechinus variegatus,* substantially lose their birefringence and grow smaller in minutes. The reaction resembles cold or colchicine treatment. Spindle birefringence and size are not appreciably reduced by injection of 100 mM KCl, buffer solution alone (100 mM KCl, 10 mM phosphate buffer pH 6.9, 0.02% Na-azide),

or pre-immune IgG in buffer; if the birefringence decreases it recovers, although some cells injected with large volumes of IgG (with or without Anti-TL) blebbed extensively and lysed after approximately half an hour. Mouse and embryonic chick brain microtubules assembling *in vitro* are also depolymerized by Anti-TL, but again not by pre-immune IgG or buffer alone. The rise of specific viscosity ceases and decays exponentially as soon as Anti-TL is added to the polymerizing solution (2.5–3 mg/ml tubulin, 0.1 M PIPES, 1 mM DTT, 1 mM EGTA, 0.5 mM $MgCl_2$, 0.1 mM EDTA, 4 M glycerol). We do not yet know whether the Anti-TL depolymerizes microtubules by shifting the microtubule-subunit equilibrium, or by directly attacking the microtubules themselves.

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Field and laboratory observations of lobster mating behavior. Elisa Karnofsky and Jelle Atema.

Courtships and mating behavior of the lobster (*Homarus americanus*) have been observed in our laboratory in large naturalistic aquaria. Field observations are now being made to compare laboratory observations to behavior in the natural environment. In the laboratory, dominant males establish stable residences and regularly displace other individuals from their burrows. There is female mate selection: females approach the dominant male burrow 1 to 5 days premolt, exhibiting ritualized behavior as do males during these entrance ceremonies. The female may leave the burrow periodically, chasing other lobsters from the area. This may be part of a generally observed premolt aggression peak. The male often lunges at the opening, preventing the female from leaving. Approximately 20 min after the female molts, the male mates with her. He then feeds on and later rejects the molt shell. Cohabitation without leaving continues for 1 to 5 days. Females may leave for short excursions before leaving permanently. Mating in the field was observed by direct and indirect clues. Two lobsters were discovered in a large burrow. The male had previously been tagged and the female had no crusher claw. This couple was observed for 8 days during which the female molted as indicated by a regenerated crusher; also, a molted seizer claw was found outside the shelter. Male entrance ceremonies were performed, as was blocking of the entrance. On the 8th day the female disappeared. The same day a tagged female appeared and cohabited for 7 days. She molted between the 1st and 3rd day. During this time the male was seen evicting another male from a nearby burrow.

This study shows that observations in naturalistic seetings contribute continuity to scant and difficult-to-obtain field observations, while field studies modify and add reality to still rather artificial laboratory observations in large aquaria.

Properties of polymerizable tubulin from isolated Spisula *spindles.* Thomas C. S. Keller III and Lionel I. Rebhun.

Spindles were isolated from activated eggs of the surf clam *Spisula solidissima* in a buffer containing 3.4 M glycerol, 100 mM PIPES, 1 mM $MgCl_2$, 5 mM EGTA, 1% NP-40 pH 6.8. Tubulin was recovered from the isolated spindles by washing them free of the glycerol into a buffer containing 100 mM PIPES, 1 mM $MgCl_2$, 5 mM EGTA pH 6.8 and placing them on ice until birefringence disappeared. The nonbirefringent spindle remnants were pelleted leaving the cold depolymerized spindle tubulin in the supernatant. Upon addition of GTP (to 1 mM) and warming to 24 or 37° C, the spindle tubulin polymerized into microtubules detectable by an increase in light scattering and by the appearance of birefringent strands. The reassembled spindle tubulin was further purified through two additional cycles of temperature dependent assembly-disassembly at either 24 or 37° C. After three cycles, the spindle tubulin cycled at 37° C contained predominantly tubulin, small amounts of a high molecular weight component (which differed in mobility from *Spisula* axoneme dyneins and bovine brain HMW's) and a doublet approximately 33,000 daltons. Tubulin cycled at 24° C contained at least 3 additional proteins normally lost at 37° C. Polymerization of the cycle-purified spindle tubulin was both concentration- and temperature-dependent with critical

concentrations of 0.09 mg/ml at 37° C and 0.24 mg/ml at 24° C. To determine whether the associated proteins were necessary for polymerization, they were removed by DEAE-cellulose and phosphocellulose chromatography. The resulting purified tubulin polymerized with critical concentrations of 0.11 mg/ml at 37° C and 0.28 mg/ml at 24° C, not significantly different from those obtained with cycle-purified tubulin containing associated proteins. Thus *Spisula* spindle tubulin polymerizes at physiological temperatures (24° C) and the extent of this polymerization is independent of associated proteins.

Preparation and characterization of phagosome membranes from Arbacia punctulata *embryo cells.* David Kew, Christine Kaznowski, and Jay Brown.

Phagosomes from dissociated *Arbacia* embryos at eight-cell and hatched blastula stage were characterized by scanning and transmission electron microscopy, by enzyme assays, and polyacrylamide gel electrophoresis. Phagosomes were isolated after allowing the single cells to phagocytize latex beads.

Cysteine/papain treated eggs were grown to eight-cell stage for dissociation in Ca^{2+}, Mg^{2+}-free sea water. Untreated eggs were grown to hatched blastula for dissociation—Ca^{2+}-free sea water washes, then shearing in sucrose/citrate/EDTA. Washed cells were incubated with 0.7 micron latex beads—3 to 4 hr for eight-cell stage, and 6 to 8 hr for blastula. Washed cells were lysed in hypotonic buffer and lightly homogenized. Phagosomes containing beads were isolated on a sucrose step gradient spun at $100{,}000 \times g$ for 90 min, at the 15 to 20% interface. Washed phagosomes contained an average of 0.6% of the homogenate protein. SEM series show progressive development of bead-size lumps in the blastula cells, and TEM sections show beads deep in the cells. Lactoperoxidase-catalyzed ^{125}I-labeling showed some major different proteins labeled on living cells compared to washed phagosomes, when the proteins were separated by PAGE on Matsudara gels. Membrane enzymes assayed were: cytochrome c oxidase for mitochondria, NADH diaphorase for smooth ER, acid phosphatase for lysosomes, 5′nucleotidase and phosphodiesterase for plasma membrane. The non-plasma membrane markers were greatly reduced in the phagosomes relative to the homogenate, except acid phosphatase was slightly increased in the blastula cells. This increase is probably due to phagosome-lysosome fusion after the long incubation. 5′nucleotidase was assayed for eight-cell stage, at 7-fold phagosome enrichment. Phosphodiesterase was assayed at blastula stage, at 10-fold phagosome enrichment. The method shows a good yield and purification of membrane recently phagocytosed. It offers an opportunity to investigate properties and changes in a developing system, and as a non-developmental system to investigate transport, turnover, or pharmacological effects.

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Adaptation of bacteria to rapidly changing environmental conditions. David Kirchman and Ralph Mitchell.

We used tidal flow in the Great Sippewissett salt marsh, Massachusetts, as a model system to determine the response of bacteria to rapidly changing environmental conditions. The number of bacteria was measured over two tidal cycles using the acridine orange direct count method (AODC). The total number of free bacteria was approximately 2.0×10^6/ml at low tide. As the tide came in, total numbers decreased to a minimum at high tide of 1.0×10^6/ml. The number of free bacteria increased again to approximately 2.0×10^6/ml as the tide left the marsh. We measured growth rates of bacteria from Buzzards Bay and from the marsh at low tide, in filtered sterilized (0.22 μm Millipore) water from Buzzards Bay and from the marsh. Growth rates were determined by measuring changes in bacterial numbers with AODC over 10 hr in 300-ml BOD bottles incubated in the marsh. Marsh bacteria grew in Buzzards Bay water at lower growth rates than in marsh water. Minimal growth of Buzzards Bay bacteria in Buzzards Bay water was detected in the 10 hr experiment. Bacteria from Buzzards Bay did grow in marsh water, but this growth does not appear to be fast enough to account for the observed changes in bacterial numbers over a tidal cycle at Great Sippewissett. While bacterial numbers doubled in 6 hr from high to low tide,

significant growth by bacteria from Buzzards Bay in marsh water was not evident until the eighth hour. The fate of marsh bacteria carried into Buzzards Bay is unclear over a longer time scale. Bacteria from Buzzards Bay do not appear to adapt to the changing environmental conditions in the marsh rapidly enough to account for the pattern in bacterial numbers over a tidal cycle. A fresh inoculum of bacteria from the sediment at each tidal cycle may explain the large population at low tide.

This work was supported in part from NOAA sea grant to Harvard University NA 79-AA-00091.

How photoisomerizable azobenzene compounds affect acetylcholine receptors of skate muscle. M. Krouse, H. Lester, and M. Weinstock.

Previously, we have studied the effects of azobenzene compounds on *Electrophorus* electroplaques. This work extends those investigations to the skate, *Raja erinacea*. Photoisomerizable drugs were applied to the depressor rostri muscle. Effects of these drugs on endplate potentials and endplate currents (recorded under voltage clamp) were examined. *Trans*-Bis-Q is a potent agonist in the eel electroplaque ($K_D = 150$ nM at -150 mV). A 30 μM solution of Bis-Q containing predominantly the inactive *cis* isomer was added to the voltage clamped muscle. Then, a light flash increased the concentration of the *trans* isomer from about 5 to 11 μM and thus induced a net inward current of 15 nA. This increased inward current was, however, transient even though both isomers are stable in the dark. Ten μM 2BQ, a competitive antagonist (in its *cis* isomer) on the eel electroplaques ($K_I = 150$ nM) reduced the amplitude of the endplate current to about 25% of its control value. This effect was seen with both the *cis*- and *trans*-configurations of 2BQ. Ten μM benzyl Bis-Q inhibits the response to carbachol in the eel but in the skate muscle the effect is to increase the amplitude of the endplate potential and to prolong its decay, suggesting that it is a cholinesterase inhibitor. A small antagonist effect would not have been seen. EW-1, a local anesthetic (in its *cis* isomer) on the eel electroplaque ($K_I = 25$ μM) has no effect on endplate currents with concentrations as high as 100 μM. While there are differences between eel electroplaque and skate muscle in regard to drug action and potency, the observations reported here serve to demonstrate that photoisomerizable compounds may be profitably used on preparations other than *Electrophorus*.

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Characteristics of the photosynthetic unit in macrophytic red algae. T. A. Kursar, D. Mauzerall, and R. S. Alberte.

Phycobilisomes were isolated from field-collected *Griffithsia* sp. and *Champia parvula*. Excitation of intact phycobilisomes with 500 nm light results in a small fluorescence emission at 576 nm from phycoerythrin and a large peak at 672 nm. *Griffithsia* sp. phycobilisomes in 0.65 M Sorensen's buffer have an uncorrected sedimentation coefficient of 83S.

The *in vivo* fluorescence of wild type *Gracilaria tikvahiae* and an orange mutant (P-7-1, obtained from J. van der Meer, NRC, Halifax, Nova Scotia) was also measured. Excitation of chlorophyll **a** with 430 nm light resulted in emission at 696 nm in both wild type and the mutant. Excitation of phycoerythrin at 550 nm in the wild type resulted in phycoerythrin emission at 577 nm, an unidentified emission at 644 nm, and a prominent shoulder at 683 nm, whereas the mutant (P-7-1) showed a decreased 644 nm emission, almost no 683 nm emission and an emission band at 700 nm. The excitation spectrum of the 690 nm emission includes phycoerythrin and a large contribution from phycocyanin and allophycocyanin in the wild type, whereas for P-7-1 the 690 nm fluorescence is principally excited by phycoerythrin with a small contribution from phycocyanin and no apparent contribution from allophycocyanin. The chlorophyll content of the wild type and P-7-1 are the same, but P-7-1 is low in carotenoids. The growth rate of P-7-1 is about half that of the wild type. Our preliminary conclusion is that the light harvesting system of photosystem I is normal in P-7-1 and that the lesion

has altered the phycobilisome or the light harvesting chlorophylls and carotenoids associated with photosystem II (or both). These results also suggest that in P-7-1 excitation energy transfer may go directly from phycocyanin to chlorophyll a and bypass allophycocyanin.

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Slow changes in the magnitude of the potassium current associated with changes in the internal perfusion solution in squid axons. David Landowne and Virginia Scruggs.

Experimentally we find it difficult to alter the internal potassium concentration in internally perfused squid giant axons. Apparently there is no inert replacement monovalent cation which does not iself reduce the potassium current. When we reduced the internal potassium, replacing it with sucrose, we find the magnitude of the potassium current declines in two phases. There is a rapid decline during the first 1 to 2 min which corresponds with the time required to change the internal solution. This is followed by a slow decline over tens of minutes. This is reversible, on switching back to a high potassium solution the current increases in two phases. The relative proportion of the two phases varies. We think this is asociated with damage to the interior of the axon, with less damage favoring more slow phase. Prolonged treatment with pronase removes the slow phase.

From these current measurements it seems that the residual axoplasmic lattice retains some of the potassium and only slowly equilibrates with the perfusion fluid when no cation is provided to exchange for potassium. It will similarly retain sodium or TMA. When the internal solution was changed from one containing sodium and potassium to one containing potassium only, and with sugar replacing sodium, the current-voltage curve retained the negative conductance region associated with the presence of sodium. A 1-min rinse with high potassium followed by return to low potassium removed the negative conductance.

Phosphocellulose purification of dogfish and skate brain tubulin. George M. Langford, Lascelles E. Lyn-Cook, and Daniel Robbins.

Dogfish shark and skate brain tubulins were purified first by two cycles of a temperature dependent assembly-disassembly procedure and then by phosphocellulose (PC) column chromatography. The cycled tubulins were capable of spontaneous assembly but the phosphocellulose purified tubulins were not. The cycled tubulins, nevertheless, contained no high molecular weight, microtubule associated proteins (MAPs). The high molecular weight proteins present in the supernatant and pellet fractions obtained after each of the assembly-disassembly steps were determined by sodium dodecyl sulfate, polyacrylamide gel electrophoresis (SDS-PAGE). The gels of the initial extract show two major high molecular weight protein bands of appoximately 280,000 and 320,000 daltons. At least two minor bands in the molecular weight range of 300,000 daltons could also be seen. One of the two major proteins, the 280,000 daltons protein, is lost in the first cold pellet. The 320,000 dalton protein which co-migrates with ciliary dynein 1, co-purifies with tubulin through the largest number of steps. Sometimes it is retained after completion of two cycles of purification but most often it too is lost in an earlier step. The twice cycled tubulins free of high molecular weight proteins were put on a phosphocellulose column. Because phosphocellulose sequesters Mg^{2+}, this ion was immediately added to each of the column fractions. The fractions containing tubulin were combined and concentrated by a Millipore immersible ultrafilter. Phosphocellulose tubulin, in the range of 0.5 to 1.0 mg/ml, did not spontaneously assemble. When mixed with cycled tubulin, PC tubulin was incorporated into microtubules after a lag time of 1 min. Electron microscopy revealed that the tubules formed from a mixture of PC and cycled tubulins were often defective. Incomplete closure of tubules resulting in regions that appear as flat ribbons of protofilaments were often seen. These data show that high molecular weight MAPs do not cycle with the fraction of tubulins obtained by temperature cycling. Since PC

tubulins do not spontaneously initiate assembly, there may be MAP fragments or lower molecular weigh proteins required for initiation of polymerization.

Tidal rhythm and tissue organization in the neural gland of Ciona intestinalis: *correlates with cellulose and fibronectin.* James W. Lash, Michael Ovadia, Charles H. Parker, and Clive N. Svendsen.

Rarely have tidal rhythms been associated with specific tissue changes. It is more common that such rhythms are expressed as behavioral changes. In the ascidian *Ciona intestinalis* tidal rhythms have been found to be correlated with specific alterations in the neural ganglion and gland. At high tide the ganglion secretes PAS positive granules, but not at low tide. At low tide the gland is organized as a parenchyma and secretes an Alcian Blue positive substance. At high tide the neural gland undergoes a marked reorganization, and the gland cells now adhere to newly appearing PAS and Alcian Blue positive fibers. The function of the gland is poorly understood and the nature of the secretory products is not known. Ganglion/gland complexes isolated from the animal undergo complete transition from low tide phase to that of high tide, but not the reverse. In an attempt to mimic the gravitational forces thought responsible for these changes, intact animals were centrifuged for one tidal interval at $1.5 \times g$. This permitted the change from low tide phase to that of high tide, but maintained the high tide phase. Various enzymes were used to obtain evidence of the constituents of the ganglion/gland complex. Hyaluronidase digested the ganglion secretory granules. Cellulase removed most of the fibers in the gland at high tide phase. Using human and hamster fibronectin antiserum and fluorescein conjugated IgG, fluorescence was seen primarily in the neural gland, coating the fibers at high tide and generally distributed around the gland cells at low tide. Thus the extra-cellular matrix of the gland appears to contain cellulose and fibronectin.

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The growth and reproduction of selected species of meiofauna in selected natural microfloral assemblages. John J. Lee and Monica J. Lee.

Previous research has shown that various species of meiofauna are highly selective in their feeding habits and that when incubated in gnotobiotic cultures with different species of algae and bacteria their growth and fecundity vary greatly. Sometimes two or more species are needed to satisfy the nutritional requirements of an animal. Enigmatically most of the meiofaunal species studied gnotobiotically are most fecund on species with low natural abundances ($\leq 3\%$). This raises the question of whether littoral benthic microbial assemblages are consumer controlled or whether they selectively control meiofaunal abundances and fecundity. Experiments carried out this summer in both the field and the laboratory provided more data on this important question.

Natural microfloral assemblages were gently sieved (38 μm) to remove all larger organisms and meiofauna. After the sieved mixtures were examined and the juveniles, nauplii, larvae, ciliates, etc. were picked out with the aid of small capillary pipettes, the mixtures were used as inocula for growth experiments. Selected meiofaunal species were inoculated into tissue culture flasks with the "natural mixtures" and incubated *in situ*. The tissue culture flasks had nylon filter (3.0 μm) covered windows cut into their surfaces which allowed free passage of sea water while retaining the algal assemblages. Vessels in assemblages without meiofauna and vessels incubated in the laboratory under constant environmental conditions served as controls.

Allogromia laticollaris, a foraminiferan, steadily increased in all the natural assemblages into which it was introduced. Populations of *Chromadorina germanica*, a nematode, reproduced vigorously and then crashed without recovery in one set of experiments and failed to reproduce in a mixture incubated later in the summer. *Rhabditis marina*, another nematode, failed to reproduce in either experiment. *Nitocra typica*, an harpacticoid copepod, reproduced after a lag of 3 weeks and maintained a high population for 7 weeks. Permanent preparations were made

of the diatom assemblages of the control and experimental vessels. Studies of these preparations are now in progress.

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The photosensitizing action of psoralens in dogfish ocular tissues. S. Lerman, J. M. Megaw, and Y. Takei.

The photosensitizing action of 8-methoxypsoralen (8-MOP) has led to increasing concern regarding potential ocular complications in patients treated with this drug. Free 8-MOP can be demonstrated in the dogfish ocular lens and retina for 24 hr following an intraperitoneal injection of 1 mg of this drug. The 8-MOP can be detected in the lens by phosphorescence spectroscopy and labelled 8-MOP (25–50 μCi ^{3}H or 15 μCi ^{14}C) by autoradiography. When the fish is kept in the dark, the free 8-MOP diffuses out of these tissues after 24 hr. However, photic stimulation by exposure to G.E. BLB fluorescent light (mainly 365-nm radiation) for 1 to 15 days, results in the formation of photoproducts involving lens proteins and 8-MOP. The extracted and purified soluble protein fractions (after repeated TCA washing) had a significant activity (both for ^{3}H and ^{14}C) compared with background levels in proteins from controls kept in the dark. Autoradiographic studies were also positive in the tissues obtained from UV-exposed fish. These data correlate well with phosphorescence, EPR, and NMR experiments which demonstrate at least one photoproduct in the soluble lens protein fraction (*in vitro* and *in vivo*) following exposure to UV radiation longer than 320 nm. We have shown that the tryptophan 8-MOP photoreaction requires O_2, in contrast with the cyclobutane photoproducts formed between 8-MOP and thymine, which are not O_2 dependent. Singlet O_2 plays a role in the 8-MOP-tryptophan photoreaction. Because the ocular lens is a completely encapsulated organ which grows throughout life and never sheds any of its cells, the photobinding of 8-MOP and lens proteins (as well as with DNA) will result in the permanent retention of new photosensitizers within this organ. This could enhance the UV-induced changes already occurring in the normal aging lens and might even have a cataractogenic potential. Furthermore, the occular lens normally acts a a UV filter, preventing UV radiation (longer than 300 nm) from damaging the retina. Thus the presence of 8-MOP in the retinas of aphakic patients could also prove hazardous.

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Possible mechanisms for inhibition of cellular activity by dyes with highly negative reduction potentials. Deborah Lipman and Seymour Zigman.

Cyanine (methine) dyes with reduction potentials (E_R) more negative than -1.0 V have been found by us to inhibit mitosis in sea urchin embryos at 10^{-5} to 10^{-6} M. Chemically similar dyes wih E_R values less than -1.0 V were ineffective. The motility of the dynoflagellate *Gymnodinium breve* was also eliminated by dyes with E_R values more negative than -1.0 V, but not with E_R values more positive (calomel electrode). Consideration of the possible mechanisms behind these varied effects and also the inhibitory effects on the synthesis of DNA and protein in sea urchin eggs by these dyes led to a hypothesis that the dyes with highly negative E_R's interfere with cellular electron transport in oxidative metabolism and with energy production. The highly negative potentials (*i.e.*, greater than -1.22 V using the hydrogen electrode) of these dyes compared to those of NAD, FAD, and the cytochrome c half cells (all considerably more positive) would predict an action due to electronic saturation. Observations of the ability of sea urchin eggs (*Arbacia punctulata*) to divide, and measurements of their ATP content (measured by the firefly tail assay) after treatment with dyes having highly negative E_R's indicated that no major reduction in ATP content resulted. These dyes may alter electron transport reactions involved in other phases of cellular oxidative metabolism, for example cytochrome c. Thus the inhibition of growth and reduction of activity of cells due to their exposure to dyes with highly negative E_R's may not result from interference with energy metabolism, but could result from electronic saturation of the components of cellular respiration with other consequences.

(Dyes supplied by Eastman-Kodak Co.)

Presynaptic calcium current and postsynaptic response generated by a presynaptic action potential; a voltage clamp study in the squid giant synapse. R. Llinás, M. Sugimori, and S. Simon.

The present study was designed to characterize the presynaptic calcium current (Pre I_{Ca}) and its relation to postsynaptic potential (PSP) and current (PSI) during a presynaptic action potential. This was accomplished by driving the command amplifier of the presynaptic voltage clamp circuit with a prerecorded spike. In addition, the experiments would test whether the properties of the Pre I_{Ca} predicted for a presynaptic spike by our model (Llinás *et al.*, 1976, *Proc. Nat. Acad. Sci.*, **73**: 2918) could be reproduced experimentally.

In the present study the waveform of a presynaptic action potential was recorded prior to the application of tetrodotoxin tetraethylammonium and 3-aminopyridine. Following addition of these agents, voltage clamp conditions at −70 mV holding potential were simultaneously set (via two independent circuits) to both pre- and postsynaptic elements in the junction in order to measure I_{Ca} and PSI (Llinás and Sugimori, 1978, *Biol. Bull.*, **155**: 454). The Pre I_{Ca} measured during the spike-like presynaptic voltage clamp depolarization started near the peak of the presynaptic "spike", and its maximum amplitude had a sigmoidal dependence on the amplitude of the presynaptic voltage transient. Both peak and total postsynaptic current and peak PSP were linearly related to peak or total presynaptic I_{Ca}. This study confirmed the linear relationship between presynaptic calcium and postsynaptic response. Furthermore, the time course and magnitude for I_{Ca} coincided with those predicted by the model and with the conclusion that synaptic transmission is mainly produced by an "off" calcium current (*i.e.*, generated at the falling phase of the action potential).

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Stimulation of testosterone production by mouse Leydig cells with factors isolated from a microorganism and Ovalipes ocellatus. Takeshi Mauro, Amy R. Segal, and S. S. Koide.

During the course of determining choriogonadotropin(CG)-like substance in tissues of marine organisms, we have demonstrated that a trypsin-like protease present in the crab (*Ovalipes ocellatus*) stomach mimics CG in the radiommunossay (RIA) and the radioreceptor-assay (RRA) systems and is capable of stimulating rat ovarian adenylate cyclase activity *in vitro*.

In this communication evidence will be presented to show that a CG-like factor is produced by a micoorganism. Extract of an acetone-dried preparation of a culture content of a microorganism tentatively named as "*Progenitor crytocides*" was prepared. The extract contained a CG-like factor as determined by RIA with the antiserum to hCGβ-COOH-terminal peptide and RRA using bovine corpus luteum membranes. The CG-like factor was purified by chromatography on Sephadex G-100, Concanavalin A-Sepharose, and DEAE-Sephadex A-50. Interference by proteases in the extract was excluded. The bacterial factor was adsorbed on ConA-Sepharose, suggesting that it contains glucose or mannose moieties. Gel filtration on Sephadex G-100 demonstrated that the purified factor was eluted at the same position as standard hCG.

When the *in vivo* biological activity of the purified factor was determined by the uterine weight and the ovarian weight assays using immature female rats, the potency of the factor was 380 IU/mg and 900 IU/mg, respectively. Moreover, it stimulated testosterone production by mouse Leydig cells. Its biological potency was estimated to be equivalent to 3400 IU/mg. The CG-like factor purified from crab stomach induced a slight stimulation of testosterone production by mouse Leydig cells. The relative biological potency was equivalent to 0.3 mIU/mg. On the other hand, trypsin and chymotrypsin did not stimulate steroid formation.

The present findings suggest two alternative hypotheses for the presence and expression of mammalian gene(s) in bacteria and invertebrates. One is that the CG gene has its origin early in evolution, possibly as a protease. The other is that it is a consequence of a natural process of recombination.

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Studies on urn cell complexes of Sipunculus nudus *(Linnaeus): influence of physiologic and pathologic mammalian sera on mucus secretion.* Luigi Mastroianni, Jr., Santo V. Nicosia, Ellen Streibel, and Gilbert Haas.

Serum may play an important role in both vertebrate and invertebrate mucus secretion. This possibility is intriguing since many factors which induce serum transudation, such as estrogen and immune complexes, are also associated with enhanced mucus release. In order to study the influence of different hormonal milieux and of altered immunologic states on the mucus-stimulating activity (MSA) of serum, urn cell complexes (UCC) of *Sipunculus nudus*, 50 to 100/exp., were incubated with unheated physiologic and pathologic sera. Sera were diluted 1:1 with filtered sea water (FSW). Mucous tails (mean μm tail length $\pm$ s.e.) were produced in response to male and female rabbit, mouse, and human sera obtained during endocrinologically different states. A significant ($P < 0.001$) enhancement of mucus release was induced by estrous mouse sera (67.22 ± 4.45 *vs.* 43.90 ± 3.06 at diestrus). Mucous tails of significantly greater length ($P < 0.001$) were also observed with sera obtained from estrous rabbits 12 to 15 hr after treatment with 5.0 mg of conjugated estrogen (45.97 ± 4.11 *vs.* 17.88 ± 1.87, before) and 20 min after a single intravenous injection of 25 μg of estradiol-17β (37.91 ± 3.75 *vs.* 14.00 ± 2.01, before). The estrogen-enhanced MSA of serum was not altered by extraction with activated charcoal and, as indicated by dialysis, the active factor had a molecular weight greater than 13,000. Mucus release did not occur with FSW alone and with FSW containing 10 μg/μl of estradiol-17β or 10 to 20 mg/ml of bovine serum albumin. UCC also released mucous tails of significantly greater length ($P < 0.001$) when exposed to pathologic sera obtained from infertile or vasectomized men exhibiting circulating antisperm agglutinins (64.12 ± 1.57 *vs.* 16.82 ± 1.83, normal serum). This secretory response was commensurate with agglutinin titers. These results indicate that mammalian sera may contain more than one fatcor capable of inducing augmented mucus secretion.

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Effects of extreme temperatures on protein synthesis in the toadfish. Rita W. Mathews and Audrey E. V. Haschemeyer.

The toadfish (*Opsanus tau*), a temperate species occurring along the eastern Atlantic from the Gulf of Maine to Cuba, tolerates a broad range of temperatures both in nature and in the laboratory. Studies of protein synthesis in liver *in vivo* have shown a moderate temperature dependency ($Q_{10} = 2.5$) from 15° to 30° C. As temperature is decreased, Q_{10} increases dramatically; however, even at 4° C all steps of protein synthesis continue to operate slowly without apparent differential breakdown in function. Rates return to normal when fish are warmed.

At temperatures above 30° C polypeptide chain elongation showed a normal rate increase with temperature (Q_{10} about 2.5), reaching 6 amino acid residues per second at 37° C, as in mammalian liver. However, total incorporation of radioactive amino acids into protein, reflecting all steps in protein synthesis, declined precipitously above 32° C. Results were obtained as fractional incorporation rate (incorporation into protein divided by total radioactivity in liver and by incubation time). At 30° C the fractional incorporation rate for ^{14}C-leucine is 0.10 min^{-1} representing 10% turnover of the intracellular leucine pool per minute. In 1-min pulse experiments carried out *in vivo* immediately after the fish was warmed to the experimental temperature fractional incorporation was found to follow an Arrhenius relationship downward with Q_{10} about 1/40 up to the lethal limit of 39° C. Fish returned to 30° C after 5-min exposure to higher tempera tures showed nearly complete recovery of synthetic rate up to an exposure temperature of 38° C. The results suggest a partially reversible lesion in the system involving initiation or recycling of ribosomal subunits.

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The size of the photosynthetic unit and its turnover time in various seaweeds. D. Mauzerall and D. Wittenberg.

The size of the photosynthetic unit (PSU), defined as the moles of chlorophyll (Chl) per mole of O_2 emitted in a single flash was determined following the classical experiment of Emerson and Arnold. Our previous measurements have shown that green seaweeds have about the same PSU size (2000 Chl/O_2) as freshwater green algae and higher plants, but that the red seaweeds have a smaller size based on Chl **a**. We have extended these measurements to grasses and to other red and brown seaweeds. *Spartina alterniflora*, short: 2100 ± 300, tall: 3600 ± 900 (A. Hogan); *Zostera marina*, 2200 (L. Mazzella); *Champia parvula*, 1500; *Ceramium rubrum*, 1100; *Gracilaria verrucosa*, high light 1600, low light 2100 (T. Kursar); *Fucus vesiculosis*, 1800; *Laminaria saccharina* 3300 (S. Schatz); *Sargassum filipendula*, 2000; *Sphacrotrichia divaricata*, 1900. Light absorption of the accessory pigments makes up for the missing Chl in the red seaweeds, but add to the absorption of the brown algae. A quantitative measure of pigment efficiency is the fraction of solar photons absorbed by the pigment. This is roughly equal for all the pigments over the full solar spectrum. This selection weakness may allow a more random play of evolution and account for the wide diversity in amount and kind of pigments observed. The turnover time is remarkably constant, near 0.5 msec, for all the species studied. This result shows a constancy in the evolution of photosynthesis that transcends the variable color and morphology of the benthic algae.

We thank Dr. J. Fiore for help in collecting and identifying the seaweeds, the named students in the program for data, and the Experimental Marine Botany Program and the NSF (grants PCM 74-11747 and 79-06638) for support.

Photosynthetic characteristics of Zostera marina *L. (Eel grass).* L. Mazzella, D. Mauzerall, H. Lyman, and R. S. Alberte.

The photosynthetic activity of *Zostera marina*, from a tidal bed in the Woods Hole area, was studied. It was found that the chlorophyll (Chl) content (mg Chl/g fresh weight and mg Chl/cm^2), oxygen evolution rates [μmol O_2/(cm^2·min) and μmol (O_2/(mg Chl·min)] and numbers of photosynthetic units (PSU) per unit leaf area varied in response to leaf age, light exposure and degree of epiphytization. These parameters were measured for different leaves from the youngest innermost leaf to the oldest outermost epiphytized leaves, and in different portions of the leaves from the youngest lowest portion to the oldest upper more epiphytized portion. The Chl content was lowest in the base of the youngest innermost leaves (0.002 mg Chl/cm^2), while the highest concentration was found in the middle portion of old epiphytized leaves (1.56 mg Chl/cm^2). The lowest photosynthetic activity [2.5×10^{-1} μmol O_2/(mg Chl·min)] was found in the base of the youngest leaves while the highest activity was observed in the tip of old epiphytized leaves [$(10.5 \times 10^{-1}$ μmol O_2/(mg Chl·min)]. In general a gradient in Chl content and photosynthetic activity from the tip to the base of all leaves was found except in epiphytized old leaves from which the epiphytes were removed. The disappearance of this gradient from the latter leaves and the highest activity seen in the tip of old epiphytized leaves suggests that a significant portion of photosynthetic activity of older leaves is due to the presence of epiphytes. A gradient in Chl content and photosynthetic activity in the younger portion of all leaves from the outermost to the innermost leaves was also observed. The different maturity of the tissue and the different exposures to light intensity could explain this gradient in the young portion of different leaves. In conclusion, the photosynthetic activity of *Zostera* leaves is regulated by three factors: first, age of tissue; secondly, light intensity exposure; and thirdly, presence of epiphytes with all other parameters constant (*i.e.* temperature, depth). For the young part of all leaves the light intensity and the maturity of tissue play an important role. For the oldest epiphytized leaves, the presence of epiphytes is more important than the other factors in the dynamics of the photosynthetic activity and biomass production.

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Interaction of lipsosomes with dogfish lens capsules. J. M. Megaw, S. Lerman, and Y. Takei.

Liposomes are microscopic vesicles composed of lipid bilayers. They have been used to deliver drugs and enzymes into tissues and cultured cells. Techniques employed to enhance the specificity of delivery have included antibody and lectin-mediated binding. We have investigated the possibility of binding concanavalin A (Con A) containing liposomes to the lens capsule of the dogfish both *in vitro* and *in vivo*. Liposomes were prepared by injection of ethanol solutions of phosphatidyl serine and phosphatidyl choline into PBS alone, or PBS containing either unlabeled or fluorescein labeled Con A. The vesicle size was increased by incubation in the presence of $CaCl_2$ followed by addition of EDTA. The liposomes were then washed by centrifugation and suspended in PBS. Whole dogfish lenses, some pre-incubated with unlabeled Con A, were incubated in the various liposome preparations and then washed in PBS. The capsules were examined by scanning electron microscopy (SEM) or by fluorescence microscopy (FM). For the *in vivo* studies, small volumes of aqueous humor were withdrawn from the eyes and were replaced with fluorescein-labeled liposomes in PBS. After 6, 23, or 29 hr, the eyes were removed and portions of the cornea, angle, iris, retina and lens were fixed for SEM or were examined by FM. SEM and FM demonstrated that liposomes containing Con A were bound to the lens capsule *in vitro*. Pre-incubation of lenses with unlabeled Con A resulted in decreased binding of Con A containing liposomes to the lens capsules. FM of eye tissues from animals injected with fluorescein-labeled Con A liposomes indicated binding of fluorescent material to the anterior lens capsule and to a lesser extent, to corneal endothelium. No fluorescence was noted in vitreous, retina or posterior lens capsule.

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Nicotinamide deamidase activity in oocytes of Spisula solidissima. Akira Momii and S. S. Koide.

We have demonstrated previously that 5 mM nicotinamide inhibits germinal vesicle breakdown (GVBD) in *Spisula* oocytes and that nicotinamide is rapidly converted to a metabolite by *Spisula* oocytes. These findings suggest that the active inhibitor of GVBD may not be nicotinamide itself, but a metabolite. The present study was performed to identify the metabolite, to determine the enzymatic system mediating the transformation, and to investigate the mechanism of GVBD inhibition induced by incubation with nicotinamide.

Packed oocytes (2.5 ml) were suspended in 9.5 ml artificial sea water (ASW) and incubated with 0.5 ml of ^{14}C-nicotinamide (25 μCi) at 22 to 23° C for 3 min. The final concentration of nicotinamide was 5 mM. The oocytes were washed with ASW three times and extracted with 2% $HClO_4$. The extract was neutralized with 1 N KOH and centrifuged. The metabolite in the supernatent was identified as nicotinic acid by PEI-cellulose and DEAE-cellulose thin-layer chromatography (TLC). No nicotinamide was detected. Since it has been demonstrated previously that nicotinic acid has no influence on GVBD in *Spisula* oocytes, it is unlikely that this metabolite is the active agent. Since ammonia is a product of the transformation, the ability of ammonium bicarbonate to influence GVBD was tested. At 15 mM, ammonium bicarbonate blocked GVBD induced by insemination (45% GVBD compared to 95% for the control). Nicotinamide deamidase activity (EC 3.5.1.19) of *Spisula* oocytes was determined. Oocytes homogenate was incubated with ^{14}C-nicotinamide at 37° C for 15 min. The resulting nicotinic acid was quantified after separation on PEI-cellulose TLC developed with H_2O for 5 cm and with 1 N HCOOH for 10 cm. The deamidase activity is greater than 700 nmoles/(mg protein·hr) and does not change after fertilization. This value is far greater than that reported for mammalian tissues or the level found in *Arbacia* eggs. *Spisula* hepatopancreas has deamidase activity equivalent to that found in *Spisula* oocytes.

It can be concluded that *Spisula* oocytes possess an active deamidase system that rapidly transforms nicotinamide to nicotinic acid. The liberated ammonia may be reponsible for the inhibition of GVBD in *Spisula* oocytes induced by nicotinamide.

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Differential solubilities of cytoskeletal proteins in squid axoplasm. James R. Morris and Raymond J. Lasek.

We have studied the forms which cytoskeletal proteins take under physiologic conditions using squid giant axon axoplasm. The axoplasm consists principally of cytoskeletal proteins (tubulin, neurofilament proteins (NFP), and actin). A cyclinder of fresh axoplasm was extruded from the giant axon directly into a physiologic axoplasmic buffer (buffer P). Using SDS-PAGE, proteins diffusing into buffer P were compared to those remaining in axoplasm. After 24 hr at 20° C in buffer P, essentially all of the NFP (95%) remain in the axoplasm while 83% of the tubulin and 75% of the actin diffused into buffer P. Most of the other axoplasmic proteins appear only in buffer P. The axoplasm maintains its cylindrical morphology throughout the experiment. The mitochondria are retained in the axoplasm. Electron microscopy shows the presence of a network primarily composed of neurofilaments (NF). Microtubules are absent. This structure is referred to as the axoplasmic ghost.

Because all the NFP remained in the ghost and electron microscopy shows the ghost to be principally NF, we conclude that essentially all axonal NFP are normally polymerized in NF. A fraction of the tubulin and actin also remain attached to the ghost. This fraction must also exist as stable polymer. Most of the tubulin and actin diffused into buffer P. This diffusable component exists either as monomers or as a polymer which is soluble under physiologic conditions. We distinguish between monomeric and polymeric forms by analyzing the kinetics of protein diffusion into buffer P. This analysis shows that the diffusion of tubulin and actin includes a fraction which is slowed when compared to physico-chemical predictions. Thus, we have quantitatively analyzed three forms of cytoskeletal proteins in axoplasm: stable polymer, soluble polymer, and diffusable monomer. NFP differ from tubulin and actin in that NFP exist solely as stable polymer while tubulin and actin exist in all three forms in the axon.

Cytoskeletons in Labyrinthula *slimeways.* Norio Nakatsuji and Eugene Bell.

Electron microscopic observations have shown the existence of three kinds of filaments in *Labyrinthula* slimeways; many actin-like 6-nm filaments, rare short filaments that are thicker than 6-nm, and numerous short 2.3-nm filaments. The latter two kinds of filaments may be myosin aggregates and individual myosin molecules respectively. Decoration of the 6 nm filaments with myosin S-1 fragments was done after mild glycerination. They made clear arrow-head structures, showing that actin is contained in the filaments. Controls with 4 mM ATP showed no decoration. Polarity of parallel filaments was not always in the same direction. Decorated filaments were frequently observed to terminate in dense plaques associated with the slimeway membrane. Immuno-fluorescent staining, SDS-polyacrylamide gel electrophoresis and arrow-head decoration of filaments support the probability that actin and myosin occur in the slimeways.

Mg^{2+} and Ca^{2+}-free sea water causes the slimeway to break into many beads. Thin sections of these beads show the attachment of 6-nm filaments to the slimeway membrane plaques clearly. Some thin sections of glycerinated slimeways suggest that 6-nm filaments are also attached to the outer surface of the unit membrane of intra-slimeway vesicles. Thus, for a model mechanism of cell translocation, we propose that actin filaments are attached to the inner surface of the slimeway membrane and to the outer surface of the membrane that is investing the spindle cells, to permit myosin-mediated sliding between these filaments.

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Marginal band systems in blood cells of marine species: visualization by indirect immunofluoresence. Iris Nemhauser, William D. Cohen, Amy Milsted, and Robert D. Goldman.

Marginal bands (MBs) and associated paired centriole-like structures ("perioles"; possible MB organizing centers) were observed in Triton-lysed red cells of the mollusc *Anadara*

transversa, using indirect FITC immunofluorescence with anti-tubulin. Only MBs and the associated perioles fluoresced, with the disposition of the latter matching that observed in the same lysed cells in phase contrast. In some cases MB fluorescence was so great as to obscure that of the perioles, though they were clearly visible in phase contract. Similar results were obtained with preparations of lysed dogfish erythrocytes (*Mustelus canis*), except that MB-associated, paired fluorescing structures were observed in a minority of cells. It is possible that they are present only in immature cells or obscured in most cells by the MBs or other structures. The results suggest that centriole-like structures may be associated with the MBs of diverse species, and that a careful search for them is warranted.

Anti-tubulin binding was also employed to follow structural alterations occurring in MBs of lobster coelomocytes (*Homarus americanus*) as they undergo changes in morphology while spreading on glass substrata (at room temperature, approximately 21° C). At t = 0 (no spreading) the cells are flattened and somewhat elliptical, with virtually all of their microtubules contained within intact circular or elliptical MBs. After spreading for 5 min (t = 5 min), most MBs are still recognizable but show signs of disorganization, with figure-8 forms prevalent. At t = 10 min MBs are no longer recognizable as such, with bundles of microtubules splayed in various directions. At t = 20 min the microtubule network has disappeared, leaving only scattered points of fluorescence visible in some cells. This is in contrast to other cell types such as those of cultured cell lines, which reorganize microtubule networks as they spread. Perhaps, in this instance, the cells are engaged in a process of self-destruction related to a clotting role *in vivo.*

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Ultrastructure of urn cell complexes of Sipunculus nudus *(Linnaeus) before and after serum-induced mucus release.* Santo V. Nicosia and Janice Sewinski.

This report analyzes the cytology of urn cell complexes (UCC), a currently used invertebrate model of humorally-regulated mucus secretion. UCC are 60–80 μm in size and are composed of one vesicle cell (VC) and one basal cell (BC) marginally joined by prominent desmosomes. VC's are thin-walled structures whose cytoplasm contains few mitochondria and scattered, sudanophilic and osmiophilic, lipid droplets. These cells surround a vesicular cavity which contains mucus-like, microfibrillar material and is continuous with the extracellular milieu through the BC-delimited opening of UCC. There is a distinct polarity in cilia distribution and secretory activity in the BC. Rows of 5 to 10 μm long cilia are present, amidst microvilli, exclusively in the outer lining of BC. These cilia are anchored by 3 to 5 μm long, curving rootlets which exhibit a periodicity of 65 nm and may have, together with desmosomes, a role in UCC plasticity during the mechanical stresses of mucus release, debris sweeping, and forward propulsion. In addition to numerous ribosomes, scattered mitochondria, and few lipid droplets, BC contain approximately 8 to 10×10^3 secretory-like granules which are uniformly electron-dense and lack a distinct limiting membrane in glutaraldehyde-osmium fixed UCC. However, they display cytochemical characteristics of mucous granules (diastase resistance, periodic acid-Schiff positivity, metachromasia, and immunoperoxidase-detectable lysozyme activity). The number of these granules is also drastically reduced after serum-induced mucus release. Typical exocytotic discharge of secretory material into the vesicular cavity of UCC is rare. More often these granules are found around or within intracellular canaliculi or sacs which extend up to the inner lining of BC where mucus release takes place. BC also contain 4 to 5 circular bundles of 14 to 17-nm wide microfilaments whose integrity, along with the UCC ability to release mucus, is disrupted by cytochalasin B. This study suggests that mucous tails are formed on the inner aspect of BC by a microfilament-mediated extrusion of mucus from storage reservoirs (intracellular canaliculi and/or the vesicular cavity) of UCC.

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Particle size selectivity in Modiolus demissus *and* Balanus balanoides. Deanna H. Olson.

In great Sippiwissett Salt Marsh, Falmouth, Massachusetts, *Balanus balanoides* settles on *Modiolus demissus,* as *M. demissus* shells comprise most of the available hard substrate in the marsh system. Particle-size selectivity of these filter feeders was studied and related to posible food competition which may limit growth in areas of the marsh. Each species was allowed to filter natural sea water in a standing culture system. The water contained a decreasing logarithmic progression of particles with increasing size. Particle concentration was determined (Coulter counter: 5–49 μm size range) before introduction of the species to the system, and after 2 and 4 hr of filtration (7–11 replications).

Mean percent removal of seven particle size classes, within the range studied, was calculated for *M. demissus* (valve length range: 71–74 mm) and for a number of individuals of *B. balanoides* which corresponded to the average observed on marsh mussels. For both species, percent removal of particles increased positively with increasing particle size. Within 2 hr, the removal of particles in each size class by *M. demissus* ranged from 68 to 94% and by *B. balanoides* from 10 to 90%. After four hr, *M. demissus* and *B. balanoides* filter 93 to 99% and 19 to 95% of available particles, respectively. Particle-removal spectra for *M. demissus* suggested generalized filtration while *B. balanoides* selectively removed larger particles. Equal biomasses of these species (obtained by adjusting barnacle numbers) yielded particle removals similar to the above. Therefore competition between these filter feeders occurs only for the larger-sized particles studied.

Ammonification and nitrification potentials of soils from a northern hardwood forest and a pine plantation. K. C. Parsons and J. M. Melillo.

In a laboratory study, rates of ammonification and nitrification were measured for organic and mineral soils from a northern mixed hardwood forest and a pine plantation. Two-normal KCl extractions of organic soil samples from both stands yielded no nitrite-nitrate after nine weeks of incubation, although more than 250 μg of ammonium-nitrogen per gram soil (dry weight) was extracted from the same samples. Only mineral soil samples from the hardwoods produced significant, albeit low, levels of nitrite-nitrate (8.0 μg $NO_2 + NO_3$-N/g soil from the upper 15 cm mineral soil, 2.5 μg $NO_2 + NO_3$-N/g soil from 15 to 30 cm mineral soil horizon). Levels of ammonium extracted from mineral soils from the hardwood stand ranged from 2.5 to 8.0 μg NH_4-N/g soil. Mineral soils from the pine plantation produced 1.5 to 5.0 μg NH_4-N/g soil.

Other soil samples were leached with distilled water periodically for thirteen weeks to give rates of nitrification. Levels of nitrite-nitrate were significantly greater than ammonium in samples from the upper 15 cm of mineral soil from the hardwoods. No nitrite-nitrate was leached from the organic soils from either stand, and none was produced by the mineral soils from the pine plantation. Ammonification was evident in all soil samples, but especially prominent in the organic horizons. When soils were fertilized with phosphorus, nitrification rates remained essentially zero. Ammonification in amended samples was not significantly different from controls in any soil type from either stand. Both systems are concluded to be ammonium-dominated. These experiments show nitrifiers not to be phosphorus-limited. Directions for further research are suggested.

The histochemistry of muscle fiber types in the regenerating claws of the lobster, Homarus americanus. Natalie G. Pascoe.

Lobsters regrow autotomized claws initially as soft limb buds, which upon the subsequent molt become hard-shelled, functional claws. Histochemical techniques developed by Mark Ogonowski and Fred Lang for the characterization of lobster muscle in respect to fast and slow properties were used to examine the state of differentiation of regenerating opener and closer muscles in claw limb buds from adult animals. Early in the development of a bud, the muscles stain darkly for NADH diaphorase, an indicator of oxidative capacity (fatigue

resistance). However, initially, myofibrillar ATPase, an enzyme that regulates speed of contraction, stains faintly. The staining patterns in mature buds are similar to those of the adult cutter and crusher claws. In the closer muscle of the mature bud alternate staining of sequential sections reveals that fibers that stain intensely for myofibrillar ATPase stain lightly for NADH diaphorase. Conversely, fibers that stain darkly for NADH diaphorase show little myofibrillar ATPase activity. Some single fibers of intermediate staining intensity are found, and they stain in an intermediate extent for both enzymes. High specific activity myofibrillar ATPase seems to exclude the property of high oxidative capacity, or vice versa.

Opener muscles show regional differentiation in respect to NADH diaphorase; however, they do not have high specific activity myofibrillar ATPase in areas of low NADH diaphorase. They may lack the potential for this.

The diurnal response of the gray seal, Halichoerus grypus, *to tide and insolation during the month of April at Muskeget Shoals, Nantucket Sound, Massachusetts, USA.* David Paton.

The integument of the gray seal undergoes a once-yearly molt during April in these latitudes. This metabolic activity increases the nutritional requirements of the animal because it remains on land. Heating of the skin by insolation augments this metabolic stress to the advantage of the animal. If the seal can spend the time of the molt cycle in air (a fluid of a lower rate of thermal conductance than water), it can sustain the molt in less time, thence saving energy that would normally be used in finding and processing food at sea. The gray seal has been observed to respond directly to the period of the diurnal tide cycle outside of the breeding and molting periods of the year. This behavior is supposed to be associated with feeding at sea.

During 5 days in April, a pod of gray seals was observed at Nantucket Sound. White light reaching the area was measured with a hand held Vivitar light meter, M90, and compared with a pyronometer, Epply 6–90, located at Woods Hole. Cloud cover, wind, sea and air temperature were also noted. Tide movement was observed on the study site and compared with published tide tables for the effects of wind fetch and current. Two gales swept the area during this time. The animals were not observed at night. A control animal, being offered its usual diet, was reported to have molted in an aquarium on Cape Cod isolated from tides. Climate diagrams were plotted for four size-color classes of model gray seals. The gray seals were observed to respond to insolation of white light rather than the period of the tide during the days of April 12th to 17th, 1979. Thermal regulatory behavior was observed. Further investigations into the bioenergetic bounding effect of this habitat on the gray seal will be continued.

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Distribution of gametophytes and sporophytes of Chondrus crispus *in the vicinity of Woods Hole.* Nancy Penncavage, Scott Schatz, Esther McCandless, and James Fiore.

The red alga *Chondrus crispus* of the North Atlantic coast derives economic importance from its high content of kappa and lambda carrageenans, sulfated galactans which differ in degree and position of sulfation and in the presence of an anhydro-ring in the former. Kappa carrageenan gels in the presence of K^+; lambda carrageenan solutions are viscous but do not gel. The variability in proportions of the two polysaccharides in carrageenan preparations was explained recently by the discovery by one of us that kappa carrageenan is produced only by gametophytes, lambda carrageenan only by sporophytes of *C. crispus* and certain other red algae. In these species, qualitative analysis for anhydrogalactose can therefore be used to determine the phase of nonsporulating plants. Northern collections of *C. crispus* have demonstrated that the percentage of sporophytes in populations increased with depth, but no information is available on relative proportions or distribution of the phases here near the southern limits of the species.

Between June 21 and August 18 we made 11 collections from 8 different Massachusetts sites: Black Rock, Cuttyhunk jetty, NMFS jetty, MBL beach, Menemsha jetty, Nahant, Nobska, and West Falmouth jetty. At NMFS jetty, Menemsha and Nobska, collections were made at several depths. A number of randomly selected plants from each collection (30 to 40) were analyzed for anhydrogalactose. Usually the proportion of sporophytes at mean low water (MLW) level was very low, approximately 5% of plants. At Menemsha this increased to 40% at −12 feet (below MLW), a distribution similar to that seen farther north. However, on both Nobska jetty and NMFS jetty the proportion of sporophytes was higher at MLW (22–28%) and reduced with greater depth (5% at −7 ft and 11% at −9 ft, respectively.) Explanation of these observations is not yet apparent. (Support of the Experimental Marine Botany program and NSF grant PCM-7906638 is acknowledged.)

Spectroscopic and chemical studies of protein-methylglyoxal complexes. Ronald Pethig and Jane A. McLaughlin.

Szent-Györgyi has drawn attention (1979, *Biol. Bull.*, **157**: 398) to the rôle of methylglyoxal (MG) in forming charge-transfer reactions with protein molecules. Such a concept has received strong experimental support from the studies of dielectric and electron spin resonance properties of protein—MG complexes (see Bone, 1979, *Biol. Bull.*, **157**: 358; Gascoyne, 1979, **157**: 369). Our purpose has been to investigate the basic chemical reaction responsible for such a charge-transfer interaction. A neutral aqueous mixture of bovine serum albumin (BSA), casein or lysozyme with twice distilled MG assumes a yellow color having an absorption peak at 330 nm and a "shoulder" at around 350 nm. Addition of $NaBH_4$ removes these absorption peaks. If the reaction is performed at pH4 the initial solution remains colorless. Adjustment of a neutral protein–MG aqueous mixture to pH3 modifies the absorption to give a new peak at 340 nm and no "shoulder" at 350 nm, and this effect is reversible on readjustment to pH7. The ϵ-amino groups of the lysine side-chains of BSA and casein have been reductively dimethylated and after this treatment the proteins no longer react with MG to form a yellow color. The blocking of the arginine side-chains of BSA with cyclohexanedione has produced no observable effect on the color reaction with MG. Studies have also been made using methanol-water mixtures.

We consider that these studies show that the relevant reaction for our studies involves the formation of a Schiff base between methylglyoxal and the protein lysine side-chains. Theoretical calculations by others show that such a Schiff base provides a good electron acceptor for charge-transfer interactions with neighboring peptide units. This forms a strong theoretical basis for the electronic desaturation concepts described by Szent-Györgyi as being relevant to the "living-state."

L-leucine transport by toadfish liver studied by the Oldendorf method in vivo. Roger Persell and Audrey E. V. Haschemeyer.

A technique originally developed by Olendorf for the study of transport in mammalian brain was extended by Pardridge (1977, *Am. J. Physiol.*, **232**: E492–E496) to amino acid transport in rat liver. A pulse injection of ^{14}C-amino acid and ^{3}HOH is given via the hepatic portal vein, and retention of amino acid relative to water is evaluated as the Liver Uptake Index (LUI).

We have examined L-leucine uptake by toadfish liver *in vivo* at two temperatures by this technique. Hepatic clearance of ^{3}HOH was followed as a function of time and the LUI for L-leucine determined. Maximal ^{3}HOH extraction was about 80% of dose, similar to the value found in rat, but washout was much slower. In the time range up to 1.5 min, $t_{\frac{1}{2}}$ was 2.5 min at 20° C and 2.8 min at 10° C, compared to 0.8 min for rat. This represents a three-fold difference in rate of portal blood flow between the two species. LUI for leucine in toadfish was 0.7 to 0.8 for the first 4 min after injection and then increased as ^{3}HOH continued its efflux from the liver. At $t = 10$ min, LUI based on free (acid-soluble) radioactivity was 2.0 at 20° C. If protein-bound radioactivity is included in the LUI, following the method of Pardridge,

LUI is as high as 5. The latter value, however, reflects protein synthesis more than transport activity.

Previous studies in toadfish liver have shown concentration of L-leucine relative to an extracellular space marker, ^{3}H-inulin. This procedure has been used to study saturation and competition for the carrier-mediated transport process. The Oldendorf technique appears to be less useful in the fish because of the slowness of blood flow. Thus, the kinetics of liver amino acid transport when analyzed relative to ^{3}HOH at the critical early times after injection are obscured by the slow water efflux.

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The relative importance of bacterial and fungal biomass and Spartina *organic matter in the nutrition of two species of salt marsh amphipods.* Neal W. Phillips.

Several recent studies question the generalization that microbes are the major food source for detritus-feeders. I examined the relative importance of microbial and non-microbial biomass in the nutrition of two species of marsh amphipods, *Orchestia grillus* and *O. spartinophila*. Assimilation efficiencies for total organic matter and for bacteria and fungi were estimated by an ash-tracer method which requires measurements of the ash fraction and of bacterial and fungal densities in food (dead leaves of *Spartina alterniflora*) and feces.

Specimens of *O. spartinophila* were fed submersed in chambers designed to minimize microbial growth on fecal pellets. Specimens of *O. grillus* were fed in moist fingerbowls from which fecal pellets were rinsed every 4 hr. After 12 hr of feeding, fecal pellets and uneaten food were homogenized and preserved for bacterial and fungal counts. Bacteria were enumerated by the acridine-orange direct count method; fungal hyphae were measured microscopically on filtered subsamples incubated with water-soluble aniline blue, a fluorescent stain. Fecal pellets from food of known ash content were dried, weighed, combusted (500° C, for 3 hr) and reweighed to obtain ash fractions.

Orchestia spartinophila assimilated 64 and 23% of total organics in two experiments. Assimilation efficiencies (5 replicates) for bacteria ranged from 23 to 75% and for fungi from 0 to 60%. The animals did not scrape fungi from leaf surfaces. *Orchestia grillus* apparently assimilated 39% of total organics and 60 to 70% of bacteria, but fungal assimilation efficiencies were highly negative. This suggests that the animals selectively ingest fungi, and that the calculated efficiency for total organics is incorrect. Bacteria averaged 0.11% of litter dry weight, and fungi 0.24%. Microbial biomass may represent a small fraction of total assimilable organic matter.

Detection of a synexin-like soluble factor in anglerfish islet tissue that aggregates islet secretory granules in the presence of small amounts of calcium. Harvey B. Pollard, Bryan D. Noe, and G. Eric Bauer.

Exocytosis is a common mechanism for the secretion of hormones and transmitters that are transiently sequestered in secretory granules, and depends in many cell types on an initial rise in the intracellular calcium concentration. Elevation in calcium appears to cause secretory granules to become closely associated with either plasma membranes or, frequently, with the membranes of other secretory granules that have already secreted but are still attached to the plasma membrane. This calcium-dependent membrane interaction may be mediated by a new protein called synexin. Synexin was recently discovered in chromaffin cells where it caused chromaffin granules to aggregate with each other and with plasma membranes only in the presence of calcium. Synexin activity can be quantitated simply by following the increase in turbidity of a granule suspension at A 420 nm that accompanies aggregation, and we have utilized this approach to search for synexin activity in crude extracts of Anglerfish (*Lophius americanus*) islet tissue. We found that the post-microsomal supernatant fraction contained a potent islet granule aggregating activity which could also be observed directly by phase microscopy. Further analysis showed that the reaction was strictly dependent upon calcium,

since neither barium, strontium nor magnesium (all 2 mM) could either substitute for or inhibit calcium action (1.2 μM). A careful titration of the calcium concentration dependence with an EGTA buffer revealed that the K (1/2) for granule aggregation was *ca.* 0.04 μM, with a Hill coefficient of *ca.* 3.5. Furthermore, the activity was stable to both dialysis and boiling, the latter observation clearly distinguishing Anglerfish islet factor from bovine synexin. Nonetheless, the general similarities of the reactions catalyzed by both factors lead us to conclude that secretion of some hormones found in islet tissue may prove to be mediated by a synexin-like activity similar in some ways to the protein previously characterized in chromaffin tissue.

Succession of five common salt marsh detritivores on Spartina alterniflora *detritus of decreasing particle size and increasing age.* CATHERINE N. POURREAU.

Detritus from the common cord grass *Spartina alterniflora* in little Sippewissett salt marsh, Falmouth, Massachusetts, is the primary source of food for five invertebrate detritivores (high marsh: *Philoscia vittata* (isopod), *Orchestia grillus* (amphipod); low marsh: *Gammarus palustris* (amphipod), *Melampus bidentatus* and *Littorina littorea* (gastropods)). Feeding preferences of these detritivores for different physical and chemical states of decomposition of *S. alterniflora* leaves were studied by observing feeding activity in a quartered petri dish. Experiments (replicated three times) consisted of observations of animal positions in the dish every 10 min. *Orchestia grillus* and *P. vittata* proved photosensitive, hence were tested in the dark. *Melampus bidentatus* fed only in the dark.

Selection for specific detrital sizes and age classes were detected in all species. Coarse particles (0.6 cm leaf fragments) were selected by *M. bidentatus* and *L. littorea*, fine particles (0.6 mm) by *G. palustris*, and very fine (0.2 mm) by *P. vittata* and *O. grillus*. Conversely, 0- to 1-month-old detritus was preferred by *P. vittata*, 2- to 5-month-old detritus by *M. bidentatus*, 6- to 9-month-old detritus by *G. palustris*, and 9 months or older detritus by *L. littorea* and *O. grillus*. Relative importance of detrital particle size versus age for detritivore was tested by offering detritus of preferred age of an unpreferred particle size, and *vice versa*. *Philoscia vittata* and *O. grillus* responded more strongly to detrital age; *G. palustris*, *M. bidentatus*, and *L. littorea* to particle size. Consideration of these results and habitats of these detritivores suggests that the detrital resource is partitioned and does not overlap.

Transmembrane movements of sulfur compounds in the squid giant axon. R. D. PRUSCH AND F. C. G. HOSKIN.

The intracellular ionic content, including anions, is closely regulated by a variety of diffusional and active processes. Isethionate, 2-hydroxyethanesulfonate, accounts for nearly half of the total anion balance in the squid giant axon. Both sulfide and cysteine (specifically the sulfur of cysteine) are involved in the biosynthesis of isethionate and hence in the maintenance of the anion balance. Thus measurements of transmembrane movements of both cysteine and sulfide are important to an understanding of the anion balance in the squid axon.

When isolated squid axons were equilibrated with ^{35}S-cysteine (10^{-5} M cysteine externally) dissolved in sea water (1 mM HEPES, 10^{-4} M DTT, pH 7) a steady state intracellular concentration of 18 μM was reached in 2 to 3 hr. Cyanide in the bathing medium (1 mM) reduced the internal cysteine concentration to 8 μM in the same time. When isolated axons were injected with ^{35}S-cysteine, an efflux rate constant of about 1.15×10^{-3} min^{-1} was attained after a 1 to 1½ hr equilibration period. A reversible increase in cysteine efflux was brought about by 1 mM CN or 1 mM cysteine in the external medium. When the experiment was performed with 10^{-5} M Na_2S, the internal concentration of sulfide after 5 hr was 10 μM. Cyanide (1 mM) reduced this to 6 μM. The rate constant for ^{35}S-sulfide efflux was found to be 2×10^{-3} min^{-1} 2 hr after injection of the isotope. This was reversibly increased to 3×10^{-3} min^{-1} by 1 mM sulfide in the external medium and to 2.7×10^{-3} min^{-1} in the presence of 1 mM CN.

In summary both cysteine and sulfide uptake are reduced by CN, and the efflux of both are increased by CN. More importantly, cysteine efflux is stimulated by external cysteine, and sulfide efflux is stimulated by external sulfide. These observations indicate that both cysteine and sulfide are transported by carrier-mediated processes.

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Chemoreception in Homarus americanus: *responses of primary receptors to secondary plant compounds.* Pamela Reilly, Charles Derby, and Jelle Atema.

Secondary plant compounds are known feeding inhibitors in terrestrial systems; their role in the marine environment, however, has only recently been examined. The major secondary compounds of terrestrial plants have generally not been found in marine plants; other compounds, such as phenolics, seem to be more important. The chemosensory basis of detection of these compounds by marine consumers is unknown. This study is a neurophysiological analysis of the sensitivity of lobster chemoreceptors to terrestrial and marine secondary plant compounds, including a comparison of responses of two chemoreceptive appendages: antennules and walking legs.

Extracellular responses from nerves of excised appendages were recorded while chemical stimuli were injected into sea water flowing over the chemoreceptive region of the appendage. Secondary plant compounds tested include those known in terrestrial environments (atropine sulfate, sinigrin, caffeine, salicin, amygdalin, morin, quinine sulfate, heliotropine) and marine environments (ferulic acid, *p*-coumaric acid, gallotannin, phloroglucinol, bromoform, diiodomethane). Mean responses (average number of spikes per trial) for these compounds were compared to those of two known excitatory stimuli: L-glutamate of equimolar concentration (10^{-5} M) and a standard mussel extract. Mean responses to L-glutamate were identical for leg and antennular chemoreceptors: 22% of the mussel extract response. The mean responses to secondary plant compounds were generally low, ranging from 0 to 60% of the L-glutamate mean response. The mean responses to these compounds were almost always higher for leg than for antennular chemoreceptors; this is due, at least in part, to the larger number of responding neurons in the legs. This implies that there is a larger population of receptors sensitive to secondary plant compounds in legs. This greater sensitivity of legs to secondary plant compounds indicates a functional difference between antennular and leg chemoreceptors, the latter being more important in the ultimate acceptance or rejection of food. This difference parallels that of smell and taste and is another argument in the functional separation of two chemoreceptor systems in aquatic vertebrates and arthropods.

Image intensification as a tool in low level fluorescence studies of living cells. Geo. T. Reynolds.

Wolniak *et al.* (1979, *Biol. Bull.*, **157**: 402) describe results obtained using a high gain image intensification system for fluorescence studies of *Haemanthus* cells in a situation where the excitation intensity level required by conventional recording techniques destroys the cell development under study. The high gain system is an area detector consisting of a 4-stage intensifier capable of photon gains up to 10^6, and the output phosphor is viewed with a plumbicon vidicon, SIT vidicon, or conventional film camera. In this work the excitation level was reduced by several orders of magnitude by means of filters. This, and even further reduction made possible by the high gain system, is also an advantage in studies in which: first, the high level excitation normally required "bleaches" the fluorescent probe; secondly, fluorescence tagging is limited in order to avoid interfering with normal cell processes; or thirdly, receptors for the probe are sparse. In observations in which background fluorescence is a problem, it is possible to utilize a very narrow band width excitation to enhance the signal to background ratio. With the low excitation levels possible using high gain image intensification, the required bandwidths can be achieved using a suitable monochrometer rather than with the relatively complex and expensive tunable dye lasers required for high intensity excitation.

Helpful discussion with Elliot Elson is gratefully acknowledged. This work is supported by DOE contract EY-76-S-02-3120.

The use of Limulus *amebocyte lysate (LAL) for the removal of lipopolysaccharide from biological reagents.* Frederick R. Rickles and Jack Levin.

We have previously reported that many reagents are contaminated by lipopolysaccharide (LPS) (or endotoxin). Contamination of materials by LPS may interfere with the interpretation of results in various biological systems since LPS can mimic the action of many reagents and inhibit the action of others. For example, concanavalin A (con A), which is a potent mitogen for mammalian mononuclear cells, is frequently contaminated by LPS, thus limiting its usefulness as a specific B-cell lectin. We report here the results of separation experiments designed to remove LPS from con A by differential absorption with a lysate made from the amebocytes of *Limulus polyphemus*. Con A was radiolabeled with ^{125}I and then mixed 1 : 1 (V/V) with LAL. After gelation and high speed centrifugation only 33% ± 9.8 (s.e.) of the radioactivity could be recovered from the supernatant. If, however, the lectin was pre-incubated with the disaccharide alpha-methyl-D-mannoside, 80.5% ± 10.6 of the radioactivity was in the supernatant. Four different preparations of con A have undergone purification by this method with complete removal of LPS. Each preparation has remained fully mitogenic when tested with human mononuclear cells (mean maximum DNA synthetic response = 7259 ± 2355 cpm before LAL absorption: 7477 ± 3410 cpm after LAL absorption). Similar results have been obtained with other protein antigens and lectins. Therefore, LAL can be used to remove LPS from a variety of proteins without loss of activity. In this way reagents can be prepared which have greater specificity and the pitfalls of LPS contamination can be avoided.

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Intracellular pH regulation in squid giant axons. J. M. Russell and W. F. Boron.

Squid axons respond to internal acid loads by returning intracellular pH (pH_i) toward normal (~ 7.3), in a process ("acid extrusion") which requires internal Cl^- and external Na^+ and HCO_3^-. Thomas (1977, *J. Physiol.*, **273**: 317–338) has proposed that external Na^+ and HCO_3^- exchange for internal H^+ and Cl^-. Alternatively, Na^+ may combine with $CO_3^=$ to form the $NaCO_3^-$ ion pair which enters in exchange for Cl^-. Both models predict a stoichiometry of 1 Na^+ : 1 Cl^- : 2 H^+ neutralized intracellularly. We have now used ^{22}Na, ^{36}Cl, and pH microelectrodes to measure the stoichiometry on internally dialyzed squid giant axons (inside: pH = 6.5, $[Cl^-]$ = 150 mM; outside: pH = 8.0, $[Na^+]$ = 425, $[HCO_3^-]$ = 12, TTX = 10^{-7}, ouabain 10^{-5}). The HCO_3^--dependent Na^+ influx was 3.1 ± 0.5 (s.e.) (n = 5) pmol/(cm²·sec). Na^+ efflux and Cl^- influx were −0.1 ± 0.3 (3) and −0.2 ±0.3 (3), respectively. Cl^- efflux, taken from 1976 experiments (similar conditions), was 3.9. The acid extrusion rate, calculated from the pH_i recovery rate after halting dialysis, was 8.8 ± 0.8 (10). These data are thus consistent with predicted 1 : 1 : 2 stoichiometry. In addition, we studied the dependence of acid extrusion rate on $[HCO_3^-]_o$ and on $[Na^+]_o$. Values for apparent Km and Vmax were 2.3 ± 0.2 mM and 1.1 ± 0.05 pmol/(cm²·sec), respectively, for HCO_3^-, and 77 ± 13 mM and 1.1 ± 0.6 for Na^+. When these acid extrusion rates were plotted as a function of $[NaCO_3^-]_o$, the data from the HCO_3^- and the Na^+ experiments fell on the same curve, with best fit values of 74 ± 3 μM for Km, and 1.1 ± 0.2 for Vmax. This is consistent with the ion pair model, but does not rule out the Thomas model. Acid extrusion was reversibly inhibited 80 to 85% by 1 mM furosemide and by 1 mM 4,4′-dinitro-2,2′-stibenedisulfonic acid.

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A comparative study on Laminaria saccharina (*Phaeophyta*) *infected by* Phycomelaina laminariae (*Ascomycotina*). Scott Schatz, David Mauzerall, and James Fiore.

A comparative study on the brown alga, *Laminaria saccharina* infected by the ascomycete, *Phycomelaina laminariae* is presented. Light microscopy reveals that fungal hyphae grow

intercellularly in the epidermis and outer cortex of host stipe tissue. Affected host cells become elongated and distended prior to cell-wall breakdown. Host plant susceptibility appears to be in part a function of the aging process with sporophytes younger than one year old displaying complete resistance to infection. The percentage of the population infected increased from 14.8 in May to 42 in August. Growth rates of infected plants was much less than that measured in healthy plants. Examination of the photosynthetic capabilities of blade tissue from healthy and infected plants shows that the photosynthetic unit sizes (psu) are similar. The psu in healthy plants is 3400 ± 100 while in infected plants the psu is 3000. However, the rate of oxygen evolution in saturating light was much greater in healthy plants than in infected plants. Significant differences were also found when transmitted light was reduced by 56, 74, and 90%. These data suggest that the dark reactions of photosynthesis are inhibited and not the light reactions. The following hypotheses are presented as possible mechanisms by which growth and photosynthesis are inhibited in infected host plants: first, the fungus serves as a sink, drawing away metabolites essential for the dark reactions of photosynthesis; secondly, the transport of photosynthetic products into the stipe is reduced resulting in a feedback inhibition of the dark reactions in the blade; thirdly, a mycotoxin produced by the fungus is transported from the stipe into the blade; and fourthly, these phenomena are simply a result of natural senescence processes typified by reduced metabolic activity thereby predisposing the plant to fungal infestation.

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Direct effect of LHRH on testicular steroidogenesis in Rana pipiens. S. J. Segal and C. A. Adejuwon.

The LHRH (luteinizing-hormone-releasing-hormone) decapeptide has been identified in hypothalami of mammals, birds, and amphibia. For all vertebrate species studied, the role of releasing hormone in controlling LH secretion by the pituitary can be demonstrated. Recently, attention has been directed toward possible extra-pituitary effects of the decapeptide in order to explain paradoxical inhibitory action of LHRH or its analogues on gonadal function in mammals.

The direct effect of synthetic LHRH and one of its analogues (d-ser(bu^t)^{6}desgly^{10} LHRH ethylamide) on steroidogenesis by the testis of *Rana pipiens* has been investigated. The study includes the influence of the polypeptides on the well-known stimulatory action of hCG on the frog testis. Individual testes of specimens of *R. pipiens* (in breeding season and producing abundant spermatozoa) were incubated in amphibian ringer's solution at room temperature. One hundred μl aliquots of incubation medium were taken at 2 or 4 hr and assayed for testosterone (T) by radioimmunoassay. Two-hour control value of T production per individual testis is 22 ng. Addition of 16 ng, 32 ng, or 64 ng hCG to the incubation medium stimulates T production to levels of 38 to 95 ng. Adding 100 ng of LHRH (without hCG) results in 2-hr T production of 103 ng. When 64 ng hCG and 100 ng LHRH are included in the 2-hr incubation together, an intermediate production level of 80 ng is obtained. Similarly, the values obtained when a dose of 100 ng LHRH is included along with 32 ng or 16 ng hCG are intermediate between the values obtained with LHRH or hCG individually. The 4-hr T production level of *R. pipiens* testis *in vitro* is 20 ng in this study. Adding hCG (16, 32, or 64 ng) raises the production to 60 to 115 ng. The T produced by incubating testis slices with 100 ng LHRH for 4-hr is 124 ng. When both hormones are added, intermediate levels are produced. Parallel results are obtained with the LHRH analogue. A dose of 100 ng stimulates T production at 2 hr to 63 ng and at 4 hr to 83 ng. When the analogue (100 ng) is added along with 16 ng, 32 ng, 64 ng hCG the stimulatory effects of the two hormones are not additive.

These results demonstrate that: first, LHRH and one of its analogues directly stimulate steroidogenesis by the *R. pipiens* testis *in vitro;* secondly, the stimulatory effect of the decapeptide or its analogue is not additive to the stimulatory action of hCG; thirdly, at the time and doses employed, the LHRH analogue is approximately equipotent to LHRH in causing T production by the frog testis *in vitro.*

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A general method, employing arsenazo III in liposomes, for the study of calcium ionophores: Results with A23187 and prostaglandins. Charles Serhan, Paul Anderson, Elizabeth Goodman, Elisabet Samuelsson, and Gerald Weissmann.

Multilamellar (MLV) and large unilamellar (LUV) lipid vesicles (liposomes) trap the metallochromic dye arsenazo III (2,7-bis(arsonophenylazo)-1,8-dihydroxynaphthalene-3,6-disulfonic acid) in their aqueous compartments. When ionophore A23187 was preincorporated into lipid bilayers of either MLV's or LUV's above 0.001 molar %, addition of Ca to the outside of liposomes produced spectral shifts (max. at 656 nm) characteristic of the AIII·Ca_2 complex. The method permitted detection of two molecules of A23187 per liposome. Liposomes with A23187 were permselective: Mg or other divalent cations were not translocated. Integrity of liposomes was monotered by addition of excess EGTA which dissociated extraliposomal AIII·Ca_2 complexes. Incorporation of A23187 did not enhance permeability of liposomes to glucose, nor did valinomycin or gramicidin provoke Ca uptake. Ca uptake was not influenced by omission of cholesterol from the usual molar lipid composition of MLV's or LUV's (phosphatidyl choline 7: dicetylphosphate 2: cholesterol 1). Since prostaglandins may act as calcium ionophores, we have incorporated into MLV's and LUV's stable prostaglandins (PGE_2, PGI_2, PGB_1), endoperoxides (PGH_2-analogues), and a water-soluble, polymeric derivative of PGB_1: PGBx. None acted as ionophores. In contrast, when added to the *outside* of preformed MLV's or LUV's PGBx, at concentrations above one micromolar, provoked permselective Ca uptake equivalent to that induced by 10^{-8} M A23187. These studies demonstrate not only that liposomes containing arsenazo III may be employed in a sensitive assay method to study agents which form channels for divalent cations, but that a water-soluble derivative of a naturally occuring fatty acid, PGBx, is a potent calcium ionophore.

Sulfate-depletion profiles and sulfate-reduction rates for a salt marsh. Susan Shen.

Sulfate reduction is a major process in salt marsh decomposition, and the resulting reduced inorganic sulfur compounds are a potential source of energy to the coastal food web. As a step toward a better understanding of the process of sulfate reduction in salt marshes, the extent of sulfate depletion and the rate of sulfate reduction were measured in several sites in the Great Sippewissett Salt Marsh. Profiles of sulfate depletion in peat over depths up to 23 cm were constructed for various types of marsh sediments: dwarf *Spartina alterniflora*, tall *S. laterniflora, S. patens*, and creek bottom. Profiles are highly variable for the sites sampled, with the exception of a creek bottom site where distinctly less sulfate is found at greater depths. Variations were also found among cores taken at the same site. There are two possible explanations for such variability in sulfate depletion profiles: first, the hydrology of these marsh sediments is very complex, affecting the time during which sulfate reduction has occurred, and secondly, the extent of sulfate reduction and re-oxidation varies with depth and vegetation type.

Sulfate reduction rates were measured in the salt marsh at sites containing homogeneous stands of dwarf *Spartina alterniflora*. A method was used to determine the rate of disappearance of sulfate in replicate cores incubated for periods of up to 2 weeks. Rates were consistently high for the top 5 cm of the peat and tended to be much lower at other depths. Although reduction rates varied greatly between similar sites and among replicate cores, they are well within the range of rates of reduction previously determined for dwarf *S. alterniflora* sediments during the summer by a radio-tracer technique. Much of this variation may be due to variation in the initial sulfate concentrations among the replicate cores.

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Repeated genomic sequences cloned from the sea urchin Lytechinus pictus. Jonathan Smith, Leslie Serunian, William Phillips, Andrew Murray, Sharon Horowitch, and Gerald Rubin.

The sea urchin genome is composed of approximately 25% repeated DNA sequences, most of which are short (average length 300 bases) and well characterized. However, 6 to 8% of the genome consists of repeated elements greater than 2 kilobases in length. Although much attention has been directed toward the tandemly repeated sequences (histone and ribosomal RNA genes), the dispersed repetitive sequences have been virtually ignored, except for studies showing that they are more highly conserved than the short interspersed repeats.

We have studied the long repeated sequences in the genome of *Lytechinus pictus* by isolating and characterizing genomic DNA fragments using recombinant DNA technology. Recombinant plasmids were constructed by ligating Hind III digested *L. pictus* genomic DNA into the Hind III site of bacterial plasmid pBR322 and were used to transform *E. coli*. Eight hundred and fifty transformants were isolated by resistance to ampicillin and screened with a ^{32}P-labeled probe enriched in long repeated sequences. This probe was prepared by reassociating DNA to low Cot values, digesting with S1 nuclease, size-selecting on sucrose gradients, and nick translating. When the S1-resistant DNA was electrophoresed on agarose gels, discrete bands, 500 and 800 nucleotides in length, were observed. Approximately 100 of the original clones hydridized to the repeated DNA probe; 48 of these were hybridized to ^{32}P-complementary DNA synthesized from polyadenylated RNA isolated from hatching blastulae and pluteus embryos. One single clone hybridized to both of these probes indicating that the genomic fragment contains both repeated and transcribed sequences. DNA was also prepared from two clones containing long repeats (10 and 13 kilobases in length). The genomic inserts represented by repeated sequences were localized by hybridization of the repetitive DNA probe to restriction fragments of clones transferred to nitrocellulose according to the method of Southern.

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A comparative study of the marginal bands in newt (Notophthalmus viridescens) *and chick* (Gallus domesticus) *erythrocytes.* Denice Smith.

Although the erythrocytes of newt and chick are like the red cells of other non-mammalian vertebrates in that they possess a microtubule-containing structure called a marginal band, these cells differ considerably in cell size and numbers of microtubules present in the marginal bands and they additionally differ in the degree to which the marginal band can be isolated from the nucleus, suggesting that the marginal bands in the two systems might be organized quite differently.

While marginal bands from chick erythrocytes are bound tenaciously to the nucleus by a fibrous matrix, newt marginal bands can be released from the nucleus (and possibly the matrix) by treatment of the erythrocytes with 0.5% Triton X-100 followed by a separation procedure in a sucrose step gradient. Analysis of these preparations by SDS PAGE reveal proteins which migrate in regions corresponding to MAPs, spectrin, tubulin, and actin when compared to standards known to contain these proteins, suggesting a possible association of marginal bands with the cytoskeleton.

Treatment of marginal bands with 0.5 M KCl, which is known to disrupt the trans-band material, permits the individual microtubules of chick marginal bands to be visualized in negative stained preparations. When newt marginal bands are treated with 0.5 M KCl for 1 hr, the marginal bands appear under phase contrast microscopy to be released from the cell, with microtubules splaying apart. Preliminary analysis by SDS PAGE suggests a selective removal of high molecular weight proteins by this procedure. Treatment of chick and newt erythrocytes with DNAase (1mg/ml) for 1 hr appears to release the marginal band from the nucleus. Although the fibrous matrix appears to be disrupted, the marginal bands retain their characteristic curvature. Preliminary analysis of the preparations by SDS PAGE suggests a selective removal of high molecular weight proteins as well as spectrin.

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Heavy metal effects on intestinal absorption of nutrients in the toadfish, Opsanus tau. Robin Socci, John Mercuro, and A. Farmanfarmaian.

Heavy metals (Cd, Hg, Cr, Co) have been shown to inhibit the activity of various enzymes in different animal and plant tissues. Less is known of the inhibitory effect of these heavy metals on membrane transport mechanisms and, in particular, intestinal epithelium which acts as the first and most important barrier to the entry of these heavy metals in higher invertebrates and all vertebrates. We have examined the effect of $CdCl_2$ on the absorption of amino acids in the intestine of the toadfish *in vivo*.

The test solution consisted of Forster Taggart fish ringer, a ^{14}C-labeled amino acid (L-alanine, L-leucine), with or without a chosen level of the heavy metal inhibitor ($CdCl_2$). A closed loop of the midgut was cannulated under anesthesia and the test solution inserted for a 10 min incubation period. This solution was mechanically mixed by an oscillating pump stirrer. Two concentrations of substrate were usually used. Tritiated inulin was included as a water marker and radioisotopes were measured by liquid scintillation spectrometry. At 40 mM L-leucine and 5×10^{-4} M $CdCl_2$, there was a reduction in the absorption rate of 36% ($P \leq 0.01$), compared to that of the control without $CdCl_2$. However, at the 6.6 mM level, no significant inhibition was observed. When the $CdCl_2$ level was reduced to 1×10^{-4} M, uptake of L-leucine was reduced by 52% ($P \leq 0.001$). At 1×10^{-5} M $CdCl_2$, there was no significant ($P \leq 0.05$) inhibition of leucine transport. Two concentrations of $CdCl_2$ (1×10^{-4} and 5×10^{-4} M) were tested with 6.6 mM L-alanine. In both cases there was no significant ($P \leq 0.05$) cadmium inhibition.

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Behavioral evidence for two populations of amino acid receptors in catfish taste. Ann Stewart, Bruce Bryant, and Jelle Atema.

From electrophysiological studies on the maxillary barbel of the catfish, it appears that there are two populations of receptors for amino acids: a generalist and a specialist (Caprio, J. and D. Tucker, 1976, *Soc. Neurosci.*, 2: 152). The generalists respond best to alanine, but also to all other amino acids, while the specialists respond only to L-arginine, its methyl-ester, and slightly to alanine. In cross-adaptation experiments, a 10^{-4} M solution of alanine raised the threshold of all amino acid responses except arginine to about 10^{-3} M; conversely an adapting arginine background had a minimal effect on the thresholds for other amino acids.

We tested behaviorally by touching the maxillary barbels of eight resting catfish (*Ictalurus nebulosus*) with cotton swabs dipped in stimulus solutions. The fish were first conditioned to not respond to the tactile stimulus of a blank swab. Then thresholds for alanine and arginine were determined. Both ranged from 10^{-6} to 10^{-5} M. When the aquaria were flooded with a 10^{-4} M background of arginine, the alanine threshold was raised by 0.7 or more log units in four fish and unchanged in four. When 10^{-4} M alanine was used as the adapting background, increases of 0.7 log units or greater were found for six fish ($P < 0.05$, sign test). Thus the alanine effect on arginine thresholds was slightly larger than the converse effect. When arginine and alanine stimuli were presented in their own background, the stimuli had to be 5 to 25% above background to elicit responses. This behavioral study confirms earlier electrophysiological work and demonstrates that catfish have an alanine and an arginine taste system which operate rather independently. Such a system would make it possible to detect arginine in high backgrounds of other amino acids. The significance for the catfish's natural environmet has yet to be determined.

Evidence for three conducting systems in the hydroid Clava. Darrell R. Stokes and Norman B. Rushforth.

Three nonpolarized conducting systems can be activated by electrical stimulation of the colonial hydroid *Clava squamata*. Two of these systems have been described within individual polyps: a Contraction Pulse System (CPS) and a Delayed Burst System (DBS). Stimulation of the CPS produces Contraction Pulses (CPs) which may occur as single events or as

bursts of potentials of facilitated amplitude (about 0.5 mV), and are correlated with polyp contraction. Stimulation of the DBS produces a program of bursts of pulses (DBPs) beginning about 20 sec after a shock. DBPs facilitate within a burst to amplitudes of about 0.5 mV and correlate with symmetrical depression of the tentacles.

Electrical stimulation of the stolons which interconnect polyps of the colony activates a third system: a Fast Pulse System (FPS). Fast Pulses (FPs), with no behavioral correlate, are of smaller amplitude (*ca.* 0.05 mV) and shorter duration (*ca.* 20 msec) than pulses from the CPS or DBS. Such pulses are conducted at a velocity of about 10 cm/sec, similar to that of the DBPs but three times faster than CPs. Production of FPs by electrical stimulation of the stolons induces CPs in adjacent polyps. In contrast, stimulation of a polyp to produce CPs does not evoke FPs in the stolons. This suggests that polarized transmission junctions occur between the stolons and the polyps.

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Statocyst cilia transmit rather than transduce mechanical stimuli. E. W. Stommel, R. E. Stephens, and D. L. Alkon.

We have investigated the role of motile cilia (hairs) in mechanotransduction by the statocyst of the nudibranch mollusc *Hermissenda crassicornis*. Movement of hairs which experience the weight of statoconia causes variance of voltage noise and membrane depolarization. Two complementary approaches were used to immobilize the cilia. Vanadate anion (V^{+5}) was iontophoretically injected into hair cells, using 0.1 nA currents through an electrode filled with 0.01 M $NaVO_3$ and 0.1 M Na-acetate. This reversible inhibitor of dynein cross-bridge cycling initially caused the cilia to lose their rigid, vibratile motility and assume a more classic, pliable beat pattern. When the concentration of vanadate approached 10^{-5} M, the cilia were arrested against the cell membrane. Voltage noise decreased as the cilia slowed and bent more extremely, nearly disappearing as motility was lost. The cell no longer depolarized upon gravitational or mechanical stimulation. Rapid reversal with norepinephrine or slow reversal with time restored both the noise and depolarization response. Cilia were also rendered rigid by covalent cross-linkage of the membrane "sleeve" to the 9 + 2 axoneme, using the photoactivated, lipophilic, bifunctional agent 4,4′-dithiobisphenyl azide. In initial stages of cross-linkage, the cilia remained vibratile but slowed and moved through wider excursions. Voltage noise decreased in frequency but increased in amplitude. When the cilia were fully arrested, voltage noise was minimized while the resting potential remained essentially constant. Since cilia which are partially arrested by vanadate undergo considerable bending but show decreased noise, neither the axoneme nor the ciliary membrane proper appear to be sites of transduction. In full vanadate arrest, the exposed plasma membrane itself shows no response to stimulation. However, in beating, stiffened cilia, voltage noise becomes amplified, implying an increased efficiency of transduction. Therefore, the basal region is the most likely transduction site, being the leverage point to which force is applied via the ciliary shaft.

The larval stages of Lepocreadium areolatum *(Linton, 1900) Stunkard, 1969, (Trematoda: Digenea).* Horace W. Stunkard.

At the General Scientific Meetings a year ago, it was reported that certain hydrozoan and scyphozoan medusae and ctenophores in the Woods Hole area harbor unencysted metacercarial stages of digenetic trematodes. Five species have been recognized and the life-cycles of three of them have been worked out and published: that of *Neopechona pyriforme* Linton, 1900) Stunkard, 1969 by Stunkard (1969, *Biol. Bull.*, **136**: 96–113), of *Lepocreadium setiferoides* (Miller and Northup, 1926) Martin, 1938 by Stunkard (1972, *Biol. Bull.*, **142**: 326–334); and of *Lintonium vibex* (Linton, 1900) Stunkard and Nigrelli, 1930 by Stunkard (1978, *Biol. Bull.*, **155**: 383–394). The discovery on 20 July 1978 of an ophthalmotrichocercous cercaria from *Nassarius trivittatus*, whose structure agreed with an unidentified metacercaria, first noted in 1973, led to the elucidation of the life-cycle. The incidence of infection by this species is very light; the cercaria had not been found before in the examination of thousands of

N. trivittatus. Only one infected snail was found in 1978 and only one in the examination of hundreds of snails in 1979. The adult worm produces very few, very large eggs. A happy ecological situation facilitated experimental procedure. Crowell (1945) reported that the hydroid, *Podocoryne carnea*, lives on the shells of *N. trivittatus*. This snail harbors the trematode infection and liberates cercariae; the hydroid liberates medusae, which are attacked and invaded by the cercariae. Medusae, infected in the laboratory and embedded in bits of squid and clam, were fed to cunners, *Tautogolabrus adspersus*. This method yielded juvenile and gravid specimens of *Lepocreadium areolatum*, reported initially from white perch and cunners by Linton (1900, *Bull. U. S. Fish Commission* (1898): 267–304; 1901, *Bull. U. S. Fish Commission* (1899): 405–492) and known also from puffers and other fishes since that time.

Investigation supported by NSF DEB-74-145340 AO1.

Pancreatic secretion granules are associated with microtubules. K. A. Suprenant and W. L. Dentler.

Secretion granules (SG) isolated from goosefish pancreatic islets specifically associate with microtubules assembled *in vitro* from chick brains. Microtubules and SG's were mixed and associations were monitored in solution by darkfield light microscopy. Associations were confirmed by electron microscopy. SG's only bound to microtubules with high molecular weight associated proteins (MAP-MT's). In the presence of 0.2 mM ATP, SG's bound to 43% of the MAP-MT's. Upon addition of 100-μM cAMP, 57% of the MAP-MT's bound SG's. Microtubules also aggregated in the presence of cAMP to form large bundles associated with granules. The addition of 5-mM ATP to the cAMP treated aggregates, released the SG's from the MAP-MT's and dissociated the MT aggregates. Only 12% of these MAP-MT's had granules bound. The release of the SG's and the dispersion of the MT's are nucleotide specific, since pyrophosphate, AMP, ADP, GTP, and ITP have no effect on the release or dissociation. Polystyrene beads did not associate with the MT's under any assay conditions. The association between SG's and MAP-MT's was strong enough to be maintained during ultracentrifugation. SG's and MAP-MT's were mixed in 2 M sucrose and centrifuged through a step gradient. The granules moved upward in the gradient with MAP-MT's associated to the SG membrane. In thin section, the association appeared to be mediated by the filamentous MAP proteins. Intact MT's from goosefish islets were isolated in the presence of hexelene glycol. SG's were attached along the length of the microtubules as assayed by negative staining.

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The living state. Albert Szent-Györgyi.

"What is life" is the main problem of biology. We can ask the same question in a more meaningful way: what is the difference between animate and inanimate. The difference is so great that we are justified to speak of the "living state" as a special physical state. Material systems can be decomposed into molecules, molecules into atoms, atoms into electrons and nuclei. Electrons and nuclei can be decomposed still further but the energies needed are far beyond biological dimensions. The great sensitivity and reactivity of living systems indicate that the smallest independently moving particles are very small. We have to look for them among electrons and nuclei.

The number of electrons of the electron cloud, is given by the atomic numbers. With this number of electrons the electronic cloud is closed, the atom is a "closed shell" atom. Closed shell atoms build closed shell molecules. In them the electrons have to be immobile. To make them mobile electrons have to be taken out. Electrons cannot be taken out without disturbing the electroneutrality. Electrons can be transferred to another atom by charge transfer, and form then a charge transfer complex which is electroneutral. In the one of the two atoms or molecules the cloud will be desaturated and electrons will have a mobility. Charge transfer has been studied on various models having methylglyoxal as electron acceptor and methylamine, or symmetrical dimethylethylenediamine. The properties of these models are

discussed. Charge transfer introduces an electronic mobility and reactivity, as shown by the optical and electron spin resonance spectra. The living state is an electronically desaturated state.

Migration rate of mud bacteria as a function of magnetic field strength. Barbara D. Teague, Michael Gilson, and Ad. J. Kalmijn.

Certain marine and freshwater mud bacteria are endowed with a permanent magnetic dipole moment. This moment is attributed to an endogenous chain of tightly coupled, single-domain magnetite crystals. When separated from the mud, these magnetic bacteria swim north, following the earth's magnetic field lines. As at Woods Hole, Massachusetts, the field lines are steeply vertically inclined, the bacteria rapidly return to the bottom substrate where they seem to thrive best. To quantify this migration, we measure the time to traverse the distance between two lines, 1 mm apart, as a function of the ambient magnetic field strength. Using dark-field illumination, we observe single organisms as they migrate in a low-oxygen hemocytometer chamber. We control the ambient magnetic field by regulating the current through a Helmholtz-coil system. At high magnetic field strengths, the bacteria follow a virtually straight path, swimming at rates around 150 μm/sec. At lower field strengths, they take a more random path which reduces their migration rate. Although they swerve moderately at the earth's magnetic field strength (0.5 gauss), the bacteria still achieve about 80% of their maximum migration rate observed at higher-gauss fields. This suggests that the bacterial dipole moments are well adapted to orientation in the earth's magnetic field. Since the strength of their magnet determines the degree to which the organisms overcome random motion, we can estimate the magnitude of their dipole moment.

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Excitation of squid giant axon membrane exposed to an identical solution intracellularly and extracellularly. Susumu Terakawa.

An electrical excitability was demonstrated in a squid giant axon membrane exposed to an identical solution intracellularly and extracellularly. The solution used for the intracellular and extracellular perfusion contained glycerol (12% in volume) and one of cobalt-, manganese-, barium-, or nickel-salts at the concentration of 2 mM. Usually, nothing else was added to the solution. The membrane potential measured by a KCl-containing glass pipette electrode stayed in the range of −10 to +10 mV during the intracellular and extracellular perfusions with an identical solution. When a constant inward current was passed through the membrane under such perfusions, oscillatory changes in the membrane potential and the membrane conductance were observed. The shape and the time course of these changes were very similar to those of repetitively fired action potentials. When calcium-, magnesium-, or strontium-salt was used as a sole electrolyte species, the oscillatory response did not appear. The oscillatory response was reversibly suppressed by 4-aminopyridine, but could not be suppressed either by tetrodotoxin or by D-600. A voltage clamp study revealed the N-shaped I-V characteristic of the membrane. Interpretations proposed by Teorell (1959, *J. Gen. Physiol.*, **42**: 847) and by Katchalsky (1968, *Q. Rev. Biophys.*, **1**: 127) for oscillatory phenomena in inanimate membranes may be helpful in understanding the results obtained.

Comparison of long-lasting hyperpolarization produced synaptically with that induced by cyclic AMP in Aplysia *pacemaker neurons.* Steven N. Treistman.

Cell R15 of *Aplysia* possesses an endogenous rhythm in which bursts of action potentials alternate with interburst hyperpolarizations. A few stimuli to the branchial nerve can silence the cell for periods of minutes to hours. We had previously shown that phosphodiesterase inhibitors could augment the hyperpolarization evoked by branchial nerve

stimulation, suggesting that cyclic nucleotides may play a role in the generation of these long-lasting potentials. Intracellular injection of cAMP derivatives, as well as GMP-PNP, an adenylate cyclase activator, caused sustained hyperpolarization of R15, strengthening this hypothesis. In this presentation, a voltage clamp paradigm is used to generate the pseudo-steadystate current-voltage curve, and compare the conductance changes produced by nerve stimulation with those induced by cAMP manipulation. Branchial nerve stimulation at high stimulus strengths caused an increased slope conductance in the R15 plot. Moreover, the negative slope region in the plot thought to be basic to generation of bursting activity was obliterated. Similar results were obtained after intracellular injection of GMP-PNP, cAMP derivatives, or cAMP in cells pretreated with the phosphodiesterase inhibitor, Ro 20–1724. These results are compatible with a cAMP mediation of the long-lasting synaptic event seen in R15 after branchial nerve stimulation.

This work was supported by a grant from the National Institutes of Health, and a Steps-Toward-Independence Fellowship from the Marine Biological Laboratory.

Mechanism of red algal bile pigment formation. Robert F. Troxler, Stanley B. Brown, and Kevin M. Smith.

The photosynthetic accessory pigment phycocyanobilin is structurally closely related to bilirubin, the end product of heme turnover in mammals. The latter process involves insertion of two oxygen atoms and ^{18}O-labeling experiments have shown that these atoms are derived from two oxygen molecules (Two-Molecule Mechanism) rather than from one oxygen molecule (One-Molecule Mechanism). The similarity in structure between bilirubin and algal bile pigments suggests that heme may be an intermediate in phycocyanobilin biosynthesis. However, since plants manufacture substantial quantities of magnesium protoporphyrin IX, it has been suggested that phycocyanobilin might arise from the magnesium branch of the porphyrin pathway.

We have performed preliminary ^{18}O-labeling experiments with the unicellular rhodophyte, *Cyanidium caldarium*, which suggested phycocyanobilin synthesis by the Two-Molecule Mechanism. However, photosynthetic production of $^{16,16}O_2$ prevented a precise quantitative interpretation of the results. In the present work, ^{18}O-labeling experiments were performed using cells in which photosynthesis was inhibited with the inhibitor, 3-(3,4-dichlorophenyl)-1,1-(dimethylurea). Quantitative measurements on the mass spectra of phycocyanobilin isolated from these cells, revealed high ^{18}O incorporation and clearly demonstrated synthesis by the Two-Molecule Mechanism. This process probably involves independent attack by two oxygen molecules followed by intramolecular rearrangement and release of an oxygen molecule containing atoms from each of the attacking oxygen molecules.

To evaluate the possible origin of phycocyanobilin via the magnesium branch of the porphyrin pathway, a chemical model system was studied in which a magnesium chlorin was converted to a dihydrobiliverdin derivative, chemically analagous to phycocyanobilin. A thin-layer chromatography system was developed by which the product could be purified and isolated for mass spectrometry. ^{18}O-labeling experiments on this model system are in progress. If the reaction occurs by the One-Molecule Mechanism, then phycocyanobilin synthesis via the magnesium branch is very unlikely. If, however, a Two-Molecule Mechanism is found, further experiments to distinguish between the magnesium and iron branches of the porphyrin pathway will be necessary.

Supported by NATO research grant no. 1721, NSF grant PCM79 24139 and CHE78 25557, and NIH grant GM 22822.

Glial-axonal protein transfer: its functional significance. Michael Tytell and Raymond J. Lasek.

In the squid giant axon, many proteins synthesized by the adaxonal glial sheath cells are actively transferred into the axon. We examined these transferred proteins and compared them with the proteins of axoplasm (AXM) and the stellate ganglion of the giant axon. Proteins synthesized by the sheath and ganglion were labeled by incubating the giant

axon and ganglion in 1 mCi/ml ^{3}H-leucine in artificial sea water for 60 to 360 min at 19 to 21° C. Then the AXM, containing labeled transferred proteins, was extruded. The empty sheath, AXM, and ganglion were each homogenized in either a physiological buffer or electrophoresis solubilization buffer. The homogenates were analyzed by ultracentrifugation, Sephadex column chromatography, and 2-dimensional polyacrylamide gel electrophoresis (2D-PAGE).

The centrifugation and column chromatography experiments revealed that the proteins transferred into the AXM were contained in a particle which was unaffected by hypotonic shock or 400 mM KI, but was partially disrupted by 1% Triton and completely disrupted by 0.1% SDS. The 2D-PAGE analysis of the transfer particle in AXM showed that it consisted of a select group of about 50 sheath proteins, one of which has been tentatively identified as actin.

Eleven of the transfer particle proteins in the 2D-PAGE analysis were coincident with and therefore, identical to major axonal proteins. Since axonal proteins are made in the soma and supplied to the axon via axonal transport, we compared the 2D-PAGE pattern of proteins synthesized in the ganglion with these 11 transfer particle proteins. All but one were also made in the ganglion. Thus, some of the major proteins of axoplasm, presumably supplied by the soma, are also selectively transferred to the axon in particulate form from the adaxonal glial cells. We propose that glial–axonal protein transfer may serve to supplement a specific fraction of axonally transported proteins.

RNA synthesis in isolated chloroplasts. Elizabeth Vierling.

Chloroplasts were isolated from *Hordeum vulgare* cv. Himalaya (barley) using 10 to 80% preformed Percoll silica sol gradients. These gradients separate intact plastids from broken plastids, mitochondria and other cellular debris. As judged by phase contrast microscopy the preparations were free of nuclear and bacterial contamination. Chloroplasts were prepared for electron microscopy using the Miller spreading technique after a brief lysis in 0.005% Triton X-100. DNA fibrils observed at the periphery of lysed chloroplasts were morphologically distinct from both eukaryotic chromatin and prokaryotic DNA. DNA strands had a diameter several times that of double-stranded DNA and were irregularly beaded. Structures which could be identified as transcriptionally active were not observed. For *in vitro* transcription, chloroplasts were incubated in the presence of ^{3}H-UTP at a chlorophyll concentration of 100 μg/ml. Incorporation of ^{3}H-UTP into an RNAase-A-sensitive product proceeded linearly for 30–45 min. Incorporation was α-amanitin insensitive at a concentration of 10 μg/ml and was not light dependent. The time course and level of RNA synthesis were identical with or without the addition of 1 mM $MnCl_2$. This observation is consistent with chloroplast but not with nuclear RNA polymerase activity. Initiation of transcription was assayed for using Hg-agarose affinity chromatography of γ-thiol ATP or GTP labelled RNA. Results indicated that 0.4% of the ^{3}H-UTP incorporated represented new transcript initiation.

This work was done in the MBL Physiology course, 1979 with funding by PHS training grant no. T 32-GM-07784.

Plasma fibronectin (CIG) of the dogfish plasma mediates attachment of phagocytes to collagen substrates. Gerald Weissmann, James W. Lash, G. E. Siefring, J. Ibers, and Laszlo Lorand.

Plasma fibronectin (cold-insoluble globulin CIG) mediates the attachment of mammalian cells to collagenous substrates: we sought evidence for a similar protein in plasma of *Mustelus canis*. Blood phagocytes of the dogfish (10^6) were exposed either to uncoated Sepharose 4B beads (10^4) or to beads coated with gelatin by means of cyanogen bromide. In heparinized, complement-sufficient plasma, 58 and 90%, of control and gelatin beads, respectively bound three or more phagocytes (30 min, 23° C); evidence that complement activation (as in human plasma) by Sepharose 4B mediates cell adhesion to polysaccharide particles. In decomplemented (30 min, 56° C) plasma, only 4% of control, but 75% of gelatin beads had phagocytes attached. When plasma was also depleted of CIG (by preincubation with gelatin beads), cells attached to 5% of control, and to only 28% of gelatin beads. Small petri dishes were prepared with

purified mammalian collagens (Types I, II, and III) with and without proteoglycan aggregates. Dogfish phagocytes in decomplemented plasma avidly adhered to each type of collagen (but not to uncoated areas of the dish) and proteoglycans diminished attachment. Dogfish CIG was isolated by affinity chromatography on Sepharose 4B·gelatin and eluted in 2 M arginine; yield: 0.38 mg/ml plasma. After dialysis, the isolated protein migrated identically to human CIG on 3.8% polyacrylamide gel electrophoresis (MW = 220,000) and dissociated to a single chain in the presence of dithiothreitol. Gelatin—but not control—beads exposed to either decomplemented dogfish plasma or purified CIG showed specific fluorescence when first reacted with goat anti-human CIG or rabbit anti-hamster fibroblast fibronectin and then with rabbit anti-goat IgG. Data suggest that CIG is conservative both in structure and function: attachment of dogfish and mammalian phagocytes is mediated by the same recognition protein.

Protein synthesis in cell free extracts of Lytechinus pictus *eggs.* Matt Winkler, Ellen Baker, and Tim Hunt.

The rate of protein synthesis increases 20- to 30-fold upon fertilization of the sea urchin egg as maternal mRNA is mobilized into polysomes. The increase in intracellular pH accompanying fertilization appears to play a major regulatory role in this activation. A cell-free protein synthesizing system prepared from *Lytechinus pictus* eggs was used to study this regulatory process. These cell-free systems show excellent incorporation of amino acids into protein after an initial, somewhat variable lag. Raising the pH shortens this lag and appears to mimic the activation of protein synthesis *in vivo*.

These systems will translate added globin, histone and TMV mRNAs at both pH's, as measured by the appearance of new labeled bands on SDS gels. The added mRNAs, like the endogenous mRNAs, are translated to a much lesser extent at pH 6.9 than at 7.4. Addition of even large amounts (up to 320 μg/ml) of histone mRNA results in at most a 2-fold stimulation of protein synthesis at pH 6.9 and some degree of inhibition at pH 7.4. Analysis of products suggests that the added mRNA competes with the endogenous mRNA at pH 7.4, while it augments existing protein synthesis at pH 6.9. Previous analysis of the protein synthetic machinery in the intact egg has been interpreted to indicate that the maternal mRNA is unavailable for translation before fertilization; but the very modest stimulation of protein synthesis by added mRNA at pH 6.9 seems inconsistent with this idea, and suggests that other components of the translational machinery may be in a dormant state before fertilization. The transit time for a specific mRNA was measured in this system by the addition of TMV mRNA and assaying for the first appearance of completed product on SDS gels. The maximum transit time for the synthesis of the 130,000 MW product is 20 min at pH 7.4, or approximately one amino acid per second.

Supported by NIH training grant no. TG-HD07098.

Low level excitation of chlorotetracycline fluorescence in Haemanthus *endosperm cells using image intensification.* Stephen M. Wolniak, Peter K. Hepler, William T. Jackson, and Geo. T. Reynolds.

Cell plate formation in dividing endosperm cells of *Haemanthus* involves the migration of calcium-rich vesicles to the equatorial region of the cell, followed by their coalescence at the site of incipient wall deposition. The calcium-membrane probe, chlorotetracycline (CTC, 10–50 μM) permits visualization of the plate-forming vesicles in living cells with fluorescence microscopy. CTC fluorescence is punctate and is localized at the spindle poles from prophase through late anaphase. Previous studies have shown that the poles are enriched in endoplasmic reticulum, mitochondria and plastids. By the onset of telophase, there is a dispersion of fluorescence at the poles and a concomitant increase in the region of the forming plate. Since visualization of fluorescence with intense near-UV light (390–410 nm) often disrupts normal divisions, we reduced incident excitation light by a factor of approximately 250 with a neutral density filter. We then employed image intensification of the fluorescence emission to study plate deposition as a dynamic process in living sells. Fluorescence micrographs were

taken at 15 min intervals during anaphase and telophase. In agreement with the high intensity fluorescence images, we first observed an increase in spindle midzone fluorescence when the poleward migration of the kinetochores was only 70 to 90% complete. At this stage of division, trailing chromatids extend through the equatorial region of the spindle. CTC-fluorescent material appears to aggregate at numerous sites in the phragmoplast, and as chromatid migration progresses, the highly fluorescent plate thickens. This technique demonstrates the feasibility of image intensification fluorescence studies on living cells that are normally adversely affected by intense short wavelength light (Reynolds, 1979, *Biol. Bull.*, **157**: 391).

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Characterization of the hemoglobin of the clam, Astarte castanea. Thomas D. Yager and K. E. Van Holde.

The hemoglobin of the clam *Astarte castanea* is a large (10^7 dalton) protein which binds oxygen non-cooperatively. At pH ≥ 9 in the absence of divalent cations, this protein dissociates into a single subunit.

Clams were placed on ice 10 min and then easily opened by severing the adductor muscles. At 4° C, hemolymph was collected from the pallial cavities by pipette, centrifuged 7 min at $13{,}000 \times g$, scrubbed with CO and dialyzed against tris or bicarbonate containing 10 mM EDTA. The dialysate is a virtually pure solution of the subunit. Polyacrylamide gel-electrophoresis in SDS revealed one band at 340,000 daltons, with a trace higher-MW contaminant (the sample having been boiled in 1% SDS/βME for 2 min and run upon 7.7×2.6 and 3.1×2.6 Laemmli slabs). Analysis in the ultracentrifuge gave $S_{20,w}^{\circ} = 11.3$ S and, for the concentration-dependence of S, $k_s = 45$ cm^3/g (with $\bar{\nu}$ assumed to equal 0.7425 cm^3/g).

Purification of the subunit was achieved by molecular sieve chromatography of the dialysate (through Bio-Rad A-1.5 m beads, mesh 200, on an 80×1.6 cm Pharmacia column). A single eluent peak was observed, for which the ratio of 414/280 nm absorbance was constant at 2.3. The trailing edge of the peak was homogeneous in sedimentation velocity and in SDS/PAGE (with the sample heated to 60° in 1% SDS/βME for 2 min, and run upon a 5.2×2.6 Laemmli slab). Sedimentation equilibrium analysis also showed a single component, with MW = 342,000. Using the $S_{20,w}^{\circ}$ and MW values from ultracentrifugation, the frictional coefficient was calculated to be 1.50. This relatively high value indicates that the subunit has some asymmetry.

This work was done in the MBL Physiology course, with funding from PHS training grant no. T32-GM-07784.

In vitro *reassembly of squid brain neurofilaments and their purification by assembly-disassembly.* Robert V. Zackroff, Anne E. Goldman, and Robert D. Goldman.

Neurofilaments from squid brain tissue were disassembled at high ionic strength and reassembled upon lowering the ionic strength to physiological levels. Brains were homogenized in and extracted with disassembly buffer (1 M KCl, 0.25 M MES, 5 mM EGTA, 1 mM PMSF, pH 6.6) and centrifuged at $250{,}000 \times g$ for 90 min at 4° C. The resulting supernatant contained soluble subunits which rapidly reassembled into neurofilaments upon dilution with 9 volumes of reassembly buffer (0.25 M MES, 5 mM EGTA, 1 mM PMSF, pH 6.6). Reassembled neurofilaments harvested by centrifugation consisted of one major polypeptide of approximately 60,000 daltons, and three minor polypeptides of higher molecular weight which were barely detectable on normally loaded (15 μg) slab gels. Further purification by a second cycle of disassembly, clarification, and reassembly resulted in retention of all four polypeptides. Negative staining of 1-cycle purified and subsequently depolymerized neurofilaments revealed the presence of *ca.* 3 nm diameter, 5 to 25 nm long subunits. Upon dilution into reassembly buffer, the turbidity increased abruptly and 10-nm diameter filaments appeared. This abrupt turbidity increase was followed by a slower turbidity rise to plateau which was

correlated with neurofilament elongation. Reassembled neurofilaments exhibited the light-scattering properties of long rods, indicating that turbidity may be used as a measure of polymer weight concentration. In addition to neurofilaments, negative staining revealed the presence of dense structures from which filaments often appeared to radiate. The dense structures were also present after salt-induced disassembly of neurofilaments, and were retained through at least two assembly-disassembly cycles. These results constitute the first successful purification of neurofilaments by assembly-disassembly. Analysis of possible homologies of neurofilament polypeptides and polypeptides of intermediate filaments from non-neural cells is in progress.

This work was supported by NSF and the Runyon-Winchell Cancer Fund.

Bacterial denitrification: a gas chromatographic study using acetylene inhibition of N_2O reductase in two hour incubations. C. M. Zacks, P. A. Steudler, and J. M. Melillo.

Current techniques for measuring denitrification potential in soil by the acetylene block method require incubation times of many hours or days. We have determined that denitrification characteristics of soil change rapidly after sampling, suggesting that misleading rates will be obtained unless the incubation is initiated within a day of collection, and is completed as soon as possible. A system was developed to measure denitrification in unaltered field samples in a 2-hr period. This was made possible by detection of N_2O in the 0.02–0.2 ppm range by electron capture, and use of N_2 instead of He as the balance gas in incubation vessels. In this range, short-term incubations required greater reproducibility of N_2O concentration values than could be obtained with a N_2O-He mixture. Improvement was noted when N_2 was used in place of He, which was attributed to improved homogeneity of the N_2O-N_2 system over N_2O-He.

In preliminary studies of a forest ecosystem, soil from clearcut and undisturbed areas was assayed without further laboratory alteration. It was found that activity in clearcut areas was up to 30 times greater than in the undisturbed control (650 pg N_2O-N/hr *vs.* 20 pg N_2O-N/(hr·g organic wt). This large difference was confined to surface layers alone. Correlations were found between denitrification potential and the concentration of mineral nitrogen ions, as determined by extraction with 2 N KCl. Nitrate-nitrite maintained a constant low level over a range of denitrification rates, suggesting rapid nitrate uptake, while ammonium concentrations were found to be high in high potential areas, and low in inactive sites. Rapid uptake of nitrate is supported by the finding that addition of this ion induced dramatic increase in activity, but further investigation is required to assess the relative contributions of nitrate and ammonium precursors to the denitrification pathway.

Fetatures of elasmobranch eye lenses relative to those of humans. Seymour Zigman and Teresa Paxhia.

Comparisons of elasmobranch lenses with those of humans reveal differences in size, visual accommodation and pigmentation and similarities in growth processes and structural protein distribution. Most similar is the age-related buildup of highly aggregated proteins that derive from lower molecular weight precursors. These aggregates are firmly bound to the fiber cell membranes, which are structurally similar in both species. Dogfish (*Mustelus canis*) lenses remain transparent in spite of the presence of these aggregated complexes which would cause light scattering in human lenses. Visibly fluorescent pigment is present at very low levels in dogfish lenses, at intermediate levels in those of surface-swimming sharks, and at very high levels in human lenses, especially in the membrane complexes. Lens fiber cells of elasmobranchs have similar shapes and knoblike interdigitations to those of man, but are several times larger. Their membranes accumulate soluble crystallins which cannot be removed by extensive washing with 8 M urea. The major SDS-solubilized membrane protein

of dogfish lenses has a molecular weight of 25,000 daltons, but this may include a certain amount of soluble crystallins. Heating in SDS does not aggregate this protein fully. Near-UV light exposure of lens homogenates in the presence of 1 mM tryptophan increased the binding of protein and non-tryptophan fluorescence to dogfish fiber cell membranes substantially, and electron microscopic examination revealed much greater electron density. The data suggests that additional binding of non-membrane protein to lens membranes could result from exposure of lenses to near-UV light, with fluorescent and pigmented tryptophan photoproducts serving as crosslinking agents.

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Continued from Cover Two

of biological journal titles is that published each year by BIOLOGICAL ABSTRACTS (BIOSIS List of Serials; most recent issue, 1976). Foreign authors, and others who are accustomed to use THE WORLD LIST OF SCIENTIFIC PERIODICALS, may find a booklet published by the Biological Council of the U.K. (obtainable from the Institute of Biology, 41 Queen's Gate, London, S.W.7, England, U.K. at £0.65 or $1.75) useful, since it sets out the WORLD LIST abbreviations for most biological journals with notes of the USASI abbreviations where these differ. CHEMICAL ABSTRACTS publishes quarterly supplements of additional abbreviations. The following points of reference style for THE BIOLOGICAL BULLETIN differ from USASI (or modified WORLD LIST) usage:

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5. *Figures.* The dimensions of the printed page, 5 by 7⅜ inches, should be kept in mind in preparing figures for publication. Illustrations should be large enough so that all details will be clear after appropriate reduction. Explanatory matter should be included separately in legends as far as possible, although the axes should always be numbered and identified on the illustration itself. Figures should be prepared for reproduction as either line-cuts or halftones; no other methods will be used. Figures to be reproduced as line-cuts should be drawn in black ink on white paper, good quality tracing cloth or plastic, or blue-lined coordinate paper; those to be reproduced as halftones should be mounted on board, and both designating numbers or letters and scale-bars should be affixed directly on the figures. We recommend that halftones submitted to us be mounted prints made at about 1½ times the linear dimensions of the final printing desired (the actual best reductions are achieved from copy in the range from 1¼ to 2 times the linear dimensions). As regards line-blocks, originals can be designed for even greater reductions but are best in the range 1½ to 3 times. *All* figures should be numbered in consecutive order, with no distinction between text and plate-figures. The author's name should appear on the reverse side of all figures, and the inked originals for line-blocks must be submitted for block-making.

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CONTENTS

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THE BIOLOGICAL BULLETIN accepts original research reports of intermediate length on a variety of subjects of biological interest. In general, these papers are either of particular interest to workers at the Marine Biological Laboratory, or of outstanding general significance to a large number of biologists throughout the world. Normally, review papers (except for a limited number of solicited review papers which may be accepted after formal refereeing), very short papers (less than five printed pages), preliminary notes, and papers which describe only a new technique or method without presenting substantial quantities of data resulting from the use of the new method cannot be accepted for publication. A paper will usually appear within four months of the date of its acceptance.

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3. ***A condensed title*** or running head of no more than 35 letters and spaces should be included.

4. ***Literature Cited.*** The list of references should be headed LITERATURE CITED, should conform in punctuation and arrangement to the style of recent issues of THE BIOLOGICAL BULLETIN, and must be typed *double-spaced* on separate pages. Note that citations should include complete titles and inclusive pagination. Journal abbreviations should normally follow those of the U. S. A. Standards Institute (USASI), as adopted by BIOLOGICAL ABSTRACTS and CHEMICAL ABSTRACTS, with the minor differences set out below. The most generally useful list

Continued on Cover Three

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A FIELD STUDY OF GROWTH AND REPRODUCTION IN *APLYSIA CALIFORNICA* [1]

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Some aspects of reproduction in the marine opistobranch *Aplysia californica* have been the subject of considerable research over the past ten years. Description of a cluster of neurosecretory cells in the central nervous system was followed by extensive work directed toward characterization of their secretory product, which was found to induce egg-laying (Arch, 1972; Arch, Earley, and Smock, 1976; Kupfermann, 1967, 1970; Pinsker and Dudek, 1977). Strummwasser, Jacklet, and Alvarez (1969) noted a yearly cycle both in egg-laying hormone production and in receptivity to the substance. Thus initial investigation of the reproductive cycle in *A. californica* was from the standpoint of its neurohormonal control.

To date, no long-term studies of natural populations of this species have been published. *Aplysia punctata,* found in the eastern Atlantic, has been more extensively investigated. A 16-month study of this species from Trearddur Bay, Anglesey (Carefoot, 1967a) led to the conclusions that *A. punctata* is an annual, breeding between May and October, with major settling in the autumn. The red alga *Plocamium coccineum* supplies a substantial part of the diet of the Trearddur Bay population (Carefoot, 1967a), in addition to supporting more rapid growth than several other algal species being consumed (Carefoot, 1967b, c).

A major difference between *A. punctata* and *A. californica* is size. While *A. punctata* rarely exceeds 80 g (Carefoot, 1967a), *A. californica* often exceeds 3000 g with a species record of 6800 g reported by MacGinitie and MacGinitie (1968). The growth rate and life span necessary to achieve such size have not been documented, nor have seasonal changes in abundance, weight, or reproductive

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activities of a natural population been reported. This paper describes the results of such an investigation.

Materials and Methods

This study was conducted between July 1972 and August 1975 at the University of California's Catalina Marine Science Center on Santa Catalina Island, 25 km off the California coast south of Los Angeles. Two field study areas were selected which were in close proximity to the marine laboratory and known to support subtidal populations of *A. californica* (subsequently referred to as *Aplysia*). Site 1 covered part of Bird Rock, a small island approximately 500 m from shore. The area investigated covered about 35 m of shoreline and extended to a depth of 8 m. The total area of ocean floor studied was roughly 5600 m^2. The sea bed sloped gently within the study area, but dropped more precipitously around the rest of the rock island.

Site 2 consisted of approximately 140 m of coastline starting inside Big Fisherman's Cove on Catalina Island, extending a short distance east along the coast and to a depth of about 8 m for a total sea bed area of approximately 2800 m^2.

Except for the collections described below, the *Aplysia* populations in these areas were protected from human disturbance.

Collections were made once each month at each site, beginning in July of 1972 at Site 1, and in June of 1973 at Site 2. A monthly collection at each site was continued through August of 1975. Each month, weather permitting, from two to five divers using SCUBA covered the bottom in a zigzag pattern between the depths of 1 and 8 m along the length of the study area (depths were approximate as indicated by divers' depth gauges). Because most specimens of *Aplysia* were found in water shallower than 5 m, on some dives the area below this depth was not searched. Each specimen of *Aplysia* encountered was collected. Divers rarely found animals weighing less than 20 g due to the searching techniques used, and the tendency of smaller animals to be camouflaged amidst algal fronds. The data on abundance and weight presented in this paper therefore exclude all newly metamorphosed specimens and most juveniles of less than 20 g.

From July of 1972 through March of 1973, the presence or absence of copulating individuals was noted during the monthly collections. From April 1973 through the end of the study period, additional data were collected on underwater slates during the dives. These included the number of animals paired or aggregated, and in many cases the number of animals in individual aggregations. Paired animals were usually copulating and were always in physical contact with one another. An aggregation was defined as a group of three or more specimens of *Aplysia* in physical contact with one another. Such groups generally included individuals engaged in egg-laying, copulation, or both. At any given time the aggregation could include (or on rare occasions be entirely composed of) animals not engaged in either of these activities (see also Kupfermann and Carew, 1974).

After collection, the animals were brought into the laboratory where each was weighed (after being placed on a paper towel for a few seconds to drain off

excess water). Beginning in August 1973, each animal was tagged with a one-half inch Floy Tag (Floy Tag and Manufacturing, Seattle, WA), colored with waterproof marker and stamped with an identifying number or letter. A tagging gun was used to insert the tag through one of the parapodia. While in the laboratory, the animals were housed in aquaria supplied with a continuous flow of fresh sea water within 1° C of ambient ocean temperature. After being tagged and weighed, the animals were returned to the study site, usually within 24 hr of the time of capture.

Histological examination of gonads from *Aplysia* was performed each month from October 1973 through September of 1975. To assure a supply of gonads throughout the year and to conserve local populations, gonads were obtained from specimens of *Aplysia* sacrificed for neurophysiological research at the California Institute of Technology in Pasadena, California. These animals were obtained (through Pacific Biomarine Supply Co.) from Palos Verdes on the California coast about 25 km north of Catalina Island. These were maintained in a recirculating sea water system without feeding on a regular schedule of 12 hr of darkness alternating with 12 hr of light. The temperature was maintained at a constant 14° C.

The animals whose gonads were examined had been maintained under these conditions for an average of 11 days. Each animal was weighed after removal of excess water as described above. A piece of gonad (3 to 5 mm^2) was removed (preliminary measurements had shown that average oocyte diameter is uniform throughout the gonad) and fixed in Bouin's solution made with sea water. The tissue was then dehydrated, cleared, and embedded in Paraplast. Non-serial (usually every tenth section was retained) 5-mm sections were stained using Cason's modification of the Mallory-Heidenhain stain (Humason, 1967). The stained sections were examined microscopically, and oocyte diameter measured with an ocular micrometer. To establish a standard point of reference for measurement, only oocytes in which nucleoli were visible were measured. According to Thompson and Bebbington (1969), the nucleolus is prominent until the *Aplysia* oocyte has completely matured, at which time it disappears. Thus a slight bias against very large oocytes may be introduced by this method. The average diameter of the first 50 oocytes in which the nucleolus was visible was determined. Fixation seemed to have little effect on oocyte size, as the diameter of the largest oocytes measured (80 μm) was approximately the same as a newly laid egg. In extremely immature gonads, oocytes were small and difficult to measure with certainty. Based on initial measurements, an average value of 7 μm was assigned to the oocytes of such immature gonads. The number of animals sampled each month ranged from 5 to 20, but in 19 of the 24 months, ten or more animals were examined.

Results

Seasonal variation in weight

Figure 1 shows size-frequency distributions of all collections at Site 1 in which more than six animals were found. Presentation of the data in this form

reveals some trends as well as the considerable variability in population structure from one year to the next. The tendency of small animals to appear in the winter and spring can be seen in 1973 and 1975. An increase in overall size during late spring and summer is evident in 1974 and 1975. Unpredictable fluctuations in size and numbers also occurred. In late winter and early spring of 1973, juveniles appeared as expected, and had begun to increase in size by May. In June, however, the population inexplicably disappeared and remained absent for the remainder of the summer and fall. A few animals appeared in December, and specimens of *Aplysia* in a range of sizes were present through the spring of 1974. The sizable component of small animals present in the spring of the preceding and succeeding years was absent.

Figure 2, which shows the trends apparent over the three-year sampling period, was generated by combining the data for each month throughout the sampling period. Each site is graphed separately, with each point representing the weighted average of the collections made during that month. Seasonal differences in the size of the animals are apparent. Highest weights were recorded during the summer months of June through August, while weights were generally lowest between February and May. There is also a significant difference in the weights of the populations found at the two study sites. These were compared by performing a

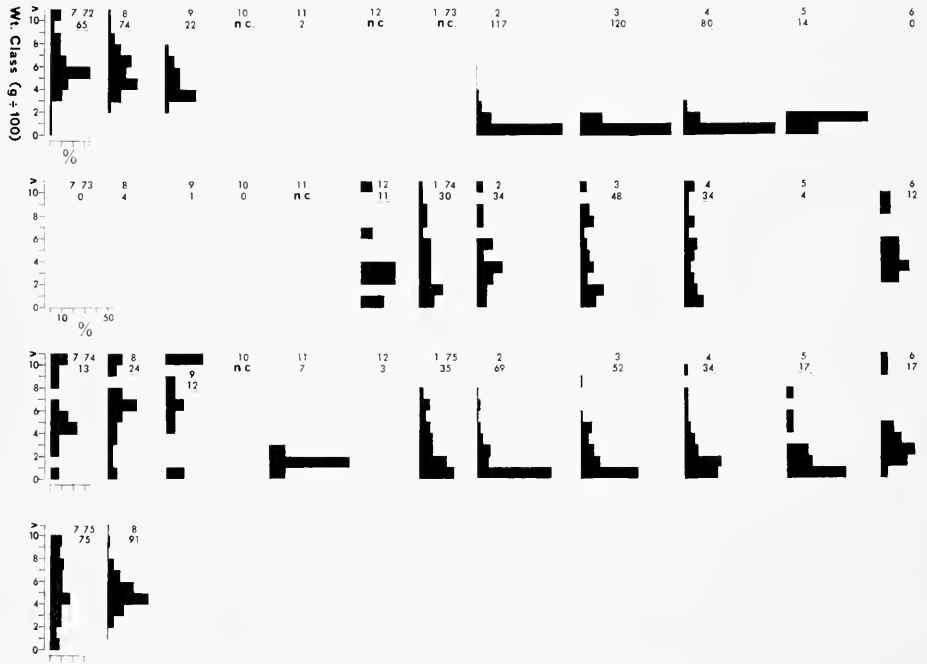

FIGURE 1. Size-frequency distribution of all collections at Site 1 in which more than six animals were found. The width of the figure corresponds to the percentage of animals found in each size class. The uppermost number is the date, while the underlined number immediately below it shows the number of specimens of *A. californica* collected that month. N.C.: no collection.

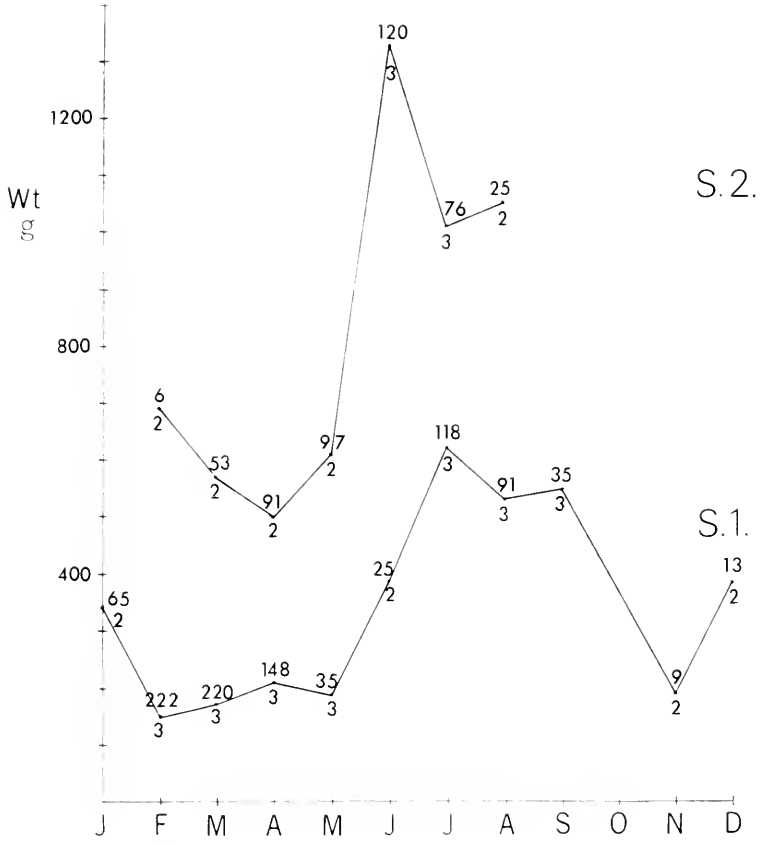

FIGURE 2. Average weights of specimens of *A. californica* collected at each study site, plotted by month. S. 1: Site 1; S. 2: Site 2. The upper of the two numbers associated with each data point is the total number of animals collected during that particular month throughout the study period. The lower number is the number of years in which samples were collected during that month. Only months in which six or more specimens of *A. californica* were collected are graphed.

paired *t*-test on the average weights for the ten individual months in which more than six animals were collected at each site (two-sided paired *t*-test; $t = 3.6$, $P < 0.01$). The two study sites differed in the availability of food algae readily consumed by *Aplysia*. Site 1 was dominated by beds of the surfgrass *Phyllospadix* to a depth of about 3 m. This plant is not normally consumed by *Aplysia* (Winkler and Dawson, 1963; personal observation). Below 3 m, the giant kelp *Macrocystis* predominated. Numerous other algal species were present on the rocky substrate including several which have been reported to serve as food for *Aplysia* (Leighton, 1966). Of these, only two were readily consumed under laboratory conditions, *Laurencia* and *Plocamium*. Neither of these algae was abundant at Site 1. Site 2 supported (among many other algal species) large beds of *Plocamium* between the depths of 1 and 4 m. Below this depth, *Macrocystis* predominated.

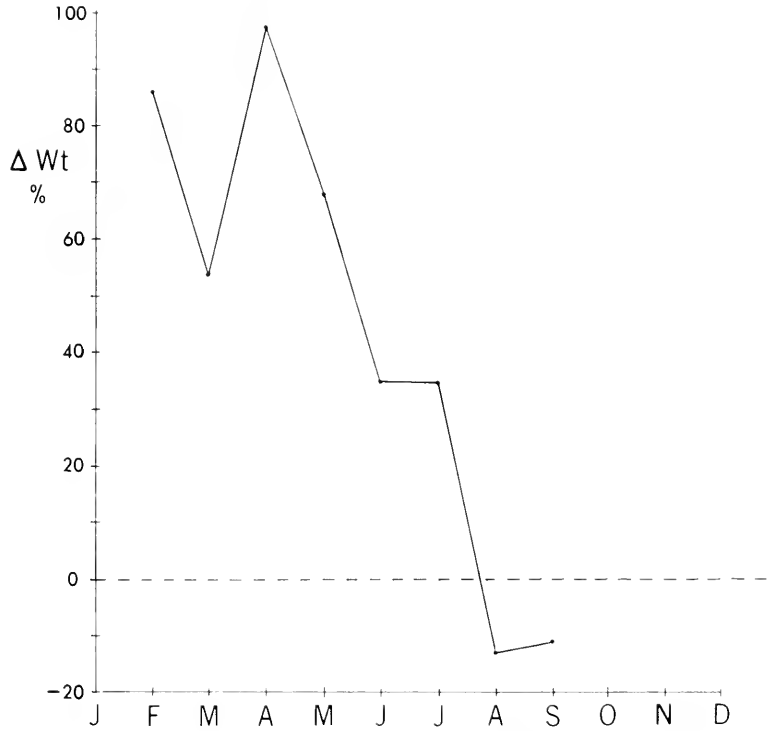

FIGURE 3. Graph of data from Table I. Changes in weight are expressed as percentages of initial body weight and plotted according to month. Data from both study sites collected throughout the tagging program are presented. Only months in which five or more animals were recaptured are graphed.

The average weights of animals used at Caltech also show seasonal variation, in spite of attempts by Pacific Biomarine Supply Co. to provide animals of roughly uniform size. Figure 6 shows that animals were smallest during late winter and early spring, while maximum weights were reached during the summer and fall. This parallels the trend seen in the populations studied on Catalina Island.

Tagging and recapture

A total of 154 specimens of *Aplysia* were recaptured between June of 1973, when the tagging program was initiated, and August of 1975, when the study was completed. Altogether, 728 animals were tagged during this period. Of those recaptured, 101 had been tagged the previous month. Only these were used to generate Figure 3, to best illustrate the growth trends typical of individual months. In Figure 3, field weight changes have been expressed as percentages of beginning body weight. Data have been combined for animals collected at both study sites, and for the same month throughout the tagging period. Only months in which five or more individuals were recaptured are graphed. Since few animals were found in winter (Fig. 4) there were few recaptures between October

and January. Again, strong seasonal trends are evident, with the maximum percent increase occurring in late winter and early spring when the animals are relatively small, and decreasing as the average weight of the population increases. A net loss of weight occurred during August and September. Table I shows the number of recaptured animals which had gained or lost weight during each month of the year. Weight loss was almost never observed between January and June, but after June, the majority of animals recaptured had lost weight.

In some instances, the same individual was recaptured more than once, providing information on the weight changes which individuals experience with age and season. These cases reveal that this species is capable of considerable growth during a single month. One animal from Site 2 gained 910 g between February and March of 1975, and an additional 1178 g between March and April of that year. Two animals which were captured first in the spring and later in the summer each showed an initial weight gain, followed by summer weight loss.

In this study, specimens of *Aplysia* were tagged with considerable success. Nine animals were recaptured after four or five months, indicating that some individuals spend a considerable time within a limited area. This species is capable of traveling relatively long distances, however. Kupfermann and Carew (1974) found that *A. californica* could easily cover a distance of 75 m (net) in a two-day period. Because many animals could have moved out of the study areas from one month to the next, recapture data from this study were not dependable for population size estimates.

Seasonal changes in abundance

It was noted during collections that the abundance of *Aplysia* seemed to vary seasonally, with a maximum during the spring, and a nearly total disappearance during the months of October and November. Abundance was estimated by determining the number of animals captured per unit effort, with one unit of effort defined as one diver-hour. The trend that emerges when data taken throughout the study are graphed by month is shown in Figure 4A. While few or no animals were found during regular monthly dives between October and December, juveniles ranging from 1 mm to 2 cm in length were found on *Plocamium*

TABLE I

Weight changes under field conditions.

Month	N	Initial Wt. (mean, g)	Wt. Change (mean, %)	No. gaining	No. losing
Jan	2	380	42	2	0
Feb	5	475	85	4	1
Mar	16	386	54	16	0
Apr	11	695	98	11	0
May	7	545	68	7	0
Jun	12	865	30	10	2
Jul	15	1060	35	7	8
Aug	31	644	−13	3	27
Sep	5	906	−11	2	3

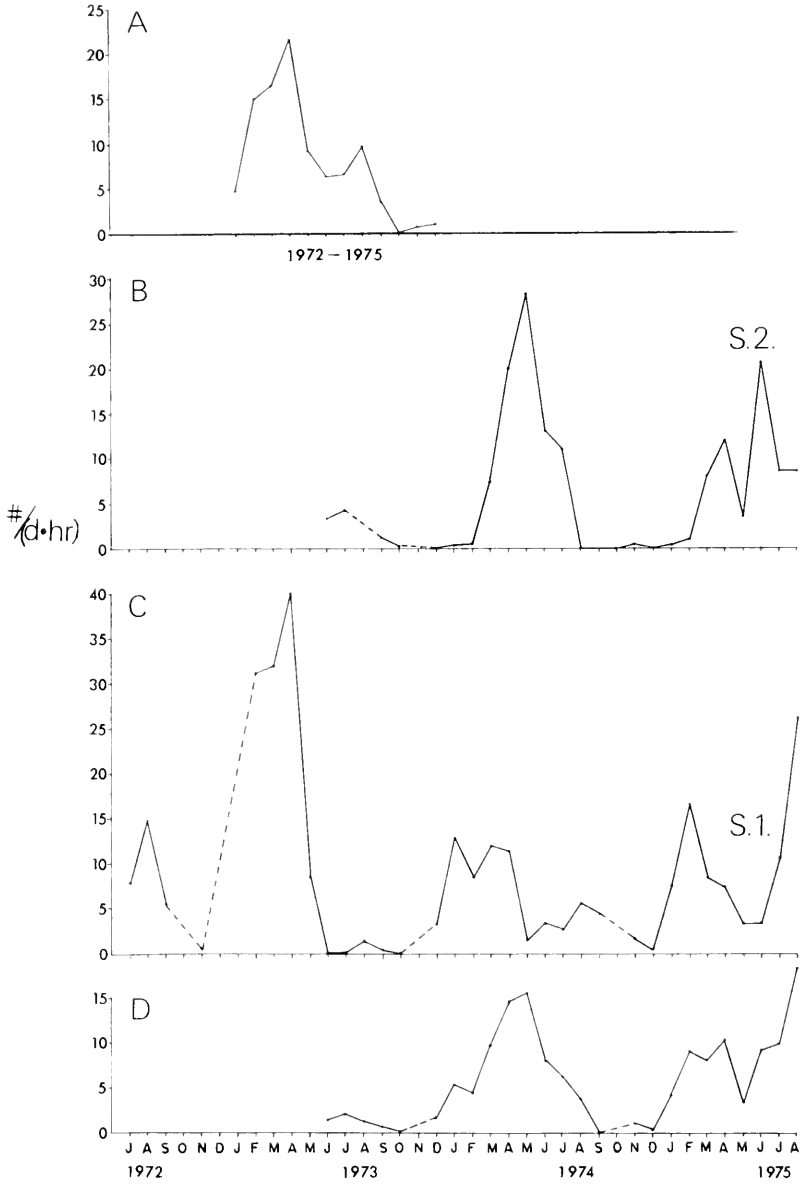

FIGURE 4. Abuandance of animals expressed as *Aplysia* per diver-hour, plotted against the month. A: data from both study sites over the entire collection period are combined in a single "composite year". B: abundance at Site 2 plotted against actual time. C: abundance at Site I plotted against actual time. D: average abundances for both sites graphed for that period during which both sites were sampled. Dotted lines indicate that one or more collections were missed during the period covered.

and *Laurencia* brought into the laboratory. Juveniles very similar in color to the red algae were found hidden among the fronds, and these small animals seemed to be especially abundant during fall, winter and early spring.

Although distinct trends emerged from the total data collected (Figure 4A), Figures 4B, C and D show the considerable variability of the two sites from year to year. For example, collections at Site 1 during June, July and August 1973 produced a total of five individuals, while collections during the same months of 1975 produced 183 animals (including 25 recaptures).

A predictable occurrence at both sites was the nearly total disappearance of animals in the fall (October through December). Many individuals collected during this period exhibited symptoms of physical deterioration. These included erosion of the free edges of the parapodia and loss of pigment resulting in pale patches on the body. Such animals frequently died in captivity, an unusual occurrence at other times of the year. Few dead animals were found in the field at any time of the year.

Reproductive activity

When collections were initiated at Site 1 in July of 1972, copulation was already in progress. The following year, no copulation or egg-laying was observed there due to the disappearance of the population in June (May was the last successful collection in 1973 at Site 1, and no mating was observed at that time.) Collections at Site 2 began in June 1973, at which time copulation was occurring. In 1974, a few large animals were first seen mating in April at Site 1, and in May at Site 2. In 1975, again a few large animals were seen mating at Site 2 as early as April, but at Site 1, copulation and egglaying were not observed until July, at which time many animals were participating.

Data on the percentage of animals aggregated, and the number of animals making up aggregations, are plotted in Figure 5. In April and May, the percentage of animals observed in pairs and aggregations was small, but increased to 80% or more during the months of July and August (Fig. 5A). Fifty-three aggregations are plotted according to the number of animals participating (Fig. 5B), which ranged from 2 to 19. Pairs of animals were observed more frequently than any other size grouping. Since *Aplysia* mates in chains of indefinite length, a pair of animals may be considered the shortest possible mating chain.

Seasonal changes in oocyte diameter

The data graphed in Figure 6 show the changes in oocyte diameter which underlie seasonal breeding in *A. californica*. Most animals collected in winter (January through March) have extremely small gonads containing few recognizable oocytes, these averaging about 7 μm in diameter. In April and May, the gonad is characterized by a mixture of oocytes of all sizes, ranging up to 80 μm, the approximate diameter of a newly laid ovum. The proportion of large oocytes increases with time, until, by the months of September, October, and November, mature animals show few of the small oocytes which predominate in the spring. The low standard error in average oocyte diameter in animals collected between January and October inidicates that oocyte maturation is relatively synchronized. In November and

December, the standard error increases due to the inclusion of some sexually immature animals. It should be noted that, during the winter months, size is not an accurate indicator of sexual maturity. Sexually immature specimens of up to 600 g have been found at this time.

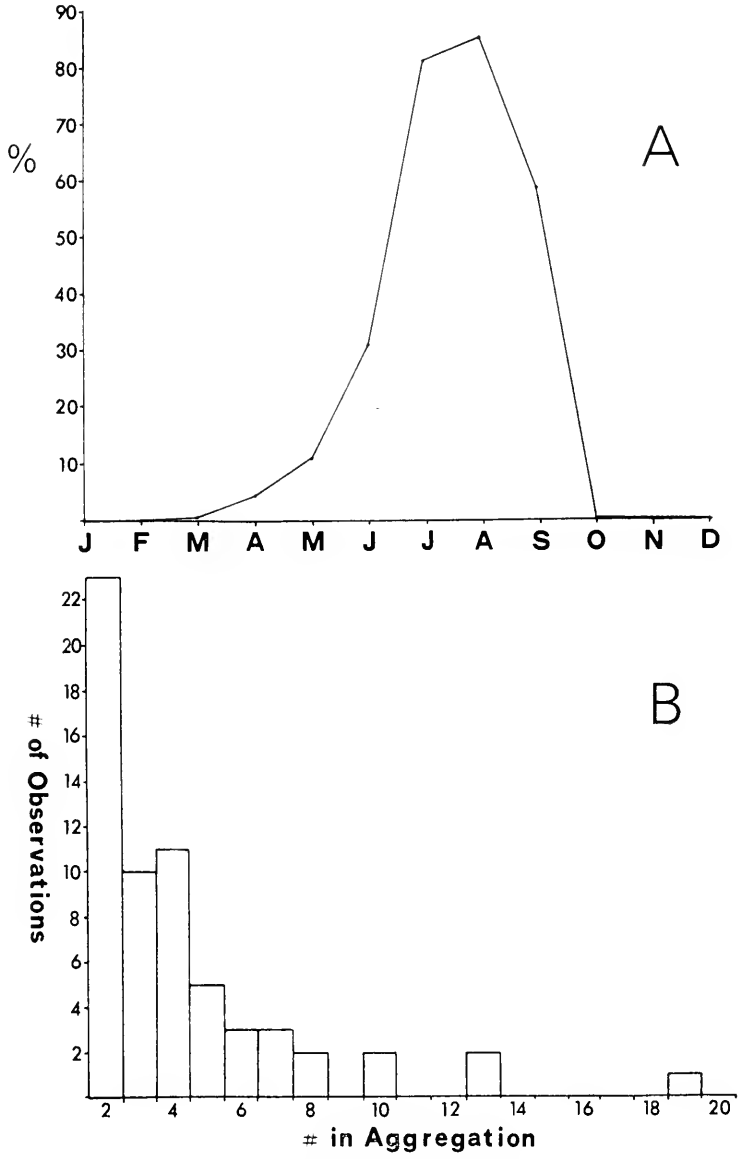

FIGURE 5. A: percentage of the total number of specimens of *Aplysia* captured each month that were paired or aggregated. Data from both study sites throughout the collection period are combined. B: frequency of observation of aggregation plotted against aggregation size.

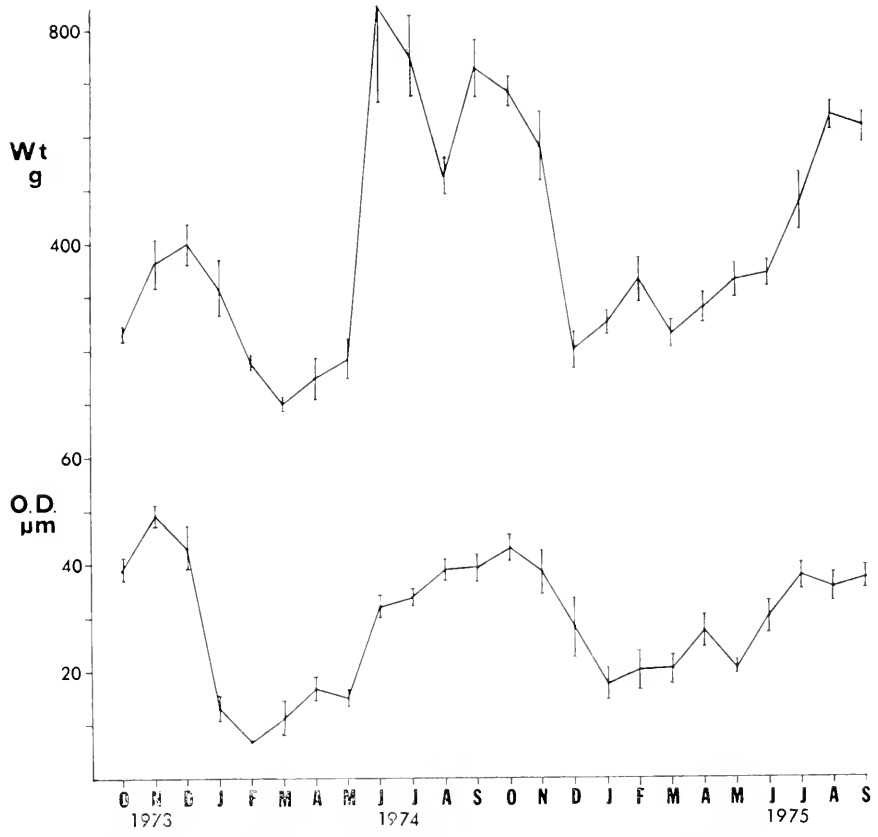

FIGURE 6. Mean weights (upper line) and oocyte diameters (lower line) of specimens of *Aplysia* plotted against time. These animals were collected at Palos Verdes, California, by Pacific Biomarine Supply Co. Most data points represent at least 10 individuals (see text). These were sacrificed throughout the month indicated, and collected an average of 11 days prior to being killed. Bars indicated standard error of the mean.

DISCUSSION

Data presented in this paper support the hypothesis that *Aplysia* have a life expectancy of approximately one year, and a single extended period of copulation and egglaying which begins late in spring and ends in the fall with the death of the sexually mature population. Although migration into deeper water and reappearance in the spring cannot be entirely ruled out, there is presently no evidence in support of this hypothesis. Animals found in the spring tend to be smaller and more abundant than those found later in the year. They appear healthy and live for long periods under laboratory conditions, in contrast to animals obtained in the fall whose skin is often eroded and who usually die after a short period of captivity.

The extended period of egg production might be expected to result in asynchrony with respect to size and sexual maturity within the population. However, there

is evidence that increasing water temperature in the spring provides a synchronizing cue for the initiation of gonadal development (Audesirk, 1976; Smith and Carefoot, 1967). Differences in survival and growth rate during different seasons might also result in greater uniformity in size than would be predicted from the long period of egglaying. Further study is required to test these hypotheses. The disappearance of nearly all animals larger than 20 g at Catalina Island as early as October is in contrast to the situation at Palos Verdes where sexually mature specimens are found until December. A comparison of environmental factors including water temperature, food availability, and wave exposure between the two locations might provide clues to this discrepancy.

The mean weights of the populations at Palos Verdes and Catalina show pronounced seasonal differences consistent with an annual species (Figures 1, 2, and 6). On Catalina, small specimens of *Aplysia,* presumably offspring of the previous reproductive season, make their appearance between January and May. Maximum size is reached in June or July, and shows a decrease in August and September. In October, November and December, few animals are found (Fig. 4). Although trends in mean weight were roughly parallel for the two Catalina Island populations sampled, a consistent and significant size difference was observed (Fig. 2). The apparent explanation for this is the relative availability of food algae at the two sites. At Site 1, no algal species known to be readily consumed by *Aplysia* under laboratory conditions was found in abundance. In contrast, Site 2 included large beds of the red alga *Plocamium* which *A. californica* will eagerly consume. In his study of *A. punctata,* Carefoot (1967a) found a strong correlation between the size of the animals and the abundance of *Plocamium.* In a comparison of eight algal species consumed by *A. punctata, Plocamium* was preferred over six other species, and supported most rapid growth (Carefoot, 1967b).

Data from tagging and recapture (Fig. 3; Table I) indicate that, at Catalina Island, *A. californica* experiences fastest growth between February and April, prior to the onset of breeding. The animal's capacity for rapid growth explains how some individuals may achieve sizes in excess of 3000 g in a single year. Growth slows as breeding intensity increases, and the average size reaches a peak in June or July. In August, when breeding is at its most intense (as indicated by the percentage of animals in pairs or aggregations in Fig. 5), a net loss of weight occurs. This loss is possibly attributable to two major causes. The first is loss of foraging time due to time spent in reproductive activities. Although the percentage of time spent in breeding has never been quantified, field observations indicate that individuals could remain in aggregations for a day or longer. Kupfermann and Carew (1974) tagged the five animals at one aggregation site and, during four visits during the next five days, always observed one or more tagged animals at the site. The animals were not individually identified, and some had certainly departed and returned, but others may have remained. Aggregations and pairs of individuals appear almost exclusively during months when copulation is observed. It is hypothesized that the animals in aggregations which are not engaged in egglaying or copulation at any given time may have recently completed one or more of these activities, or may be about to engage in them. Further study is needed to test this hypothesis. During the present study, at Site 1 nearly all the mating aggregations were discovered in dense beds of the surfgrass *Phyllo-*

spadix, whose closely spaced blades seem to present an ideal anchoring site for the tangled egg masses. Since they do not consume this plant, the animals at Site 1 probably did not eat during the time spent in aggregations. Kupfermann and Carew (1974) also never observed feeding by animals in breeding aggregations.

The second major factor contributing to weight loss is the massive egg production by these hermaphrodites (MacGinitie, 1934) which requires large energy expenditures.

Although seasonal trends in abundance are evident, these are superimposed upon dramatic fluctuations from one year to the next. Beeman (1977) noted that opisthobranch populations "tend to be erratic and sporadically explosive," a generalization with some applicability to *Aplysia.* Eales (1921) and Carefoot (1967a) reported similar fluctuations in populations of *A. punctata.*

Like *A. punctata,* which is found dead or dying in the field between September and December (Carefoot, 1967a), *A. californica* shows physical deterioration manifested externally as tissue erosion during the same months. A related phenomenon was reported by Lickey, Wozniac, Block, Hudson, and Augter (1977), who noted that specimens of *A. californica* in the laboratory seldom die between December and July, but rarely survive more than a month between August and November. For unknown reasons, dead animals were rarely found in the field at Catalina Island. In the laboratory, however, death was common during these months.

The brief life cycle of *A. californica* and the dramatic changes in its physiology and behavior as it progresses from sexual immaturity through the reproductive state to senescence have important implications for investigations of *Aplysia* neurobiology. Changes in neurohormones with respect to season have already been noted (Strummwasser, Jacklet, and Alvarez, 1969). The formation of breeding aggregations, copulation, and egglaying are dramatic behavioral changes correlated with sexual maturity. The imminent death of mature animals obtained in the fall must also be taken into account during investigations of *Aplysia* behavior and nervous system function.

Summary

1. Observations of field growth rates, reproductive activities, and abundance of *Aplysia californica* were made over a three-year period on Santa Catalina Island off southern California.

2. The mean weight of the population was found to vary with the location in which the animals were collected, presumably as a result of differing availability of food.

3. Seasonal weight differences were also apparent. In general, small specimens of *A. californica* appear between February and May. Mean weight reached a maximum between June and August. Considerable variability was encountered from year to year.

4. Tagging and recapture showed that growth rates reached a maximum in the spring just prior to breeding. The rate decreased thereafter until weight loss was experienced in August and September.

5. *A. californica* was usually most abundant in the spring, with numbers decreasing during the summer. The animals almost completely disappeared during the months of October, November, and December with the exception of extremely small specimens found on algae.

6. Breeding activity was occasionally observed as early as April and reached its greatest intensity during July and August when at least 80% of the animals collected were in breeding aggregations.

7. Histological examination of gonads showed maximum oocyte diameter between June and October, and minimum between January and March.

8. Data are consistent with an annual species whose extended summer breeding period is terminated by the death of mature individuals during the fall.

LITERATURE CITED

Arch, S., 1972. Polypeptide secretion from the isolated parietovisceral ganglion of *Aplysia californica*. *J. Gen. Physiol.*, **59**: 47–59.

Arch, S., P. Earley, and T. Smock, 1976. Biochemical isolation and physiological identification of the egg-laying hormone in *Aplysia californica*. *J. Gen. Physiol.*, **68**: 197–210.

Audesirk, T., 1976. The Role of Seasonal Periodicity, Chemical Communication, and Chemoreceptive Organs in Reproduction in *Aplysia californica* Cooper; *Ph.D. thesis, University of Southern California*, Los Angeles, California, 201 pp.

Beeman, R. D., 1977. *Gastropoda: Opisthobranchia*. Pages 115–180 in A. C. Giese and J. S. Pearse, Eds., *Reproduction of Marine Invertebrates, Vol. IV*. Academic Press, New York.

Carefoot, T. H., 1967a. Studies on a sublittoral population of *Aplysia punctata*. *J. Mar. Biol. Assoc. U.K.*, **47**: 335–350.

Carefoot, T. H., 1967b. Growth and nutrition of *Aplysia punctata* feeding on a variety of marine algae. *J. Mar. Biol. Assoc. U.K.*, **47**: 565–589.

Carefoot, T. H., 1967c. Growth and nutrition of three species of opisthobranch molluscs. *Comp. Biochem. Physiol.*, **21**: 627–652.

Eales, N. B., 1921. *Aplysia*. Liverpool Marine Biological Memoirs No. 24. *Proc. Trans. Liverpool Biol. Soc.*, **35**: 183–266.

Humason, G. L., 1967. *Animal Tissue Techniques*. W. H. Freeman and Co., San Francisco, 569 pp.

Kupfermann, I., 1967. Stimulation of egg-laying; possible neuroendocrine function of bag cells of abdominal ganglion of *Aplysia california*. *Nature*, **216**: 814–815.

Kupfermann, I., 1970. Stimulation of egg-laying by extracts of neuroendocrine cells (bag cells) of abdominal ganglion of *Aplysia*. *J. Neurophysiol.*, **33**: 877–881.

Kupfermann, I., and T. J. Carew, 1974. Behavior patterns of *Aplysia californica* in its natural environment. *Behav. Biol.*, **12**: 317–337.

Leighton, D. L., 1966. Studies on food preferences in algivorous invertebrates of southern California kelp beds. *Pac. Sci.*, **20**: 104–113.

Lickey, M. E., J. A. Wozniac, G. D. Block, D. J. Hudson, and G. K. Augter, 1977. The consequences of eye removal for the circadian rhythm of behavioral activity in *Aplysia*. *J. Comp. Physiol.*, **118**: 121–143.

MacGinitie, G., 1934. The egg-laying activities of the sea hare *Tethys californicus* (Cooper). *Biol. Bull.*, **67**: 300–303.

MacGinitie, G., and N. MacGinitie, 1968. *Natural History of Marine Animals* (Second Edition) McGraw-Hill, San Francisco, 523 pp.

Pinsker, H. M., and F. E. Dudek, 1977. Bag cell control of egg-laying in freely behaving *Aplysia*. *Science*, **197**: 490–493.

Smith, S. T., and T. H. Carefoot, 1967. Induced maturation of gonads in *Aplysia punctata* Cuvier. *Nature*, **215**: 652–653.

STRUMWASSER, F., J. W. JACKLET, AND R. B. ALVAREZ, 1969. A seasonal rhythm in neural extract induction of behavioral egg laying in *Aplysia*. *Comp. Biochem. Physiol.*, **29**: 197–206.

THOMPSON, T. E., AND A. BEBBINGTON, 1969. Structure and function of the reproductive organs of three species of *Aplysia* (Gastropoda: Opisthobranchia). *Malacologia*, **7**: 347–380.

WINKLER, L. R., AND E. Y. DAWSON, 1963. Observations and experiments on the food habits of California sea hares of the genus *Aplysia*. *Pac. Sci.*, **17**: 102–105.

Reference: *Biol. Bull.* **157**: 422–433. (December, 1979)

SALT AND WATER BALANCE IN TWO MARINE SPIDER CRABS, *LIBINIA EMARGINATA* AND *PUGETTIA PRODUCTA*. II. APPARENT WATER PERMEABILITY

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In studies of salt and water balance, permeability is an important parameter since knowledge of it, together with a knowledge of the appropriate driving force, permits predictive statements concerning net fluxes. A complication in this matter is that permeability may vary. A reduction in tracer water exchange rate has been found in a number of osmoregulating decapod crustaceans transferred to a dilute medium (*Rithropanopeus harrisii,* Smith, 1967; Capen, 1972: *Carcinus maenas,* Smith, 1970; Berlind and Kamemoto, 1977: *Hemigrapsus nudus,* Smith and Rudy, 1972; *Palaemonetes pugio,* Roseijadi, Anderson, Petrocelli and Giam, 1976). However, not all osmoregulating decapods respond to a dilute salinity in this fashion (*Palaemonetes varians,* Parry, 1955: *Carcinus maenas,* Rudy, 1967: *Penaeus duorarum* and *Uca* spp., Hannan and Evans, 1973), nor does the same species necessarily respond in the same way under similar conditions. Also, there are alternative explanations for changes in water exchange other than a true change in permeability (Smith, 1970, 1976). However, the adaptive value of a reduction in water permeability when the osmotic gradient is increased is quite clear and, on the basis of the available evidence, a strong argument can be made in favor of changes in water permeability.

There is, perhaps, little reason to predict that an osmoconforming crab would respond to dilute salinity with a reduction in permeability; however, when specimens of *Libinia emarginata* were transferred from 100 to 80% sea water, there was a reduction of 30% in water exchange after the first hour (Cornell, 1973). It can be argued that a reduction in water permeability is also an adaptive response to dilute salinity in an osmoconformer, but the argument is open to question. In the present study, some aspects of apparent water permeability have been examined in the osmoconforming crabs *Libinia emarginata* and *Pugettia producta* and in the osmoregulating crab *Carcinus maenas.* An attempt is made to identify some factors which may be important in changes in water exchange rates.

Materials and Methods

General procedures

Specimens of *Pugettia producta* were maintained at 10–12° C in filtered sea water (SW), about one crab per 4 liters, at the University of California, Berkeley, California. Specimens of *Carcinus maenas* and *Libinia emarginata* were maintained at 19 to 21° C in running SW at the Marine Biological Laboratory, Woods

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Hole, Massachusetts. The SW used at Berkeley was obtained from the Bodega Marine Laboratory, Bodega Bay, California and had an osmotic pressure of about 1015 milliosmols, while that at Woods Hole was about 945 milliosmols.

Exchange rates of tracer water were measured using D_2O according to the technique described by Welsh, Smith and Kammer (1968). Specimens of *Carcinus* and *Libinia* were immersed for 11 and 15 min, respectively, in aerated 3-liter solutions of sea water (SW) plus 2.0 to 2.5% D_2O maintained at constant temperature (21 ± 0.5° C) in a bath of running SW. Samples of blood were withdrawn by puncturing an arthrodial membrane with a drawn-out Pasteur pipet.

Heart rate was determined by implanting chronic platinum electrodes which just penetrated the hypodermis over the region of the heart. The electrodes were cemented to the carapace with quick-setting ("five-minute") epoxy glue, after scraping away the epicuticle from the region where the bond was to be made. Electrodes were implanted at least 24 hr prior to an experiment.

Measurements of pressure changes caused by the beat of the scaphognathite were made with a Statham model P23BC pressure transducer connected by polyethylene tubing (P.E. 190, 0.047 inch = 1.2 mm inside diameter) to the cut-off tip of an 18-gauge hypodermic needle. A hole, positioned by external landmarks, was drilled through the carapace, and the needle tip was inserted through the hole and into the branchial chamber, just posterior to the scaphognathite. The needle tip was cemented in place with quick-setting epoxy. Measurements of heart and scaphognathite rates were recorded on a Grass Instrument Co. model 7a polygraph.

Isolated gills from *Libinia* were first flushed and then perfused with a solution containing inorganic ions in ratios given for *Cancer* perfusion fluid (Welsh, Smith and Kammer, 1968) and dextrose (1.5 mM). The solution was buffered to pH 7.2 with "Tris" (about 25 mM) and the final solution was adjusted to 945 milliosmols. A peristaltic pump, either a Buchler Instruments Polystatic Pump or a Sage model 375A, controlled the rate of perfusion and recirculated fluid between the gill and a small reservoir from which samples were taken. The total fluid volume of the system was about 1.5 to 2.0 ml. Polyethelene tubing was fitted to the afferent and efferent vessels and secured with silk thread. A small clamp, placed about 5 mm from where the tubing entered the gill, maintained the proper spatial relationship between the two tubes. A rod, attached to the clamp, suspended the preparation in a beaker containing 300 ml of aerated SW plus 3% D_2O maintained at 20 ± 0.1° C. Constant stirring was provided by a magnetic stirrer.

A practical approach to estimates of apparent water permeability

Two different measurements of water permeability can be made, osmotic permeability and diffusive permeability.

Osmotic water permeability (P_{osm}) = $L_pRT/\bar{V}_w$, where L_p is the hydraulic conductivity, R is the gas constant, T is the absolute temperature, and $\bar{V}_w$ is the partial molar volume of water. L_p is a measurement of permeability and, simply stated, is a flux divided by a driving force. The advantage of converting L_p into P_{osm} is that by doing so, it is possible to compare P_{osm} with estimates of diffusive water permeability, since both can be expressed in the same units.

Unfortunately, the conditions under which L_p may be determined are incompatible with most whole-animal experiments. However, the concept of expressing a net flux divided by a driving force is useful and has been adopted here. The symbol L_p* will be used to indicate a net flux divided by osmotic pressure and values will be expressed in $\mu l/(g \cdot hr \cdot osmol)$. P_{osm}* will be expressed in $cm^3/(g \cdot sec)$ in keeping with the more conventional units $[cm^3/(cm^2 \cdot sec) = cm/sec]$. When expressed in $cm^3/(g \cdot sec)$, L_p* is in the units of $cm^3/(g \cdot sec \cdot atm)$, $R = 82.04\ cm^3/(atm \cdot mole \cdot degree)$, T is absolute temperature in degrees K, $\bar{V}_w = 18\ cm^3/mole$. Since permeable surface area is unknown in a whole animal, weight has been substituted and allowances must be made for this factor in comparisons where large weight differences occur.

In a decapod L_p* will reflect not only water permeability, but other factors as well. L_p* only provides an adequate description of water movements across an ideal semi-permeable membrane which separates two solutions containing non-electrolytes. The characterization of a membrane which is permeable to solutes is more complex. When the solute is composed of univalent ions, the phenomenological approach based on irreversible thermodynamics requires the determination of six membrane coefficients (see review by House, 1974). Since water movement is influenced by the presence of permeant ions, L_p must be measured in the absence of an osmotic gradient and current flow, and in the presence of a driving force of hydrostatic pressure. Clearly, the measurement of net water fluxes under these conditions would be difficult in an intact decapod crustacean. It is believed, despite some objections, that the present approach provides a meaningful way of dealing with the problem of making measurements of "water permeability" in a whole animal.

Diffusive water permeability $(P_d) = kv/A$, where v is the volume of the water pool, A is surface area and k is a rate constant. In a well-stirred, two-compartment system in a steady state, the tracer concentration, f(t), in the very small compartment which initially contains no tracer is given by $f(t) = C(1 - \exp -kt)$, where t is time, C is the concentration in the very large compartment, and k is a rate constant. The value of k is proportional to the diffusive water permeability, and in similar animals the constant is similar, so that k should provide a reasonable basis on which to make comparisons of permeability. In the present study, the results of the experiments with tracer water will be expressed in terms of the rate constant k in (1/hr), where $k = (1/t)\ \log_e[C/(C - f(t))]$. The symbol P_d* in $cm^3/(g \cdot sec)$ will indicate the analog to the more familiar P_d in cm/sec.

Results

*Estimates of L_p**

For an animal in a steady state, L_p* may be estimated from the urine production rate and the osmotic difference between the blood and the medium. This approach is well suited to osmoregulators, but in an osmoconformer, the osmotic difference can be small (about 2 milliosmols in *Pugettia*) and thus subject to large errors in measurement. An alternative approach is to determine the rate of weight gain at time 0, when an animal is transferred to a dilute test medium.

TABLE I

Estimates of L_p in normal and nephropore-occluded specimens of* Libinia emarginata *and* Pugettia producta. *Analysis of covariance of the regressions of the transformed weights (see text)* vs *time indicates that there are significant differences between the initial rates of weight gain for normal specimens of* Libinia ($N = 19$) *and* Pugettia ($N = 10$) ($P < 0.001$) *and for nephropore-occluded specimens of* Libinia ($N = 7$) *and* Pugettia ($N = 7$) ($P < 0.05$).

Animal	*Libinia*		*Pugettia*	
State of nephropores	Normal	Occluded	Normal	Occluded
Average weight (g)	152	144	121	117
Rate of weight gain at $t = 0$, expressed in volume [μl/(g·hr)]	30	33	70	67
Osmotic gradient at t = 0 (osmols)	0.189	0.189	0.203	0.203
L_p* [μl/(g·hr·osmol)]	159	174	345	330

At this moment, urine production is equal to that in the normal medium, the osmotic gradient is known, and the rate of weight change can be assumed to equal the net flux of water.

Empirically, it has been found that the equation $f(t) = Ct \exp -jt$ (where $f(t)$ is the weight at time t, C is a constant and j is a rate constant) provides a good description of weight changes in a number of decapods transferred to a dilute medium. At time 0, the rate of change of $f(t)$ can be determined from the first derivative and is simply C. Thus, by plotting the transformed weight data (transformed by $\log_e[((W_t/W_o) - 1)(1/t)]$, where t is time and W_t and W_o are the weights at times t and 0, respectively) against time, the y intercept can be determined, and the value of C is equal to antilog$_e$ of the y intercept. The resulting plots are seldom strictly linear, but they are sufficiently so that it is an easy task to estimate the intercept.

Using the above technique, the rates of weight gain were obtained from data on normal and nephropore-occluded animals (Cornell, 1976). Values for L_p* were calculated from these rates and the changes in osmotic gradients. The results, presented in Table I, suggest that *Pugettia* is about twice as permeable to water as *Libinia*. The average for normal and nephropore-occluded animals is 166 and 338 μl/(g·hr·osmol) for *Libinia* and *Pugettia,* respectively. From these estimates, it is possible to calculate the expected osmotic difference necessary to produce an influx of water equal to the rate of normal urine production (2.1 and 2.5 μl/(g·hr) for *Libinia* and *Pugettia,* respectively, Cornell, 1979); thus, $2.1/166 = 13$ milliosmols and $2.5/388 = 7.4$ milliosmols for *Libinia* and *Pugettia,* respectively. The estimate of 7.4 is greater than the observed value of 2 milliosmols in *Pugettia.* The osmotic difference between the blood and the medium is not known for *Libinia;* however, in both cases, these estimates are greater by about an order of magnitude than the colloid osmotic pressure (Mangum and Johansen, 1975). These discrepancies raise questions concerning the mechanism for the entry of water in an osmoconforming crab. One interpretation of the present results is that, whatever the mechanism, the driving force is equivalent to an osmotic pressure of between 7 and 13 milliosmols.

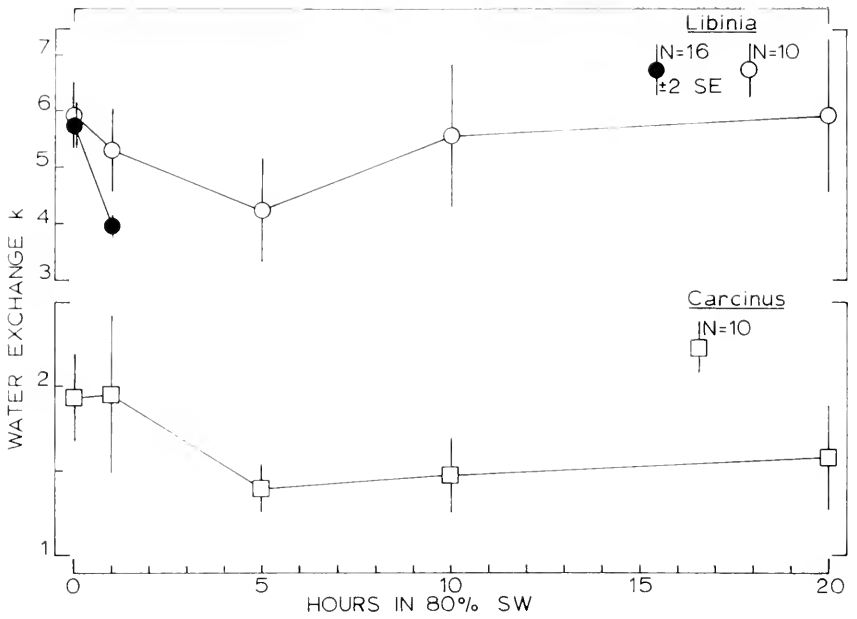

FIGURE 1. Changes in D_2O water exchange rates in specimens of *Libinia emarginata* and *Carcinus maenas* transferred from 100 to 80% SW, expressed as k in (1/hr), the hourly water exchange fraction. For specimens of *Libinia*, the results from two experiments are shown: the first, is indicated by the solid circles (average weight of crabs, 168 g); the second, is indicated by the open circles (220 g). For specimens of *Carcinus*, the results are indicated by the open squares (22 g).

I have calculated L_p* for specimens of *Pugettia* during the first several hours after transfer to 80% SW. The calculations suggest that there is a reduction in L_p* of about 60% during the first hour; however, this may be an artifact. The calculations, based on measurements which have relatively large variances (weight gain, urine production, and the osmotic gradient, Cornell, 1976), become increasingly sensitive with time to errors in measurement. Since a plot of the logarithm of the osmotic gradient between the blood and the medium versus time is not significantly curvilinear, a large reduction seems unlikely. Perhaps the safest conclusion is that the data are inadequate to indicate whether or not a short-term change in L_p* occurs.

Etimates of k

The results of two experiments in which specimens of *Libinia* were transferred from 100 to 80% SW are shown in Figure 1. For practical reasons, the points at time 0 were actually determined 24 hr prior to the transfer of animals to 80% SW. In the first experiment, k in (1/hr) was 5.68 for animals in SW and 3.99 for the same group after one hour in 80% SW, a reduction of 30% ($P < 0.001$, paired t-test). The second experiment is similar to the first, but was carried out for a longer period of time. In this experiment, k, measured after one hour of

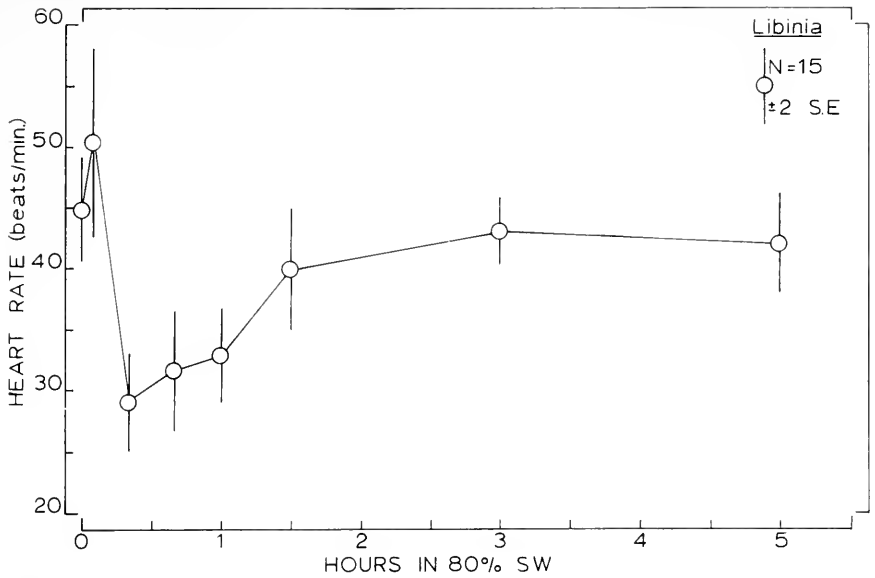

FIGURE 2. Changes in heart rate of specimens of *Libinia emarginata* transferred from 100 to 80% SW. Rates were determined from 2-min intervals (average weight of crabs, 150 g).

exposure to 80% SW, was not significantly different from k in 100% SW, but after five hours there was a significant decrease ($P < 0.005$, paired t-test). The reduction in k appears to be a transient response, since k returned to normal after ten hours. The cause of the difference in k between the two groups of animals is unknown.

While the reduction in k in *Libinia,* an osmoconforming crab, appears to be a transient response, long-term reductions have been reported for a number of osmoregulating crabs transferred to dilute salinities. The time course for this change has been reported for *Rithropanopeus* (Capen, 1972). In this crab, there is a reduction in k within the first hour of transfer from 75 to 10% SW. The initial phase of this reduction is not unlike that in *Libinia,* both in the initial rate of change and in the final extent of the change. It is of some interest to know if the time course in *Rithropanopeus* is similar to that in other osmoregulating crabs that have a reduced k in dilute media. To determine this, k was measured in specimens of *Carcinus maenas* transferred from 100 to 80% SW. The results of this experiment appear in Figure 1. For specimens of *Carcinus* in 100% SW, k was 1.94. After one hour of exposure to 80% SW, there was little change in k, but after five hours of exposure, the value had decreased by about 30% ($k = 1.41$, $P < 0.005$, paired t-test).

In the present study, k was determined in animals which were in a steady state, and in animals which were adapting to 80% SW and were not in a steady state. Therefore, it seems appropriate to ask if the reduction in k can be attributed solely to a change in state. The answer is probably "no." Two variables will be considered, surface area and volume of the water pool. The

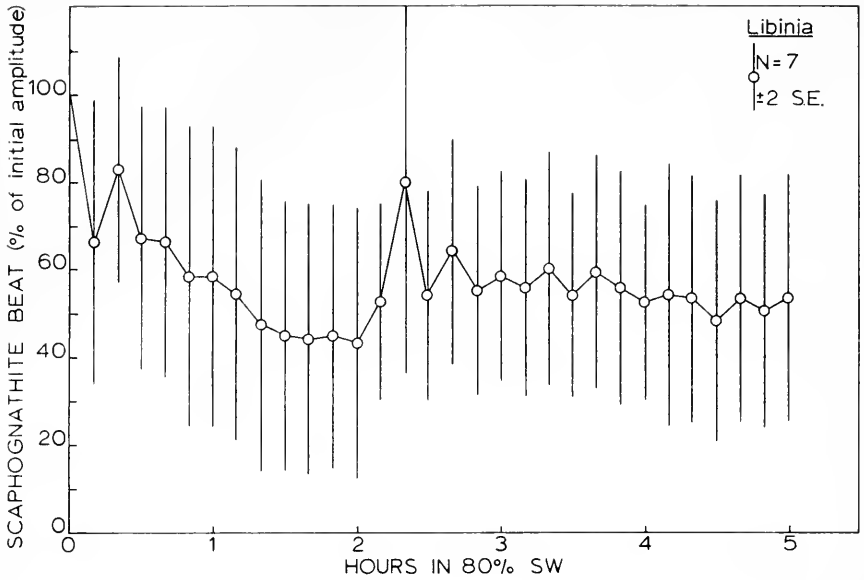

FIGURE 3. Changes in the amplitude of scaphognathite beat in specimens of *Libinia emarginata* transferred from 100 to 80% SW. Results are expressed as a percentage of the pressure developed by beats at time 0. The average amplitude of beat at time 0 was 0.7 cm H_2O and the average rate, 180/min (average weight of crabs, 163 g).

first, although difficult to evaluate, may be dismissed at the gross level on the grounds that whatever tendency for change exists is for an increase in surface area—a direction opposite to that necessary to account for the reduced k. The second may be dismissed on the grounds that, although an increase in volume occurs, and this would tend to reduce the value of k, the extent of the volume change (0.56% body weight in *Libinia* and much less in *Carcinus*) is insufficient to change the volume of the water pool (about 70% body weight) by an amount necessary to account for the reduction. The presence of more than one water compartment can also be considered; although this was not found in preliminary studies in *Libinia*, it has been reported for *Carcinus* (Rudy, 1967). Rudy found that the fast compartment was 60 to 80% of the total volume of exchangeable water; if all of the increase in volume occurred in this compartment, it would not account for the decrease in k.

Two possible mechanisms for changes in k

There is some similarity in the initial change in k in specimens of *Carcinus*, *Libinia* and *Rithropanopeus* transferred to a dilute salinity. Although this similarity may be superficial, there could be a common mechanism. There are, of course, many possible changes which could account for a reduction in k, but for present purposes, we will consider changes which occur in the unstirred layers at a liquid-solid interface. The concentration of a substance within an unstirred layer may be quite different from its concentration in the free medium, and under some condi-

TABLE II

Comparison of P_{osm} and P_d* for some crabs. References are indicated by superscripts: a, this report; b, Born (1970); c, urine production from Shaw (1961), osmotic gradient from Smith (1970); d, urine production from Binns (1969), osmotic gradient from Smith (1970); e, Rudy (1967); f, Smith (1970).*

Animal	Medium (% SW)	$P_{osm}^* = L_p^* \frac{RT}{\bar{V}_W} \times 10^4$ [cm³/(g·sec)]	$P_d^* = \frac{kv}{g} \times 10^4$ [cm³/(g·sec)]
Libinia emarginata	100	28[a]	11[a]
Pugettia producta	100	56[a]	36[b]
Carcinus maenas	100–94		1.5[a], 4.6[f], 3.7[a]
	80–70	5.3[c], 5.4[d]	1.5[e], 5.3[f], 2.7[a]
	50–40	4.3[c], 4.4[d]	1.4[e], 3.9[f]
	25–35	5.1[c], 7.5[d]	3.4[f]

tions, the thickness of this zone is an inverse function of the free stream velocity. Unstirred layers have a large influence on the rate of tracer water movement across very permeable membranes, since as permeability increases, diffusion through the unstirred layers becomes rate limiting.

For several crabs (*Rithropanopeus,* Capen, 1972; *Uca,* Hannan and Evans, 1973), it has been shown that 86 to 90% of tracer water exchange occurs in the gill chambers. Thus a change in blood circulation, or external irrigation of the gills could have a major influence on total water exchange. When specimens of *Libinia* were transferred to 80% SW there was a reduction in heart rate (Fig. 2). This could indicate a reduction in the blood circulation in the gills, and might account for the reduction in k. This idea received some support when it was found that water exchange was a function of the perfusion rate in isolated gills of *Libinia* maintained in SW: after 10 min of perfusion at 25, 47, and 90 ml/hr, the concentrations in the perfusion fluid, expressed as a percentage of the external medium, were 6.3 ± 2.84 (6), 14.2 ± 4.56 (6)*, and 18.2 ± 10.5 (5) % D_2O (mean $\pm$ SD (N), * = $P < 0.001$, paired *t*-test), respectively.

These is also evidence for a reduction in the rate of irrigation of the gills. In a typical 150-g specimen of *Libinia* in SW, the scaphognathite, or gill bailer, beats 150 to 200 times per minute. When this crab was transferred from 100 to 80% SW, there was a somewhat erratic, but definite reduction in the amplitude of the pressure pulse (Fig. 3), and a small tendency for a reduction in the rate of beat. Furthermore, in some animals, the scaphognathite appeared to stop for periods of several minutes. Most recordings were carried out for a 5-hour period, at which time the scaphognathite beat had not returned to normal. In one 15-hr recording, the scaphognathite beat returned to normal at about 9 hr. Considering the time courses, the change in the amplitude of the scaphognathite beat is better correlated with the change in k than the change in heart rate.

DISCUSSION

Comparative studies of decapod crustaceans, using tracer water (Rudy, 1967; Thompson, 1970; Hannan and Evans, 1973) and other techniques (Nagel, 1934; Gross, 1957; Herreid, 1969), have shown that there is a relationship between

"permeability" and habitat. In general, permeability decreases sequentially in animals from marine, brackish-water, and fresh-water habitats. Among closely related osmoconformers, permeability, as indicated by weight gain, seems to decrease with increasing euryhalinity (Davenport, 1972). *Libinia emarginata* and *Pugettia producta* provide an additional example of this type of relationship, since the lower apparent permeability of *Libinia* (Table II), can be correlated with its great salinity tolerance; *Libinia* can be adapted to 40% SW (Gilles, 1970) while *Pugettia*, to only 50 to 55% SW. These differences between *Libinia* and *Pugettia* are possibly related to the fact that environmental salinity fluctuations are greater on the east coast of the United States, where *Libinia* is found, than on the west coast of the United States, where *Pugettia* is found.

The relationship between tracer water exchange rate (k) and habitat reinforces the belief that k is a meaningful measure of permeability; however, some reservations about the meaning of small changes may be necessary. Generally among decapod crustaceans, changes in salinity have been found to affect k by about 20 to 40%. This change in k is small enough that factors other than permeability could be responsible. One such factor is a change in unstirred layers. In specimens of *Libinia* exposed to a dilute medium, the transient reduction in k, which initially resembles the long-term reduction in *Rithropanopeus harrisii* (Capen, 1972) and *Carcinus maenas* (this report), can be correlated with a reduction in the amplitude of the beat of the scaphognathite. In other studies, there are also indications of reductions in gill irrigation of crabs exposed to a dilute medium (*Libinia* and *Maja verrucosa*, King, 1965; *Carcinus maenas*, Hume and Berlind, 1976). A temporary reduction in gill irrigation could provide a simple mechanism for short-term reductions in k and might confuse the issue of permeability changes since the scaphognathite is not only affected by salinity changes, but oxygen tension (McMahon and Wilkens, 1975) and tidal cycle (Arudpragasam and Naylor, 1964) as well.

Changes in blood flow could also affect k, but the evidence for this is not strong. Reductions in the heart rates of *Libinia* and *Carcinus* were thought by Cornell (1973, 1974) to provide a possible explanation for reductions in k; however, further data make this possibility less likely. In addition, Hume and Berlind (1976) found that the heart rate in *Carcinus* increases in dilute media. The cause of this discrepancy between the results of Hume and Berlind and my own is not known.

It is generally believed that the effects of unstirred layers can be much greater on estimates of diffusive water permeability (P_d) than on osmotic water permeability (P_{osm}) (House, 1974). However, much of the discrepancy between these estimates may be eliminated by adequate stirring (Dainty and House, 1966). Thus, one possible way of evaluating the effects of unstirred layers comes from the comparison of P_{osm} and P_d.

It should be pointed out that it is difficult to justify the necessary assumptions in comparing P_{osm} and P_d in something as "simple" as an isolated sheet of epithelium, let alone an intact animal. But, we are guided by the words of House (1974, p. 341): "In view of the dubieties about errors in P_d and L_p values one has no proper right to compare them. Nevertheless, I shall do so." Table II shows P_{osm}* and P_d* for *Carcinus*, *Libinia* and *Pugettia*. In *Libinia* and *Pugettia*, P_{osm}* was determined from the rate of weight gain in 80% SW at time 0 and

the osmotic gradient caused by the transfer from 100% SW. In *Carcinus*, P_{osm}* was determined from published values of urine production rates, and osmotic gradients, for animals in a steady state in dilute SW. For estimates of P_d*, it was assumed that the exchangeable water in all crabs was 70% body weight. P_{osm}*/P_d* is greater than unity in all cases. Interpreting these results is a matter for speculation, but I suggest that these data are consistent with the possibility that changes in unstirred layers could have a major influence on water exchange rates in all of these crabs since the ratio of P_{osm}*/P_d* does not differ greatly among *Carcinus, Libinia* and *Pugettia*.

Thus, a transient increase in the thickness of unstirred layers surrounding the gill might explain the transient reduction in k in a crab such as *Libinia*. Such a mechanism could also affect the initial phases of a long-term reduction in k in a crab such as *Carcinus*, but the evidence suggests that the maintenance of long-term reductions in k are the result of some other mechanism, which may be under hormonal control (see Tullis and Kamemoto, 1974, and Berlind and Kamemoto, 1977). The existence of this other mechanism is demonstrated by the fact that the isolated gills of *Callinectes spadius* and *Cancer irroratus* from crabs adapted to 40% SW have a lower water exchange rate than do those from crabs adapted to 100% SW (Cantelmo, 1977).

In the strictest sense, it is possible to state that a change in water permeability has not been demonstrated beyond doubt in any decapod crustacean. But regardless, a process that can reduce the effect of an osmotic gradient is potentially of adaptive value. Thus, we should ask if a reduction in k reflects the action of such a process. A definitive answer to this question is not yet available for *Libinia, Pugettia*, and many other decapod crustaceans.

This paper constitutes part of a doctoral dissertation submitted to the Department of Zoology, University of California, Berkeley. It is a pleasure to thank Dr. Ralph Smith for his encouragement and guidance during the course of this work. I am grateful to Dr. Cadet Hand and the laboratory staff for their assistance and for the use of the facilities of the Bodega Marine Laboratory, Bodega Bay, California. I thank Dr. Robert Josephson for the opportunity to pursue my work while acting as a course assistant in the Experimental Invertebrate Zoology Course at the Marine Biological Laboratory, Woods Hole, Massachusetts. Financial assistance in the form of a one-year University Fellowship was greatly appreciated. My wife, Mary, deserves special thanks.

Summary

1. Estimates of osmotic water permeability suggest that *Libinia emarginata* [166 μl/(g·hr·osmol)] is less permeable to water than *Pugettia producta* [338 μl/(g·hr·osmol)]. Data on tracer water exchange supports this conclusion and the differences in apparent permeability can be correlated with differences in habitat.

2. Short-term changes in D_2O water exchange were examined in specimens of *Carcinus maenas* and *Libinia emarginata*. When these crabs were transferred from 100 to 80% sea water, there was an initial reduction in k in (1/hr), the

hourly water exchange fraction, of from 1.9 to 1.4 and from 5.8 to 4.3 in *Carcinus* and *Libinia,* respectively. In both crabs, the initial response to the dilute medium is similar; however, in *Libinia,* the reduction in k is transient while in *Carcinus* it is a long-term response.

3. Estimates of osmotic (P_{osm}*) and diffusive (P_d*) water permeabilities for *Carcinus, Libinia* and *Pugettia* indicate that the ratio of P_{osm}*/P_d* is about 2, which suggests that unstirred layers could have a major influence on tracer water movement in all of these crabs. It is proposed that the initial changes in k, which occur during adaptation to a dilute medium, are at least partly the result of an increase in thickness of the unstirred layers surrounding the gills, caused by a reduction in the flow of medium through the gill chamber.

LITERATURE CITED

ARUDPRAGASAM, K. D., AND E. NAYLOR, 1964. Gill ventilation volumes, oxygen consumption and respiratory rhythms in *Carcinus maenas* (L.). *J. Exp. Biol.,* **41**: 309–322.

BERLIND, A., AND F. I. KAMEMOTO, 1977. Rapid water permeability changes in eyestalkless euryhaline crabs and in isolated, perfused gills. *Comp. Biochem. Physiol.,* **58A**: 383–385.

BINNS, R., 1969. The physiology of the antennal gland of *Carcinus maenas* (L.). II. Urine production rates. *J. Exp. Biol.,* **51**: 11–16.

BORN, J., 1970. Changes in blood volume and permeability associated with molting in a marine crab, *Pugettia producta. Ph.D. Thesis, University of California,* Berkeley, 106 pp. (University Microfilms/Dissertation Abstracts International No. 71–748).

CANTELMO, A. C., 1977. Water permeability of isolated tissues from decapod crustaceans. I. Effect of osmotic conditions. *Comp. Biochem. Physiol.,* **58A**: 343–348.

CAPEN, R. L., 1972. Studies of water uptake in the euryhaline crab *Rithropanopeus harrisi. J. Exp. Zool.,* **182**: 307–319.

CORNELL, J. C., 1973. A reduction in water permeability in response to dilute medium in the stenohaline crab *Libinia emarginata* (Brachyura, Majidae). *Biol. Bull.,* **145**: 430–431.

CORNELL, J. C., 1974. A reduction in water exchange rates in an osmoconforming crab. *Am. Zool.,* **14**: 1259.

CORNELL, J. C., 1976. Aspects of salt and water balance in two osmoconforming crabs *Libinia emarginata* and *Pugettia producta* (Brachyura: Majidae). *Ph.D. Thesis, University of California, Berkeley,* 243 pp. (University Microfilms/Dissertation Abstracts International No. 77–15, 646).

CORNELL, J. C., 1979. Salt and water balance in two marine spider crabs, *Libinia emarginata* and *Pugettia producta.* I. Urine production and magnesium regulation. *Biol. Bull.,* **157**: 221–233.

DAINTY, J., AND C. R. HOUSE, 1966. "Unstirred layers" in frog skin. *J. Physiol. (Lond.),* **182**: 66–78.

DAVENPORT, J. D., 1972. Volume changes shown by some littoral anomuran Crustacea. *J. Mar. Biol. Assoc. U.K.,* **52**: 863–877.

GILLES, R., 1970. Osmoregulation in the stenohaline crab *Libinia emarginata* Leech [Leach]. *Arch. Int. Physiol. Biochim.,* **78**: 91–99.

GROSS, W. J., 1957. An analysis of response to osmotic stress in selected decapod Crustacea. *Biol. Bull.,* **112**: 43–62.

HANNAN, J., AND D. H. EVANS, 1973. Water permeability in some euryhaline decapods and *Limulus polyphemus. Comp. Biochem. Physiol.,* **44A**: 1199–1213.

HERREID, C. F., 1969. Integumental permeability of crabs and adaptation to land. *Comp. Biochem. Physiol.,* **29**: 423–429.

HOUSE, C. R., 1974. *Water transport in cells and tissues.* Edward Arnold Ltd., London. 562 pp.

HUME, R. J., AND A. BERLIND, 1976. Heart and scaphognathite rate changes in a euryhaline crab, *Carcinus maenas,* exposed to a dilute environmental medium. *Biol. Bull.,* **150**: 241–254.

KING, E. N., 1965. The oxygen consumption of intact crabs and excised gills as a function of decreased salinity. *Comp. Biochem. Physiol.*, **15**: 93–102.

MANGUM, C. P., AND K. JOHANSEN, 1975. The colloid osmotic pressures of invertebrate body fluids. *J. Exp. Biol.*, **63**: 661–671.

MCMAHON, B. R., AND J. L. WILKINS, 1975. Respiratory and circulatory responses to hypoxia in the lobster *Homarus americanus*. *J. Exp. Biol.*, **62**: 637–656.

NAGEL, H., 1934. Die Aufgaben der Exkretionsorgane und der Kiemen bei der Osmoregulation von *Carcinus maenas*. *Z. Vgl. Physiol.*, **21**: 468–491.

PARRY, G., 1955. Urine production by the antennal glands of *Palaemonetes varians* (Leach). *J. Exp. Biol.*, **32**: 408–422.

ROSEIJADI, G., J. W. ANDERSON, S. R. PETROCELLI, AND C. S. GIAM, 1976. Osmoregulation of the grass shrimp *Palaemonetes pugio* exposed to polychlorinated biphenyls (PCBs). I. Effect on chloride and osmotic concentrations and chloride- and water-exchange kinetics. *Mar. Biol.*, **38**: 343–355.

RUDY, P. P., 1967. Water permeability in selected decapod Crustacea. *Comp. Biochem. Physiol.*, **22**: 581–589.

SHAW, J., 1961. Studies on ionic regulation in *Carcinus maenas* (L.). I. Sodium balance. *J. Exp. Biol.*, **38**: 135–152.

SMITH, R. I., 1967. Osmotic regulation and adaptive reduction of water permeability in a brackish-water crab, *Rithropanopeus harrisi* (Brachyura, Xanthidae). *Biol. Bull.*, **133**: 643–658.

SMITH, R. I., 1970. The apparent water-permeability of *Carcinus maenas* (Crustacea, Brachyura, Portunidae) as a function of salinity. *Biol. Bull.*, **139**: 351–362.

SMITH, R. I., 1976. Apparent water-permeability variation and water exchange in crustaceans and annelids. Pages 17–24 in P. S. Davies, Ed., *Perspectives in experimental biology*, Vol. 1. Pergamon Press, Oxford and New York.

SMITH, R. I., AND P. P. RUDY, 1972. Water-exchange in the crab *Hemigrapsus nudus* measured by use of deuterium and tritium oxides as tracers. *Biol. Bull.*, **143**: 234–246.

THOMPSON, L. C., 1970. Osmoregulation of the fresh water crabs *Metopaulias depressus* (Grapsidae) and *Pseudothelphusa jouyi* (Pseudothelphusidae). *Ph.D. Thesis, University of California*, Berkeley, 101 pp. (University Microfilms/Dissertation Abstracts International No. 71-9938.)

TULLIS, R. E., AND F. I. KAMEMOTO, 1974. Separation and biological effects of CNS factors affecting water balance in the decapod crustacean *Thalamita crenata*. *Gen. Comp. Endocrinol.*, **23**: 19–28.

WELSH, J. H., R. I. SMITH, AND A. E. KAMMER, 1968. *Laboratory exercises in invertebrate physiology*. Burgess Publishing Co., Minneapolis, Minnesota, 219 pp.

Reference: *Biol. Bull.* 157: 434–444. (December, 1979)

UPTAKE OF AMINO ACIDS BY MARINE POLYCHAETES UNDER ANOXIC CONDITIONS

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Representative species of at least twelve different families of the class Polychaeta have served as experimental organisms in studies of transepidermal transport of small organic solutes in several laboratories. These studies include many published reports dealing with specific aspects of this phenomenon (reviewed by Stephens, 1972; Jørgensen, 1976) as well as unpublished work in this laboratory. Since marine annelids are often conspicuous members of the infauna, are easy to collect and maintain for brief periods in the laboratory, and live in habitats relatively rich in dissolved organic resources, they continue to be used for such work (Ahearn and Gomme, 1975; Rice and Chien, 1978; Stephens, 1975). Despite this attention, basic characteristics of this influx remain unexamined. We undertake to explore two aspects of influx of amino acids in two representative polychaetes.

First, the relation between influx of labeled substrates and net movement of substrate into or out of the animal is still in question. Stephens (1975) showed net influx of known amino acids in *Nereis diversicolor* and *Capitella* sp. using a fluorometric procedure to follow disappearance of added substrate. However, labeled substrates were not employed in this work. Details of the relation between influx of substrate followed radiochemically and net movement of substrate followed chemically have been described and analyzed for molluscs (Wright and Stephens, 1977, 1978; Crowe, Dickson, Otto, Colon and Farley, 1977) and for enchinoderms (Stephens, Volk, Wright and Backlund, 1978). The only report concerning annelids is that of Johannes and Webb (1970) in which they describe influx of ^{14}C-labeled glycine accompanied by net efflux of amino acids in *Clymenella*. Since many of the reports concerning influx of ^{14}C-labeled substrates overtly or tacitly assume that such influx represents net removal of substrate from the medium by the animal and draw conclusions on this basis, the subject merits investigation.

Second, infaunal polychaetes undergo periods of relative oxygen deprivation or anaerobiosis. If net influx of small organic molecules is to be invoked as a potential supplementary form of nutrition for these animals, the response of the process to relative or complete anoxia is important.

Stephens (1963) reported that influx of ^{14}C-labeled amino acids in *Clymenella* was unimpaired after long exposure to sea water through which gaseous N_2 was bubbled. However, no direct measurements of P_{O_2} were made. Several authors have used inhibitors of aerobic metabolism and reported partial inhibition of influx rates in some cases, and no clear response in other cases. None of this work includes independent criteria to assess inhibitor effects, and in most cases does not demonstrate reversibility of effects reported.

The present work reports the relation between influx and net flux of glycine in two genera of polychaetes. This relation is reported over a range of P_{O_2} including

anoxic conditions. The effects of anoxia are compared to effects of cyanide inhibition of aerobic metabolism.

Materials and Methods

Specimens of *Marphysa sanguinea* (Eunicidae) were collected intertidally from Upper Newport Bay, Newport Beach, California. Animals were found in muddy sediment under and between intertidal rocks. Specimens of *Pareurythöe californica* (Amphinomidae) were collected subtidally from a shallow lagoon at Point Mugu Naval Base near Port Hueneme, California. The animals occurred in sandy sediment just below mean low tide. Animals were maintained in the laboratory in aerated containers at 16° C. Specimens of both genera were selected in the wet weight range of 0.7 to 1.4 g for observations. Experiments were carried out at room temperature (23° C); animals were acclimated to the temperature change for several hours before use.

Influx of ^{14}C-glycine into animals was followed by periodic sampling of the medium in which animals were incubated. Experimental media were prepared from ^{14}C-gly (UL) at 20 to 40 μCi/liter plus sufficient ^{12}C-gly to achieve the desired chemical concentration. All media were prepared in artificial sea water (MBL) prepared according to Cavanaugh (1956) filtered through a Millipore filter (0.45-μm pore size). Duplicate 0.2-ml samples of medium were taken periodically, acidified to drive off CO_2, and added to a toluene-based scintillation cocktail containing a detergent. Radioactivity was determined using a Beckman CPM-100 scintillation counter. Details of sampling protocol vary according to the experimental procedure.

Net change in ambient glycine concentration was followed using fluorescamine to determine changes in primary amines in solutions in which animals were incubated. The procedure has been described (Stephens, 1975; Stephens *et al.*, 1978). The reagent, fluorescamine, reacts with primary amines to produce a fluorescent product with an absorption maximum at 390 nm and emission peak at 480 nm. Fluorescence was measured using a Perkin-Elmer spectrophotofluorometer. Initially, fluorescence reflects glycine concentration since glycine is the only primary amine present in the medium. After incubation, fluorescence represents remaining glycine plus any primary amines which may be present as a result of efflux from the animal. Fluorescence is expressed in units of equivalent glycine concentration.

Influx and net flux of glycine under anaerobic conditions was measured as follows: Several hundred ml of MBL sea water was placed in a large culture flask with a port at the base in which an oxygen electrode (YSI) was mounted. Nitrogen gas was passed through acid pyrogallol and bubbled through the culture flask using a breaker stone. Oxygen content was monitored with the oxygen meter until anoxic conditions were achieved; approximately 5 min were required to reach the same oxygen reading as that obtained for sea water chemically deoxygenated with dithionite. Four samples of 50 ml each were then siphoned into four flasks, each containing one worm. Two of these flasks were placed in the N_2 gas train and maintained anoxic. The other two were reoxygenated using an air pump and served as controls. Worms were allowed to adapt to these conditions

TABLE I

Rates of uptake of glycine in specimens of Pareurythöe *and* Marphysa *under aerobic and anaerobic conditions. In all cases, initial concentration of glycine was* 20 μM. *Influx was calculated from the rate of depletion of* ^{14}C*-glycine from the medium; net influx refers to the rate of disappearance of total primary amines from the medium, and is expressed in terms of glycine-equivalents.*

Experimental condition	Influx [moles $\times 10^{-7}$/(g·hr)]	Net influx [moles $\times 10^{-7}$/(g·hr)]
Pareurythöe		
Anaerobic	2.9	3.2
	3.0	3.2
	2.0	1.3
	2.5	2.3
	Average 2.6 ± 0.3 (s.d.)	2.5 ± 0.8
Aerobic	5.4	5.7
	4.6	5.2
	8.9	8.2
	7.8	8.0
	Average 6.7 ± 1.7	6.8 ± 1.3
Marphysa		
Anaerobic	2.2	—
	1.9	—
	1.2	1.2
	3.1	3.3
	Average 2.1 ± 0.7	2.3
Aerobic	7.4	—
	8.5	—
	4.9	4.9
	5.2	4.9
	Average 6.5 ± 1.5	4.9

for 15 to 30 min. Substrate (0.5 ml) was then added using a hypodermic syringe to initiate the experimental period. Samples were withdrawn periodically using a hypodermic syringe and were used for duplicate determinations of radioactivity and duplicate determinations of fluorescence at each time point.

Observations on influx and net flux of glycine in *Pareurythöe* were also carried out using a flow system. Filtered MBL sea water containing ^{14}C-labeled glycine (20 μm) was placed in a flask to serve as a medium reservoir. A metering pump was used to produce a flow of medium through a chamber with sintered glass discs at either end (internal diameter = 16 mm, length = 75 mm, approximate volume = 15 ml). Medium from the reservoir was led to the chamber via a sampling port which permitted monitoring P_{O_2} as it passed into the chamber. The medium reservoir was initially deoxygenated and then reoxygenated to the desired level. By using a relatively large volume of medium in the reservoir with a small free surface, negligible drift in P_{O_2} was encountered during the course of observations. Flow rate was calculated to be sufficiently rapid that oxygen consumed by the worm would represent a depletion of 10% or less of the oxygen content of sea water in equilibrium with air. This represents a necessary compromise which allowed for a minimal flow rate sufficient to permit reliable determinations of the difference between inflow and outflow concentrations. Actual flow rates ranged from 3 to 6 ml/min depending on the weight of the worm. At

each level of P_{O_2} tested, medium was pumped through the chamber for 15 min prior to sampling. Samples were then taken each 5 min for the following 30 min and analyzed in duplicate for radioactivity as described. In some experiments the fluorescence at inflow and outflow ports was also determined.

In all cases where medium depletion was followed with time, a straight line was obtained when log radioactivity or log fluorescence was plotted against time, indicating that depletion followed first order exponential kinetics. Uptake rates are presented as moles glycine/(g wet weight·hour) from an ambient concentration of 20 μM. Data from the first hour of sampling was used to establish the rate constant. Uptake rates obtained using the flow system were calculated from the average difference between inflow and outflow samples as described and are expressed in the same units.

Results

Table I presents rates of influx and net rates of influx for *Parcurythöe* and *Marphysa* under anaerobic and aerobic conditions. There is no significant difference

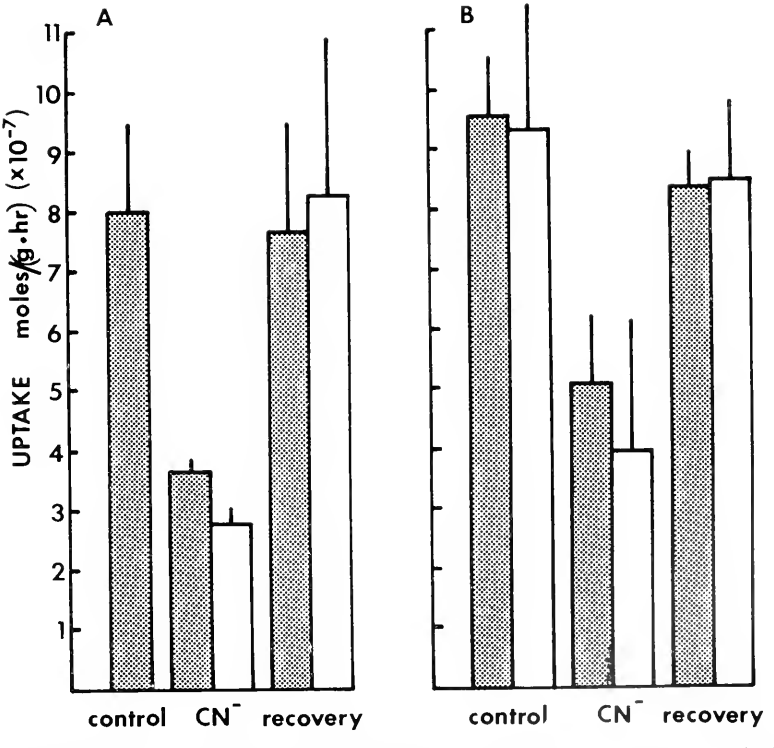

FIGURE 1. Effect of cyanide on the influx and net influx of glycine into A) *Marphysa* and B) *Parcurythöe*. Influx (shaded bars) was calculated from the rate of depletion of ^{14}C-glycine from the experimental medium; net influx (open bars) was determined from the rate of depletion of total primary amines. In all cases the initial concentration of glycine was 20 μM; the concentration of KCN was 2 mM. Bars represent +1 standard deviation.

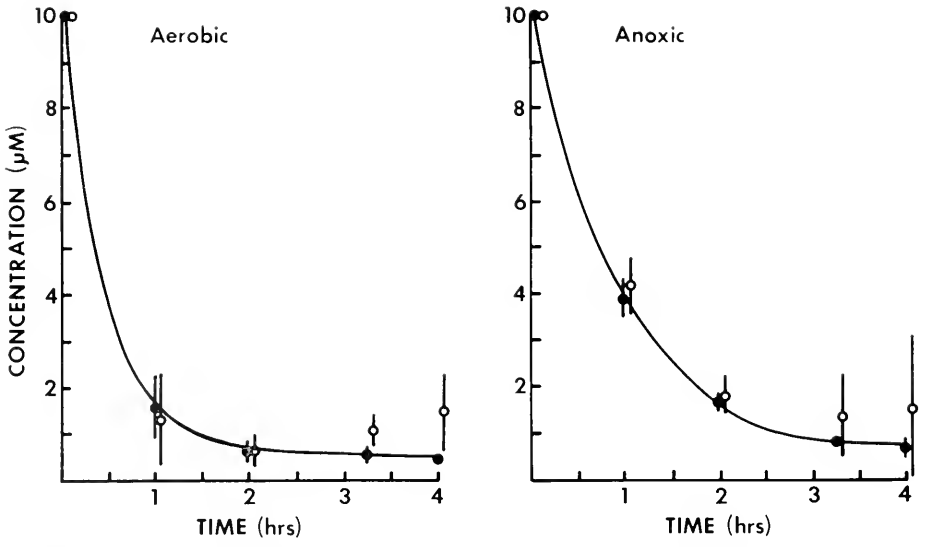

FIGURE 2. Effect of anoxia on the time course of influx and net influx of glycine into *Pareurythöe*. Solid circles represent measured levels of ^{14}C-glycine; open circles are concentrations of total primary amines expressed as equivalent glycine concentration. Anoxic conditions were produced by bubbling N_2 through experimental media containing 2 mM KCN. Each point is the mean of three separate determinations with individual worms; bars represent ± 1 s.d..

between rates of influx (^{14}C depletion) and net influx (decrease in primary amines). Hence there is a net transfer of substrate from the medium to the animal at a rate accurately estimated by either procedure. Rates of uptake under anaerobic conditions are reduced to levels 32 to 46% of aerobic rates.

Figure 1 presents data for influx and net influx of glycine in the presence and absence of 2 mM KCN. Oxygen consumption for three individuals of *Marphysa* was measured using an oxygen electrode. Exposure to 2 mM KCN for 30 min inhibited oxygen consumption to 13% of control values. Normal rates of oxygen consumption were restored when animals were permitted to recover for 24 hr. Influx and net flux of glycine into three individuals of each species before, during and after exposure to 2 mM KCN was measured on two separate occasions. Influx and net flux were reduced in the presence of cyanide to levels essentially the same as those observed under anaerobiosis (40 and 46% of control values). Recovery was complete.

Figure 2 presents data for *Pareurythöe* showing the time course of influx of ^{14}C-glycine and net flux of total primary amines under aerobic and anoxic conditions. The data for each experimental condition are mean values of separate studies on three worms. Anoxic conditions were produced by bubbling N_2 through experimental media also containing 2 mM KCN. Similar results were obtained using *Marphysa*. Note the close correspondence between influx as determined by depletion of radioactivity and net disappearance of substrate as indicated by determination of fluorescence. This is the case under both sets of conditions. In

both cases, determinations of fluorescence begin to diverge from determinations of radioactivity after two hours. In both cases, radioactivity does not continue to decline indefinitely. This does not represent a limitation of the transport system since ^{14}C-glycine at concentrations of 2 to 4×10^{-7} M is removed exponentially by both species.

Figure 3 presents data for two individuals of *Parcurythöe* in which influx and net influx of glycine was measured in the flow system. Error bars represent ± 1 standard deviation of the average difference of seven determinations of radioactivity or fluorescence at inflow and outflow ports. Actual differences range from 0.6 to 7% of inflow. Since P_{O_2} was measured at the inflow, the values are systematic overestimates of the average P_{O_2} experienced by the animal during the observation period. Six such experiments were performed. In all cases there was a sustained high rate of influx, essentially comparable to that observed in aerobic depletion experiments, until ambient P_{O_2} was reduced to approximately 10% to 20% of air saturation values. In some cases, there was some decrease with decreasing P_{O_2} prior to the more conspicuous decrease observed at very low P_{O_2}. The data are too variable to justify mathematical treatment but suffice to demonstrate that influx and net influx are not linearly related to P_{O_2} over this range of oxygen concentrations. In these observations, influx and net influx of glycine were inhibited to a greater extent under anoxic conditions than the inhibition

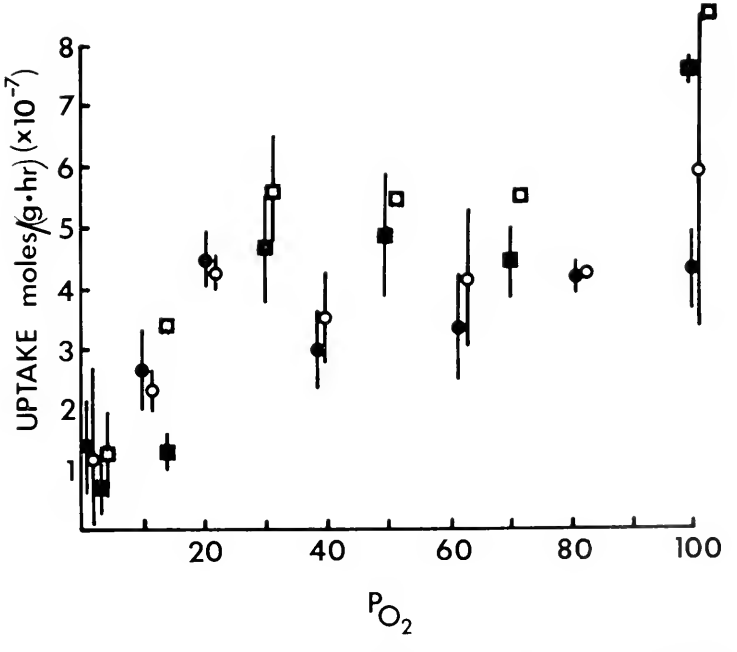

FIGURE 3. Effect of P_{O_2} on influx and net influx of glycine into *Parcurythöe*. Circles and squares represent separate experiments on two individual worms. Closed symbols represent influx of ^{14}C-glycine; open symbols represent net influx of total primary amines. Bars are ± 1 s.d.; those cases in which open symbols have no error bars indicate that the variability was too large to include effectively in the figure.

TABLE II

Nutritional role of amino acid uptake in Pareurythöe *and* Marphysa: *examination of critical parameters. See text for the basis of the calculations.*

	Pareurythöe	*Marphysa*
Concentration of free amino acids in environment (μM)	123	131
Oxygen consumption [ml/(g·hr)]	0.13	0.15
Influx [moles $\times 10^{-7}$/(g·hr)] (*cf.* Table I, for 20 μM)	6.7	6.9
Contribution for 20 μM gly (μg)	50.5	44.6
% oxygen requirement	17.4	13.8
Glycine concentration for 100% oxygen requirement (μM)	115	145
Mixed amino acid concentration for 100% oxygen requirement (μM)	52	65

observed in depletion experiments. Anaerobic influx rates were approximately 15% to 20% of those observed at higher P_{O_2}.

DISCUSSION

There is no significant difference between rates of ^{14}C-glycine influx in *Marphysa* and *Pareurythöe* estimated from radiochemical measurements and rates of net influx of glycine estimated from fluorometric determinations from an ambient glycine concentration greater than 10 μM (Figure 2, Table I). There is a slow efflux of primary amines (fluorescamine-positive material) with time in both aerobic and anoxic conditions (Figure 2). These findings agree with similar studies of molluscs (Wright and Stephens, 1977, 1978) and echinoderms (Stephens *et al.*, 1978). In all of the soft-bodied marine invertebrates examined adequately, influx of ^{14}C-labeled amino acids reflects net influx quite accurately at concentrations greater than 10 μM; however, this process is accompanied by a slow efflux of primary amines.

These results agree with the reports of Johannes, Coward and Webb (1969) and Johannes and Webb (1970) using the flatworm, *Bdelloura candida* and the polychaete, *Clymenella*. They supplied labeled substrates at low concentrations (0.6 μM and 1.0 μM in the studies cited) and observed a slow net efflux of amino acids with time at rates broadly comparable to those reported here and those reported by Stephens (1968). However, they interpreted their data as evidence for exchange diffusion and questioned the occurrence of net influx of substrate into marine invertebrates, suggesting that earlier literature might represent a misinterpretation of such exchange diffusion. However, exchange diffusion is clearly excluded as a mechanism of efflux by data showing that efflux is essentially independent of ambient concentrations, as appears to be the case in the examples previously cited. Beyond this, there is currently no evidence concerning the route or mechanism of efflux.

Table II presents measurements of naturally occurring primary amines in the immediate habitat of *Pareurythöe*. Data were obtained by expressing interstitial water from freshly collected sediment cores in the sandy subtidal habitat of *Pareurythöe*. Total free amino acids were estimated by the fluorescamine technique. Some of these data are reported in Stephens *et al.* (1978) where methodological details are presented. The samples ranged from 54 to 244 μM (average 123 $\pm$ 54

μM, n = 13). Free amino acids in the habitat of *Marphysa* were estimated in a similar fashion. However, it was not possible to obtain cores in the immediate area of the rocks where the animals were found. Therefore, cores were taken from otherwise comparable muddy sediment in immediately adjacent areas of Newport Bay. Average free amino acids ranged from 88 to 180 μM (average 131 ± 35 μM, n = 7). Table II also presents data for oxygen consumption of *Pareurythöe* in the size range employed in these experiments [0.13 ml/(g·hr) ± 0.02, n = 6]. For *Marphysa*, oxygen consumption was 0.145 ± 0.25 (n = 8). Table II also includes average rates of glycine influx (average of influx and net influx from Table I) for the two worms. Given these data, it is possible to calculate numbers to estimate the significance of amino acid influx as compared to metabolic requirements estimated from oxygen consumption. Complete oxidation of 1.0 mg of glycine requires 2.23 ml O_2 (STP). One can calculate the percentage contribution of glycine influx from an ambient concentration of 20 μM (where measurements were made) for the two cases. By making the assumption that influx at higher concentrations is linearly related to concentration, the glycine concentration in the medium which would provide sufficient reduced carbon to account for oxidative metabolism can be calculated. In fact, interstitial amino acids from the immediate habitat of *Pareurythöe* have been identified chromatographically (Stephens *et al.*, 1978). Glycine is present as well as seven other identifiable amino acids accounting for approximately 85% of total primary amines as estimated by fluorescamine. Thus it is more realistic to use a conversion factor of 1 ml of O_2 required for complete oxidation of a mixture of amino acids provided one assumes that rates of glycine influx are comparable to influx rates for other amino acid substrates. The table also includes the ambient free amino acid concentration (based on these assumptions) required to provide reduced carbon equivalent to oxygen consumption.

These data and calculations indicate that influx of free amino acids from ambient solution occurs at rates that are of the same order of magnitude as the requirement for reduced carbon to sustain oxidative metabolism; *i.e.* the process of transepidermal influx represents a potentially important source of supplementary nutrition for these animals. This conclusion is based on the assumption that bulk concentrations of free amino acids measured in interstitial water of the sediment habitat are a reasonable estimate of concentrations available to the animals. There is no direct information on this point, though Stephens (1975) has provided evidence that irrigation activity of infaunal annelids may increase local free amino acid concentration in interstitial water.

These data and calculations are not relevant to conditions during periods of anaerobiosis which infaunal worms may undergo periodically. Under such circumstances, influx rates for glycine in *Pareurythöe* and *Marphysa* decrease to 32 to 46% of aerobic rates. Requirements for reduced carbon will be considerably increased during periods of anaerobiosis. However, net influx continues from ambient concentrations in the range found in the habitat under anoxic conditions for up to 4 hr. Thus the process continues though its rate and overall contribution to metabolic requirements are both considerably reduced.

The presence of 2 mM KCN in the medium almost completely inhibits aerobic metabolism (oxygen consumption was reduced by 83%). This effect would be predicted based on the binding of CN^- to heme groups and the resulting interdiction

of electron transport. Thus the concentration used and the time of incubation employed were sufficient to force dependence on anaerobic metabolism. The resulting decrease in influx (40 to 45% of control values) agrees well with the decrease observed under anoxic conditions. Cyanide inhibition, both of influx and of oxygen consumption, were reversible; no mortality was encountered in these experiments. Thus this is additional evidence for the ability of these animals to tolerate periods of anaerobiosis. There is no evidence for a specific inhibitory effect of cyanide on the transepidermal transport system.

Mangum (1976) reviews respiratory adaptations of annelids. In her view irrigation of the burrow is one of the major adaptations to the sediment habitat in this group. Although data for *Marphysa* and *Pareurythöe* are not reported, it is reasonable to assume that they also irrigate their burrows. Mangum tabulates data comparing average P_{O_2} in the microhabitat (*i.e.* in the burrow) with P_{O_2} in the water column. The former ranges from 73 to 104 mm Hg when the overlying water column is essentially saturated. Thus the ability of the annelids examined in the present work to sustain normal rates of net influx at partial pressures of oxygen below saturation (Fig. 3) is of interest. There is little decline in influx rate until P_{O_2} drops below 20% of saturation. Since these estimates of the P_{O_2} actually encountered by the worms in the flow chamber are systematic overestimates (measured at the inflow end of the chamber), this suggests that the worms are functioning as aerobes at quite low P_{O_2}'s and hence at realistic levels of oxygen availability under normal circumstances. The depression of influx under anoxic conditions was greater in these experiments than was the case in the medium depletion experiments (15 to 20% rather than 32 to 46% of control values). The flow chamber was large in diameter compared to the worms. Under conditions of very low P_{O_2}, the worms typically coiled tightly. In contrast, medium depletion experiments were done with continuous gentle agitation by the gas stream (N_2 or air). It is possible that the greater depression of influx in the flow system is simply the result of this behavior and the reduction in surface exposed to the medium by the worms in the two situations.

The limitations of the use of oxygen consumption as a measure of metabolic requirements should be reemphasized. These limitations are based on the necessary assumption that metabolism is aerobic (clearly not always the case for annelids) and on the fact that requirements for organic material which must support growth and balance losses by other pathways do not enter the estimation process. It would be far more appropriate to undertake measurement of heat production (Pamatmat, 1978) and estimate minimum requirements on that basis; however, this has yet to be done for annelids. The calculations presented in Table II indicate that influx of naturally occurring free amino acids may provide a major input under aerobic circumstances. However, the significance of influx under anaerobic conditions will certainly be less, both by virtue of the reduction in rate and because of the increased requirements for substrate. In any case, the conclusion drawn from this work is that uptake of amino acids is a supplement. Both worms certainly have other feeding methods at their disposal.

The metabolism of facultatative anaerobic invertebrates is complex and involves a variety of end products (de Zwaan, Kluytmans, and Zandee, 1976). Some of the pathways demonstrated produce amino acids such as alanine and succinate

and these compounds might be expected to accumulate under anoxic conditions (Hochachka, Fields, and Mustafa, 1973). Though such reactions may well be involved in facultative anaerobiosis in annelids, the resulting amino acids are not perceptively lost to the medium in *Marphysa* and *Pareurythöe*; rates of efflux of primary amines are not increased under anoxic conditions (Figure 2).

The extent to which the two species examined in this work actually experience anoxic periods is probable very different. *Marphysa* was collected from a population in the intertidal from an area characterized by fine-grained sediment of high organic content. At low tide, animals almost certainly experience anoxia. *Pareurythöe* was collected from a shallow sub-tidal population in an area of coarser sediment. It is not clear why *Pareurythöe* would ever experience anoxia so long as burrow irrigation continues. Both species tolerate anoxic periods well and behave similarly in the present observations. Mangum (1976) suggests that the circulatory system in annelids may play a more important role in acquisition of oxygen from the environment than in distribution to deeper tissues; in many species deeper tissues are virtually avascular. Possibly the widespread tolerance of anoxia among annelids may reflect the fact that deeper structures may be poorly supplied with oxygen even in aerobic conditions.

Finally, the relation of this work to earlier reports of influx of ^{14}C-labeled substrates into marine polychaetes should be mentioned. Since it is now quite simple to examine influx and net flux of amino acids, further work in this area should include such examination. However, the coupling of influx as estimated radiochemically and net influx examined chemically has now been reported for several major groups of marine invertebrates and in each case examined, influx has been shown to be a close estimate of net flux provided concentrations are realistic and initial rates are compared. It seems increasingly likely that the early reports can be accepted provisionally as providing evidence for the existence of transport systems capable of net transport of substrate from the medium into the animal. The potential contributions of such transport will depend then on environmental availability of substrate and will no doubt vary among species. The wide distribution of transepidermal transport systems among marine invertebrates indicates that further work is desirable to describe the extent to which energy flow by this pathway may contribute to the trophic organization of marine communities.

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Summary

1. The effect of anoxia on influx and net flux of amino acids from dilute solutions into two species of marine polychaetes was studied.

2. Rates of influx and net flux correspond quite closely at ambient concentrations greater than 10 μM. Anoxic conditions, produced by incubating specimens of *Marphysa* and *Pareurythöe* in solutions containing 2 mM KCN or through which N_2 was bubbled, did not affect the tight correspondence between influx and net flux, though rates were reduced by approximately 50%.

3. The effect of P_{O_2} on influx and net flux was examined using a continuous

flow system. Influx and net influx remained at control rates down to P_{O_2}'s 10 to 20% of air saturation values.

4. Comparisons of rates of net flux to measured values of O_2 consumption indicate that these animals can acquire sufficient reduced carbon to account for their oxidative needs if their surfaces are exposed to amino acid levels on the order of 50 to 65 μM.

5. Primary amines in the interstitial water of sediments in the immediate vicinity of populations of these worms averaged between 123 and 131 μM.

6. *Marphysa* and *Pareurythöe* live in habitats that are relatively rich in amino acids, and they possess transport systems capable of the net accumulation of these compounds at rates sufficient to provide a significant supplement to other forms of feeding. The uptake process continues during periods of anoxia, though its rate and overall contribution to metabolic requirements are reduced.

LITERATURE CITED

Ahearn, G. A., and J. Gomme, 1975. Transport of exogenous D-glucose by the integument of a polychaete worm (*Nereis diversicolor* Muller). *J. Exp. Biol.*, **62**: 243–264.

Cavanaugh, G. M. (Ed.), 1956. *Formulae and Methods, IV, of the Marine Biological Laboratory Chemical Room.* Marine Biological Laboratory, Woods Hole, Massachusetts, 61 pp.

Crowe, J. H., K. A. Dickson, J. L. Otto, R. D. Colón, and K. K. Farley, 1977. Uptake of amino acids by the mussel *Modiolus demissus*. *J. Exp. Zool.*, **202**: 323–332.

Hochachka, P. W., J. Fields, and T. Mustafa, 1973. Animal life without oxygen: basic biochemical mechanisms. *Am. Zool.* **13**: 543–555.

Johannes, R. E., S. J. Coward, and K. L. Webb, 1969. Are dissolved amino acids an energy source for marine invertebrates? *Comp. Biochem. Physiol.*, **29**: 283–288.

Johannes, R. E., and K. L. Webb, 1970. Release of dissolved organic compounds by marine and freshwater invertebrates. Pages 257–273 in D. Hood, Ed., *Organic Matter in Natural Waters,* Univ. Alaska Inst. Mar. Sci. Occasional Pub. #1.

Jørgensen, C. B., 1976. August Pütter, August Krogh, and modern ideas on the use of dissolved organic matter in aquatic environments. *Biological Reviews,* **51**: 291–328.

Mangum, C. P., 1976. Primitive respiratory adaptations. *In:* R. Newell Ed., Adaptation to Environment: Essays on the Physiology of Marine Animals. Butterworths, London. pp. 191–278.

Rice, M. A., and P. K. Chien, 1978. The effects of divalent cadmium on the uptake kinetics of glycine by the polychaete, *Neanthes virens*. *Wasmann J. Biol.*, **35**: 137–143.

Stephens, G. C., 1963. Uptake of organic material by aquatic invertebrates. II. Accumulation of amino acids by the bamboo worm, *Clymenella torquata*. *Comp. Biochem. Physiol.* **10**: 191–202.

Stephens, G. C., 1968. Dissolved organic matter as a potential source of nutrition for marine organisms. *Am. Zool.*, **8**: 95–106.

Stephens, G. C., 1972. Amino acid accumulation and assimilation in marine organisms. Pages 155–184 in J. W. Campbell, L. Goldstein, Eds., *Nitrogen Metabolism and the Environment.* Academic Press, N.Y.

Stephens, G. C., 1975. Uptake of naturally occurring primary amines by marine annelids. *Biol. Bull.*, **149**: 397–407.

Stephens, G. C., M. J. Volk, S. H. Wright, and P. S. Backlund, 1978. Transepidermal accumulation of naturally occurring amino acids in the sand dollar, *Dendraster excentricus*. *Biol. Bull.*, **154**: 335–347.

Wright, S. H., and G. C. Stephens, 1977. Characteristics of influx and net flux of amino acids in *Mytilus californianus*. *Biol. Bull.*, **152**: 295–310.

Wright, S. H., and G. C. Stephens, 1978. Removal of amino acid during a single passage of water across the gill of marine mussels. *J. Exp. Zool.*, **205**: 337–351.

Zwaan, A. de, J. H. F. M. Kluytmans, and D. L. Zandee, 1976. Facultative anaerobiosis in molluscs. *Biochem. Soc. Symp.* **41**: 133–168.

Reference: *Biol. Bull.* 157: 445–452. (December, 1979)

PARTHENOGENESIS IN *COPTOPTERYX VIRIDIS*, GIGLIO TOS (1915) (DYCTIOPTERA, MANTIDAE)

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The mantid *Coptoteryx viridis* has been studied for several years in our laboratory. As its behavior is similar to that of other species of the sub-order Mantodea, we considered the possible existence of a parthenogenetic reproduction mechanism. These animals are solitary and sedentary, and the female often kills the male before copulation takes place. The average adult life of the female is twice as long as that of the male (Guerrero, Maggese and Cukier, 1977).

Observations made by other workers indicate that *Brunneria borealis* reproduces exclusively by parthenogenesis (White, 1948a) and *Miomantis savignii* (Adair, 1925) reproduces both by parthenogenesis and by being fertilized. The present investigation was designed to determine the existence and type of parthenogenesis in *C. viridis*.

Materials and Methods

Oothecas of *Coptoteryx viridis* were gathered in the vicinity of the slaughterhouse of Lisandro de la Torre in Buenos Aires city, and were kept in separate flasks. The nymphs which emerged from these oothecas were kept in individual cages for their whole life, at room light and temperature. The cages and the method of feeding were as described by Guerrero and De Carlo (1976).

Male nymphs of the 5th and 6th nymphal stages were employed for determination of the karyotype, and females of the 7th nymphal stage were used to corroborate the results. The testes and ovaries were immersed in an hypotonic solution of 0.7% Na citrate, fixed in Carnoy and stained with Giemsa.

Levan's classification was used to determine the karyogram where the centromeric index is $Ic = 100\ s/c$ where s is short arm and c is whole length. Chromosomes with values of the index (Ic) of 50 are termed metacentric (type M); of from 50 to 37.5, metacentric (type m); 37.5 to 25, submetacentric (type Sm); 25 to 12.5, subelocentric (type st); 12.5 to 0, acrocentric (type t); and of 0 are termed telocentric (type T).

Study of the embryos involved fixation in Bouin, followed by manual elimination of the chorion, embedding in parafin, sectioning at 7 μ thickness, and staining with hematoxylin-eosin.

Results

Seven of the 13 females which arrived at the adult stage laid oothecas without fertilization. The number of oothecas laid by a female varied from 1 to 7, the variation depending on the time they lived as adults and on the time of the year in which they attained adulthood.

TABLE I

Animal No.	Date of birth	Date of molting to imago	Total nymphal life (days)	Time between the arrival to the adult stage and the first laying (days)	Number of oothecas per female	Interval between layings (days)	Total life time (days)	Date of death	Date of the last laying	Time between the last laying and death (days)
I(17)	22-Sep-75	26-Dec-75	95	80	2	9	203 108	12-Apr-76	24-Mar-76	19
IX(1)	15-Sep-75	22-Dec-75	98	77	1	—	178 80	11-Mar-76	8-Mar-76	3
X(3)	26-Sep-75	26-Dec-75	91	62	7	14 6 14 8 11 11	217 126	30-Apr-76	30-Apr-76	0
X(19)	3-Oct-75	26-Dec-75	84	77	3	19 7	199 115	19-Apr-76	7-Apr-76	12
X(22)	6-Oct-75	16-Jan-76	102	59	6	8 15 12 15 13	231 129	24-May-76	17-May-76	7
X(30)	13-Oct-75	19-Jan-76	98	69	1	—	167 69	29-Mar-76	29-Mar-76	0
X(37)	22-Oct-75	8-Mar-76	137	—	—	—	152 15	23-Mar-76	—	—
XII(82)	13-Oct-75	2-Mar-76	137	30	4	11 7 14	205 168	15-Aug-76	3-May-76	106

A total of 22 oothecas was laid by the 7 females (Table I). The time that elapsed between the achievement of the adult stage and the laying of the first ootheca varied from 30 to 80 days. The female that had laid her first ootheca on the 30th day had become an adult towards the end of the summer (2-Mar-76), whereas the one that delayed 80 days in laying her first ootheca had attained adulthood at the beginning of the summer (26-Dec-75). The rest of the females laid their first ootheca towards the end of the summer, having attained the adult stage at the beginning of that season. In general, the sooner they became adults the longer they delayed in laying their first ootheca.

The first ootheca was laid on 26-Feb-76 and the last one on 4-May-76, a span of two and a half months. The intervals of time between one laying and the next for all the females was from 6 to 19 days. The average intervals between successive layings per female varied between 9 and 13 days and the general average of intervals for all the oothecas laid by the 7 females was 11 days.

As a rule the females died immediately after their last laying, or a few days after it. The exception was one female (XII-82) which had arrived at the adult stage at the end of the summer, and lived 106 days after her last laying. As this laying took place at the beginning of May, it might be asked whether cessation of laying was due to low temperature, photoperiod or any other reason. Her ovaries were full of mature eggs.

The female IX-I died 3 days after laying her last ootheca, with her ovaries full of mature eggs. They were not vestigial eggs since the last ootheca laid was of an enormous size. The female X-22 died 7 days after her last laying, and she had her ovaries full of mature eggs. The female X-30 laid her last ootheca on the day of her death, and her ovaries had no mature eggs. The female X-37 lived 15 days of adult life and her ovaries were full of mature eggs. She did not lay oothecas.

According to the present observations (Table I) it seems that the female would need a stimulus to start laying oothecas. This stimulus could be the presence of a male, if there was one; otherwise the female would wait until the last moment to lay her oothecas. Other probable stimuli could be environmental factors such as temperature and photoperiod. Once the process of laying started, it would not cease until the female died, or the environmental conditions turned unfavorable. It seems that the laying would depend on a certain period of the year more than on the chronotropic age of the individual. These data also indicate that about 30 days are needed to begin laying and that the yolk replacement is significant.

The next step was to wait until the oothecas hatched, since we were not sure that they contained viable eggs. The first nymph hatched 6 months and 18 days after laying, on 11-Oct-76 (Table II) and the last one on the 15-Jan-77.

According to our data for non-parthenogenetic populations, hatching never occurs after this date (Guerrero, Maggese, Cukier, 1977). The average interval between the laying of the ootheca and the first hatching was 7.5 months. From the 22 oothecas laid, 9 hatched nymphs, and 8 of the remaining 13 oothecas exhibited exposed cells that caused the eggs to dry. The number of nymphs ecloded per ootheca was very low, between 1 and 5. A total of 23 nymphs were hatched.

TABLE II

Individual N	Ootheca N	Number of hatched nymphs	Sex	Date of laying	Date of the first hatching	Time between laying and hatching	Date of the last hatched nymphs
X-22	1	2	female	15-Mar-76	3-Nov-76	7 months and 19 days	13-Dec-76
X-3	1	4	female	17-Mar-76	22-Nov-76	8 months and 8 days	25-Nov-76
X-22	2	3	female	23-Mar-76	11-Oct-76	6 months and 18 days	15-Jan-77
1–17	1	5	female	24-Mar-76	17-Nov-76	7 months and 24 days	13-Dec-76
X-3	2	1	female	31-Mar-76	7-Dec-76	8 months and 7 days	7-Dec-76
X-19	1	2	female	31-Mar-76	29-Nov-76	8 months	7-Dec-76
XII-82	1	1	female	3-May-76	29-Dec-76	7 months and 24 days	29-Dec-76
X-19	2	1	female	7-Apr-76	6-Dec-76	8 months	6-Dec-76
X-22	3	4	female	15-Apr-76	22-Oct-76	6 months and 3 days	13-Dec-76

The nymphs were all females, with a highly restricted viability since none of them passed the second nymphal stage (they lived between 0 and 14 days). Due to these results, the oothecas were kept for observation.

At the beginning of May, some of the parthenogenetic oothecas were opened and eggs in perfect state were found inside them. These eggs were fixed, embedded in paraffin and stained, revealing a very early stage of development. They presented a blastoderm along the surface with a ventral thickening that would represent the beginning of the development of the germinal band.

Oothecas collected from nature after the hatching period (3 months) also contained fresh eggs in the same stage of development. In these last oothecas it was found that several of those that had yielded a low progeny had a great number of empty chorions.

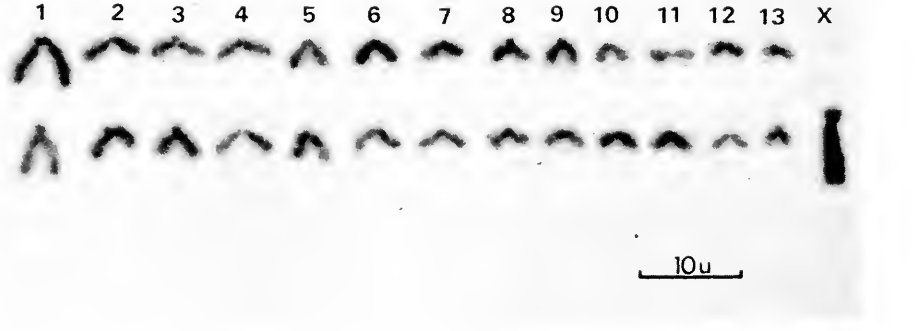

FIGURE 1. Diploid karyogram of *Coptoptery.x viridis*. This was made from two metaphases of the second meiotic division.

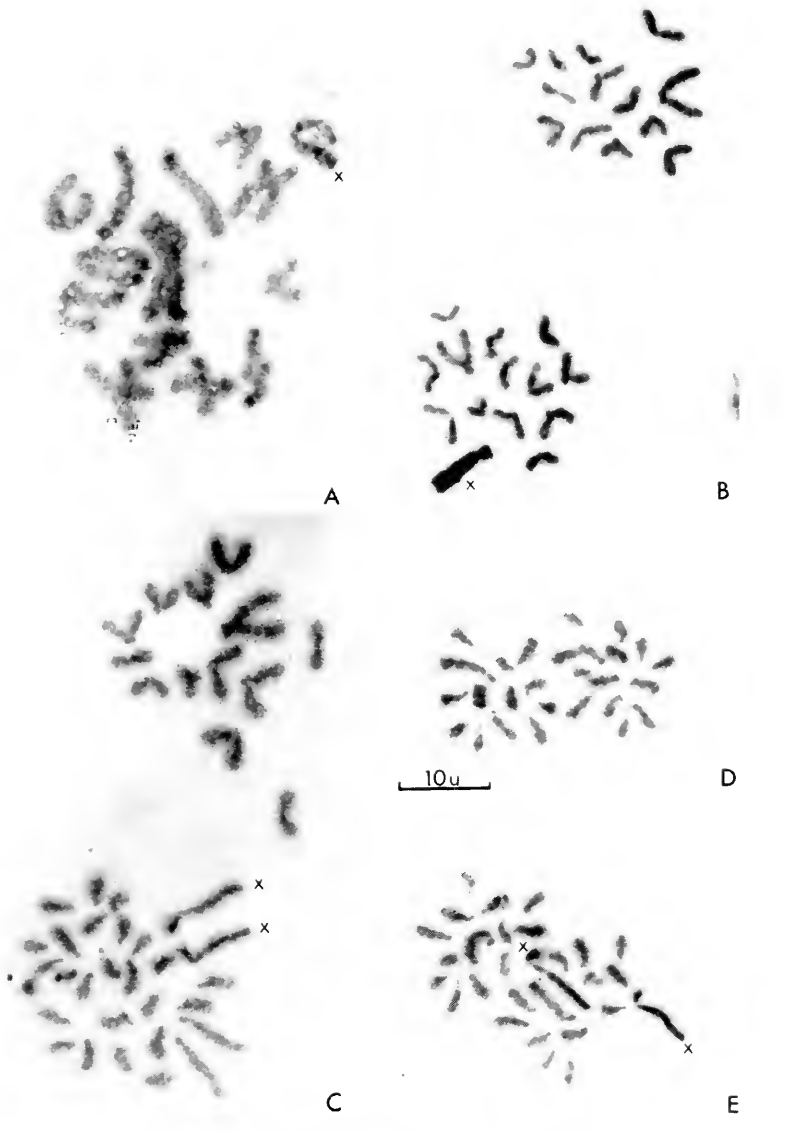

FIGURE 2. Chromosomes of *Coptopteryx viridis*. A) Diakinesis. B) two metaphases of the second meiotic division (with 13 and 13-X chromosomes). C). A metaphase of the second meiotic division with 13 chromosomes and an anaphase with 28 chromosomes. D) Telophase with 13 and 13 chromosomes. E) Telophase with 13 and 13-X chromosomes.

This evidence gave rise to two possibilities: either the existence of an annual-biennial cycle gave rise to two types of eggs, some with a slower development than others, or the development of the eggs after a certain stage had ceased for some reason (the structure of the ootheca would have allowed them to remain fresh). Kume and Dan (1968) observed in *Paratenodera aridifolia* that in the diapause

coincident with hibernation the embryos were in the same stage of development as ours.

It was decided to wait until the next spring to see which of the two alternatives was correct. The first proved to be valid, since on 22-Nov-77 nymphs started hatching from the parthenogenetic oothecas laid during the Summer-Autumn period of 1976 and which had already hatched nymphs in the Spring-Summer of 1976–1977; nevertheless the birth frequency was very low.

The next step was to determine the karyotype of the non-parthenogenetic individuals of this species. It was found that the male has 13 autosomes and a sexual chromosome as haploid number. Figure 1 shows the diploid karyogram of the species. It can be seen that it has acrocentric autosomes of the "t" type according to the classification of Levan, Fredga and Sandberg (1964), and the X chromosomes is subtelocentric of the "st" type according to the same classification.

This karyogram was built from two metaphases of the second meiotic division, with 13 and 13-X chromosomes respectively. As can be seen in the diakinesis of Figure 2a and in many others observed in this study, no trivalent chromosomes were found, which led us to the conclusion that this species belongs to the XX system for the female and XO for the male. Figure 2b shows two metaphases of the second meiotic division, with 13 and 13-X chromosomes respectively.

Figure 2c shows a metaphase of the second meiotic division with 13 chromosomes and an anaphase with 28 chromosomes which is the result of the separation of chromatids from the metaphase of the previous figure (2b). Figure 2d and 2e show two telophases, one with 13 and 13 chromosomes and the other with 13 and 13-X chromosomes respectively, indicating the end of the meiosis seen in figure 2c. All of these, and other figures studied, made us think that this species belongs to the type XX-XO with a diploid number of 27 chromosomes for the male and 28 for the female.

Discussion

The only species studied with this type of reproduction has been *Brunneria borealis*, from Central Texas and North Carolina, which reproduces exclusively by parthenogenesis (White, 1948) and *Miomantis savignii* (Adair, 1925) from Egypt, which reproduces both by parthenogenesis and after fertilization.

It has been seen in *Coptopteryx viridis,* that the beginning of oviposition varies within a very wide range and is related to the moment in which the animals attain the adult stage. This laying would be related to a certain period of the year more than to the age of the individual. This might explain why the female that reached the adult stage at the beginning of summer delayed 80 days in laying her first ootheca, whereas the one attaining maturity at the end of the same season took 30 days in doing so (Table I). From the analysis of the ovaries studied it is not clear whether or not the growth of the oocytes is synchronous with the process of nuclear maturation (meiosis).

The study of the oogenesis of this species will help to clarify this point. It was observed that the chromosome morphology of *Coptopteryx viridis* differs from that of other species of mantids described by White (1941) and Hughes-

Schrader (1943). These authors studied species from Europe, Africa and Central and North America and found that in most of them the autosomes are metacentric or "M" type, according to the classification of Levan (1964), with some of the submetacentric or "sm" type, according to the same classification. Nevertheless, the sexual chromosomes have the same configuration. This shows a marked difference with the chromosomes of *Coptopteryx viridis* where the autosomes are acrocentric or of the "t" type and the X chromosome is subtelocentric or of the "st" type according to Levan (1964). These data suggest that this is a diploid parthenogenesis, since haploid parthenogenesis never yields females. From this point, two possibilities arise: that the parthenogenesis is automictic or apomictic. In the first case the parthenogenesis would be exclusively thelytoky because of the sexual configuration of the species. In the second case, and due to the low number of hatched nymphs, it could be a thelytoky or amphitoky parthenogenesis. This last type of parthenogenesis would yield males by abnormal meiosis, as occurs with the Aphides (Homoptera) which have a XX-XO sexual system (Lees, 1961). This did not occur in *Coptopteryx viridis,* therefore we are inclined to think that this is a case of diploid parthenogenesis, automictic or apomictic, but exclusively thelytoky. Nevertheless, due to the low viability of the indviduals, it is possibly an automictic parthenogenesis, since homocygosis favors the expression of deletereous genes.

The authors express thanks to Dr. C. Naranjo for photographic assistance.

Summary

Several years of observations of the behavior of the mantid *Coptoteryx viridis* suggested evidence of parthenogenesis in this species. *C. viridis* is a solitary, sedentary animal, where the female often kills the male before copulation takes place, and the average male adult life is half that of the female.

Virgin females were reared in our laboratory from their hatching to the end of their lives; these laid oothecas. From these oothecas, parthenogenetic nymphs were born, all of the female sex and with a very low viability. The karyotype of the non-parthenogenetic individuals of this species was found to be XO-XX with a diploid number of 27 chromosomes for the male and 28 for the female. The autosomes were acrocentric or "t" type while the X chromosome was subtelocentric or "st" type, according to Levan's classification.

LITERATURE CITED

Adair, E. W., 1925. On parthenogenesis in *Miomantis savignii* Saussure (Orth). *Bull. Soc. R. Entomol. Egypte,* **8**: 104–148.

Guerrero, G. A., and J. M. De Carlo, 1976. Contribución al conocimiento del aparato genital femenino y fases del desarrollo de *Coptopteryx viridis* (Insecta, Mantodea). *Physis (B. Aires),* **35** (#90 C) 125–137.

Guerrero, G. A., M. C. Maggese, and M. Cukier, 1977. Estudio de una población de laboratorio de *Coptopteryx viridis* Giglio Tos (1915) (Insecta, Mantodea). *Physis (B. Aires),* **36** (#92 C) 295–303.

Hughes-Schrader, S., 1943. Meiosis without chiasmata in diploid and tetraploid spermatocytes of the mantid *Callimantis antillarum* Saussure. *J. Morphol.,* **73**: 111–141.

KUME, M., AND K. DAN, 1968. *Invertebrate Embryology*. Printed in Yugoslavia by Prosveta, Belgrado, 467 pages.

LEES, A. D., 1961. Clonal polymorphism in Aphids. *Symp. R. Entomol. Soc. Lond.*, 1: 68–79.

LEVAN, A., K. FREDGA, AND A. A. SANDBERG, 1964. Nomenclature for centromeric position on chromosomes. *Hereditas*, 52(2): 201–220.

WHITE, M. J. D., 1941a. The evolution of the sex chromosomes. I. The XO and XXY mechanism in praying mantids. *J. Genet., India*, 42: 143–172.

WHITE, M. J. D., 1948a. The chromosomes of the parthenogenetic mantid *Brunneria Borealis*. *Evolution*, 2: 90–93.

Reference: *Biol. Bull.* **157**: 453–463. (December, 1979)

DEVELOPMENTAL PATTERN AND ADAPTATIONS FOR REPRODUCTION IN *NUCELLA CRASSILABRUM* AND OTHER MURICACEAN GASTROPODS[1]

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An extensive literature about development in muricacean gastropods from the northern hemisphere and tropical coasts has been developed; however, many of these works are strongly descriptive and do not always present an adaptive interpretation of features observed. In recent years a valuable advance has been achieved by Spight, based on his extensive field experience with thaiidids. Some muricid developmental rules proposed by Spight refer to factors influencing pre-hatching time, ecology of hatching size, hatching type in relation to latitude and habitat conditions as well as factors conditioning selection of spawning sites (Spight, 1975, 1976b, 1977a, c).

To determine how far these rules are applicable in controlling the evolution of developmental patterns in muricaceans requires more extensive comparative knowledge of species from geographical areas not yet explored. In this sense, the coast line of Chile is very promising, as it comprises an extensive latitudinal range accompanied by great diversity in habitat conditions. Some years ago, a comparative study of reproduction in Chilean muricids was begun. Preliminary interest was focused on the commercially important species *Concholepas concholepas* (Gallardo, 1973; Gallardo, in press). Later, egg masses, embryo feeding and hatching type of *Chorus giganteus* were also analyzed; these results have been discussed in relation to habitat conditions (Gallardo, in press). In the present paper, studies on egg masses and embryos of the intertidal snail *Nucella crassilabrum* from the locality of Mehuín, a small bay near Valdivia, are reported; this information is complemented with field observations on habitat, spawning sites, embryo mortality and pre-hatching time at two different seasons. A discussion follows in order to interpret some of these features; hatching type and hatching size are analyzed in relation to the rules earlier set out by Spight. Emphasis is given to embryo feeding patterns and their possible adaptive significance within the holobenthic muricaceans, a question still not completely answered.

Materials and Methods

Egg capsules of *Nucella crassilabrum* were collected on the intertidal rocky shore of Mehuín (39° 25′ S, 73° 10′ W) from March, 1976 until April, 1977. Capsules of one cluster were separated and monitored to record capsule size composition as well as synchronism of development between different capsules in each group. Development of embryos was followed by opening capsules in different

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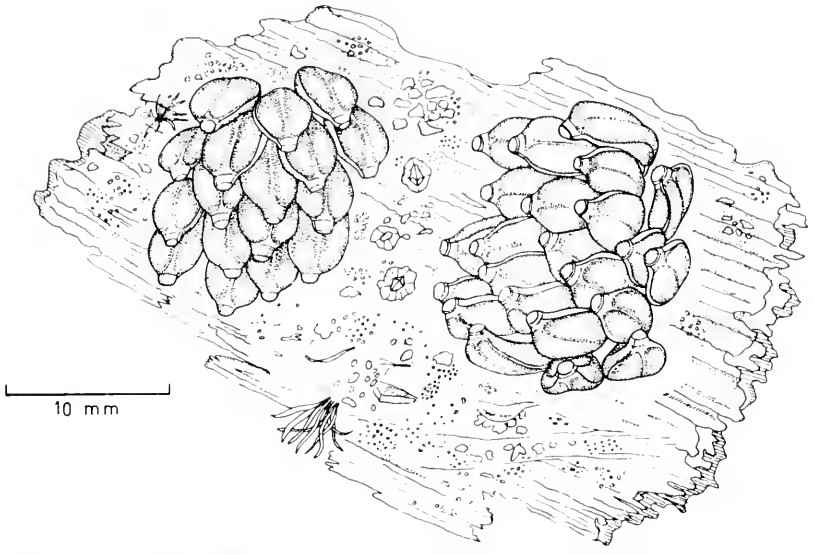

FIGURE 1. *N. crassilabrum*. Egg masses attached on vertical rocky substrate at the intertidal of Mehuín.

embryonic phases. Measurements and drawings were made from living material observed under a stereo-microscope provided with a micrometer eyepiece. Number of nurse eggs ingested per embryo was estimated from capsules in which all nurse eggs had been eaten; careful dissections of embryos in early trochophore phase made it possible to determine the number ingested and its variation.

Pre-hatching time was recorded in clusters that were spawned at different seasons of the year. For this purpose, two egg masses containing eggs in early cleavage stage were selected and tagged. One of these was spawned during late autumn and the other during late spring. Each spawning site was periodically visited on tidal exposure periods until the snails had hatched; on each visit, 3 or 4 capsules were collected for further examination at the laboratory. Water temperature of the sea is recorded daily at the Laboratory of Mehuín; this is the information used for the present paper. Spawning sites used by *N. crassilabrum* were inspected to record habitat preferences. Embryos killed by physical stresses were identified by their pink color.

RESULTS

Egg capsules and masses

Shape of the egg capsule of *Nucella crassilabrum* resembles that of its congener, *N. lapillus* (Ankel, 1937). Each flattened capsule has concave and convex sides; when seen from either the convex or concave side, the structure above the peduncle appears nearly oval in shape, with a gradual increase in breadth toward the top. At the top of the capsule there is a circular exit hole which is closed by a

prominent plug. It is possible that, as observed in other neogastropods (Hyman, 1967), this plug weakens and dissolves as the embryos reach the hatching stage. The exit hole diameter is approximately 875 to 1200 μm. Inside the capsule, eggs and mucus-like fluid are contained in a thin transparent sac. Each capsule possesses a short stalk; stalks of various capsules are cemented to the substrate in a continuous band. The capsule wall is fairly transparent, showing the embryos inside. Clusters are yellowish, due to the yellowish eggs when freshly laid, and fade to dull grey with the development of the larvae. Size of egg capsules depends on size of the female producing them. The length of the capsules we have observed, excluding the stalk, varies between 5.0 to 12.8 mm.

The capsules are laid very close to each other with a distance of approximately 2.0 to 2.5 mm between stalks. Field observations suggest that, as typical in certain muricids, communal spawning is also the rule in this species. The clusters (Fig. 1) are laid close to each other, making it difficult to ascertain the number of egg capsules laid by a single female. Nevertheless, in certain cases, the orientation and capsule size allow one to distinguish egg masses laid by different females; such clusters may contain up to 60 egg capsules. Capsules of the same cluster are arranged in a definite pattern, all of them facing the same direction; capsules arranged in a given row alternate in position with respect to those from a contiguous row as seen in Figure 1. Direction in which the different clusters are oriented on the substrate appears random.

Spawning sites

Egg masses are attached to rocky substrates, most frequently in crevices and on vertical surfaces and least frequently in tidepools and on horizontal surfaces. In general, permanently wet and shaded intertidal sites, from extreme low water of spring tides to about mean low water of neap tides, are preferred. Capsules at this last intertidal level were found in a group of rocks partially buried in the soft sandy beach. In this area, extreme seasonal sand fluctuations of about 80 or 100 cm are observed and a great quantity of sand is deposited during the summer, greatly reducing the rocky surface merging above the soft bottom. In that case, many egg masses of *Nucella crassilabrum* are covered by the sand. Furthermore, the retreating tide regularly exposes them to dry air and wind, especially throughout late spring and summer; snails become exposed to air longer and more frequently than lower on the shore and many egg masses do not complete development, probably owing to the effect of these environmental stresses. Capsules with embryos killed by physical stresses change from the normal yellow color to pink or purple. The most favorable situation for subsequent hatching of capsules was at those sites where they were permanently submerged in the sea water.

Eggs and embryos

The eggs are creamy white, their diameter varying between 204 and 293 μm with a mean of 240 μm. The number of eggs per capsule varies from 134 to 1116. A significant correlation observed between capsule length and number of eggs per capsule (Fig. 2) accounts for this variation. Besides the normally viable eggs there are many others that undergo atypical development and serve as nurse eggs.

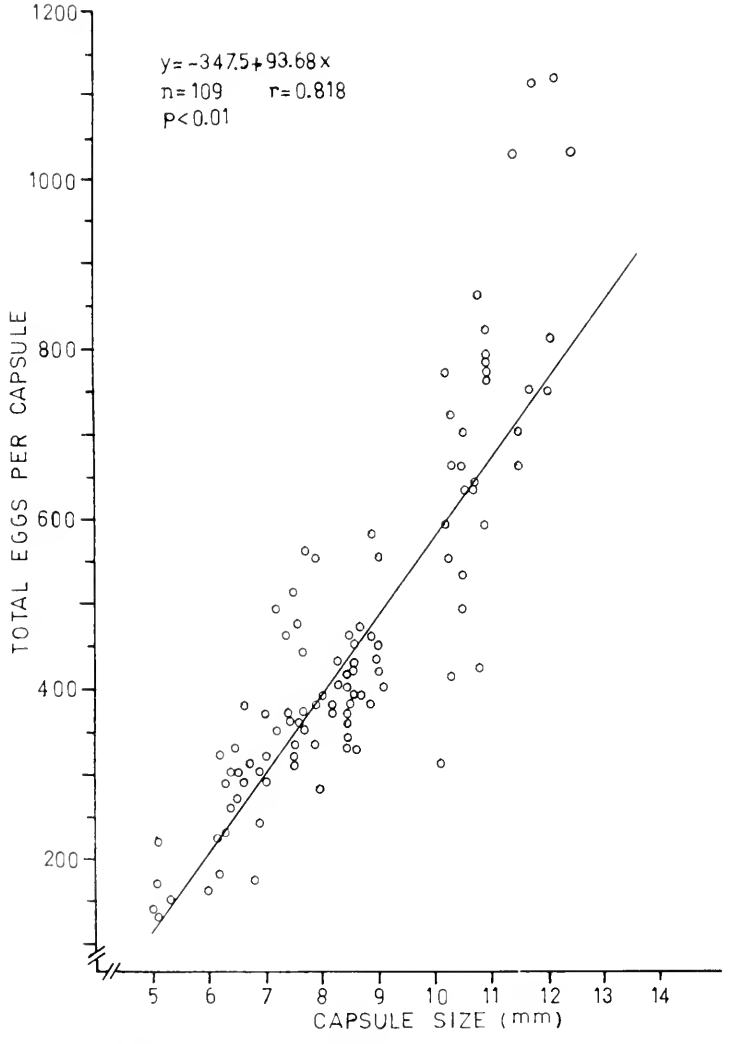

FIGURE 2. *N. crassilabrum*. Relationship between number of eggs per capsule and capsule size.

Counts in three egg capsules of different sizes (Table I) reveal that only 6.6 to 7.9% of the total eggs in a capsule are viable. The number of embryos also increases in proportion to capsule size (Fig. 3) ; it varies between 10 and 122.

The intracapsular development of *N. crassilabrum* is of direct type. In viable embryos it follows the normal spiral pattern of cleavage. On the other hand, atypical cleavage is observed in non-viable nurse eggs which appear very variable in shape. The atypical development of these nurse eggs is finally arrested. The fertile larvae begin ingesting nurse eggs when they attain an early trochophore stage. By then, the mouth, esophagus and body expand to enclose entire nurse eggs as they are pushed down the digestive tract by the cilia lining it. The

TABLE I.

Nucella crassilabrum. *Percentage of viable embryos and nurse-eggs in three different capsules.*

Capsule size (mm)	Total nurse eggs	Viable embryos and percentage
7.9	406	32 (7.3%)
6.8	342	24 (6.6%)
8.6	397	34 (7.9%)

ingested eggs are visible as distinct bulges in the body wall (Fig. 4b) and will spill out intact from an embryo opened during the feeding period. In the succeeding stages (Figs. 4c, d) and intracapsular veliger is gradually developed; by then, the embryos show the appearance of the shell and foot and the velar lobes are partially expanded. The embryo is now clearly divided into head (anterior to the shell), foot (ventrally), and visceral hump (covered by the shell); nurse eggs ingested still obscure details of internal structure (Fig. 4e). The posterior

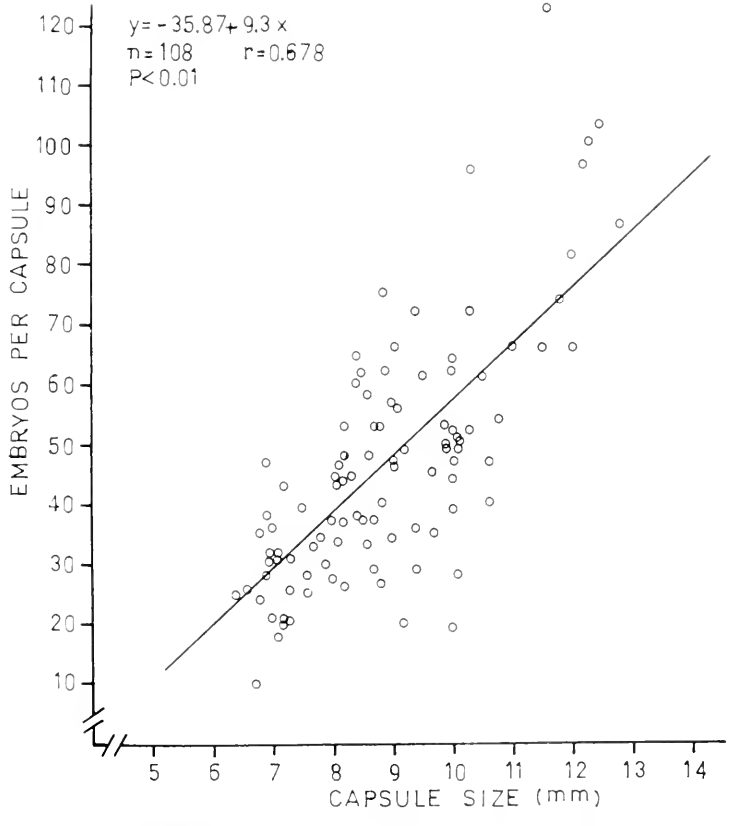

FIGURE 3. *N. crassilabrum.* Relationship between number of viable embryos per capsule and capsule size.

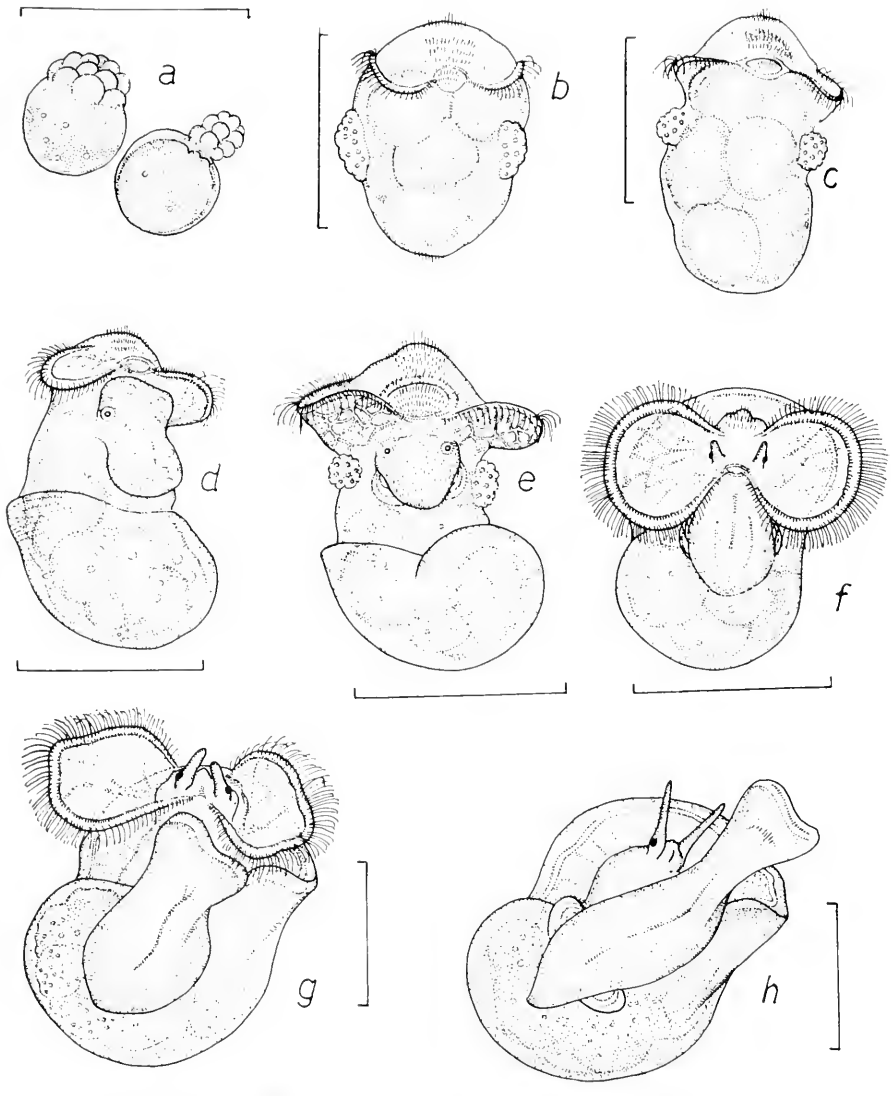

FIGURE 4. *N. crassilabrum.* Different stages of intracapsular development. a) a fertile egg (left side) next to a nurse-egg, in cleavage stage. b) trochophore stage with the outlines of whole nurse-eggs seen through the body wall. c–d) early veliger stage. e–f) mean and advanced veliger stage. g) pre-hatching stage with the velum in reabsorption process. h) hatching juvenile stage. The lines equal 500 μm.

face of the foot gradually differentiates a small, thin operculum. On either side of the foot a spherical statocyst is visible. Within the velar rim and around the stomodaeum, rudiments of the adult head are now beginning to become organized and a pair of black eyespots is visible on the base of recently developed tentacles

(Fig. 4f). Ingestion and feeding activity may continue in some capsules up to the veliger stage illustrated in Figure 4e.

The next stages of the intracapsular veliger (Figs. 4g, h) show a gradual and extensive increase in size, especially of the shell, foot and tentacles. The yolk gradually disappears in the visceral hump with accompanying differentiation of the viscera. A columella muscle faintly visible on the left side of the visceral hump is able to effect withdrawal of both head and foot into the shell. By the end of intracapsular development, the lateral lobes of the velum have been resorbed and this pre-hatching juvenile shows an active crawling foot and large tentacles.

Embryonic feeding and rate of development

During intracapsular development, some embryos acquire more nurse eggs than their capsulemates and this is reflected in the size distribution of embryos and hatchlings. Embryos in trochophore phase as small as 450 μm were found together with others as large as 775 μm after all nurse eggs had been eaten. By this time, the number of eggs eaten by each larva varies from 3 to 20 as shown in Figure 5 (left side) for three capsules with different numbers of capsulemates. This figure also shows the frequency at which embryos ingest different numbers

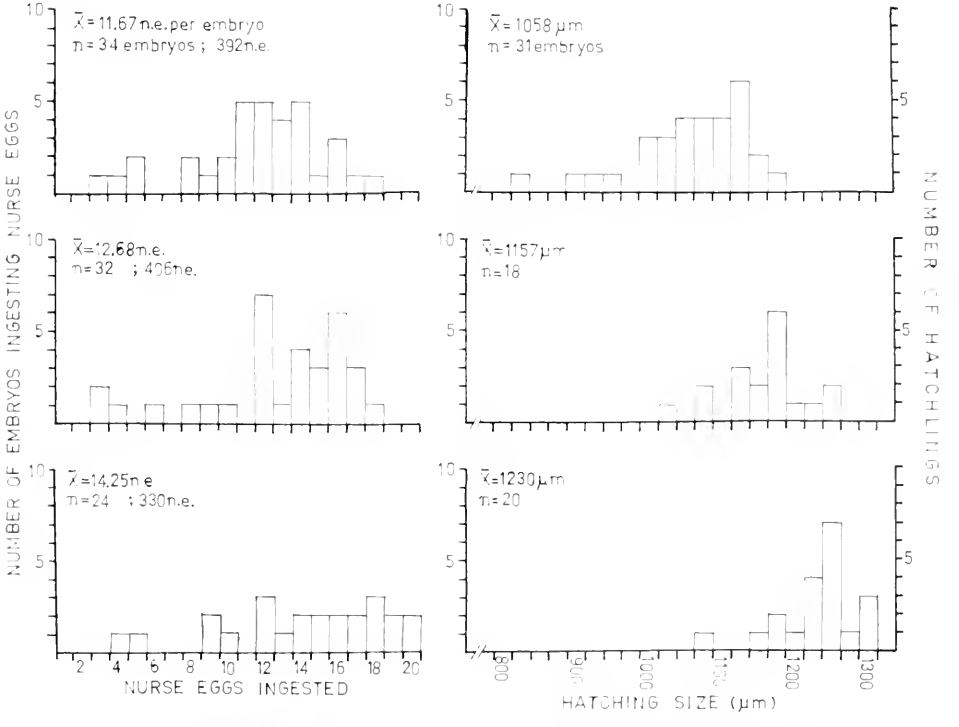

FIGURE 5. *N. crassilabrum*. Left, frequencies of embryos ingesting different numbers of nurse-eggs in three different capsules. Right, hatching size frequencies between embryos of three different capsules. n.e. = nurse-eggs.

of nurse eggs. Hatching size distributions for juveniles from three different capsules are shown in Figure 5 (right side). While some juveniles attain 825-μm shell length at the moment of hatching, others are as large as 1300 μm. Mean number of nurse eggs ingested per embryo per capsule as well as mean hatching size, seem to change regularly as the number of embryos per capsule decreases. The effect of haphazard distribution of embryos among capsules on hatching size, has been previously studied by Spight (1976a) in the muricids *Thais emarginata* and *Acanthina spirata*. While embryo counts are less predictable in these species, nurse eggs per capsule are distributed more regularly. Some embryos share their yolk supplies with many more capsulemates than others and this factor is most important in determining a variable hatching size between nurse egg feeders' embryos (Spight, 1976a).

Time required to complete intracapsular development was estimated from two egg masses spawned at different seasons of the year. At the time the observations began, the egg capsules were in an early cleavage stage. An egg mass controlled during autumn and winter months took between 70 and 80 days to fully develop and hatch; mean of monthly water temperature from May to August, 1976 at Mehuín, varied between 9.67 and 10.60° C. In other masses that developed during late spring and early summer, the time was significantly reduced to approximately 55 to 65 days; in this case, mean of monthly water temperature from November, 1976 to January, 1977 varied between 11.40° and 14.10° C. Doubtlessly, differences in developmental rates in nature are well accounted for by seasonal changes in sea water temperatures at Mehuín.

Discussion

Nucella crassilabrum shows the most evolved pattern of development known for Chilean muricids if we compare it with observations in *C. concholepas* (Gallardo, 1973) and in *Ch. giganteus* (Gallardo, in press). The type of development of *N. crassilabrum* agrees with that reported for its congener *N. lapillus* (Pelseneer, 1910). Such pattern of development is expected in high latitude muricaceans inhabiting interidal rocky shores (Spight, 1977a). Spight establishes that, among the rocky intertidal muricaceans, the hatching type evolves markedly according to a latitudinal gradient; while in this habitat all tropical species maintain planktonic larvae, all high latitude species metamorphose before hatching. In this sense, *N. crassilabrum,* whose geographic distribution extends along the Chilean coast to the Magellan Strait, may be included within the muricid group showing such reproductive tendency. In a paper about hatching type of *Chorus giganteus* (Gallardo, in press), a case of coincidence with Spight's predictions for muricaceans from another type of habitat has also been reported. According to Spight (1977a), muricaceans with an intermediate hatching type (a non-feeding veliger near to metamorphosis) had been found to inhabit a shallow water sand habitat; the findings in *Ch. giganteus* agree, as this sand bottom muricid also possesses such hatching type. Causes for these tendencies in muricid developmental patterns when related to latitude and habitat conditions are unknown.

All reproductive patterns should involve adaptations to maximize survival of embryos and, consequently, their reproductive fitness. Some of these adaptations

should include ability of adults to discriminate and choose good spawning sites, as well as an appropriate developmental time and hatching size. Our field observations in *N. crassilabrum* reveal that mortality of embryos is of common occurrence at least at the upper shore spawning sites. Probably some sites offer more protection than others; for instance, one would expect physical stresses to be minimal in tidepools, but they are used less frequently than vertical surfaces exposed to repeated dehydration during low tides. According to observations of the intertidal muricid *Thais lamellosa* (Spight, 1977c), spawning sites selected would reflect the conflicting demands of different life history stages. According to Spight, vertical surfaces are least accessible to predators and often are covered with food (barnacles), but provide little protection from physical stresses. Crevices offer some protection from physical stresses and usually are covered with barnacles but are accessible to predators. Understone surfaces and tidepools offer protection from stresses but are accessible to predators and are often distant from food sources. With these considerations in mind, Spight concludes that *T. lamellosa* females deposit capsules most frequently on the surfaces that are most suitable when the demands of all life history stages are considered. Studying the congeneric species *N. lapillus,* Feare (1970) found that physical stresses operating during exposure to air are responsible for hatching success of 0% at mean tide level and 57% at mean low water of neap tides. Our field observations in *N. crassilabrum* suggest that this species also uses poor spawning sites regularly and it probably does so for the same reasons that *T. lamellosa* does. For instance, different environmental stresses seem to be influencing survival of *N. crassilabrum* adults when closely located sites are compared (Gallardo, in preparation); demands of this life history stage could be in part conditioning the selection of spawning sites that are most suitable.

Hatching size of intertidal muricaceans is shorter (0.6 to 1.3 mm; $\bar{X} = 1.01$ mm) than that of species living in subtidal habitats (0.6 to 2.5 mm; $\bar{X} = 1.54$ mm) or on coral reef flats (1.1 to 1.9 mm; $\bar{X} = 1.40$ mm) [Spight, 1976b; ($\bar{X}$) = mean values calculated from a few species where data are available]. According to this, the hatching size of *N. crassilabrum* (0.82 to 1.30 mm) corresponds with the habitat this muricid occupies. To attain the appropriate hatching size, holobenthic muricaceans have followed two evolutionary paths: provision of nurse eggs or increase in size of a self-sufficient fertile egg. Now the question arises: which selective forces favor one or the other of these developmental options? It has been hypothesized that providing much of the yolk as nurse eggs may allow a snail to have a large hatching size and at the same time a relatively brief developmental time (Spight, 1975). In order to prove the validity of this hypothesis between muricaceans, we may use information available about developmental time, hatching size and embryonic feeding source both in *N. crassilabrum* (this paper) as in other species (Spight, 1975, 1976b); this information is compared in relation to habitat. We may see that among intertidal muricaceans adaptation for nurse egg feeding is of common occurrence; in a few species of this habitat, energy for developing embryos is totally obtained from larger fertile eggs (*Thais lamellosa* 590 μm; *T. canaliculata,* 620 μm). *T. lamellosa* reveals a developmental time of 67 to 91 days at 9.6° to 11° C, more or less similar to that of *N. crassilabrum,* although the upper limit of its range, 91 days, slightly exceeds that of *N. cras-*

silabrum; 80 days at 9.6° to 10.6° C. On the other hand, subtidal species usually require a large hatching size. To attain it, they usually have evolved a large fertile egg (*Ceratostoma foliatum*, 720 μm; *Torvamurex territus*, 675 μm); numerous nurse eggs per embryo are consumed in species of this habitat showing such feeding mechanism (*Murex senegalensis*, 35 n.e. per embryo; *M. quadrifrons*, 91 n.e. per embryo). Subtidal species whose egg exceeds 600 μm in diameter, reveal a markedly slow developmental rate; for instance, *T. territus* takes 90 days to hatch at 20° C and *C. foliatum* takes 120 days to hatch at 10° to 12° C. Our observations in *N. crassilabrum* and those of Spight (1977c) and Feare (1970) reveal that one of the most important sources of embryo mortality in muricaceans laying in the intertidal, are the physical stresses operating at this level; an embryo of *T. lamellosa* cannot tolerate even one two-hour exposure (Spight, 1977a). Similar observations have been made by Pechenik (1978) in prosobranchs of the family Nassidae. Pechenik found that egg capsules of *Nassarius obsoletus* did not afford substantial protection against desiccation; 0.5-hr exposures to 75% relative humidity killed 17.5% of the embryos. It is reasonable to assume that such mortality would be reduced by laying the egg capsules in protected micro-environments, as *N. obsoletus* clearly does (Pechenik, 1978), or by shortening developmental time of clusters spawned at more exposed sites of the intertidal. When, for reasons considered above, an intertidal muricid does not always use good spawning sites for embryos, the possibility that it is shortening developmental time should be preferred. An evolutionary option in this sense may be to change to another form of embryonic feeding, that is the provision of nurse-eggs. But, how to explain nurse-egg feeding by some subtidal muricaceans? In this case, this embryonic feeding adaptation seems to have been specially favored to attain an extremely large hatching size. Relatively large muricid eggs range from 675 to 920 μm in diameter (*T. territus*, *Thais lima*); nevertheless the hatchlings developed from such eggs do not exceed from 1.3 to 1.5 mm in length. In subtidal species, usually requiring a larger hatchling (1.6 to 2.0 mm), each embryo is provided with numerous nurse eggs (*M. quadrifrons*, *M. senegalensis*). These preliminary considerations suggest that a delicate compromise between at least the optimal hatching size and developmental time, could be orienting evolution of embryonic feeding adaptations among holobenthic muricaceans. However, further work is necessary to reinforce this hypothesis. The results for the small number of species considered in this discussion cannot, by themselves, support it, but they can add to the data for future comparative studies. Information about developmental time, hatching size, habitat, and mortality sources is still lacking in various muricids. An optimal material to evaluate the advantages of evolving nurse eggs is offered by *T. canaliculata*; this intertidal snail appears to be in the course of evolving from one reproductive mode (self-sufficient large egg) to the provision of nurse-eggs for embryos (Spight, 1977b).

Summary

1. Eggs of *Nucella crassilabrum* range from 204 to 293 μm in diameter (mean = 240 μm). Only 6.6 to 7.9% are fertile; the remaining are ingested as nurse eggs.

2. Embryos metamorphose before hatching. Pre-hatching time ranges from 55 to 80 days according to seasonal temperature fluctuations.

3. Hatching size varies from 0.82 to 1.3 mm, depending on number of nurse-eggs ingested per embryo (from 3 to 20). Number of fertile embryos per capsule (10 to 122) depends on capsule size.

4. Hatching type and hatching size shown by *N. crassilabrum* agree with those of other muricaceans living in similar habitat conditions.

5. Pre-hatching time and hatching size data of various muricaceans are analyzed to determine to what extent they influence embryonic mode of nutrition, namely the presence of nurse-eggs or alternatively large and fertile self-sufficient eggs. Provision of nurse-eggs for embryos is of common occurrence among intertidal muricaceans and this mode of nutrition seems to have been favored in such habitats to reduce developmental time. Providing the yolk as nurse-eggs seems also to contribute to a larger hatching size, as suggested by some subtidal muricaceans with such embryo support patterns.

LITERATURE CITED

Ankel, W. E., 1937. Der feinere Bau des Kokons der Purpurschnecke *Nucella lapillus* (L.) und seine Bedeutung für das Laichleben. *Verh. Dtsch. Zool. Ges.*, **1937**: 77–86.

Feare, C. J., 1970. Aspects of the ecology of an exposed shore population of dogwelks *Nucella lapillus* (L.). *Oecologia*, **5**: 1–18.

Gallardo, C. S., 1973. Desarrollo intracapsular de *Concholepas concholepas* (Brugiere) (Gastropoda Muricidae). *Museo Nacional de Historia Natural, Santiago, Chile. Publ. Ocas.*, **16**: 1–16.

Gallardo, C. S., 1979. El ciclo vital del muricido *Concholepas concholepas* y consideraciones sobre sus primeras fases de vida en el bentos. *Biol. Pesq. Chile* (In press).

Gallardo, C. S., 1979. Postura y estadio de eclosión del gastrópodo Muricidae *Chorus giganteus* (Lesson, 1829). *Studies on Neotropical Fauna and Environment* (In press).

Hyman, L. H., 1967. *The Invertebrates.* Vol. VI. *Mollusca I.* McGraw-Hill Book Company, New York, 792 pp.

Pechenik, J. A., 1978. Adaptations to intertidal development: studies on *Nassarius obsoletus*. *Biol. Bull.*, **154**: 282–291.

Pelseneer, P., 1910. Recherches sur l'embryologie des Gastropodes. *Mem. Acad. R. Belg.*, Ser. 2, **Vol. 3**: 1–167.

Spight, T. M., 1975. Factors extending gastropod embryonic development and their selective cost. *Oecologia*, **21**: 1–16.

Spight, T. M., 1976a. Hatching size and the distribution of nurse eggs among prosobranch embryos. *Biol. Bull.*, **150**: 491–499.

Spight, T. M., 1976b. Ecology of hatching size for marine snails. *Oecologia*, **24**: 283–294.

Spight, T. M., 1977a. Latitude, habitat and hatching type for muricacean gastropods. *The Nautilus*, **91**(2): 67–71.

Spight, T. M., 1977b. Is *Thais canaliculata* (Gastropoda: Muricidae) evolving nurse eggs? *The Nautilus*, **91**(2): 74–76.

Spight, T. M., 1977c. Do intertidal snails spawn in the right places? *Evolution*, **31**: 682–691.

Reference: *Biol. Bull.* **157**: 464–477. (December, 1979)

EARLY POST-METAMORPHIC GROWTH, BUDDING AND SPICULE FORMATION IN THE COMPOUND ASCIDIAN *CYSTODYTES LOBATUS*

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Compound ascidian colonies are comprised of many individuals wholly embedded in a common tunic. Budding is accomplished by active epidermal synthesis and constriction, with the regenerating buds moving through this common tunic to form a new system (see Berrill, 1951, 1961 for reviews of early papers; Sebastian, 1957; Levine, 1960; Freeman, 1971; Nakauchi, 1966a, b, c, 1970, 1977; Nakauchi and Kawamura, 1974a, b, 1978). *Cystodytes,* unlike other genera in the suborder Aplousobranchia, family Polycitoridae, is distinguished by large numbers of calcareous spicules surrounding the abdomen of each zooid, forming a spicular sac separating each individual from neighboring zooids (Ritter, 1900; Van Name, 1945). During budding this spicular sac must be reorganized and reformed around the abdomen of each bud, but the way in which this is accomplished is completely unknown. The common tunic overlying the spicular layer contains numerous large, closely packed, acid-filled bladder cells (Abbott and Newberry, 1980); any rupture of these cells results in immediate dissolution of nearby spicules and evolution of CO_2. The spicules might be dissolved and reformed during budding or just reallocated in some way among the buds; there appear to be no life history studies on any species of the genus *Cystodytes,* even though the genus is common and widespread (Van Name, 1945; Millar, 1975). Therefore, the present study is an examination of the general ecology and method of budding in *Cystodytes lobatus* (Ritter, 1900), a species that occurs abundantly in the low intertidal and subtidal zones along the central California coast. The time course of tadpole release, spicule formation in oozooids (the individuals developing from newly-settled tadpoles before the first budding), and the method of budding with each zooid isolated in its own spicular sac are discussed here. Included is the discovery that the spicules are contained within a discrete extra-cellular membrane. This membrane has been named the tunic spicular lamina and is concluded to form an organic matrix for spicule formation.

Materials and Methods

This work was carried out at the Hopkins Marine Station, Pacific Grove, California, between February and August, 1978. Animals were collected from rocks in front of the marine station and at Pt. Pinos, about two miles away. Three color variants exist: white, pink and orange. Since the taxonomy of these variants has not been studied, and since Ritter (1900) based his original description of the species on the white form, only white colonies were used throughout this study.

Colonies were maintained in unfiltered running sea-water aquaria in the laboratory; between February and August the temperature varied only from 13° to 16° C. Colonies usually lived at least a month in the laboratory, and if they reattached to the bottom of the aquarium they survived even longer.

Colonies with tadpoles were easily recognized by the presence of the bright pink yolky embryos. Since it is known that some colonial ascidians release their tadpoles in response to light (*e.g.*, Watanabe and Lambert, 1973), the colonies were kept in the dark at night. Tadpole collection was accomplished by removing the colonies in the morning to a clear plastic aquarium supplied with running sea water. Drainage was through a hose penetrating the aquarium at one end, with water flow adjusted so that the water level was at the hose exit. In this way, as tadpoles were released from the colonies and swam upwards they were skimmed off and carried through the hose to a tadpole collector, consisting of a short piece of polyvinyl chloride pipe with 300-μm Nytex glued to the bottom of it and resting in a small dish. Tadpoles were released throughout the day. Periodically they were removed from the collector to a petri dish with a glass slide in the bottom of it centered over a piece of black plastic under the dish to induce settling of the tadpoles in the darkest area. These slides were then placed in a plexiglass slide holder and submerged in a large cement tank in the laboratory filled with running unfiltered sea water where they were maintained for several months. Slides were removed from the holder and placed in a sea water-filled petri dish for examination of the living zooids, using an American Optical dissecting microscope with phototube. All photographs were taken with an Olympus OM-2 35-mm SLR camera with microscope adapter.

A Beckman Expandomatic IV pH meter equipped with a rapid-response MI-410 combination pH probe (Microelectrodes, Inc., Londonderry, N. H.) was used for all pH determinations. Bladder cell pH was determined in two ways. Adult colonies were washed several times in distilled water, blotted dry with Kimwipes, then either the pH electrode was carefully inserted about 2 to 4 mm into the superficial layer of the tunic or bladder cells in the upper tunic layer were broken by agitation with a fine probe and then the pH electrode was immersed directly into the resulting pool of (mostly) bladder cell fluid. Results were the same for both methods. Bladder cell contents were analyzed for the presence of chloride and sulfate ions by probing them directly with hand-made finely drawn out glass micropipettes under a dissecting microscope. The fluid collected was expelled into a watch glass, and a drop of either silver nitrate or barium chloride was added. ($AgNO_3$ forms a precipitate of $AgCl_2$ in the presence of chloride ions, $BaCl_2$ forms a precipitate of $BaSO_4$ in the presence of sulfate ions.)

Because alcohol dehydration and even fixation in buffered formalin did not prevent rupture of bladder cells and dissolution of spicules, razor blade sections of living colonies were made and stained supravitally with PAS, aldehyde fuchsin, alcian blue pH 2.5, alcian blue pH 1.0, sudan black B, 0.1% toluidine blue, or neutral red, using the methods in Pearse (1968), in order to analyze the tunic for acid mucopolysaccharides and other structural materials.

A few 4-wk-old oozooids settled on slides were relaxed in 100 ml sea water to which had been added 1 drop of menthol-saturated 95% ethanol, according to the method of Abdel-Malek (1951). When total relaxation was achieved in 3 to

6 hr. 10% formalin was added drop by drop while stirring, until the animals were dead. The zooids were transferred to 10% formalin for 24 hr, then removed from their slides, washed, dehydrated in alcohols and embedded in the Polysciences JB4 plastic embedding medium. After 24 hr the plastic blocks were trimmed and sectioned at 1 to 2 μm. Sections were removed one at a time, placed in a drop of 1% ammonia on a slide to flatten out, then flame-dried and stained with methylene blue or 0.1% toluidine blue.

Results

General biology

Cystodytes lobatus grows in large mats, up to a half meter across or more; whether each mat is one colony or many that have fused or abutted was not determined. Colonies by the Hopkins Marine Station averaged 5 mm in thickness. However, material collected elsewhere and in D. P. Abbott's private collection may be up to 1 cm or more in thickness (Abbott and Newberry, 1980).

Colonies always occurred in the low intertidal or subtidal; a −0.6 ft tide or lower was necessary to collect intertidally. The best intertidal sites were under overhanging rocks and to a lesser extent on the north side of vertical rocks, away from direct sunlight and (possibly) from competition with plants.

Large colonies were commonly observed to have overgrown many barnacles and polychaete worm tubes, resulting in a mat with many superficial ridges and knobs. Cross sections indicated colonies to be of variable thickness; some ridges covered overgrown barnacles while others were present without any underlying irregularity of the substratum. Other compound ascidians, common in the same area as *Cystodytes*, were *Aplidium californicum* and *Archidistoma psammion;* colonies of both these species also attained large size, and a dynamic situation appeared to be present of overgrowth of one ascidian by another—either overgrowth of *Cystodytes* by *Aplidium* or *Archidistoma* or vice versa.

Very few predators were observed feeding on *C. lobatus;* those animals found on or near colonies in the field were the starfish *Patiria miniata* and the gastropods *Calliostoma ligatum, Tegula funebralis, Megathura crenulata,* and *Lamellaria diegoensis.* However, these observations were made intertidally at low tide when *Cystodytes* was out of water; high tide observations might be different. When *Cystodytes* is out of water it forms a slimy coating of an (apparently) mucus-like material that can be seen hanging in long strands and dripping off the colonies. This material is not present in submerged colonies until the surface is rubbed. The significance of this is as yet unknown.

Laboratory feeding observations in the above-mentioned animals found on or near colonies showed that all but *Tegula funebralis* would eat *Cystodytes* in the laboratory. Stomach contents of subtidal *Calliostoma ligatum* and *Megathura crenulata* collected on or near *Cystodytes* yielded chunks of colony with the spicules mostly intact (Sellers, 1977 and personal communication). *Lamellaria diegoensis* was maintained in the laboratory from March 10 until August 31 on a diet of nothing but *Cystodytes lobatus*; during this period its weight increased from 2.2 grams (May 3) to 6.0 grams (August 21). A study of feeding of

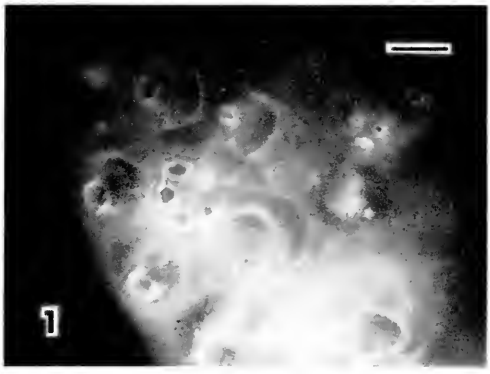

FIGURE 1. Two *Cystodytes lobatus* systems comprised of 5 and 3 zooids. Scale bar 1 mm.

Lamellaria on *Cystodytes*, including a calorific analysis of *Cystodytes* colonies utilizing a semimicro bomb calorimeter, will be published separately (Lambert, 1979).

Other animals associated with *Cystodytes* as well as other colonial ascidians are the clam *Mytilimeria nuttalli* and the amphipod *Polycheria osborni*, both of which live embedded in the ascidian test (Abbott and Newberry, 1980). Very little is known of the relationship of these species to their ascidian hosts (Skogsberg and Vansell, 1928; Yonge, 1952).

Colony organization and tadpole release

The zooids of *Cystodytes* (Fig. 1) are arranged in systems (Van Name, 1945) with a mean of 4 or 5 zooids per system (Fig. 2). The atrial siphons open separately at the surface (Fig. 1). The abdomens are surrounded by a layer of overlapping calcareous spicules (Figs. 3, 4) and the test matrix is filled with bladder cells (Van Name, 1945) tightly packed and ranging in size roughly from 35 to 80 μm. These cells are filled with sulfuric and possibly hydrochloric acids; tests were positive for the presence of both sulfate (Abbott and Newberry, 1980)

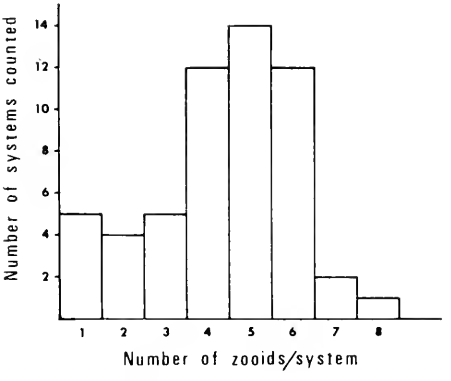

FIGURE 2. Number of zooids per system. n = 55 systems, $\bar{x}$ = 4.36 zooids/system, s.d. = 1.69.

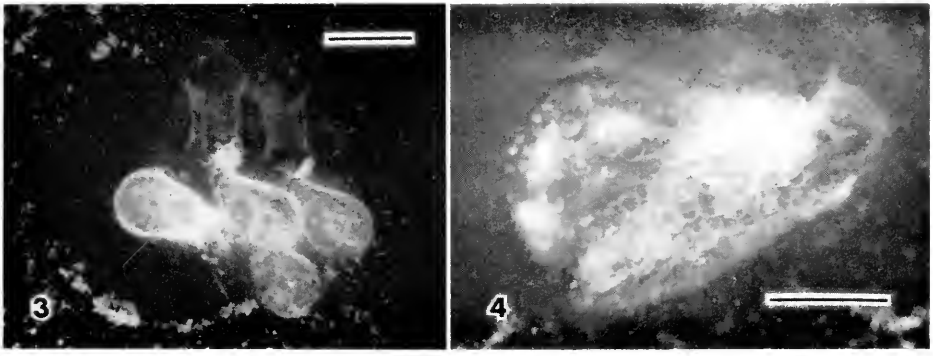

FIGURE 3. Underside of a *C. lobatus* system. Scale bar 1 mm.
FIGURE 4. *C. lobatus* oozooid 32 days old. Scale bar 0.5 mm.

and chloride ions. The pH of bladder cell contents was determined to be 1.3.

Embryos develop within the atrial chamber of the adult zooids (Van Name, 1945). Because of their bright pink color they could be observed easily and were recorded in nearly all colonies collected between March 7 and August 18.

Tadpoles were collected in the tadpole collector and settled on glass in order to observe the growth of zooids in the laboratory, the time course of spicule and bladder cell formation, and the method of budding. The time course of tadpole release (Fig. 5) reveals that more tadpoles were released after 3 to 4 hr in the light following overnight dark adaption under black plastic than at any other time, and tadpoles were released more or less continuously all day. Colonies left in an uncovered aquarium overnight in order to observe their reaction to natural dawn also released larvae sporadically throughout the day.

Cystodytes tadpoles are large, with a mean body length of 1.27 mm ($n = 25$, s.d. = 0.113), tail length of 2.59 mm ($n = 25$, s.d. = 0.141), and tail width of

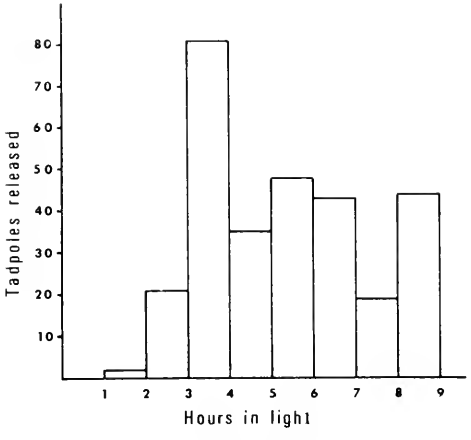

FIGURE 5. Tadpole release as related to duration of light period after darkness. Numbers are totals for 10 days of observations.

0.76 mm (n = 25, s.d. = 0.049). The heart beats somewhat erratically but does reverse, as in the tadpoles of *Distaplia* and *Diplosoma* (Cloney, personal communication) and *Pycnoclavella stanleyi* (Trason, 1963). Bladder cells are already densely packed in the test matrix of the tadpole. The pH of six individuals homogenized in a few drops of distilled water was 2.85. A few tadpoles metamorphosed within 15 min of being released, though most had a free larval life of 1 to 3 hr. Settlement was greatly enhanced by using slides that had been soaked in sea water for several days.

Post-metamorphic growth and budding

Young oozooids begin to feed 3 to 4 days after settlement (as determined by the presence of food pellets in the gut) and by one week of age the gut has differentiated into five well-defined regions similar to those in many aplousobranchs: esophagus, stomach, post-stomach, mid-intestine, and intestine or rectum. The stomach and intestine are orange-brown; the rest of the zooid is colorless or nearly so. By 24 hr or so after settlement, oozooids have four rows of stigmata, the same as the adult blastozooids. This is similar to the closely related polycitorid *Archidistoma ritteri* (Levine, 1960) in which both oozooids and blastozooids have three rows. In the polyclinid *Amaroucium multiplicatum* (Nakauchi, 1966a), in contrast, the oozooids have four rows but after budding the blastozooids have six or seven rows of stigmata. In *C. lobatus* there are usually four stigmata per side in the anteriormost row at metamorphosis; at budding this row has 17 or 18 stigmata per side. With successive buddings the blastozooids orient vertically with the abdomen directly beneath the thorax rather than curved around it is in the oozooid, but otherwise the oozooids and blastozooids appear to be the same morphologically. Whether oozooids ever form gonads, however, was not determined.

There is great variation among oozoids in the quantity of spicules produced, even those arising from tadpoles from a single colony reared on the same slide (compare Fig. 4 with Figs. 6 and 8). A number of tadpoles were collected on May 25 and allowed to settle on three slides which were maintained in the

TABLE I.

Relationship between spicule density and time to budding, number of abdominal buds and time until buds begin feeding in C. lobatus.

	n	$\bar{x}$	s.d.
Days from settlement to budding for zooids with few spicules	32	29.72	3.0
Days to budding for zooids with many spicules	18	37.33	6.28
Number of buds, few-spiculed zooids	32	3.59	0.61
Number of buds, many-spiculed zooids	18	2.89	0.47
Days from budding until buds begin feeding in few-spiculed zooids	24	5.58	0.50
Days from budding until buds begin feeding, many-spiculed zooids	15	5.67	0.62

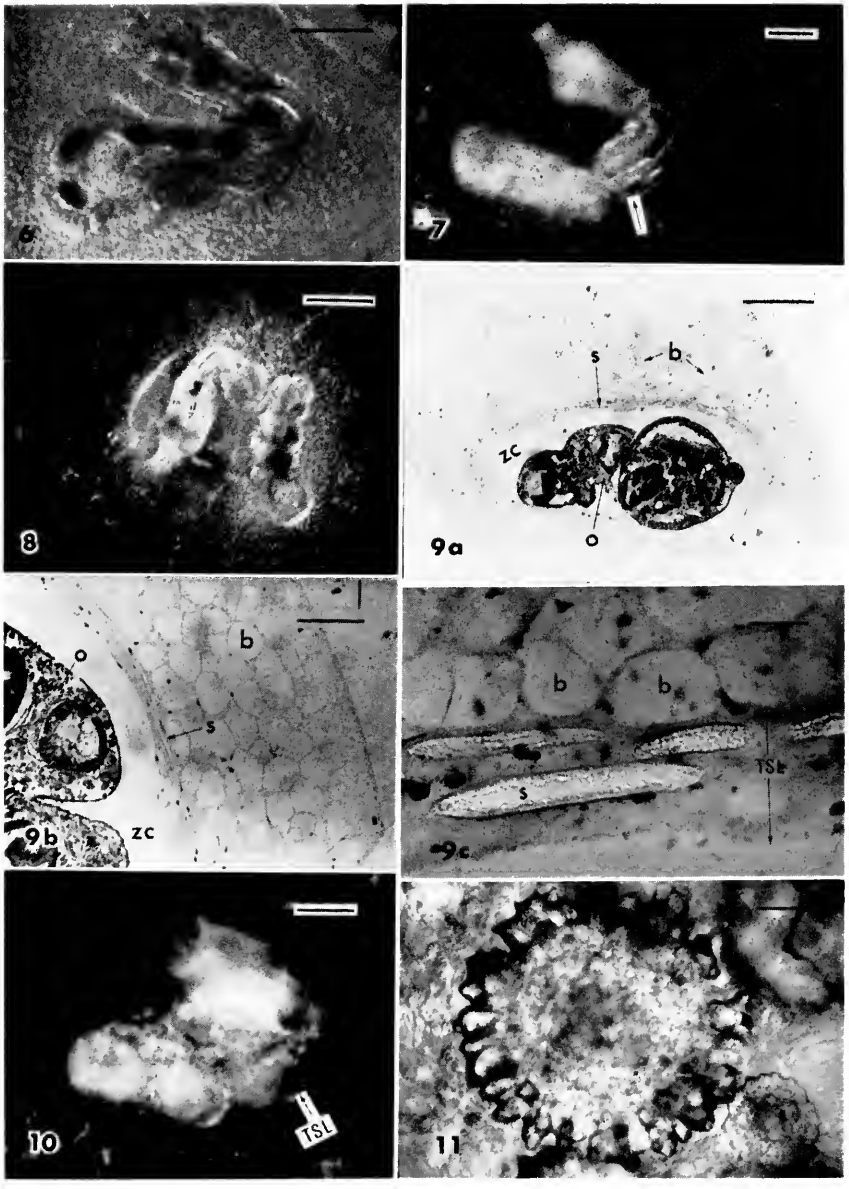

FIGURE 6. Twenty-five-day-old oozooid. Arrow indicates position of new stomach. Scale bar 0.5 mm.

FIGURE 7. Thirty-one-day-old oozooid. Arrow indicates new stomach at posterior end of mother bud. Scale bar 0.5 mm.

FIGURE 8. Thirty-three-day-old oozooid; budding is nearly complete. Scale bar 1 mm.

FIGURE 9. Four-week-old oozooids, 1 μm sections. (a) entire zooid; scale bar 250 μm. (b) Enlargement of tunic region; scale bar 100 μm. (c) TSL region of tunic; scale bar 20 μm. b = bladder cell, o = oozooid, s = spicule, TSL = tunic spicular lamina, zc = zooid cavity.

FIGURE 10. Thirty-six-day-old oozooid forming 3 abdominal buds; scale bar 0.5 mm. B = bud, TSL = disrupted tunic spicular lamina.

FIGURE 11. Spicule from an adult *C. lobatus* colony. Scale bar 50 μm.

laboratory until the end of August. Table I compares oozooids that developed few spicules with those that developed many. Zooids with many spicules took longer to bud (37 days as compared with 30 days) and produced fewer buds. (These are lab times; colonies occurring naturally in the field grew more rapidly than those raised in the lab, and achieved larger size). Without exception, buds always produced the same (subjective) quantity of spicules as the mother had: all buds of a many-spiculed oozooid developed a heavy coating of spicules, and all buds of a few-spiculed oozooid developed only a sparse coating of spicules. This may explain why in some colonies in the field all the zooids have few spicules, while in other colonies all zooids have many spicules.

An attempt was made to study growth rate in a group of zooids by removing slides from the slide holders every other day for measurement of the zooids settled on them. However, this had an obviously detrimental effect on the zooids: the time to budding was longer, only two buds were produced per oozooid, and nearly all the animals ultimately degenerated, so these data were discarded. Other workers contemplating using this technique should be aware of these possible effects.

Although the process of budding has not previously been described for *Cystodytes*, it is in fact similar to budding in other polycitorids (Oka, 1942; Oka and Usui, 1944; Berrill, 1947, 1948; Levine, 1960; Nakauchi, 1966b, c) and in the polyclinids (Nakauchi, 1966a, 1970, 1974, 1977). *Cystodytes* exhibits Nakauchi's (1966a) Type I budding (abdominal; all buds receive some digestive and epicardial tissue). As in *Amaroucium yamazii* (Nakauchi, 1970), budding in *Cystodytes* is preceded by an elongation and enlargement of the posterior end of the esophagus which begins at least 10 days prior to budding (Fig. 6). This esophageal enlargement will become the new stomach of the mother bud (Figs. 7, 8). A few hours before budding begins, the orange-brown stomach and intestine elongate greatly, as does the epicardium. Feeding ceases, the thorax contracts, and all fecal pellets in the digestive tube collect at the end of the rectum. Budding proceeds posteriorly, with the first constriction occurring at the anterior end of the old stomach. Sometimes the heart of this first bud (called the mother or thoracic bud) can be seen beating at this time. Three or four abdominal buds form, with the terminal bud receiving the mother's old heart. All buds receive some of the orange-brown stomach and intestinal tissue as well as some epicardial tissue. After budding is completed and the mother bud's digestive tube has fully regenerated, the thorax relaxes, the old fecal pellets are ejected, and feeding resumes. Buds begin feeding after 5 to 6 days, and the next budding occurs in about 3½ weeks (lab time). Buds usually orient to the mother but one or two might join a neighboring system; this is probably the reason for the large range in number of zooids per system in Figure 2.

Spicule formation and reallocation at budding

As mentioned earlier, a major difference from the Polyclinidae and from other genera in the Polycitoridae is the formation of calcareous spicules in *Cystodytes*. Using a dissecting microscope at 40 or 80× one can distinguish the first spicules as early as 5 days after settlement. At first, the spicules are very tiny and cannot be individually discerned; only a white spot is noticeable constituting the

entire amount of spicular material. Spicule formation always begins on the inner side of the abdomen at the junction between abdomen and thorax. Gradually the spicules appear to migrate as they increase in number and size until at the end of 4 weeks (laboratory growth time) a zooid's abdomen is completely covered with somewhat overlapping non-birefringent spicules (Fig. 4). These spicules are not attached to the abdomen, though; they are embedded in the innermost layer of tunic that lines the zooid cavity. This layer has been named the "tunic spicular lamina" (TSL). Figure 9 shows the newly forming spicules embedded in the middle of the TSL. Above it are the closely packed bladder cells extending all the way to the outer tunic surface, with the common tunic consisting of the material between the bladder cells. The TSL appears to be completely extracellular, because when a razor blade cross section is made through an adult colony, the zooids can be removed easily from the tunic leaving all the spicules behind. The TSL thus forms a spicular sac in the tunic surrounding but separated from the abdomen of each zooid.

The fate of the spicules at the time of budding was next studied, to determine whether they are dissolved and reformed or simply divided up among the buds in some way. A careful examination at 2 to 3 hr intervals of a few budding zooids showed that first the abdominal epidermal constrictions separated the buds within the zooid cavity. When this process was at least partially completed, the spicular sac began to constrict, resulting ultimately in the abdominal portion of each bud being surrounded by a small spicule sac (Fig. 10). At no time was any dissolution of spicules observed; some of the larger spicules could be followed as they migrated to the buds. During the first stages of spicule "reallocation" many of the spicules were disoriented from their previously regular overlapping pattern. Instead of lying parallel to the abdominal epidermis, they might now be perpendicular to it. This would be expected if the spicules were embedded in a membrane and the entire membrane were constricting. A few spicules were sometimes "left behind" in the test, where they remained isolated. However, active and rapid synthesis of new spicules was also occurring during this time, and approximately 3 to 4 days after the onset of budding all buds were close together but separated from one another by their own spicular sacs, with the spicules regularly aligned parallel to the inner edge of the tunic lining the zooid cavity.

New spicules continued to be added as the buds grew, and the old spicules increased in diameter. Indeed, in adult colonies some spicules may be 1 mm or more in diameter; these might have been carried through a number of generations. They exhibit a complex configuration of knobs not found on small, newly formed spicules (Fig. 11).

Histochemistry of the tunic spicular lamina

Hunt (1970) indicated that in molluscs, the formation of calcium carbonate may depend upon induction by an organic matrix with the presence of sulfated acid mucopolysaccharides being important to this process. Recent papers on tunic composition listed these and other substances found in ascidian tunic and the stains appropriate for their detection (Deck, Hay and Revel, 1966; Smith,

TABLE II.

Histochemistry of C. lobatus *tunic.*

Stain	Specific for	Time (min.)	Tunic region	
			TSL*	Common tunic
0.1% toluidine blue in 30% ethanol	Sulfated acid mucopolysaccharide	10–30	++++ B-metachromatic	++
Aldehyde fuchsin	Sulfated acid mucosubstances	5–15	++	++
Alcian blue pH 2.5	Sulfated acid mucopolysaccharide	14–40	++++	++
Alcian blue pH 1.0	Sulfated acid mucopolysaccharide	15–30	+++	++
Periodic acid-Schiff	Cellulose-like compounds	10	++	++
Sudan black B	Lipid	40	+++	++

*TSL = tunic spicular lamina.

1970; Stiévenart, 1970, 1971). The techniques described by Pearse (1968) for making and using these stains were applied to fresh thin razor blade slices of adult *Cystodytes lobatus* colonies; the results are listed in Table II. Alcian blue and toluidine blue, two stains specific for sulfated acid mucopolysaccharides, stained more heavily around the spicules and inner edge of tunic lining the zooid cavities than elsewhere in the tunic, thus definitely delineating the tunic spicular lamina (TSL). Even those spicules isolated in the tunic during budding and no longer associated with any particular zooids retained this darkly staining membrane around them. (The fact that aldehyde fuchsin did not stain this region in a similar fashion supports Pearse's (1968) statement that it is not the specific stain it has been considered to be).

DISCUSSION

Cystodytes is one of the few genera of ascidians containing mineral concretions that persist in the sediments after the animal's death (Herdman, 1884). In several cases new fossil species of *Cystodytes* have been described solely on the basis of the spicules (Bonet and Benveniste-Velásquez, 1971; Monniot, 1970a; Monniot and Buge, 1971). The taxonomic significance of these spicules depends on the determination of whether or not the spicules form in a species-specific fashion. Monniot (1970b) concluded that polycitorid and didemnid spicules form as a "physico-chemical precipitation of aragonite in the tunic independent of cellular action" and could be used taxonomically only in a general way, to indicate the ascidian group. However, she did intimate that the tunic must play some part in spicule formation due to its fibrous or lamellar structure, but she did not elaborate. The present study proves the existence of an organic matrix for spicule formation (the TSL), differing from the surrounding common tunic in the concentration of sulfated acid mucopolysacharides. Also, the newly forming spicules have a lumen and are incompletely mineralized (Fig. 9c), while the spicules of adult *C. lobatus* are completely mineralized; this may indicate

another organic matrix for mineralization within each spicule (Lowenstam, personal communication). Cellular action is implicated by the fact that the spicules form in a particular region of the animal, at the junction between abdomen and thorax, and migrate out from that point to the TSL. In addition, a genetic component is implied by the great variation among colonies in the extent of spicule formation. Lafargue and Kniprath's (1978) paper proves the cellular origin of spicules in the Didemnidae. They identified the organ of spicular origin and also found that the spicules are surrounded by a discrete double-layered membrane. Thus their study and this study contradict the findings of Prenant (1925), Pérès (1948) and Monniot (1970b). Pérès was obviously troubled by his inability to determine the reasons for tunic stratification in the Polyclinidae, which he stated was especially noticeable around the periphery of zooids in addition to the thin outer cuticle.

Examination of the spicules in *Cystodytes lobatus* was difficult because nearly any treatment (fixation, sectioning, staining, even relaxation of live zooids) usually resulted in some disruption of the bladder cells and partial dissolution of the spicules. This is why all of the staining was done supravitally, as suggested by Pearse (1968). Nevertheless, some stains (aldehyde fuchsin and alcian blue pH 1.0 especially) changed the spicules into needle-like clusters within a few minutes. Fixation in alcohol or buffered formalin also disrupted bladder cell membranes, and after being left for a few weeks in fixative the surface irregularities disappeared from many spicules in adult colonies. Indeed, some *Cystodytes* colonies after several years on a museum shelf have few if any recognizable spicules left, a fact which undoubtedly has led to erroneous calculations of the abundance and type of spicules when the colonies were alive (Ritter, 1900; Van Name, 1945; Millar, 1962). Because of these difficulties, taxonomic descriptions of *Cystodytes* and didemnid species should include photographs or at least descriptions of spicules from live colonies if possible.

The question remains of how the tunic spicular lamina can "bud" when the zooid buds, a problem especially puzzling since the lamina appears to be completely extracellular. At a magnification of 675×, it is possible that a membrane was indistinctly seen in the 1-μm sections between the outer edge of the tunic spicular lamina and the beginning of the bladder cell region. If this is so, perhaps this membrane somehow separates from the rest of the tunic during budding. It will be necessary to embed and section zooids in the process of budding in order to examine this further.

My most sincere thanks go to Dr. Donald P. Abbott of the Hopkins Marine Station for suggesting this topic, sharing his laboratory with me, and offering me continued help and encouragement. I also thank P. Adams, F. Chapman, J. Cooper, Y. Fadallah, C. Harrold, A. Hines, C. Patten, V. Scofield, and R. Sellers for their assistance. Discussions with Drs. Todd Newberry and Richard Cloney greatly aided this work, and I especially want to thank Dr. Newberry for his excellent translation of a number of pages of French. Dave Behrens identified the *Lamellaria;* Drs. Donald Abbott, Roger Seapy and Heinz Lowenstam considerably improved the manuscript by their critical comments and valuable sug-

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Summary

1. The colonial ascidian *Cystodytes lobatus* has a long breeding season (at least 6 months) and releases tadpoles sporadically throughout the day, indicating a long period of recruitment.

2. Tadpoles of *C. lobatus* were settled and reared in the laboratory in order to observe early growth, budding and spicule formation.

3. Budding is preceded by the formation of a new stomach at the posterior end of the esophagus and fits Nakauchi's Type I budding pattern.

4. Spicule formation begins within 5 days after settlement. The spicules appear to form in a particular region at the anterior end of the abdomen and migrate over the abdomen to form a single or slightly overlapping layer embedded in a "tunic spicular lamina." This lamina lies between the common tunic and the zooid cavity and forms a spicular sac in the tunic surrounding but separated from the abdomen of each zooid. It stains especially heavily for sulfated acid mucopolysaccharide; the spicules are concluded to form by cellular action in this organic matrix.

5. There is great variation among zooids in the quantity of spicules formed. These differences are maintained in the buds, resulting in colonies in which all zooids either have few or many spicules, and are therefore probably genetic in origin.

6. During budding the spicular sac becomes disrupted and appears to bud, resulting in a reallocation of the spicules to the buds and formation of separate spicular sacs around the abdomen of each bud. At budding there is apparently no disruption of bladder cell membranes in the tunic and no dissolution of spicules by the acids contained in the bladder cells.

LITERATURE CITED

Abbott, D. P., and A. T. Newberry, 1980. Urochordata: The Tunicates. In Morris, R., D. P. Abbott, and E. C. Haderlie, *Intertidal Invertebrates of California.* Stanford University Press, Stanford, California (in press).

Abdel-Malek, E. T., 1951. Menthol relaxation of helminths before fixation. *J. Parasitol.*, **37**: 321.

Berrill, N. J., 1947. The structure, development and budding of the ascidian, *Eudistoma. J. Morphol.*, **81**(2): 269–281.

Berrill, N. J., 1948. Structure, tadpole and bud formation in the ascidian *Archidistoma. J. Mar. Biol. Assoc. U. K.*, **27**: 380–388.

Berrill, N. J., 1951. Regeneration and budding in tunicates. *Biol. Rev.*, **26**: 456–475.

Berrill, N. J., 1961. *Growth, Development, and Pattern.* Chap. 14, pp. 358–402: Regeneration and total development: ascidian buds. W. H. Freeman Co., San Francisco.

Bonet, F., and N. Benveniste-Velásquez, 1971. Espiculas de ascidias fosiles y actuales. *Revista del Instituto Mexicano del Petrolco*, **3**(4): 8–35.

Deck, J. D., E. D. Hay, and J. P. Revel, 1966. Fine structure and origin of the tunic of *Perophora viridis. J. Morphol.*, **120**: 267–280.

Freeman, G., 1971. A study of the intrinsic factors which control the initiation of asexual reproduction in the tunicate *Amaroucium constellatum. J. Exp. Zool.*, **178**: 433–456.

Herdman, W. A., 1884. The presence of calcareous spicules in the Tunicata. *Proc. Geol. Soc. Liverpool*, **5**: 42–45.

HUNT, S., 1970. *Polysaccharide-Protein Complexes in Invertebrates.* Academic Press, New York. 329 p.

LAFARGUE, F., AND E. KNIPRATH, 1978. Formation des spicules de Didemnidae (ascidies composeés). 1. L'apparition des spicules chez l'oozoide après la métamorphose. *Mar. Biol.,* **45**: 175–184.

LAMBERT, G., 1979. Predation by the prosobranch mollusk *Lamellaria diegoensis* on *Cystodytes lobatus,* a colonial ascidian. *Veliger,* **22**: (in press).

LEVINE, E. P., 1960. Studies on the structure, reproduction, development, and accumulation of metals in the colonial ascidian *Eudistoma ritteri* Van Name, 1945. *Ph.D. thesis, Stanford University,* Stanford, California. 192 pp. Diss. Abs. 21: 3235.

MILLAR, R. H., 1962. Further descriptions of South African ascidians. *Ann. S. Afr. Mus.,* **46(7)**: 113–221.

MILLAR, R. H., 1975. Ascidians from the Indo-West-Pacific region in the Zoological Museum, Copenhagen (Tunicata, Ascidiacea). *Steenstrupia,* **3(20)**: 205–336.

MONNIOT, F., 1970a. *Cystodytes incrassatus* n. sp., Ascidie fossile du Pliocène breton. Nouvelle interprétation des *Neanthozoites* Deflandre-Rigaud. *C. R. Acad. Sci. Paris,* **271**: 2280–2282.

MONNIOT, F., 1970b. Les spicules chez les tuniciers aplousobranches. *Arch. Zool. Exp. Gen.,* **111**: 303–311.

MONNIOT, F., AND E. BUGE, 1971. Les spicules d'ascidies fossiles et actuelles. *Ann. Palcontol. Invertebres,* **57**: 93–105.

NAKAUCHI, M., 1966a. Budding and colony formation in the ascidian, *Amaroucium multiplicatum. Jpn. J. Zool.,* **15(2)**: 151–172.

NAKAUCHI, M., 1966b. Budding and growth in the ascidian, *Archidistoma aggregatum. Rep. Usa Mar. Biol. Stn.,* **13(1)**: 1–10.

NAKAUCHI, M., 1966c. Regeneration in the zooid of *Polycitor mutabilis* (a compound ascidian). *Sci. Rep. Tokyo Kyoiku Daigaku,* Sec. B, **12(184)**: 151–189.

NAKAUCHI, M., 1970. Asexual reproduction in *Amaroucium yamazii* (a colonial ascidian). *Publ. Scto Mar. Biol. Lab.,* **17(5)**: 309–328.

NAKAUCHI, M., 1974. Development and budding in the oozooids of polyclinid ascidians. 1. *Sidnioides snamoti. Rep. Usa Mar. Biol. Stn.,* **21**: 1–18.

NAKAUCHI, M., 1977. Development and budding in the oozooids of polyclinid ascidians. 2. *Ritterella pulchra. Annot. Zool. Jpn.* **50(3)**: 151–159.

NAKAUCHI, M., AND K. KAWAMURA, 1974a. Behavior of buds during common cloacal system formation in the ascidian, *Aplidium multiplicatum. Rep. Usa Mar. Biol. Stn.,* **21**: 19–27.

NAKAUCHI, M., AND K. KAWAMURA, 1974b. Experimental analysis of the behavior of buds in the ascidian, *Aplidium multiplicatum.* I. *Rep. Usa Mar. Biol. Stn.,* **21**: 29–38.

NAKAUCHI, M., AND K. KAWAMURA, 1978. Additional experiments on the behavior of buds in the ascidian, *Aplidium multiplicatum. Biol. Bull.,* **154(3)**: 453–462.

OKA, H., 1942. On a new species of *Polycitor* from Japan with some remarks on its mode of budding (Ascidiae Compositae). *Annot. Zool. Jpn.,* **21(3)**: 155–162.

OKA, H., AND M. USUI, 1944. On the growth and propagation of the colonies in *Polycitor mutabilis* (Ascidiae Compositae). *Sci. Rep. Tokyo Bunrika Daigaku,* Sec. B, **7**: 23–53.

PEARSE, A. G. E., 1968. *Histochemistry Theoretical and Applied.* 3rd ed., vol. 1, Little, Brown and Co., Boston, 759 pp.

PÉRÈS, J. M., 1948. Recherches sur le sang et la tunique commune des ascidies composeés. I. Aplousobranchiata (Polyclinidae et Didemnidae). *Annals Inst. Oceanogr. Monaco,* **23**: 345–473.

PRENANT, M., 1925. Contributions a l'etude cytologique du calcaire. II. Sur les conditions de formation des spicules de Didemnidae. *Bull. Biol. Fr. Belg.,* **59**: 403–435.

RITTER, W. E., 1900. Some ascidians from Puget Sound, collections of 1896. *Ann. N. Y. Acad. Sci.,* **12(14)**: 589–616.

SEBASTIAN, V. O., 1957. Studies on an ascidian of Madras coast. *Ph.D. thesis, University of Madras,* Madras, India. G. C. Press, 55 pp.

SELLERS, R. G., JR., 1977. The diets of four species of *Calliostoma* (Gastropoda, Trochidae) and some aspects of their distribution within a kelp bed. *M.S. Thesis, Stanford University,* Stanford, California. 31 pp.

Skogsberg, T., and G. H. Vansell, 1928. Structure and behavior of the amphipod, *Polycheria osborni*. *Proc. Calif. Acad. Sci.*, 4th series, **17**(**10**) : 267–295.

Smith, M. T., 1970. The blood cells and tunic of the ascidian *Halocynthia aurantium* (Pallas). I. Hematology, tunic morphology, and partition of cells between blood and tunic. II. Histochemistry of the blood cells and tunic. *Biol. Bull.*, **138** : 354–388.

Stiévenart, J., 1970. Recherches histologiques sur la tunique de deux ascidiaces phlebobranches : *Ascidiella aspersa* Mull. and *Phallusia mammillata* Cuv. *Ann. Soc. R. Zool. Belg.*, **100** : 139–158.

Stiévenart, J., 1971. Recherches sur la morphologie et etude histochimique de la tunique d'*Halocynthia papillosa* Gun. (Ascidie stolidobranche). *Ann. Soc. R. Zool. Belg.*, **101** : 25–56.

Trason, W. B., 1963. The life cycle and affinities of the colonial ascidian *Pycnoclavella stanleyi*. *Univ. Calif. Publ. Zool.*, **65**(**4**) : 283–326.

Vam Name, W. G., 1945. The North and South American ascidians. *Bull. Am. Mus. Natl. Hist.*, **84** : 1–476.

Watanabe, H., and C. C. Lambert, 1973. Larva release in response to light by the compound ascidians *Distaplia occidentalis* and *Metandrocarpa taylori*. *Biol. Bull.*, **144**(**3**) : 556–566.

Yonge, C. M., 1952. Studies on Pacific coast mollusks. V. Structure and adaptation in *Entodesma saxicola* (Baird) and *Mytilimeria nuttallii* Conrad. *Univ. Calif. Publ. Zool.*, **55** : 439–450.

Reference: *Biol. Bull.* **157**: 478–493. (December, 1979)

CHARACTERISTICS AND REGULATION OF FISSION ACTIVITY IN CLONAL CULTURES OF THE COSMOPOLITAN SEA ANEMONE, *HALIPLANELLA LUCIAE* (VERRILL) [1]

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The diversity of reproductive modes within the Cnidaria is greater than that of most other metazoan groups. This is particularly evident among the sea anemones, for which Chia (1976) has compiled a list of reproductive modes, and has speculated on their evolution and adaptive significance. Reproduction in *Haliplanella* (= *Diadumene*) *luciae* Verrill is of special interest because *H. luciae* has greatly expanded its geographical range since the turn of the century (Uchida, 1932; Stephenson, 1935; Hand, 1955; Shick and Lamb, 1977). This species now occurs intertidally on boreal (Uchida, 1936; Williams, 1973), temperate (Stephenson, 1935; Hand, 1955) and tropical (Dunn, personal communication; Belem and Monteiro, 1977) coasts.

The ability of *H. luciae* to establish new populations is extraordinary since, unlike other intertidal invertebrates, *H. luciae* has never been observed to produce larvae as agents of dispersal (Davis, 1919; Shick, 1976). All observed reproduction has been asexual, through longitudinal fission (Hargitt, 1912; Davis, 1919), and less commonly by pedal laceration (Atoda, 1954; Johnson and Shick, 1977). Although a single fission event infrequently produces multiple individuals, most fission events are binary, and analogous to cytokinesis (Atoda, 1976; Minasian, 1976).

Longitudinal fission permits the rapid establishment of intertidal clones (Chia, 1976; Francis, 1976). The strategic advantages of fission have been recently discussed by Francis (1976) and Shick and Lamb (1977). Hoffmann (1976) and Shick and Lamb (1977) described the genetic composition of different conspecific clones of anemones, and have made valuable inferences concerning advantages of asexual reproduction in contrast to sexual reproduction.

Understanding the contribution of asexual reproduction to the success of *H. luciae* makes necessary the analysis of asexual reproductive rates and their regulation. However, few studies have evaluated asexual reproductive rates of sea anemones, partly due to a lack of standardized methods.

Researchers of hydroid development have calculated exponential rates of increase (symbolized "k") in numbers of polyps in laboratory cultures (Loomis, 1954), and have related these rates to environmental variables (Fulton, 1962; Davis, 1971). Minasian (1976) determined k (fission rate) in laboratory populations of *H. luciae,* and quantified the effect of feeding frequency on k. The occurrence of fission in *H. luciae* is affected by temperature (Miyawaki, 1952) and fluctuating

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temperature-emersion treatments (Johnson and Shick, 1977); but the effect of temperature on k was not quantified in those studies.

The present study quantifies the combined effects of temperature and feeding frequency on k in *H. luciae*, and provides a comprehensive analysis of longitudinal-fission activity in a sea anemone. This includes previously undescribed characteristics of fission activity, essential to the understanding of the regulation of longitudinal fission. In addition, we describe a method for establishing and maintaining permanent *in vitro* clonal cultures of *H. luciae*. This has enabled us to obtain a range of possible values of k in a clone of *H. luciae* from northwestern Florida.

Materials and Methods

Establishment of clonal cultures

Specimens of *H. luciae* were collected on 2 October from a sand-flat intertidal region adjacent to the Florida State University Marine Lab near Turkey Pt., on the N. W. Florida Gulf coast. These anemones occur on the undersides of sedimentary stones, accompanied by barnacles (*Chthamalus fragilis*) in the upper littoral region, and oysters (*Crassostrea virginica*) in the mid-littoral region. *H. luciae* cloned from this location (29° 54.8′ N, 84° 30′ W) have a brownish-green column with 12 to 24 yellowish-orange longitudinal stripes in large individuals. Shick and Lamb (1977) found a sample of striped *H. luciae* from this population to consist of two different genotypes (*i.e.* clones). The specimens of *H. luciae* cloned in this study are presumed to belong to the same genotype, since they are phenotypically (sex and color) identical and phenotypically distinguishable from the second striped clone present at the collection site.

To establish clonal cultures, three individual anemones of average diameter (about 5 mm) were isolated in three covered glass storage dishes (Corning #3250), each containing approximately 200 ml natural sea water (28 to 30‰). These cultures were maintained at room temperature (22 to 25° C) under a 14/10 hr light-dark cycle provided by a fluorescent (cool-white) light source, and fed to repletion (30 to 60 min) with newly hatched *Artemia* nauplii every second day. All culture dishes were rinsed briefly with distilled water and refilled with fresh sea water daily. The number of anemones in each culture increased rapidly through longitudinal fission.

After 3 months each clonal culture was divided into two cultures, producing a total of six clonal cultures. These served as stock cultures for the experiments, and were kept under the same photoperiod at a temperature of 16 to 18° C; feeding frequency was reduced to twice per week. Every 4 to 6 weeks each clonal culture was transferred to clean glassware. Clonal cultures were maintained under this standardized regimen for at least 4 months before being used in experiments. Anemones from stock cultures were less than 7 mm in diameter; histological examination showed that none of these anemones bore gonads.

Culture experiments

For each experiment, six duplicate cultures were set up by placing 10 anemones from each stock culture in glass bowls (glass stacking dish, Wheaton #350134)

which were 11.5 cm in diameter, and contained approximately 150 ml of sea water. Sets of six cultures were then placed under one of nine different experimental regimens. These regimens, evaluating combined temperature and feeding effects upon fission activity, were as follows: fed once every 2 days at 26, 21 or 16° C; fed once every 4 days at 26, 21 or 16° C; starved at 26, 21 or 16° C. Thus the entire experiment involved a total of 54 clonal cultures, each initially containing 10 anemones. In addition, fission activity in stock cultures was evaluated by setting up six experimental cultures as above, and observing fission activity for 3 months under maintenance conditions (fed twice per week at 16 to 18° C). Experimental cultures were exposed to the new temperature for 2 days before beginning the experiment, providing time for attachment of anemones to culture bowls. The second day after transfer to culture bowls (day 0) marked the start of the experiment; for experimental groups receiving food, day 0 was the day of the first feeding. These cultures were also under a 14/10 hr light-dark cycle. Anemones were counted daily, at which time they were fed, if necessary, and the sea water changed.

Statistical analysis

Fission rates were calculated from the least-squares regression equation of the natural log number of anemones as a function of time in culture: $\ln N_i = kt_1 + \ln N_o$. Here the rate of increase in log number of anemones ($\ln N_i$) on a given day (t_i) is defined by the fisson rate (k), which is the slope of the regression line. The number of polyps present at the start (day 0) of the culture interval, and Y-intercept of the regression line, is $\ln N_o$. This equation was employed by Loomis (1954) and Fulton (1962) to calculate the exponential rate of increase (k) for asexual production of hydroid polyps, in which $N_i = N_o e^{kt_i}$.

Since exogenous factors can impose a variable delay period prior to the initiation of fission, it was necessary to recognize two distinct parameters for fission rate, defined as follows. The overall fission rate (k) is the rate of increase during the entire culture interval, including the initial delay before the onset of fission. The second parameter, k_{adj}, gives the rate of increase subsequent to the initial delay period. Adjusted fission rate (k_{adj}) is calculated by the same method as k, except that the first data entry, day 0, is the day prior to the first occurrence of fission. Hence, k_{adj} designates the rate of increase only after the initiation of fission, rather than during the entire culture period, and better estimates the rate of active, sustained fission. Where the delay before onset of fission is absent or very short, $k = k_{adj}$. Both k and k_{adj} were calculated for each culture, except where fission activity was not sustained and considered to be nonexponential. The delay before the onset of fission also was calculated for each culture.

The effects of temperature and feeding on k and k_{adj} were evaluated by means of a two-way analysis of variance (ANOVA). Paired comparisons between statistical means were performed using t-tests.

The percentage of anemones undergoing fission per day was calculated as follows $(N_i - N_{i-1}) \cdot 100/N_i$, where N_i is the number of anemones on a given day, and N_{i-1} is the number present on the previous day. An angular transformation was performed on these percentage data, and means and standard deviations

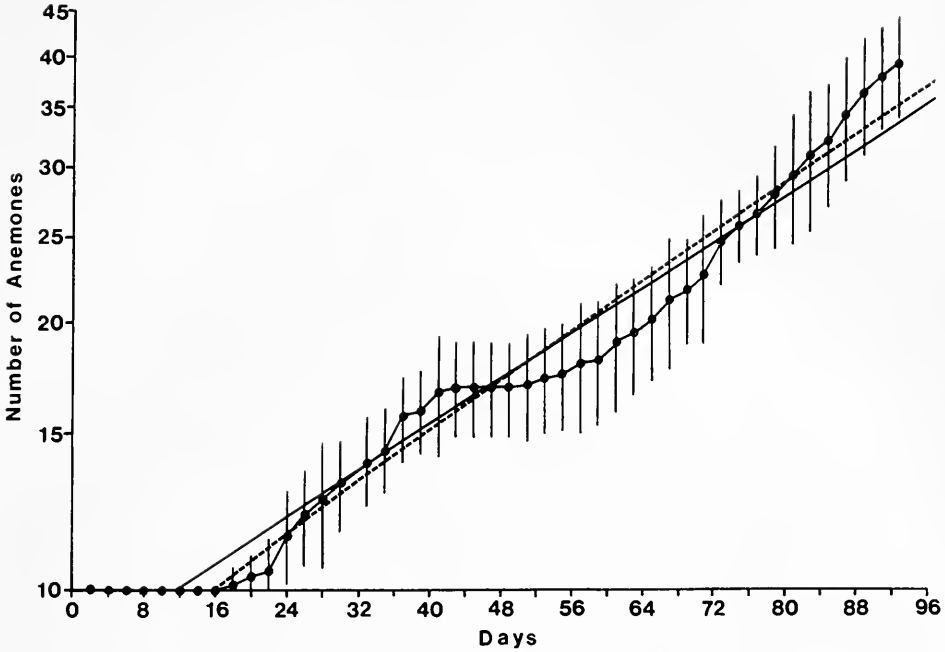

FIGURE 1. Semilogarithmic plot of numerical increase through longitudinal fission in cultures of *H. luciae*, reared under conditions of routine maintenance ($17 \pm 1°$ C, fed twice per week). Day 0 is 2 days after transfer to culture bowls from stock cultures. Each point is a mean of six cultures, each intially containing 10 anemones; error bars are standard deviations. The slope of the regression equation, $\ln N_t = kt_1 + \ln N_o$, is the fission rate, k (see Materials and Methods for further explanation). The slope of the solid line (k) is 0.0149 ± 0.0017 (mean $\pm$ s.d.); the slope of the broken line, k_{adj} (0.0162 ± 0.0010), is corrected for the initial delay period prior to the start of fission activity.

calculated for each regimen. On plots of the temporal pattern of fission pulses, the scale of the ordinate conforms to the transformation, whereas the units on the ordinate are actual, untransformed percentages. This transformation is necessary to assume normality (Sokal and Rohlf, 1969). Means and standard deviations were calculated for relative maxima of all fission pulses (pulse maxima), and for relative minimal values between fission pulses (pulse minima); these were evaluated using a multiple-range (Student-Neuman-Keuls) test. Lengths of intervals between fission-pulse maxima were also determined. A G-test for independence (Sokal and Rohlf, 1969) was performed on frequency data for pulse intervals. Test statistics corresponding to probabilities of less than 0.05 were regarded as significant.

RESULTS

Characteristics and exogenous regulation of fission activity

Figure 1 illustrates prominent characteristics of fission activity in clonal cultures reared under the standard maintenance regimen. These characteristics

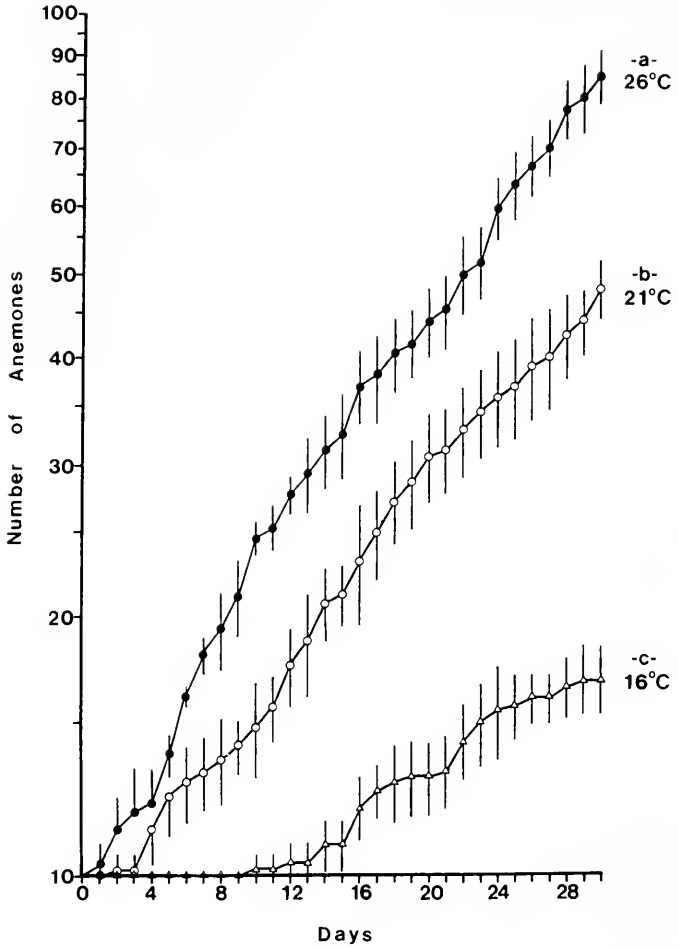

FIGURE 2. Semilogarithmic plot of numerical increase through longitudinal fission in cultures of *H. luciae* fed once every 2 days. Each point represents a mean of six cultures, each initially containing 10 anemones; error bars are standard deviations. Three experimental culture temperatures were used: (a), 26° C; (b), 21° C; (c), 16° C.

included: first, an initial delay period before the onset of fission; second, an exponential fission rate followed by a temporary cessation of fission; and third, resumption of the exponential fission rate. The variable delay lasted for 21.33 ± 4.46 (mean $\pm$ s.d.) days, although one culture initiated fission as early as day 18 (Fig. 1). This delay was initiated by the mechanical disturbance imparted in transferring anemones to new culture bowls. For the entire culture interval of 92 days, $k = 0.0149 \pm 0.0017$ (mean $\pm$ s.d.); with the delay period omitted from calculation, $k_{adj} = 0.0162 \pm 0.0010$. The calculation for k_{adj} usually increased the estimate of fission rate in addition to shifting the regression line to the right.

Figures 2 and 3 demonstrate the numerical increase in cultures of *H. luciae*

which were fed *Artemia*. At 26° C little or no delay occurred prior to the onset of fission; hence k and k_{adj} were essentially identical. Only when anemones were fed once every two days at 16° C was k_{adj} significantly larger than k (t-test, $P < 0.05$).

Values of k_{adj} in experimental cultures ranged from 0.0278 (doubling time = 24.9 days) at 16° C to 0.0728 (doubling time = 9.5 days) at 26° C, in cultures fed every second day. Analyses of temperature and feeding effects showed both factors to significantly affect k and k_{adj}, based upon comparisons between 21° and 26° C regimens (Table I). Temperature coefficients (Q_{10}) for k_{adj} over the 21 to 26° C temperature range were 1.6042 and 1.6759 for feeding frequencies of 2 and 4 days, respectively (Table II). Thus, the difference in feeding frequency did not influence the effect of temperature upon k_{adj} in this range. Since the Q_{10} for the 16 to 21° C range was much larger (Table II), the greatest effect of temperature on k_{adj} occurred below 21° C.

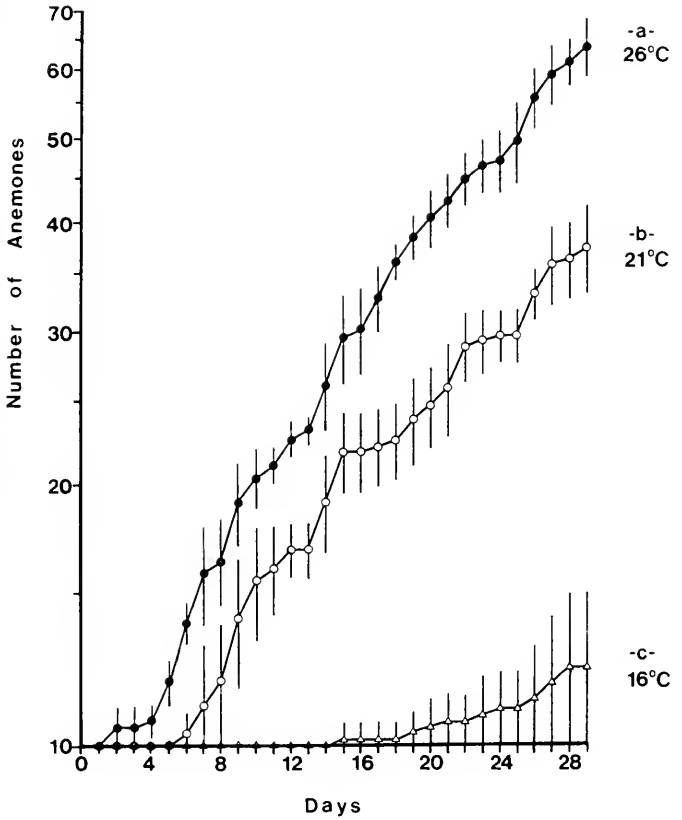

FIGURE 3. Semilogarithmic plot of numerical increase through longitudinal fission in cultures of *H. luciae* fed once every 4 days. Each point represents a mean of six cultures, each initially containing 10 anemones; error bars are standard deviations. Three experimental culture temperatures were used: a), 26° C; (b) 21° C; (c), 16° C.

TABLE I

Means ± standard deviations for values of k *and* k_{adj} *for* H. luciae *reared at different temperatures and feeding frequencies. Each experimental mean consisted of six bowls, each initially containing 10 anemones. The resulting two-way ANOVA was performed on four means (21° vs. 26° C, fed every second or fourth day). The ANOVA results were the same for both* k *and* k_{adj}*, and indicate if an effect upon* k *was statistically significant (+) or not significant (−) at the 5% level; n.c. = fission activity not sufficient to calculate* k.

Feeding frequency	Temperature		
	16 ± 1° C	21 ± 1° C	26 ± 1° C
	Values of k		
2 days	0.0207 ± 0.0036	0.0569 ± 0.0040	0.0728 ± 0.0035
4 days	n.c.	0.0518 ± 0.0031	0.0695 ± 0.0032
	Values of k_{adj}		
2 days	0.0278 ± 0.0047	0.0574 ± 0.0035	0.0727 ± 0.0036
4 days	n.c.	0.0533 ± 0.0045	0.0690 ± 0.0025

ANOVA: Effects for 21° vs. 26° C

Temperature	Feeding	Interaction
(+)	(+)	(−)

Both temperature and feeding significantly affect length of the delay period prior to the initiation of fission (Table III). The duration of delay periods varied from 1 day or less at 26° C, to over 21 days at 16° C. Table III shows that decreasing the feeding frequency from 2 to 4 days further lengthened this delay. Temperature and feeding frequency acted synergistically upon the pre-fission delay, as indicated by the significant ANOVA interaction term (Table III). For example, at 26° C halving the feeding frequency (from every second to every fourth day) increased the mean delay period by 1.3 days; at 16° C, halving the feeding frequency increased the delay by over 10 days (Table III).

Fission activity in starved cultures at 26° C and 21° C was limited to two major pulses of fission activity (Fig. 4); starved anemones at 16° C did not

TABLE II

Temperature coefficients (Q_{10}) *for fission rate* (k_{adj}) *in* H. luciae *at two different feeding frequencies; n.c. = insufficient data for calculation.*

Feeding frequency	Temperature Range		
	16°–21° C	21°–26° C	16°–26° C
2 days	4.3567	1.6042	2.6436
4 days	n.c.	1.6759	n.c.

TABLE III

Means ± standard deviations for delay to onset of fission (days) in H. luciae *at different temperatures and feeding frequencies. Each experimental mean consisted of six bowls, each initially containing 10 individual anemones. The two-way ANOVA result indicates if an effect on fission delay is statistically significant (+) or not significant (−) at the 5% level.*

Feeding frequency	Temperature		
	16 ± 1° C	21 ± 1° C	26 ± 1° C
2 days	11.6667 ± 2.3381	1.8333 ± 0.9832	0.0000 ± 1.0954
4 days	>21.0	5.5000 ± 1.3784	1.3333 ± 1.5055

ANOVA: Effects for 21° vs. 26° C

Temperature	Feeding	Interaction
(+)	(+)	(+)

undergo fission. Nonetheless, these cultures remained healthy throughout the experiment.

Temporal pattern of fission activity

After long delay periods a major pulse of synchronous fission occurred, followed by a temporary cessation of fission (Figs. 1, 2c). The resumption of fission after this initial pulse was either less synchronous and without additional major plateaus (Fig. 1), or continued to show additional, brief cessations of fission (Fig. 2c).

H. luciae exhibited distinct pulses of increased fission activity, with relative maxima (peaks) and minima (troughs), shown in Figures 5 and 6. These occurred

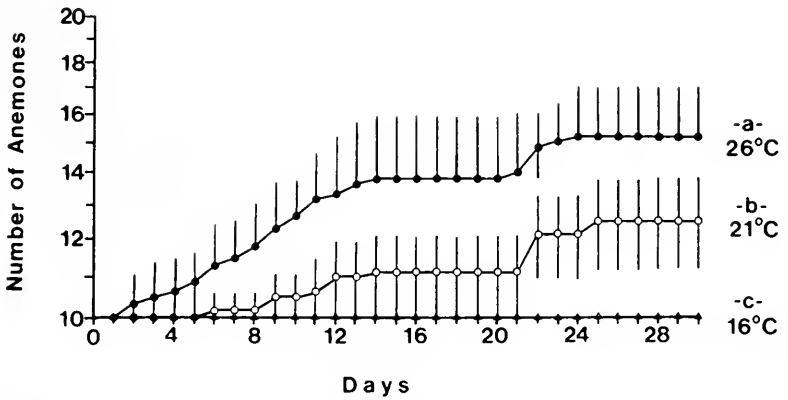

FIGURE 4. Semilogarithmic plot of numerical increase through longitudinal fission in starved cultures of *H. luciae*. Each point represents a mean of six cultures, each initially containing 10 anemones; error bars are standard deviations. Three experimental culture temperatures were used: (a), 26° C; (b), 21° C; (c), 16° C.

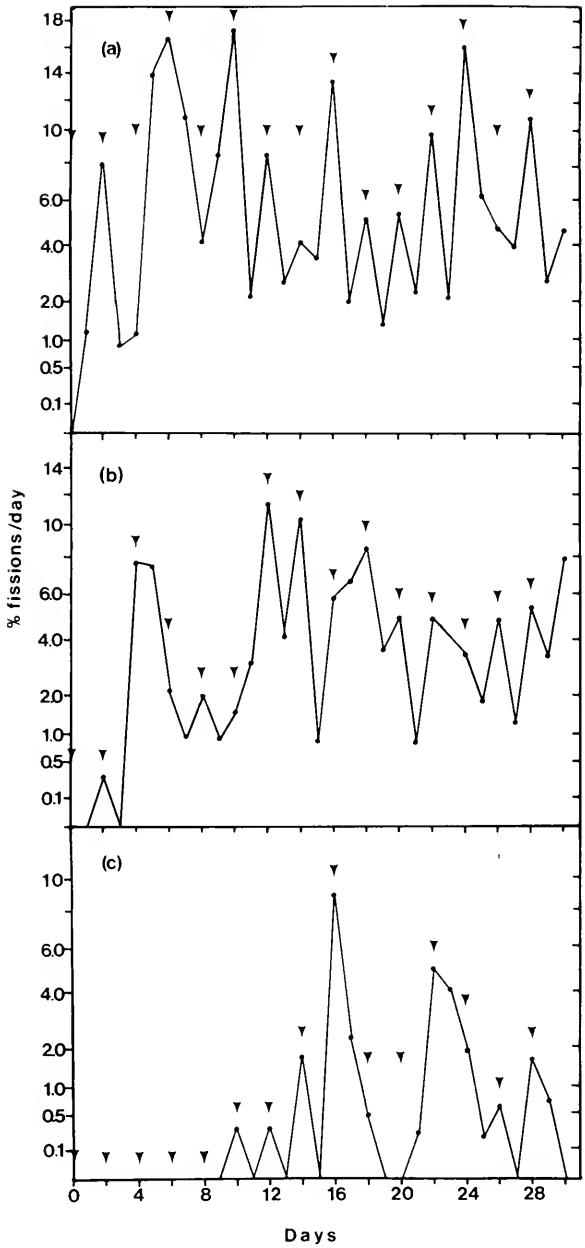

FIGURE 5. Percentages of *H. luciae* undergoing fission per culture per day in cultures fed once every 2 days. Each percentage is a mean of six cultures; arrowheads indicate feeding days. Data was subjected to angular transformation prior to statistical computations; plotted means are transformed data. The ordinate indicates actual, untransformed percentages. Three culture temperatures are represented: (a) 26° C; (b) 21° C; (c) 16° C.

TABLE IV

Different lengths of time periods between peak values of fission activity (pulse maxima), observed in H. luciae *culture populations. Cultures initially consisted of 10 anemones each and were reared under different temperature and feeding regimens for 1 month. Six clonal cultures were reared under each different culture regimen. The percentage of fissions occurring each day were then averaged, and the peaks of fission activity determined. Occurrence of the various lengths of pulse intervals depend upon culture conditions (G-test for independence, $P < 0.05$).*

Culture regimen	Length of period between pulse maxima (days)			
	2	3	4	>4
26° C, fed every 2 days	7	0	3	0
26° C, fed every 4 days	0	5	1	1
21° C, fed every 2 days	5	0	4	0
21° C, fed every 4 days	1	3	2	0
16° C, fed every 2 days	4	0	1	1

in cycles, with intervals between pulse maxima usually lasting for 2 to 4 days. These pulses of fission had a phasic dependence upon the feeding regimen. In cultures which were fed every second day, pulse maxima occurred only on feeding days, although not on all feeding days (Fig. 5). Therefore, periods between pulse maxima were multiples of 2 days, in cultures fed every second day. At 26° and 21° C most pulse maxima had a 2-day periodicity, with fewer pulse maxima being 4 days apart (Fig. 5a, b). In the 16° C cultures, a 6-day period between pulse maxima occurred at one point, although 2-day periods were still most frequent (Fig. 5c).

In cultures fed at 4-day intervals pulse maxima often occurred on days other than feeding days, and were usually 3 or 4 days apart (Fig. 6). Thus, the periodicity of pulse maxima was longer than that in cultures receiving food at

TABLE V

Means and standard deviations for fission-pulse maxima and minima observed in cultures of H. luciae *reared under different culture regimens. Each determination was taken from angular-transformed, averaged data from six clonal cultures. Means are for pulse maxima or minima occurring over a one-month period. Sample sizes are in parentheses. Asterisks denote means which are significantly different from all other means in the same column (Student-Neuman-Keuls test, $P < 0.05$).*

Culture regimen	Pulse maximum†	Pulse minimum†
26° C, fed every 2 days $k_{adj} = 0.0727$	18.359 ± 4.601 (11)	8.823 ± 1.893 (11)*
26° C, fed every 4 days $k_{adj} = 0.0690$	16.967 ± 5.039 (8)	6.486 ± 3.790 (8)
21° C, fed every 2 days $k_{adj} = 0.0574$	13.393 ± 5.014 (10)	6.828 ± 3.467 (10)
21° C, fed every 4 days $k_{adj} = 0.0533$	15.602 ± 7.650 (8)	3.658 ± 3.226 (8)*
16° C, fed every 2 days $k_{adj} = 0.0278$	7.920 ± 5.390 (7)*	0.417 ± 1.021 (6)*

† angular transformation (arcsin-$\sqrt{\%}$ fissions/day) is necessary to assume normality.

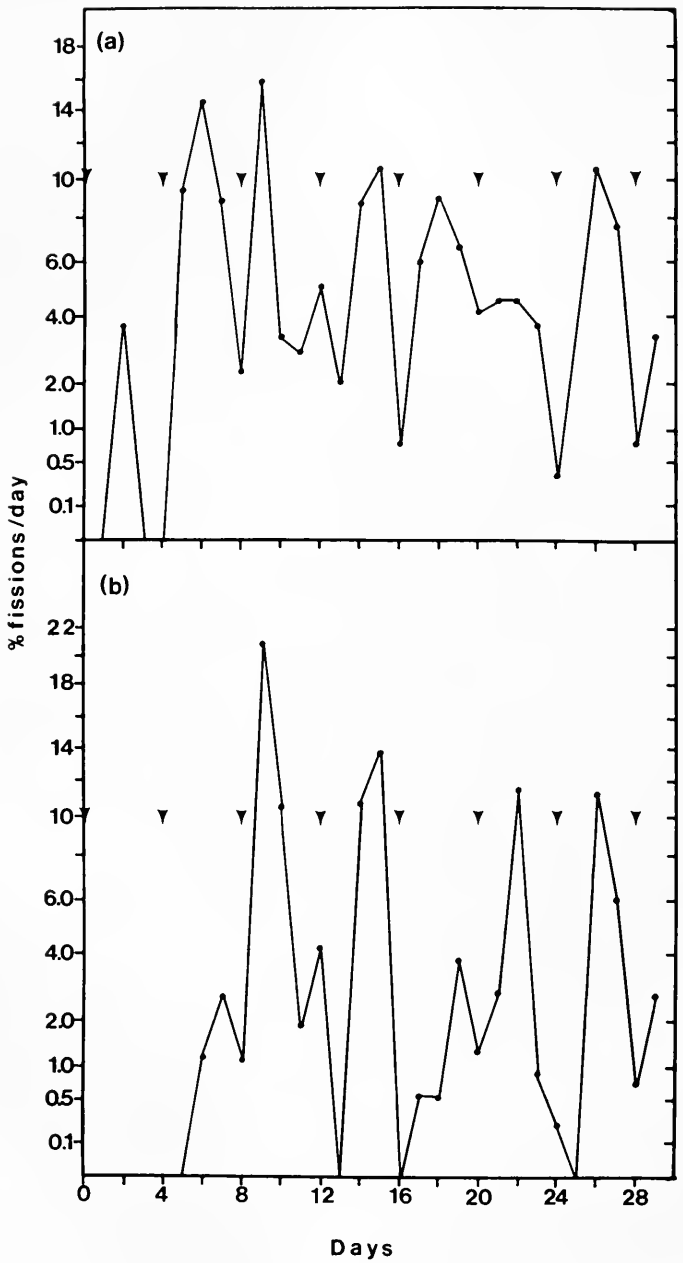

FIGURE 6. Percentages of *H. luciae* undergoing fission per culture per day in cultures fed once every 4 days. Each percentage is a mean of six cultures, each containing 10 or more anemones; arrowheads indicate feeding days. Data was subjected to angular transformation prior to statistical computations; plotted means are transformed data. The ordinate indicates actual, untransformed percentages. Two culture temperatures are represented: (a) 26° C; (b) 21° C.

2-day intervals. Only one period of 2 days was observed at the 4-day feeding frequency (Fig. 6b). Table IV summarizes the occurrence of pulse periodicity, which is dependent upon culture conditions (G-test, $P < 0.05$).

Pulse maxima often attained 10 to 20% of the culture population undergoing fission per day (untransformed percentages are given on the ordinate in Figs. 5 and 6). Pulse minima usually dropped below 4% fissions per day in cultures exhibiting lower values of k (Table V). Mean values of pulse maxima were generally indicative of values of k_{adj}. For example, a mean pulse maximum of 13.393 (arcsin-$\sqrt{\%\text{fissions/day}}$) corresponded to a mean k_{adj} of 0.0574, while a significantly lower (Student-Neuman-Keuls, $P < 0.05$) mean pulse maximum of 7.920 (arcsin-$\sqrt{\%\text{fissions/day}}$) accompanied a k_{adj} of 0.0278 (Table V). Likewise, significantly lower values of k_{adj} accompanied lower values for pulse minima (Table V).

Discussion

The distinction between k and k_{adj} is important for the analysis of fission activity in *H. luciae*. Fluctuations in population size and density are best considered in terms of k rather than k_{adj}, since periods of fission delay, as opposed to active periods of fission, are not easily distinguished in field studies. Moreover, fission-related morphological variability (Minasian, 1979) is best understood in relation to the total number of fissions over an entire interval (k), rather than the sustained or maximal rate of fission during part of that interval (k_{adj}). The parameter k_{adj} is a more accurate indication of active fission than is k. Hence, k_{adj} should be used to examine experimental effects upon fission rate. The calculation of k_{adj} requires that data be collected at frequent, preferably daily intervals.

The present study reveals three different effects of temperature upon fission activity. The first effect is upon fission rate. The Q_{10} value for k_{adj} in the 16 to 21° C range was three times greater than the Q_{10} value for the 21 to 26° C range. Sassaman and Mangum (1970) similarly found that oxygen consumption in *H. luciae* showed disproportionately large Q_{10} values over a range of 10 to 17.5° C. The second effect of temperature, a lengthening of the delay period, was also greatest below 21° C. Temperature also affected fission-pulse patterns: in cultures fed every second day, a 10° C decrease in culture temperature caused a 40% drop in the frequency of fission pulses, and a signficant decrease in values of pulse maxima and minima.

The great response of fission activity to small changes in temperature indicates that temperature is the foremost exogenous regulator of k. Miyawaki (1952) believed that a temperature threshold of approximately 15° C, below which no fission occurred, existed for *H. luciae*. He observed no longitudinal fission at temperatures below 20° C. The present study shows that values of k for this Florida clone decrease sharply as temperatures descend to 15° C, and in this respect agrees with Miyawaki's (1952) observations on Japanese *H. luciae*. However, a temperature threshold which limits fission in *H. luciae* is dependent upon other parameters which also affect k, such as food availability. Thus, k is best regarded as having a graded response to temperature, rather than an absolute threshold of 15° C.

The effect of feeding frequency upon k appears to be temperature-dependent. Although halving the feeding frequency resulted in reductions of k by only 4.5% at 26° C, and 8.9% at 21° C, a relatively large effect of decreased feeding frequency upon k occurred at 16° C, where the delay period was greatly lengthened. A temperature-dependent effect of feeding on the morphology of *H. luciae* reflects such interaction of effects on k (Minasian, 1979).

Exogenous factors determine sensitivity to mechanical stimuli which interrupt fission activity. Transferring anemones to new culture vessels (when setting up experimental cultures) imparted a mechanical disturbance, which caused little or no fission delay at high temperature and feeding frequency; but at low temperatures and feeding frequencies this delay may last for several weeks. Johnson and Shick (1977) demonstrated that fluctuating immersion-emersion cycles decrease fission activity in *H. luciae*. The mechanism underlying this effect may involve mechanically induced delays.

The synchrony of fission cessation following initial fission activity in low-temperature cultures (Fig. 1) is reminiscent of a synchronized cell culture (*e.g.*, Zeuthen and Scherbaum, 1954; Zeuthen, 1964), and implies the existence of relatively constant periods between fissions among individuals within the culture population. This long-term fission synchrony is most evident at low temperatures because periods between fission events are lengthened. When exogenous factors permit only short periods between fissions, such long-term synchronization is not observed. Studies on the lengths of inter-fission periods must be made on individual anemones to ascertain if inter-fission periods are relatively constant in duration, and how they change due to exogenous influences. The inter-fission period may simply be a delay in response to the previous fission event, which itself constitutes a mechanical disturbance.

The coincidence of fission pulses with feeding days, in cultures fed every second day, points to a regulatory role for other exogenous, and possibly endogenous factors. Photoperiodic and feeding-digestion cycles appear to be involved. For example, cultures of *H. luciae* are more active during dark portions of the photoperiodic cycle, and usually exhibit greater fission activity in the dark (Minasian, unpublished data). Similarly, Batham and Pantin (1950) observed pedal locomotory activity of the sea anemone, *Metridium senile,* to occur only at night. Although feeding causes long-term enhancement of fission, Torrey and Mery (1904) suggested that fission in *H. luciae* is inhibited by feeding. Thus, interaction of photoperiodic rhythms and short-term inhibition by feeding produces a 2-day pulse pattern in cultures fed at 2-day intervals: fission is inhibited during the first dark period subsequent to feeding, followed by a release from inhibition and increased fission activity during the second dark period after feeding. This inhibition of fission during hours subsequent to feeding explains the even-day duration of periods between fission-pulse maxima in cultures fed at 2-day intervals.

In cultures fed at 4-day intervals, periods between fission-pulse maxima are longer, with pulses of fission activity often beginning during the 24 hr subsequent to feeding. Minasian (1976) similarly observed that *H. luciae* undergo more synchronous fission activity in response to renewed feeding when starved for longer periods. Thus, cultures fed at different frequencies have inherent differences in the response of fission activity to feeding. If there exists an endogenous influence

on the fission-pulse pattern, it may permit synchronization of fission pulses with 2-day feeding intervals, but not with 4-day intervals.

The extreme sensitivity of fission activity to temperature indicates that the clone of *H. luciae* examined in these culture experiments must achieve recruitment primarily when temperatures exceed 20° C. Below 20° C, low-temperature inhibition is reinforced by tidal cycles, which can limit food availability and impart mechanical disturbance through immersion-emersion effects. Hence, below 20° C k will be small, and delays between major pulses of fission activity will be long. Previous studies (Shick and Lamb, 1977; Minasian 1979) have stated that the absence of gametogenesis (*i.e.*, absence of sexual reproduction) in *H. luciae* is associated with small size (and hence high k). Thus, the 15° to 20° C temperature range probably marks a crucial transition from small, sterile and strictly asexual anemones exhibiting high k, to larger, sexually reproductive anemones which exhibit only infrequent fission. Under present culture conditions, periodic, pulsed increments of fission activity are best interpreted in relation to two periodic, exogenous stimuli: photoperiod and feeding. In natural populations of *H. luciae*, periodic stimuli include photoperiod and tidal fluctuation, of which the latter involves both feeding and a mechanical (immersion-emersion) effect (Johnson and Shick, 1977). Therefore, it is possible that such pulses of fission activity have a phasic dependence upon tidal and photoperiodic cycles in intertidal populations of *H. luciae*. An investigation of fission activity under field conditions may elucidate this relationship.

The authors thank Dr. D. A. Meeter for consultation regarding the statistical analyses, and Dr. W. H. Heard and R. W. Seaton for comments on the manuscript.

Summary

1. Permanent cultures of a clone of *H. luciae* from N. W. Florida were reared under different temperature and feeding regimes in order to identifiy and quantify parameters of asexual reproduction.

2. The principle components of fission activity include fission rate, a delay period following a mechanical disturbance, and periodic pulses of increased fission activity; all components are regulated by temperature and feeding frequency.

3. A distinction is made between fission rate including the delay period (k), and fission rate following the delay period (k_{adj}).

4. Fission rates (k_{adj}) ranged from 0.0162 (doubling time = 42.8 days) at 17° C to 0.0727 (doubling time = 9.5 days) at 26° C.

5. Temperature is the foremost regulator of k; the greatest influence of feeding frequency was upon periodic pulses of fission activity.

6. Culture data indicate that recruitment in natural populations of this clone is restricted by seasonal temperature; below 20° C there is a sharp reduction in k. It is suggested that inhibition of k by temperatures below 20° C favors a transition from asexual to sexual reproduction.

7. The pulsatile, periodic character of fission activity is prominent in laboratory cultures, and suggests that such activity in natural habitats may have a phasic dependence upon tidal and photoperiodic cycles.

LITERATURE CITED

Atoda, K., 1954. The development of the sea anemone, *Diadumene luciae*, reproduced by the pedal laceration. *Sci. Rep. Tohoku Univ., Ser. IV (Biol.)*, **20**: 123–129.

Atoda, K., 1976. Development of the sea anemone *Haliplanella luciae*. V. Longitudinal fission and the origin of mono-, di, and tri-glyphic individuals. *Bull. Biol. Stat. Asamushi*, **15**: 133–146.

Batham, E. J., and C. F. A. Pantin, 1950. Phases of activity in the sea-anemone, *Metridium senile* (L.), and their relation to external stimuli. *J. Exp. Biol.*, **27**: 377–399.

Belém, M. J. C., and D. C. Monteiro, 1977. Contribuições ao conhecimento de fauna de cnidários do Rio de Janeiro. II. *Haliplanella luciae* (Verrill, 1898) (Actiniaria, Acontiaria), uma nova occurrencia no Brazil. *Univ. Fed. Rio de J., Inst. Biol., Dept. Zool.*, **26**: 1–19.

Chia, F. S., 1976. Sea anemone reproduction: patterns and adaptive radiations. Pages 261–270 in G. O. Mackie, Ed., *Coelenterate ecology and behavior*. Plenum Press, New York.

Davis, D. W., 1919. Asexual multiplication and regeneration in *Sagartia luciae* Verrill. *J. Exp. Zool.*, **28**: 161–263.

Davis, L. V., 1971. Growth and development of colonial hydroids. Pages 16–36 in H. M. Lenhoff et al., Eds., *Experimental coelenterate biology*. University of Hawaii Press, Honolulu.

Francis, L., 1976. Social organization within clones of the sea anemone *Anthopleura elegantissima*. *Biol. Bull.*, **150**: 361–376.

Fulton, C., 1962. Environmental factors influencing the growth of *Cordylophora*. *J. Exp. Zool.*, **151**: 61–78.

Hand, C., 1955. The sea anemones of central California. Part III. The acontiarian anemones. *Wasmann J. Biol.*, **13**: 189–251.

Hargitt, C. W., 1912. The Anthozoa of the Woods Hole region. *Bull. U. S. Bureau Fish.*, **32**: 223–254.

Hoffmann, R. J., 1976. Genetics and asexual reproduction of the sea anemone *Metridium senile*. *Biol. Bull.*, **151**: 478–488.

Johnson, L. L., and J. M. Shick, 1977. Effects of fluctuating temperature and immersion on asexual reproduction in the intertidal sea anemone *Haliplanella luciae* (Verrill) in laboratory culture. *J. Exp. Mar. Biol. Ecol.*, **28**: 141–149.

Loomis, W. F., 1954. Environmental factors controlling growth in *Hydra*. *J. Exp. Zool.*, **126**: 223–234.

Minasian, L. L., Jr., 1976. Characteristics of asexual reproduction in the sea anemone, *Haliplanella luciae* (Verrill), reared in the laboratory. Pages 289–298 in G. O. Mackie, Ed., *Coelenterate ecology and behavior*. Plenum Press, New York.

Minasian, L. L., Jr., 1979. The effect of exogenous factors on morphology and asexual reproduction in laboratory cultures of the intertidal sea anemone, *Haliplanella luciae* (Anthozoa: Actiniaria) from the coast of Delaware. *J. Exp. Mar. Biol. Ecol.*, **40**: 235–246.

Miyawaki, M., 1952. Temperature as a factor influencing upon fission in the orange-striped sea anemone, *Diadumene luciae*. *J. Fac. Sci. Hokkaido Univ., Ser. IV, Zool.*, **11**: 77–80.

Sassaman, C., and C. P. Mangum, 1970. Patterns of temperature adaptation in North American Atlantic coastal actinians. *Mar. Biol.*, **7**: 123–130.

Shick, J. M., 1976. Ecological physiology and genetics of the colonizing actinian *Haliplanella luciae*. Pages 137–146 in G. O. Mackie, Ed., *Coelenterate ecology and behavior*. Plenum Press, New York.

Shick, J. M., and A. N. Lamb, 1977. Asexual reproduction and genetic population structure in the colonizing sea anemone *Haliplanella luciae*. *Biol. Bull.*, **153**: 604–617.

Sokal, R. R., and F. J. Rohlf, 1969. *Biometry*. W. H. Freeman and Co., San Francisco, 776 pp.

Stephenson, T. A., 1935. *The British sea anemones, vol. II*. The Ray Society, London, 426 pp.

Torrey, H. B., and J. R. Mery, 1904. Regeneration and non-sexual reproduction in *Sagartia davisi*. *Univ. Calif. Publ. Zool.*, **1**: 211–226.

UCHIDA, T., 1932. Occurrence in Japan of *Diadumene luciae*, a remarkable actinian of rapid dispersal. *J. Fac. Sci., Hokkaido Imp. Univ., Ser. IV*, **2**: 69–85.

UCHIDA, T., 1936. Influence of the currents upon the distribution of races and frequency of asexual reproduction in the actinian, *Diadumene luciae*. *Zool. Mag., Tokyo*, **48**: 895–906.

WILLIAMS, R. B., 1973. Are there physiological races of the sea anemone *Diadumene luciae*? *Mar. Biol.*, **21**: 327–330.

ZEUTHEN, E., 1964. The temperature-induced division synchrony in *Tetrahymena*. Pages 99–158 in E. Zeuthen, Ed., *Synchrony in cell division and growth*. Wiley Interscience, New York.

ZEUTHEN, E., AND O. SCHERBAUM, 1954. Synchronous divisions in mass cultures of the ciliate protozoon *Tetrahymena pyriformis*, as induced by temperature changes. Pages 141–157 in J. A. Kitching, Ed., *Recent developments in cell physiology*. Academic Press, New York.

Reference: *Biol. Bull.* 157: 494–505. (December, 1979)

LOCOMOTOR AND LIGHT RESPONSES OF LARVAE OF THE HORSESHOE CRAB, *LIMULUS POLYPHEMUS* (L.)

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The American horseshoe crab, *Limulus polyphemus* is an abundant and conspicuous member of the estuarine fauna of the Atlantic coast of the United States and the Gulf of Mexico. Its importance as a laboratory animal for neurophysiological, biochemical and medical research is well established (Wolbarsht and Yeandle, 1967; Cohen, 1979). Despite these facts, remarkably little has been recorded on the ecology and natural history of the species. The limited information available (Shuster, 1958; Sokoloff, 1978; Rudloe, 1979; Rudloe, 1978; Rudloe and Herrnkind, 1976) has primarily been concerned with the adult phase of the life cycle, especially the emergence of breeding crabs onto sandy beaches.

The juvenile and larval phases of the life cycle are of equal interest, however. Juveniles are important predators of the sandy intertidal community in areas adjacent to adult breeding beaches (Green and Hobson, 1970; Rudloe, 1978), while the resemblance of the larval instar to certain trilobites has long been a source of comment and is sometimes cited as an indication of the phylogenetic antiquity of the species.

Larvae hatch approximately 5 weeks after the female lays her eggs near the high tide mark. The time may vary depending on ambient temperature, and other environmental factors (Jegla and Costlow, 1979). The embryological development of *Limulus* eggs is well described by Kingsley (1892). However, field studies of this phase of the life cycle are limited to observations by Shuster (1958) on nematode, oligochaete and maggot activity in the nests, and Hummon, Fleeger and Hummon (1976) who described the interaction of beach meiofauna with developing eggs.

Newly laid eggs are sticky and occur as tightly clumped balls. When the eggs hatch, the larvae remain in distinct aggregations at depths comparable to those of newly laid eggs. The larvae eventually reach the surface of the sand and emerge into the water column. When larvae emerge from the nest or when they are removed and exposed to water, they exhibit a "swimming frenzy" reminiscent of neonate sea turtles, swimming vigorously and continuously for hours. They are also, like the larvae of many other marine invertebrates, strongly positively phototactic, orienting immediately to any available light source (Thorson, 1964; Rudloe, 1978). Loeb (1893) first mentioned the response of *Limulus* larvae to light, while the development of the swimming behavior has been described by Pearl (1904) as a continuum from embryo to larvae. More recently, French (1977) and French and Doliner (1978) have also described aspects of larval light responses in the laboratory.

While juvenile and adult horseshoe crabs are very similar in morphology, the larval (post hatching) instar is morphologically specialized and unlike all sub-

sequent stages. This larval instar is confronted with the necessity of moving successfully from a nest buried several centimeters beneath the sand surface at a high level of the beach out into the marine environment. Once this transition is achieved, the animal will not re-enter the upper beach environment until it is a sexually active adult. At that time it will be vastly changed, both behaviorally and morphologically, from the larval stage. The timing and mechanisms of release of larval specimens of *Limulus,* as well as the characteristic behavioral patterns of the larvae that contribute to achieving this movement away from the nest and which differentiate it from other phases of the life cycle were the objectives of this study.

Materials and Methods

Field studies

Activity of larvae was monitored at Mashes Sands Beach, Wakulla County, Florida from May through November, 1977, June and July, 1978, and in June, 1979. Nests in this area occurred in a distinct zone at the level of extreme high tide and could be easily located by digging at that height on the beach. A series of nests was dug open and the depth from the center of the nest to the surface was measured at periodic intervals ranging from 3 to 7 days between May and November, 1977, for a total of 21 samples. The number of nests measured on each date varied from 10 to 25. Digging was always done within three hours of the daylight high tide.

In addition, the depths of six nests chosen haphazardly each hour were checked over an 11-hr period on the night of full moon in June, 1978. Different nests were measured at each hour, for a total of 66 nests. The nests were dug open by hand and the depth from the center of the larval aggregation to the surface was measured. Nests remained tightly packed after hatching so that accurately locating the center was not difficult. Sampling was also done at the hour of high tide at seven-day intervals thereafter to ascertain nest depths at high tides on the following new moon spring tide and at the following two neap tides. Hourly sampling of six nests was conducted on the night of new moon in June, 1979, using the method described above. Nests were checked from low tide until the hour of high tide, for a total of 48 nests.

Surface plankton tows, each of 4-min duration, were made parallel to the beach at a depth of 1 m for day and night high tides on 128 tides between May and November, 1977, using a coarse mesh net constructed of wire window screening. This mesh retained the macroscopic *Limulus* larvae while passing smaller zoo- and phyto-plankters. The captured larvae were counted and released.

Laboratory analysis of locomotor activity rhythms

Larvae were examined in the laboratory for locomotor activity rhythms using time-lapse photography. An automatically triggered motordrive 35-mm camera using black and white negative film was used, as was an 8-mm movie camera set for timed exposures. The still camera recorded a frame once every 20 min, whereas the 8-mm camera recorded every 2 min. In all cases the data were grouped into 1-hr intervals.

Activity was recorded under various light regimes. Ambient light (with care taken to insure that no artificial light source was present) with an approximate light regime of fifteen hours of light and nine hours of darkness (LD) and water temperatures ranging between 28° C and 34° C was used to approximate conditions of light and temperature encountered in the upper intertidal zone. Constant light and temperature (LL) and constant darkness and temperature (DD) were also used.

Five hundred larvae were used in each of three DD trials, and 1000 larvae were used in all other trials. These densities did not result in any significant mortality, but, assessed visually, did approximate concentrations observed in the field. Larvae were collected from the field prior to each trial on the nights of full moon, new moon, and at 7-day intervals before and after (the lunar quarters), by digging nests of hatched but unreleased animals. All trials were run until the larvae molted into the morphologically distinct first juvenile instar, usually for 6 to 8 days. Animals held under DD did not molt this rapidly and were tested for 12 days.

An additional four sets of 1000 larvae were tested for 1 week each over 1 lunar month, from full moon to full moon in August, 1978. Trials were initiated on the nights of full moon, full moon plus 7 days, new moon, and new moon plus 7 days, and continued until molting of the larvae as above. Larvae were collected from the field 8 hr prior to the start of each trial, by digging nests of hatched but unreleased animals. An ambient light and temperature regime was employed, and activity was recorded as described above, using time-lapse photography. Lunar variability in the positive response of larvae to a light source was also tested during these trials by exposing the larvae to a constant light gradient during the hours of darkness. This light was provided by an overhead source (Tensor Hi Intensity Lamp #C3812), at a distance of 1 m from the water surface. This illuminated 25% of the test aquarium with white light. The remainder of the aquarium was shaded from the light with an opaque cover, creating a steep gradient of light intensity between the lighted quadrant and the rest of the aquarium.

All trials were conducted in 20-gal glass aquaria with a sand substrate into which animals buried when they were inactive. A closed system of circulation was employed with subsand filtration. Temperatures ranged from 28° to 34° C during the ambient light regime trial and were held at 24° C under constant light and darkness. Salinity was held at 20‰ in all trials.

Results

Field studies

Nest depths in the field showed no consistent change from the beginning to the end of the 8-month breeding season. The mean depth for larvae (13.2 ± 2.9 cm) was not significantly different than that for newly laid and immobile eggs (14.6 ± 1.9 cm) although the standard deviation was somewhat larger ($t = 1.25$, n.s.). However, there was a pronounced short term movement of larval nests upward from a mean depth of 17 cm at low tide to the surface 6 hr later at high tide on the night of full moon (Fig. 1). This was followed by movements of the larval nests back down to depths of approximately 14 cm by the time of the next low tide. Nests could be observed at the surface for 30 min prior to and 30

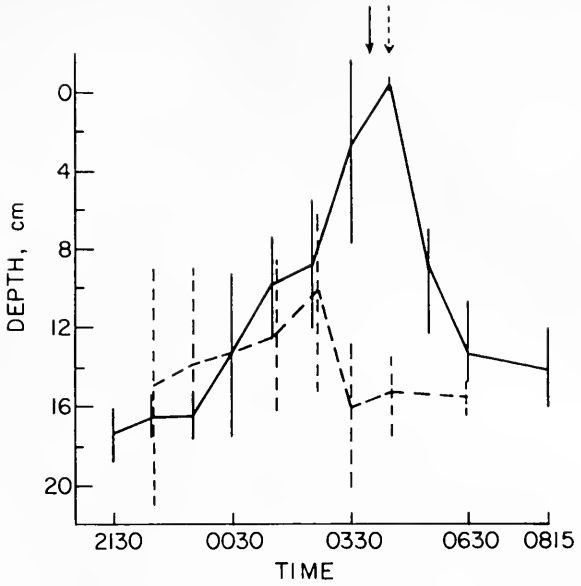

FIGURE 1. Movement of larval nests to and away from the surface of the beach during nights of full moon (solid line) and new moon (dotted line). The arrow indicates the time of high tide in each case. Bars indicate 1 s.d. Movement of nests to the surface on the night of full moon was not repeated on the night of new moon.

min after the high tide. Usually, some 15 to 20 individual larvae become partially exposed at the surface, with the rest of the nest remaining immediately below the surface. Each wave washed away the surface animals and carried them down the beach into the water. Many were cast back up onto the beach and stranded as the tide receded, where they formed drift lines of larvae on the beach. Most of these animals remained at the surface until daylight, after which they disappeared, falling prey to shore birds at dawn. The willet, *Catoptrophorus semipalmatus*, was a major predator. Reburial was probably not of great significance since scattered individuals were only occasionally found beneath the surface. Thus, larvae were seen to move in the field from their original burial depths to the surface and back down again over a 12 hr tidal cycle on the night of full moon. Such pronounced movements to the surface did not occur on the night of new moon. There was no significant difference between mean nest depth at low tide and mean nest depths at high tide on the night of new moon in 1979.

The depths of nests at the hour of high tide on the subsequent neap tide, new moon spring tide and second neap tide of the lunar month in 1978 are presented in Figure 2. Only on the night of the full moon spring tide did larvae come to the surface. The water did not reach the level of the nests at any time other than during spring tides at full and new moon.

The results of the plankton sampling adjacent to the breeding beach on day and night tides throughout the breeding season are provided in Figure 3. The major releases occurred on the night of full moon with two exceptions. And, as with breeding adults (Rudloe 1978), a strong nocturnal activity rhythm is evident.

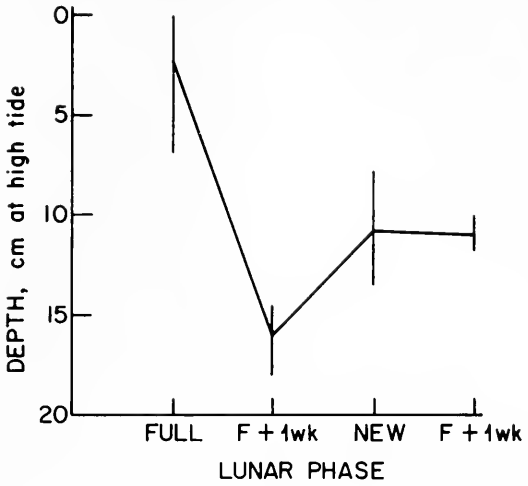

FIGURE 2. Mean depth of larval nests at the hour of high tide at each lunar phase for one month. Bars indicate 1 s.d.

There was also a major release of larvae whenever localized storms with strong onshore winds coincided with high tide, producing an unusually heavy surf. Under these circumstances, larvae were released by day as well as by night. Following a massive storm release in July, the following full moon did not show the usual peak of emergence. The larvae that would have emerged then may have been washed out in the preceding storm. Thus, the occurrence of storm conditions that coincide with a sufficiently high tide are an additional release mechanism.

Laboratory studies

When larvae were maintained under ambient light and temperature regimes, they displayed strong nocturnal activity peaks for the duration of the larval instar (Fig. 4). Larvae remained buried below the surface of the sand substrate during daylight hours. The abruptness with which activity appeared at the same hour each evening and terminated at the same hour each morning suggests that activity might be triggered very precisely by some as yet undescribed factor such as light intensity.

That the nocturnal rhythmicity of behavior might be endogenous in nature is suggested by the results of the trials in which activity was recorded under constant conditions of darkness and temperature. In Figure 5 larval activity is seen to occur during the hours corresponding to normal darkness almost exclusively. It was also noted that none of the larvae used in these trials molted as rapidly to the first juvenile instar as did those maintained under ambient light and temperature conditions.

When maintained under LL conditions and constant temperature, the larvae substained slight nocturnal activity peaks for the first 3 days (Fig. 6) of the trial, although the amplitude of the variation was considerably reduced, and substantial numbers of larvae remained active during daylight hours. Overall activity was well

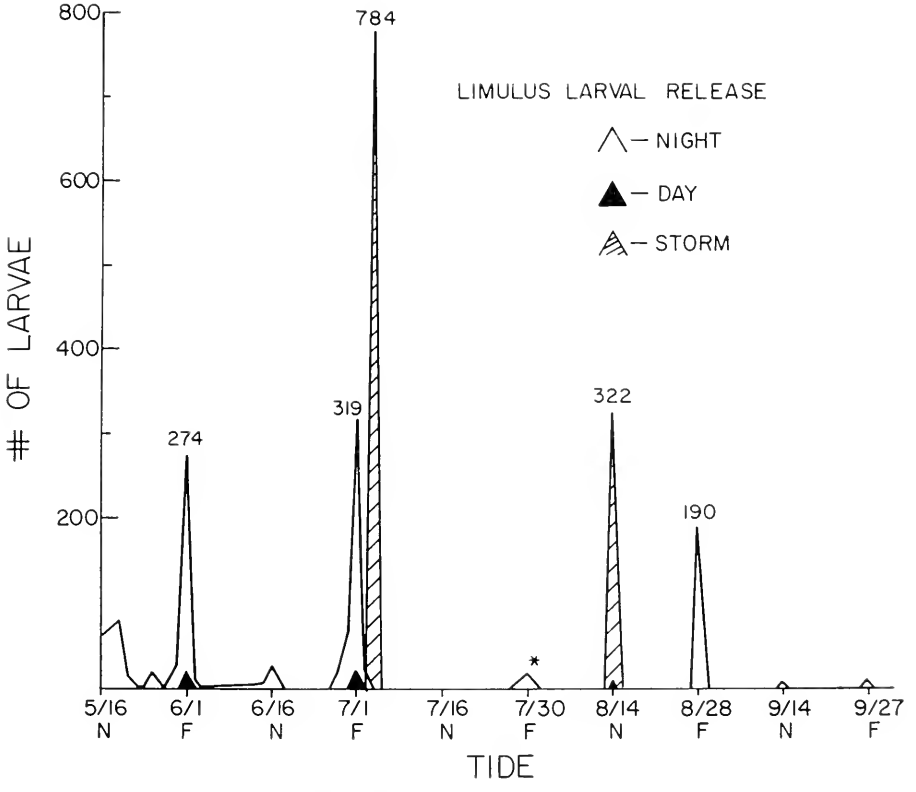

FIGURE 3. Appearance of free-swimming larvae in plankton samples adjacent to a breeding beach throughout a breeding season. F = full moon; N = new moon. Open peaks represent night high tides, closed peaks represent day high tides, and shaded peaks represent storm release. The asterisk denotes the July full moon, when larval release was not significant. This may be due to the extreme release of larvae on the preceding full moon when a massive storm release coincided with the lunar release.

below that under DD and ambient light regimes, however, and reached a level of zero by the fifth day of the trial. When light was removed for 30 min on the fifth night, swimming activity resumed within 5 min of the onset of darkness. After 30 min of darkness, light was restored and activity showed a steady decline throughout the rest of the night. This experiment was terminated thereafter due to the molting of the larvae.

A comparison of levels of activity and light responses of the larvae tested for 7 days each for each week of a lunar month are presented in Table I. Larvae were more active during weeks of spring tides (new and full moon) than on the intervening weeks of neap tides ($t = 5.49$, $P < 0.01$). Differences in levels of activity for the nights of new and full moon in the laboratory were not significant, however.

Although there was no significant difference in over-all first night activity between new and full moon, there was a marked difference in the pattern of activity

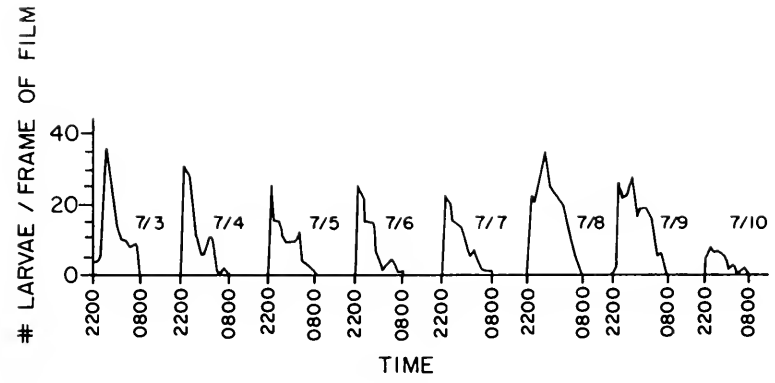

FIGURE 4. Activity patterns of 1000 *Limulus* larvae maintained in the laboratory under conditions of ambient light and temperature. Swimming activity began at 2200 hr each night and terminated at 0800 hr each morning. Larvae remained buried in the sand substrate when not actively swimming. Data is present as number of larvae visible in 1 frame of film at each hour.

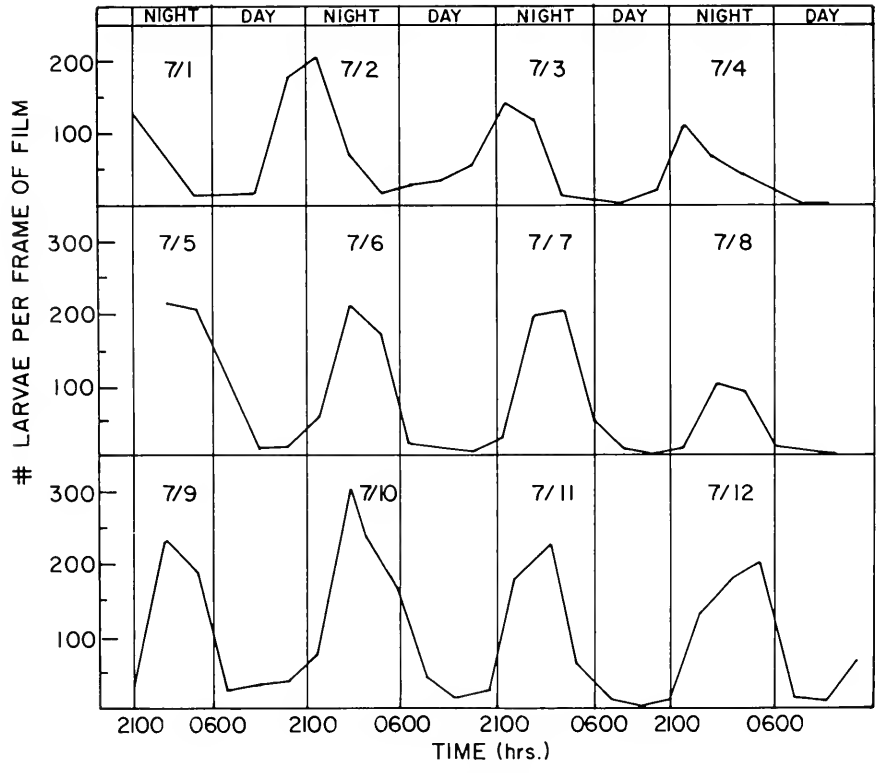

FIGURE 5. Swimming activity of 500 *Limulus* larvae maintained in the laboratory under conditions of constant temperature and darkness. One trial is presented with a duration of 12 days, after which the larvae molted.

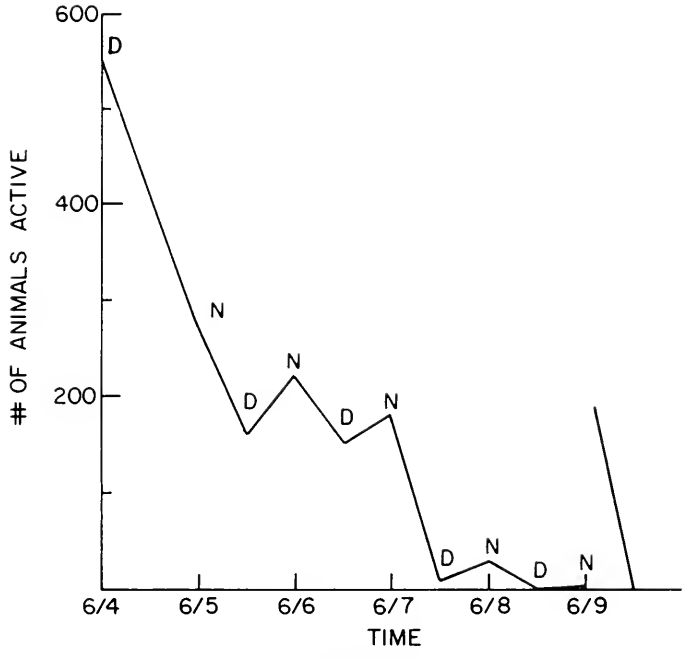

FIGURE 6. Swimming activity of 1000 *Limulus* larvae maintained in the laboratory under conditions of constant temperature and light. Activity terminated on the fifth night but was resumed immediately when light was removed for 5 min at 0100 hr. With restoration of the light, activity again declined. Animals molted during the next 24-hr period and the experiment was terminated. D is daylight at 1200 hr, N is night at 2400 hr. Number of animals swimming was plotted hourly.

during these nights. During the course of the full moon night, activity increased by a factor of 114% between 10 PM and 7 AM while it declined by 29% during the same period during the course of the new moon night. It declined even more steeply, by factors of 79% and 81% on the nights of neap or quarter moon tides. Activity also declined steeply from first to last nights of the session for the full moon (82%) and neap tide (78, 89%) weeks, but declined less sharply from first to last night during the new moon week (30% decline in activity).

Larvae were significantly attracted to light in all lunar weeks (Table I) except that of the new moon. Although larvae were active and swimming on the night of new moon, they showed no significant response to light as measured by concentration of individuals in the lighted quadrant of the aquarium. This lack of responsiveness was maintained throughout the entire week of the new moon trial.

DISCUSSION

The use by *Limulus* of the intertidal sandy beach as a breeding site and the burial of nests well below the surface affords a great deal of protection for developing eggs, and most survive to hatching. The occurrence of larval nests in a distinct zone in the upper intertidal in the northeastern Gulf of Mexico may be related

TABLE I

Lunar variation in larval swimming activity and light responses under laboratory conditions. Four sets of 1,000 larvae, one for each lunar week, were tested. The larvae responded to light at all times other than the week of new moon.

	Lunar phase			
	Full	New	Full − 1 week	Full + 1 week
Mean activity, first night	580	485	329	393
% decline in activity, first night	(increased)	29	79	81
% decline in activity, first to sixth night	82	30	78	89
% responding to light, first night	63	31	78	89
% decline in light response, first night	31	—	38	57
% decline in light response, first to sixth night	32	—	(increased) 113	38
X^2, light response, first to sixth night	49.8 $P < 0.01$	9.44 N.S.	49.8 $P < 0.01$	64.6 $P < 0.01$
X^2, light response, first night	246 $P < 0.01$	6.52 N.S.	69.5 $P < 0.01$	131.7 $P < 0.01$

n = 1000 in each trial.

to the small tidal amplitude of approximately 1 m. According to Shuster (personal communication), nests are found in Delaware Bay, where the spring tide range is approximately 2 m, over a much wider band—from the high tide line through 60% of the exposed intertidal. In Cape Cod Bay, where the range is 3 m or more, nests are also widely distributed but tend to be most numerous in the mid tidal area.

In addition to protection, this nesting pattern also creates substantial problems of emergence and dispersal of the young into the aquatic environment. However, *Limulus* larvae are a behaviorally and morphologically specialized phase of the life cycle, that cope well with the problems of nest release. With an initial swimming frenzy and positive phototaxis, and with lunar and circadian activity rhythms that synchronize activity with water levels on the beach, the larvae are well adapted for emergence from the nests and dispersal. After this transition is achieved, larvae molt into the substantially different morphology and behavior patterns characteristic of the benthic portion of the life cycle.

Although further work is required to be conclusive, the nocturnal swimming of *Limulus* larvae under DD conditions suggests an endogenous rhythm. The peaks of activity appear to occur progressively later during the night as the experiment progresses, and these peaks are approximately associated with the time of low tide in the field. Whether this is in fact reflective of an advancing endogenous tidal cycle or is a shifting of the circadian rhythm is not established at this time. The observed suppression of activity under LL is characteristic of both aquatic and terrestrial nocturnal species (Aschoff, 1960).

Semi-lunar reproductive rhythms are well known among intertidal crustaceans, snails, and bivalves (Gifford, 1962; Warner, 1967; Russell-Hunter, Apley and

Hunter, 1972; Wheeler, 1979). The field emergence of larvae on the full moon and the absence of larval release on the new moon therefore poses an intriguing question. Is it reflective of an edogenous lunar rhythm, or is moonlight a key stimulus in orienting the movement of larvae to the surface and into the water column?

Based on the activity of larvae in the laboratory, there appears to be a lunar rhythm of responsiveness to light that is somewhat different from the lunar locomotory rhythms observed. While larvae were active at both full and new moons (*i.e.* on spring tides), they were responsive to light only at full moon. This is consistent with the lack of field larval release during the spring tides of the new moon.

Furthermore, neurophysiological sensitivity to light several hundred times dimmer than moonlight has been recorded for the *Limulus* median ocellus by Lall and Chapman (1973) who obtained responses to levels of illumination as low as 1.4×10^{-10} $\mu W/(cm^2 \cdot nm)$ at 360 NM. Moonlight intensity of 10^{-3} $\mu W/(cm^2 \cdot nm)$ has been reported by Kampa (1970). Therefore moonlight is well within the sensitivity range of adult specimens of *Limulus*.

A model to explain the observed pattern of larval release in the field is presented. It is based on the comparison of field release with the activity and light response patterns seen in the laboratory. The following factors are apparently of significance: an endogenous readiness to respond to a dim light source that peaks at the time of full moon and is minimal at the time of new moon; the availability of an appropriate light source in the form of a full moon; a water level reaching the nests only during periods of spring tides; and the suppression of activity by the bright light of day that overrides afternoon spring high tides. This pattern of larval release is supplemented by occasional mass releases caused by storms that coincide with high tides at times other than full moon.

This model seems to be most consistent with the data presently available. In particular, the full/new moon differences in response to light in the laboratory correspond to the lack of field release at new moon. However, whether moonlight or the lack of it is detectable to larvae buried several centimeters in the sand prior to high tide has not been determined. If it is not detectable at the depth of the nests, then some other factor may be instrumental in initiating movement toward the surface.

If inactive larvae are removed from a nest and placed in water, swimming activity is immediately initiated. Increasing interstitial moisture associated with the rising tide might conceivably initiate activity, for instance, with moonlight becoming the dominant stimulus only as the animals approach the surface. The apparent partial movement of larvae toward the surface on the night of new moon in June, 1979, suggests the existence of some stimulus in addition to moonlight. The identification of that stimulus, should it exist, as well as testing of the other components of the proposed model, awaits additional research.

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this work. This research was funded by a grant from the Griffis Foundation and the American Littoral Society.

Summary

The horseshoe crab *Limulus polyphemus* lays its eggs on sandy beaches at the level of the highest high tide in the northeastern Gulf of Mexico, buried approximately 18 cm below the surface. When they hatch, the larvae must move from the buried nest site into the marine environment.

In the field, nests of larvae move to the sand surface and emerge at the spring high tide on the night of full moon. They may also be released by heavy surf associated with storms. No release occurs on the spring high tides associated with new moon.

In the laboratory, larvae are seen to be nocturnally active, both under ambient and DD photoperiods. Activity peaks at times of full and new moons, and larvae are positively phototatic at all lunar phases except new moon.

A model to account for observed field behavior in light of laboratory activity and light responses is presented.

LITERATURE CITED

Aschoff, J., 1960. Exogenous and endogenous components in circadian rhythms. *Cold Spring Harbor Symp. Quant. Biol.*, **25**: 11–28.

Cohen, E., Ed., 1979. *Biomedical applications of the Horseshoe Crab (Limulidae)*. Alan R. Liss, Inc., New York, 688 pp.

French, K., 1977. An effect of light on the hatching pattern of *Limulus polyphemus*. *Biol. Bull.*, **153**: 425.

French, K., and S. Doliner, 1978. Photospositive behavior of young *Limulus*. *Biol. Bull.*, **155**: 437–438.

Gifford, C. A., 1962. Some observations on the general biology of the land crab *Cardiosoma guanhumi* (Latreille) in South Florida. *Biol. Bull.*, **123**: 207–223.

Green, R. H., and K. D. Hobson, 1970. Spatial and temporal structure in a temperate intertidal community, with special emphasis on *Gemma gemma*. (Pelecypoda: Mollusca). *Ecology*, **51**: 999–1011.

Hummon, W. D., J. W. Fleeger, and M. R. Hummon, 1976. Meiofauna-macrofauna interactions. I. Sand beach meiofauna affected by maturing Limulus eggs. *Chesapeake Sci.*, **17**: 292–298.

Jegla, T. C., and J. D. Costlow, 1979. The *Limulus* bioassay for ecdysteroids. *Biol. Bull.*, **156**: 103–104.

Kampa, E. M., 1970. Underwater daylight and moonlight measurement in the eastern North Atlantic. *J. Mar. Biol. Assoc. U.K.*, **50**: 397–420.

Kingsley, J. S., 1892. The embryology of *Limulus*. *J. Morphol.*, **7**: 35–68.

Lall, A., and R. Chapman, 1973. Phototaxis in *Limulus* under natural conditions: evidence for reception of near ultraviolet light in the median dorsal ocellus. *J. Exp. Biol.*, **58**: 213–224.

Loeb, J., 1893. Uber kunstliche Unwandlung positiv heliotropischer Tiere in negativ heliotropische und umgekehit. *J. Arch. ges. Physiology*, **54**: 81–107.

Pearl, R., 1904. On the behavior and reactions of *Limulus* in early stages of development. *J. Comp. Neurol. Psychol.*, **14**: 138–164.

Rudloe, A., 1978. Some ecologically significant aspects of the behavior of the horseshoe crab, *Limulus polyphemus*. *Ph.D. dissertation, Florida State University, Tallahassee, Florida.* 246 pp.

Rudloe, A., 1979. *Limulus polyphemus:* a review of the ecologically significant literature. Pages 27–35 in E. Cohen, Ed., *Biomedical Application of the Horseshoe Crab (Limulidae)*. Alan R. Liss, Inc., New York.

RUDLOE, A., AND W. F. HERRNKIND, 1976. Orientation of *Limulus polyphemus* in the vicinity of breeding beaches. *Mar. Behav. Physiol.*, **4**: 75–89.

RUSSELL-HUNTER, W. D., M. L. APLEY, AND R. D. HUNTER, 1972. Early life history of *Melampus* and the significance of semi-lunar synchrony. *Biol. Bull.*, **143**: 623–656.

SHUSTER, C. N., JR., 1958. On morphometric and serological relationships within the Limulidae, with particular reference to *Limulus polyphemus* (L.). *Ph.D. dissertation, New York University, New York.* Diss. Abstr., **18**: 371–372, 287 pages.

SOKOLOFF, A., 1978. Observations on populations of the horseshoe crab, *Limulus* (= *Xiphosura*) *polyphemus*. *Res. Popul. Ecol. (Kyoto)*, **19**: 222–236.

THORSON, G., 1964. Light as an ecological factor in the dispersal and settlement of larvae of marine botton invertebrates. *Ophelia*, **1**: 167–208.

WARNER, G. F., 1967. The life history of the mangrove tree crab, *Aratus pisoni*. *J. Zool.*, **153**: 321–335.

WHEELER, D. E., 1979. Semi-lunar hatching periodicity in the mud fiddler crab *Uca pugnax* (Smith). *Estuaries*, **1**: 268–269.

WOLBARSHT, M., AND S. YEANDLE, 1967. Visual processes in the *Limulus* eye. *Annu. Rev. Physiol.*, **29**: 513–542.

Reference: *Biol. Bull.* **157**: 506–523. (December, 1979)

DEVELOPMENT OF BIOLUMINESCENCE AND OTHER EFFECTOR RESPONSES IN THE PENNATULID COELENTERATE *RENILLA KÖLLIKERI*

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In benthic marine invertebrates with planktonic larval forms, the conversion from larva to adult usually involves significant morphological and physiological changes. The morphological changes can involve destruction and resorption as well as elaboration and reorganization of larval structures, the production of new tissues and structures, and renewed differentiation of adult organ rudiments (see, for example, Bonar, 1978; Cameron and Hinegardner, 1974, 1978). Despite a wealth of information concerning morphological changes which occur at metamorphosis of marine invertebrate larvae, little is known about the physiological changes at this critical time. In particular, little is known about functional changes in the nervous and effector systems during larval maturation and transformation to the adult form.

The ideal preparation for studying changes in larval neuroeffector function is one in which the organization of nervous and effector elements is simple, the systems in question can be easily monitored, the adult neuroeffector systems are well understood, and the larval stages are relatively large and easy to raise through metamorphosis. These conditions are approached in the octocoral *Renilla köllikeri*. The nervous elements of *Renilla* are arranged in a diffuse neural network (Satterlie, Anderson and Case, 1976). This nerve net is through-conducting, that is, an impulse initiated in one area of the net is transmitted throughout the remaining portion without decrement (Anderson and Case, 1975; Satterlie, Anderson and Case, 1976). Effector function in *Renilla*, and other anthozoans, is based on perhaps the simplest integrative mechanism, peripheral frequency dependent facilitation (Pantin, 1935; Parker, 1920; Nicol, 1955a, b) and is therefore relatively predictable. Although larvae of *Renilla* are not large enough for electrophysiological recording, they possess an effector system, the bioluminescent system, which is easily monitored photometrically. Furthermore, in live preparations, the luminescent cells (photocytes) fluoresce when illuminated with the proper exciting wavelength of light. The luminescent system can therefore be monitored both morphologically and physiologically in live, intact specimens. The neuroeffector systems of adult *Renilla* colonies, including the luminescent system, have been examined in some detail (Anderson and Case, 1975). Electrical impulses were recorded from a colonial conduction system which controls bioluminescence, polyp withdrawal and colonial contraction. Semi-autonomous polyp conduction systems are also active (Anderson and Case, 1975).

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Our studies indicate that the development of *Renilla köllikeri* is similar to that of *Renilla reniformis* (Wilson, 1883) and other octocorals (Matthews, 1916; Gohar, 1940a, b; Chia and Crawford, 1973). The eggs of *Renilla* are large and yolky, and are usually spawned in large numbers. A swimming planula settles and metamorphoses into a primary polyp. Secondary polyps bud from the primary polyp to form the colony (see Wilson, 1883).

We have followed the development of *Renilla* from the fertilized egg to the mature primary polyp during three reproductive seasons in order to describe the onset of effector form and function, with particular reference to three activities: ciliary swimming, muscular activities, and bioluminescence. The observations of effector function have been utilized to describe the functional development of the nervous system(s) that underlie effector activation during the various stages of larval life. In another report, these observations will be supplemented by an ultrastructural investigation of the developmental stages.

Materials and Methods

Colonies of *Renilla köllikeri* Pfeffer were collected in shallow water off Zuma Beach, California and in the Santa Barbara Channel by divers. Healthy specimens were kept in large aerated aquaria at 18 to 20° C and checked several times daily for spontaneous spawning. Colonies that had been dark conditioned for 1 to 2 days in running seawater aquaria frequently spawned following transfer to lighted battery jars of still sea water. Larvae were reared in 3-liter flasks or beakers of aerated sea water, changed twice daily. Late swimmers (see results for description) were transferred to 250-ml beakers with or without sand. The lecithotrophic eggs permitted development to the primary polyp stage without feeding.

Fluorescence

Living larvae were observed in one of three types of squash preparations. Light and medium squashes involved bridging a coverslip across two other coverslips (No. $1\frac{1}{2}$ thickness) on a slide. For light squashes, the slide was flooded with sea water and for medium squashes, the animal was placed in a small drop. In heavy squashes, which frequently resulted in tissue damage, a coverslip was placed directly on a small drop of sea water containing the specimen. A Zeiss Universal Research Microscope was used with mercury lamp illumination, BG 12 and BG 38 exciting filters, and a 500 nm cut-off barrier filter. Larvae were anesthetized in a 1 : 1 solution of 0.37 M $MgCl_2$: sea water for photography.

Bioluminescence

Bioluminescence was detected with an EMI 9601B end-window photomultiplier tube operated at −950 V, giving a high signal-to-noise ratio. A Uniblitz 100–2 electric shutter was threaded onto the window end of the tube and operated from outside a dark experimental box. Signals from the photomultiplier tube were amplified with a Keithley 427 current amplifier leading to a 7P1F DC amplifier of a Grass 79D polygraph.

The larvae were tested in laminated plexiglass blocks with 2.5 cm diameter and

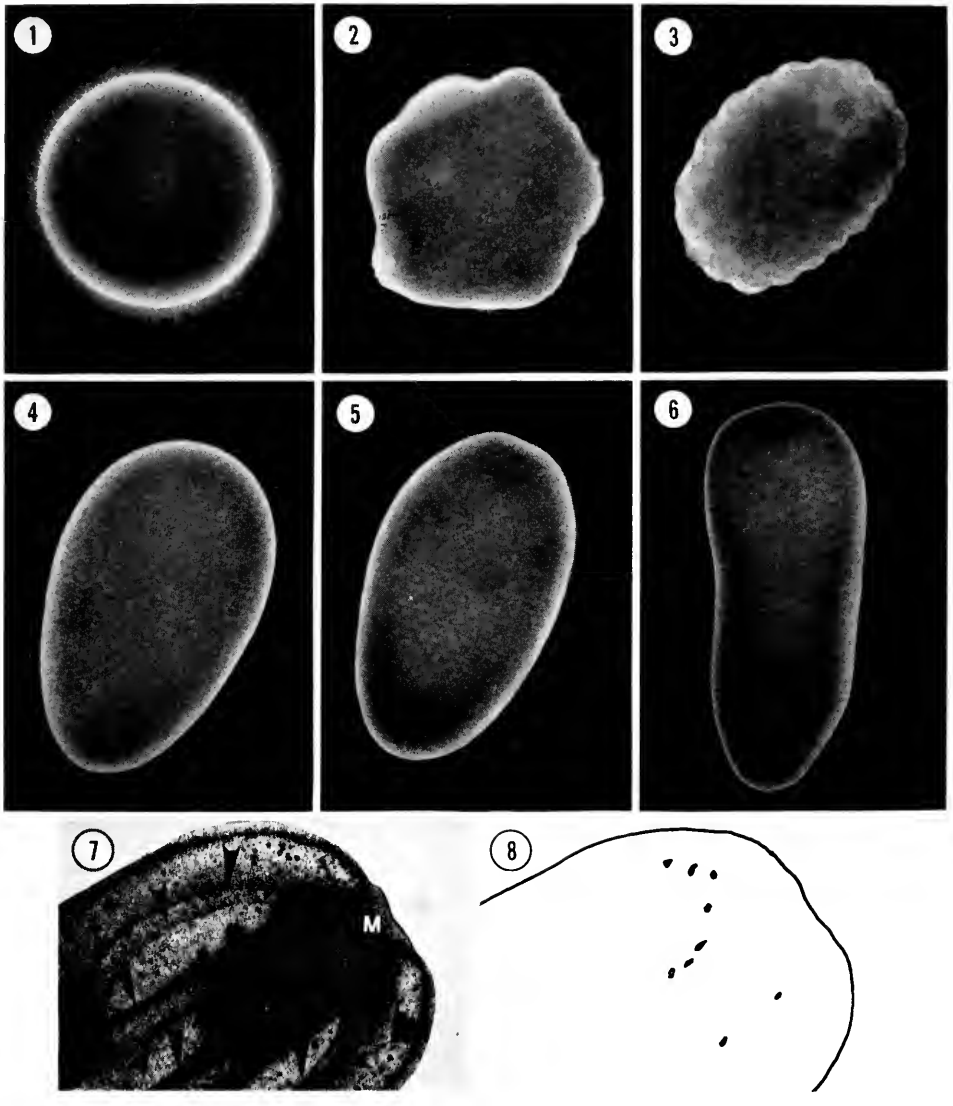

FIGURE 1. Fertilized egg approximately 15 min after release. The ciliary coating is made up of thousands of sperm adhering to the egg surface (verified by E.M.). ×76, light squash.

FIGURE 2. About 8-cell stage. The initial cleavages are irregular and apparently incomplete. At this stage, most of the sperm coating is gone. One hour embryo. ×76, light squash.

FIGURE 3. About 64-cell stage. Three hour embryo. ×76, light squash.

FIGURE 4. Pre-swimmer planula. At this stage, the endoderm is fully formed and the gastric cavity begins to appear. Ciliary activity is weak so the pre-swimmer merely spins in circles on the bottom. ×76, light squash.

FIGURE 5. Early swimmer planula. Ciliary activity is sufficient to lift the planula off the bottom. No muscular activity is evident. ×76, light squash.

1-cm-deep center wells (same diameter as shutter opening) which were positioned to allow a 3-cm specimen-to-pm tube window distance. Chemicals could be introduced via PE 50 intramedic polyethylene tubing penetrating the wall of the chamber. Single larvae were placed in 1 ml of sea water in the center well, which could be centered under the shutter pm tube by touch. One milliliter of test solution was slowly injected. The test solutions were 0.53 M KCl, 0.1 M $CaCl_2$, 3% H_2O_2, and sea water controls. The electrical test chamber consisted of a similar plexiglass block with a pair of horizontal 180 mesh silver screens (Unique Wire Weaving Co., Hillside, N. J.), leading to a Grass S9 stimulator. Test animals were placed on the bottom screen with enough sea water to cover them but prevent swimming. The upper screen was then pressed down to contact the larva or sea water. Large stimulating voltages were frequently required due to the shunting effect of the sea water. All luminescence experiments were carried out in a dark box within a darkroom at ambient air temperature (20° to 25° C).

Results

Pennatulid colonies have three distinct parts: the peduncle, rachis, and polyps. The basal peduncle is an anchoring device which is inserted into the substratum by peristaltic movements. The rachis, or main tissue mass, supports an array of two types of secondary polyps, the siphonozooids and the autozooids. The rachis and peduncle are derived from the primary polyp, and the colony is formed by budding of the secondary polyps (Wilson, 1883). The rachis of *Renilla köllikeri* is flattened and leaf-shaped. The ventral surface is devoid of polyps and normally lies in the substratum. The dorsal surface bears scattered autozooids, inhalent siphonozooid clusters, and a single exhalent siphonozooid.

Renilla köllikeri colonies are dioecious. Gametes are borne on the autozooid septal filaments. When mature, the eggs are tan in color and approximately 0.3 to 0.4 mm in diameter. In male colonies, sperm are packed in follicles which are the same size as the eggs, but white in color. Both eggs and sperm follicles are covered by a single layer of ciliated endodermal cells until spawning takes place. During the reproductive season, the septal filaments of each autozooid contain more than 20 sperm follicles or eggs at different stages of development.

Spawning

The reproductive season for *Renilla köllikeri* in the Santa Barbara area extends from May to late July or early August, a period centered around the summer solstice. During this period colonies may spawn many times, in small groups or *en masse*. In female colonies, spawning begins with an extreme inflation of the

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Figure 6. Swimmer planula. The planula is now hollow and the septa begin to form by outgrowth of the endoderm. Swimming is active. Local muscular contractions can be elicited. ×76, light squash.

Figures 7–8. Swimmer planula, approximately 10 to 15 hr older than in Figure 6. The same animal was used for both bright field (7) and fluorescence (8, traced from original to show position of dimly fluorescent photocytes) micrographs. The first sign of fluorescence is evident at this time. Note that the anthocodial septa are well formed (Fig. 7, arrows). The mouth (M, Fig. 7) is not yet open. ×56, heavy squash.

rachis, in conjunction with accelerated and pronounced rachidial peristalsis. In a 9.5 cm colony, each peristaltic wave passed across the rachis in 45 to 60 sec, a conduction velocity of 0.16 to 0.21 cm/s (21° C). This is compared to a rate of 0.11 to 0.13 cm/s (23° C) in non-spawning *Renilla*. Two and sometimes three waves are present on the rachis of spawning colonies at all times. During spawning, the autozooids remain extended with the tentacles bent slightly in an aboral direction. In female colonies, the shedding of eggs appears to result from the peristaltic movements. In one instance, three waves were required to squeeze an egg out of the autozooid mouth.

When released, the eggs are oblong, but become sperical in 15 to 30 min (Fig. 1). They are neutrally buoyant, and float at all levels in an aquarium of still water. All released eggs, as well as some dissected from a spawning colony, were already fertilized, indicating that fertilization occurs before release. Sperm follicles are not released intact during spawning. The follicles rupture within the autozooids, and the sperm are released through the autozooid mouths and the exhalent siphonozooid.

TABLE I

Summary of early development of Renilla köllikeri *with observations on effector function (25° C). The numbers in parentheses indicate the corresponding text figures.*

Hours from spawning	Stage	Ciliary swimming	Muscle activity	Fluorescence	Bioluminescence
0	Fertilized egg (1)	—	—	—	—
½–3	First cleavages (2)	—	—	—	—
1–3	About 64-cell (3)	—	—	—	—
7	Stereoblastula	—	—	—	—
32	Pre-swimmer (4)	Spinning on bottom	—	—	—
38	Early swimmer (5)	Swimming	—	—	—
60	Swimmer (6)	Swimming	Local contractions	—	—
84	Swimmer (7)	Swimming	Conducted contractions	First sign (8)	—
118½	Late swimmer (9)	Swimming, sinking	Conducted contractions	+ (10)	—
131	Settled (12)	Ceases	Conducted contractions	++ (11)	— (20B)
136½	Settled (13)	—	Separate responses	+++ (13) as in adult	First Sign (20C)
191	Tentacle buds (14)	—	Separate responses	+++	+ (20E)
274	Pinnules (15)	—	Separate responses	+++ (15, 16)	++ (20F)
323	Primary polyp	—	Separate responses	+++ (17)	+++ (20G)

Larval development

The early cleavages and larval development of *Renilla köllikeri* are similar to that described by Wilson (1883) for *Renilla reniformis*. The intial cleavages are extremely irregular and variable. Seldom can 2-, 4-, or 8-cell stages be recognized. The first detectable cleavages (*Renilla köllikeri*) occur 20 min to 3 hr after spawning, at which time embryos of 8 to approximately 64 cells are observed (Fig. 3). Prior to this stage the embryos are very irregular in shape (Fig. 2).

Early development progressed more rapidly at 25° C than at lower temperatures tested. Cooler temperatures, down to 6° C, retarded development without apparent harmful effects. A timetable for the development of *Renilla köllikeri* (25° C) is shown in Table I, and all further mention of age will refer to this table and temperature.

Just prior to cleavage, neutral buoyancy is lost, and the embryos settle to the bottom of the aquarium. No ciliary activity is apparent. Subsequent cell divisions give rise to stereoblastulae. Endoderm formation is followed by gradual disappearance of the central, yolky cells. Swimming planulae are formed 38 hr after spawning (Figs. 4, 5). The planulae are barrel-shaped and swim at the air-water inter-

FIGURE 9. Late swimmer planula. The eight anthocodial septa (one indicated, large arrow) are visible, two of which extend to form the peduncular septum (small arrow). ×101, light squash.

FIGURE 10. Late swimmer planula. Tracing of a fluorescence micrograph indicating the position of the dimly fluorescent photocytes, which are arranged in rows along the septa. ×47, medium squash.


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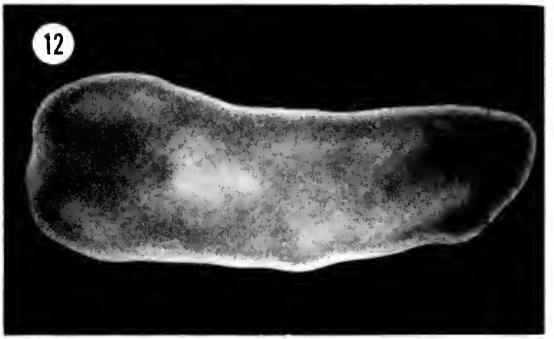
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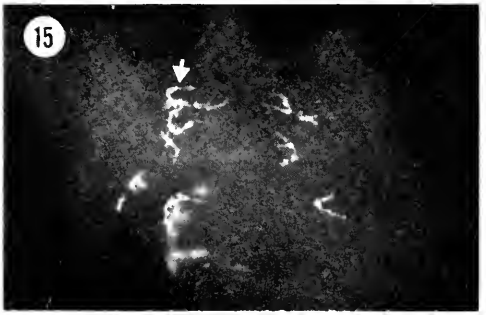
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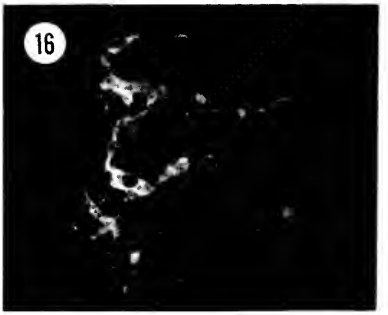
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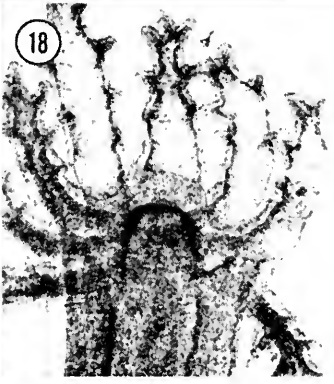
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face (early swimmers, Table 1). During the swimming period (4 to 6 days) the planulae gradually elongate (swimmers, Figs. 6, 7) and undergo considerable cellular differentiation. During this time the peduncular septum, as well as the eight anthocodial septa are formed (Fig. 7). The differentiation of muscle and nerve cells begins early in the planula stage (early swimmers; Satterlie and Case, in preparation).

When 5 days old, the planulae (late swimmers, Fig. 9) begin to sink to the bottom of the aquarium. The larvae settle oral end first on clean glass or in sand (Fig. 12). Settlement is achieved more rapidly when a sand substratum is provided, as opposed to clean glass, the difference being as much as 8 hr. Once attached to the substratum by the oral end, the larvae "roll" to the side and eventually attach by the aboral peduncle. The oral end is then raised. When settled in sand, the larvae move between sand grains and in some cases burrow in approximately a half-centimeter.

Tentacle buds first appear as conical projections on the anthocodium at 8 days (Fig. 14). The tentacles gradually elongate and sprout lateral pinnules (Fig. 15). At 13 days, the juveniles represent structurally complete primary polyps (Figs. 18, 19). The juveniles survived as single polyps for up to 3 weeks in the laboratory, but did not bud secondary autozooids. See Wilson (1883) for an account of colony formation in *Renilla reniformis*.

Development of effector systems

Ciliary swimming. The ectoderm of adult specimens of *Renilla* is not heavily ciliated except for the invaginated pharynx of each polyp. However, for a short time during development, the planulae rely on a dense ectodermal ciliary coat for locomotion. Ciliary activity is continuous, and no ciliary reversals or arrests could be demonstrated. When a planula encountered a solid object, for instance the side of a container, it would continue to swim in a forward direction (aboral end first) until it eventually slid away. The swimming motion produces a rotation about the oral-aboral axis, and in the late swimmer, which maintains a bend in the aboral end, produces a cork-screw-like motion. All ectodermal ciliary activity ceases upon settlement. If larvae are immediately dislodged, however, ciliary activity reappears. This ability is not apparent after the larvae have remained

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FIGURE 11. Freshly settled planula. Fluorescence micrograph showing the photocytes arranged along the septa. ×56, heavy squash.

FIGURE 12. Freshly settled planula, longitudinally contracted. ×78, light squash.

FIGURE 13. Settled juvenile. This animal produced a luminescent response similar to that in Figure 20C. The fluorescent cells are as bright as those in the later stages. Note the constriction in the "neck" region. The anthocodium is inflated, and the peduncle deflated, demonstrating the separation in responses in the two areas. ×58, medium squash.

FIGURE 14. Tentacle bud stage. Again note the inflated anthocodium and contracted peduncle. ×80, light squash.

FIGURE 15. Tentacled polyp at stage of pinnule development. Note the photocyte processes. ×66, medium squash.

FIGURE 16. Higher magnification fluorescence micrograph of the photocyte cluster marked by the arrow in Figure 15. ×214, medium squash.

FIGURES 17–18. Anthocodial region of a primary polyp. The photocytes are found in the lateral sides of the tentacle bases. ×43, medium squash.

FIGURE 19. Full view of a primary polyp. Two siphonozooids are visible (arrows). ×14, medium squash.

settled for an hour or more. If the larvae are not allowed to settle, the late swimmer stage is prolonged and further morphological changes, such as tentacle budding, are delayed.

Muscular activity. Obvious muscular activity is lacking until the swimmer stage (60 hr), after elongation is underway. At this point a mechanical or electrical stimulus to any part of the planula produces a local contraction which pulls the stimulated tissue away from the probe. This muscular contraction is not conducted circularly or longitudinally. Within 2 to 5 hr, similar stimuli produce a more widespread circular constriction, and the planulae contract longitudinally to about two thirds of the relaxed length. At this time, the septa are well formed (Fig. 7). By the late swimmer stage, the larvae are capable of bending movements as well as "protective" longitudinal contractions. These conducted muscular events are not observed when the planulae are placed in excess Mg^{++} for 5 min. In the anesthetized state, stimulation only produces local twitches as in the earlier planulae.

At the time of planula attachment, a division between polyp and peduncle reactions is evident. A stimulus to the peduncle produces a conducted contraction, but not always with an accompanying polyp contraction. Similarly, polyp stimulation does not always produce peduncular contractions. As soon as tentacle buds

are apparent, the primary polyp behaves much like the autozooids of a mature colony. An electrical or mechanical stimulus to the polyp causes an inversion of the anthocodium and an overall contraction of the polyp.

Peristaltic movements are not apparent until settlement, when the peduncle begins rapid, strong peristaltic contractions. If an animal is dislodged, the peristaltic contractions cease until resettlement occurs. As with muscular reactions, a definite separation is evident between the anthocodium and the peduncle, and each is capable of separate peristaltic and bending movements.

Development of the bioluminescent system

Fluorescence. The first sign of fluorescence occurs in the swimmer stage approximately 80 hr after spawning. Extremely dim fluorescent cells are evident on the eight anthocodial septa (Figs. 7, 8). The cells are oval to round, 5 to 10 μm in maximum length, and without noticeable processes. The size of the fluorescent cell bodies does not appear to change much as the larvae grow. Cell processes are first observed as small, stubby projections after settlement has taken place. By the time of tentacle pinnule development, the cells appear morphologically similar to photocytes of mature autozooids (Figs. 15, 16).

The intensity of fluorescence increases until around 140 hr, when it is approximately as intense as in mature autozooid photocytes. No subsequent increase in fluorescence intensity is apparent. If larvae are prevented from settling, fluorescence increases in intensity at the normal rate, but the appearance of the photocyte cytoplasmic processes is delayed. The increase in fluorescence is not affected by the type of settling substratum.

TABLE II

Summary of chemical bioluminescence tests expressed as number of successful trials/number of trials. The data on substrate effects are from a separate experiment using KCl as the stimulant.

Larval stage	KCl	$CaCl_2$	H_2O_2
64 Cell stage	0/30	—	0/5
Early swimmer	0/33	—	0/5
Swimmer	0/26	0/1	0/5
Late swimmer	0/34	0/2	0/5
Settled (oral attachment)	1/24	0/2	0/5
Settled (peduncle)	13/16	1/2	3/5
Swimmers (same age as peduncle settled)	0/14	—	—
Tentacle buds	23/23	2/2	4/5
Swimmers (same age as tentacle buds)	8/12	—	—
Mature tentacles	11/11	5/5	5/5

Substratum effect on KCl response—peduncle settled larvae approximately 160 hr from spawn

Glass settled (no sand)	5/32
Sand settled	19/20
Glass settled (sand in container)	5/8

As the tentacles begin to form, the fluorescent cell groups appear more peripherally in the septa (compare Figs. 8, 10, 11, 13, 15, and 17). When the tentacles are fully formed, the fluorescent cells are found between the tentacle bases (Fig. 17) as in mature autozooids.

Bioluminescence. The bioluminescent capability of *Renilla* was tested with three chemicals known to induce luminescence in adult animals. Potassium chloride, isotonic with sea water, produces transient flashing presumably by randomly depolarizing cells. H_2O_2 produces similar results. Hypotonic $CaCl_2$ was used by Anderson and Cormier (1973) to induce luminescence from lumisomes, isolated subcellular particles which contain all components of the luminescent reaction. In severed autozooids from adult colonies, 0.1 M $CaCl_2$ produces steady glowing for up to 45 min.

No bioluminescence was measurable up to the time of planula settlement (Table II and Fig. 13). Even when 12 late swimmer larvae, ready to settle, were stimulated together with KCl, luminescence was still not recordable. For comparison, *Obelia sp.* hydroids were tested with KCl. In *Obelia* the photocytes, which are fluorescent, are scattered in the stolons and uprights. Thus a piece of tissue can be dissected which contains only one photocyte. The record from a test of such a piece of *Obelia* is shown in Figure 20A, and represents a base of comparison for the *Renilla* luminescence tests.

Freshly settled planulae do not luminesce (Fig. 20B). One to three hours after settlement, the first light is recorded (Fig. 20C). At this stage, the juveniles are attached by the peduncle with the anthocodium raised, and the fluorescence intensity of the photocytes is equal to that of mature autozooid photocytes. In general, no bioluminescence is recorded from animals in which the fluorescence is less intense than in adult autozooids. If larvae are detached prior to rolling over to the peduncular attachment, and prevented from settling, luminescent competence is delayed by up to 24 hr (Table II).

The substratum is important to the development of luminescent ability (Table II). Late swimmers will settle on the sides and bottom of glass beakers if no other substratum is available. Appearance of bioluminescence in glass settled juveniles is delayed just as if settlement is prevented. Occasionally, planulae settled on the glass sides of a sand-substratum container, in which case luminescence developed normally.

The waveform of chemically-induced luminescence is initially irregular (Figs. 20C, D). When approximately 190 hr old, the juveniles exhibit a luminescent waveform similar to that of the adult, namely a rapid rise to peak followed by a slower decay (Figs. 20E–G). At the tentacle bud stage, light emission takes the form of several individual peaks (Fig. 20E) possibly indicating the sequential stimulation of photocytes or photocyte groups. By the tentacled stage, the emission consists of broad flashes up to 4 sec in duration (Fig. 20G). Multiple flashes are frequently encountered, with the initial flash normally being the brightest (Fig. 20F). In general, the intensity of bioluminescence increases with age of the juvenile. The above descriptions of the stages of bioluminescent competence represent the norm of multiple tests. The developmental rates of individuals vary slightly.

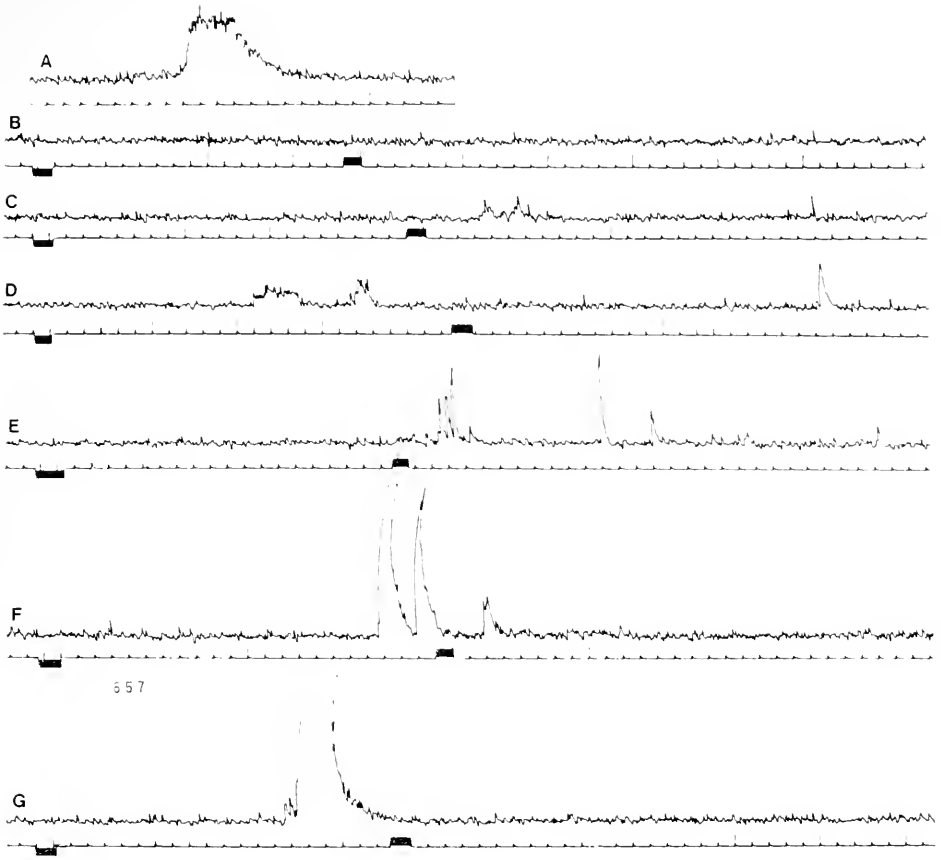

FIGURE 20. Chemically induced bioluminescence in *Renilla* larvae (B–G, see Table II). (A)—KCl induced bioluminescence from a piece of *Obelia sp.* upright containing only one photocyte. The gain is the same for all records. An event marker, superimposed on the time trace (1-second intervals), was used to indicate the beginning (downward mark) and end (upward mark) of the KCl injection. (B)—Newly settled planula; (C)—Settled planula, attached by peduncle; (D)—Juvenile settled for 12 hr; (E)—Tentacle-bud stage; (F)—Pinnule development stage; (G)—Primary polyp. Bioluminescence could not be induced prior to planula settlement.

Settled juveniles luminesce in response to electrical stimulation. Up to the tentacle bud stage (Figs. 21A, B), the voltages required (60 to 80 V/5 ms) result in tissue damage. The responses, however, are similar to those of the KCl tests (Figs. 21A ~ 20C, D; 21B, C ~ 20E; 21D ~ 20F; 21E, F ~ 20G). At the tentacle bud stage and later, the luminescent flashes occur in a 1:1 ratio with stimuli, although in some cases several stimuli are required to initiate the response (Fig. 21C). In subsequent stages, the responses begin with the first or second stimulus (Figs. 21D, E). Stimulus thresholds are on the order of 20 to 40 V/5 ms. Facilitating responses (Fig. 21F), characteristic of adult specimens of *Renilla*, are obtained when the tentacles are fully formed, 320 to 370 hr after spawning

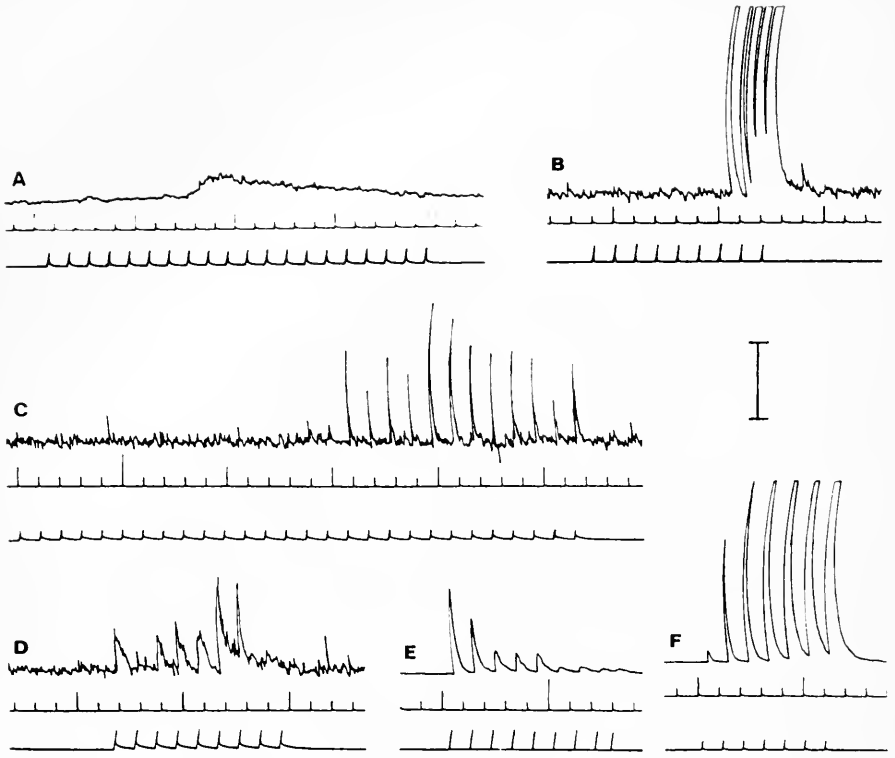

FIGURE 21. Electrically induced bioluminescence in *Renilla* larvae. The top trace represents light output; the center trace, time (1-second intervals); and the bottom trace, stimulus markers (1/s). The scale bar represents the amplitude of the single *Obelia* photocyte luminescent response (Fig. 20A). The gain for traces (A–D) are the same as in Figure 20. The gain for (E) is reduced by 2.5 times, and that for (F) by 5 times. (A)—Settled planula: (B, C)—Tentacle bud stage; (D)—Pinnule development stage; (E, F)—Primary polyp. Bioluminescence responses are initially irregular, eventually following stimuli 1:1. The final stage of maturation of the bioluminescent system is the appearance of facilitating responses (F) similar to those of adult colonies.

(Figs. 18, 19). The stimulus threshold for bioluminescent responses of the mature primary polyp is as low as 5 V/1 ms.

DISCUSSION

The initiating stimuli for spawning in *Renilla köllikeri* are unknown although light seems to be important. Wilson (1883) found that *Renilla reniformis* spawned between 5 AM and 7 AM in the laboratory. Although *Renilla köllikeri* spawned at all times of the day, most spontaneous spawning occurred between 12 noon and 3 PM. By altering the light regime, small spawns could be induced. Released sperm are swept into the female colonies and circulated by the water vascular system, into which the ripe gonads extend. Fertilization is probably internal and perhaps

stimulates female spawning. This behavior could serve to increase the probability of egg fertilization, which would otherwise be jeopardized by the surging currents in which *Renilla* colonies are found (Kastendiek, 1976).

Dispersal of *Renilla* larvae has two phases. The neutral buoyancy of the fertilized eggs allows them to be swept away from the parent colonies. Following loss of neutral bouyancy in the early cleavage stages, the swimming planulae re-enter the water column for 4 to 6 days.

The ciliary swimming behavior of *Renilla* planulae is noteworthy in that a coordinated ciliary reversal or arrest response is lacking. The forward propulsive beat continues regardless of obstacles. A reversal or arrest would require some form of cell-to-cell communication, be it mechanical or bioelectrical. In larvae of many organisms (see Spencer, 1974), as well as in many adult coelenterates (Mackie, 1965, 1975, 1976; Mackie and Passano, 1968; Spencer, 1971, 1975, 1978) impulses are conducted in excitable epithelia. Such a conduction system seems not to be involved in the swimming behavior of *Renilla* planulae unless in the normal swimming metachronism. Intercellular gap junctions could not be found in any of the larval stages of *Renilla* (Satterlie and Case, in preparation).

Muscular activity is first apparent in the swimmer stage, mostly as local responses. The coordinated contractions of late swimmers suggest the presence of a functional conduction system. Both nervous and muscular elements are present by the swimmer stage, and may represent components of this conduction system (Satterlie and Case, in preparation). It is also possible, however, that coordination is due to electrically or mechanically coupled muscle or epithelial cells. Mechanical coupling is counterindicated by the magnesium sensitivity of the muscular responses, which suggests neural control of musculature at this early stage. At least one neural conduction system is apparently present and functional prior to settlement and metamorphosis.

A proper substratum promotes planula settlement. Chia and Crawford (1973) found that sand grain size was not important to the settlement of the pennatulid *Ptilosarcus gurneyi,* but that an organic film on the sand grains was essential to induce settlement. Müller (1973) found that in the hydrozoan *Hydractinia* settlement and metamorphosis is triggered by a lipid produced by marine bacteria. Our findings suggest that a similar situation exists in *Renilla* since settlement of planulae is delayed if clean glass is the only available substratum. As in other octocorals, prevention of attachment prevents or delays metamorphosis. In several xeniid and gorgonian octocorals, delayed settlement increased the number of abnormalities, such as retarded tentacle development (Gohar, 1940b) and axial skeleton formation (von Koch, cited in Gohar, 1940b). Chia and Crawford (1973, 1977) found that planulae of *Ptilosarcus* would continue to swim indefinitely if the proper substratum was not available. Comparing planulae in which settlement was prevented to primary polyps of the same age, they noted significant differences in ultrastructure despite the identical ages of the two groups. Of 9 cell types in the planulae, and 12 cell types in the polyps, only 7 were common (Chia and Crawford, 1977). This reorganization in cellular composition can be attributed to settlement-metamorphosis. Similar settlement-induced changes may parallel the physiological changes which are apparent in the three effector systems

of *Renilla* at the time of peduncle attachment and metamorphosis. The first of these changes is that the ectodermal ciliation becomes inactive and the cilia are apparently resorbed. A second change is that peristaltic movements of the peduncle become intense and a separation can be seen between the muscular activities of peduncle and anthocodium. A separation of conduction systems, into peduncular (future colonial) and anthocodial (future polyp), is therefore suggested. This separation was shown electrophysiologically in adult *Renilla* by Anderson and Case (1975). The metamorphosis-induced changes in neuromuscular organization of *Renilla* larvae probably involve regional differentiation of the planula conduction system since the reactions of the anthocodium and peduncle do not change. Stimuli to either area still produce conducted muscular activity in the stimulated region. During the third change, the juveniles gain the ability to bioluminesce (when about 5½ days old). The green fluorescent protein (see Morin, 1974; Cormier, Hori and Anderson, 1974; Cormier, Lee and Wampler, 1975 for reviews of *Renilla* bioluminescence biochemistry) is produced prior to settlement, and its manufacture is not drastically altered during metamorphosis. The photocytes of the primary polyp are already differentiating prior to settlement, although some morphological changes do occur at this time, such as growth of photocyte processes. The cause of the immediate appearance of bioluminescent competence is not known. The possibility exists that the bioluminescent system was functional prior to settlement, and we were unable to detect the light. However, the multiple animal tests tend to refute this claim. Also, it would be difficult to explain why delays in settlement would also delay the onset of measurable light output.

Colonial bioluminescent responses in adult *Renilla* colonies are controlled by a colonial nerve net (Anderson and Case, 1975; Satterlie, Anderson and Case, 1976). Light emission generally occurs in a 1:1 ratio with stimuli, after the first, and exhibits a facilitation in intensity which is dependent upon interpulse interval (Nicol, 1955a, b). The primary polyps do not exhibit similar responses until tentacle maturation. The sequence of luminescent responses represented in Figures 21C–F could reflect maturation of the neuro-effector hook-ups (within the polyp system), of the conduction systems (colonial and polyp), of the connection between the two conduction systems, or a combination of such developments. Maturation of the polyp neuro-effector system appears to occur between the tentacle bud stage and the tentacled stage, as indicated by the shift from short multiple flashes to "coordinated" broad flashes in the KCl tests. In the latter stage, the presence of a conduction system common to the eight septa, and thus linking the photocyte groups, can be inferred.

The responses of the tentacle-bud stage juveniles are reminiscent of recordings obtained by Nicol (1955a) during bridge experiments on adult *Renilla*. With a small bridge of tissue separating two lobes of the rachis, several stimuli were required before luminous waves were observed in the distal lobe. Interneural facilitation (Pantin, 1935) within the colonial conduction system was proposed as a vehicle for the response. Similar experiments on two other pennatulids, *Stylatula* and *Virgularia*, using electrophysiological recordings of colonial nerve net activity, support this interpretation (Satterlie, Anderson and Case, in prepara-

tion). Immature juvenile conduction systems, or incomplete connections between systems could give rise to local responses. If so, the maturation of the conduction systems or connections, as well as that of the neuro-effector junctions could be represented by a gradual shift to a 1 : 1 stimulus-to-response ratio with a facilitating intensity output (Figs. 21C–F).

There are two colonial conduction systems in adult specimens of *Renilla,* in addition to semi-autonomous polyp conduction systems (Anderson and Case, 1975). We have demonstrated that at least one conduction system is present in the swimming planula prior to settlement. This conduction system mediates muscle activity of the entire larva (future polyp and colony tissue), and may represent the future colonial nerve net. At the time of settlement and metamorphosis the separation between the polyp and peduncular systems that becomes evident probably involves separation and elaboration of the polyp conduction system(s) and formation of colony-polyp connections. In light of the morphological reorganization that occurs in other pennatulids (*Ptilosarcus,* Chia and Crawford, 1977) at metamorphosis, reorganization of conduction systems in *Renilla* is certainly possible. The final stage in development of the neuro-effector systems, the appearance of facilitating responses, probably involves maturation of the neuro-effector junctions themselves. The role of the second colonial conduction systems of adult *Renilla* colonies is uncertain, and this report does not contribute to its elucidation.

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Summary

1. The development of the pennatulid coelenterate *Renilla köllikeri* was followed from fertilized egg to primary polyp stage, including observations on the development of effector responses such as ciliary swimming, muscular reactions and bioluminescence.

2. Ciliary swimming is first apparent in the early planula, 32 hr after the spawn. Ciliary activity persists until settlement, at approximately 130 hr.

3. Muscular reactions are first evident as local contractions in the swimmer stage (about 60 hr). Conducted contractions can be elicited at 80 hr, suggesting that a functional conduction system is present in the planula larva. At settlement, separate peduncular and anthocodial muscular responses and peristaltic contractions are evident.

4. The future photocytes first fluoresce at about 80 hr, and thereafter fluorescence intensity increases until the time of settlement. The first sign of bioluminescence follows settlement, and is delayed if settlement is prevented. Bioluminescent responses do not exhibit normal facilitation until the primary polyp stage, and responses of the preceding stages may reflect maturation of the

colonial and polyp conduction systems as well as of connections between the two systems.

5. Settlement and metamorphosis are delayed when planulae are reared in containers without sand.

LITERATURE CITED

Anderson, J. M., and M. J. Cormier, 1973. Lumisomes, the cellular site of bioluminescence in Coelenterates. *J. Biol. Chem.*, **248**: 2937–2943.

Anderson, P. A. V., and J. F. Case, 1975. Electrical activity associated with luminescence and other colonial behavior in the pennatulid *Renilla köllikeri*. *Biol. Bull.*, **149**: 80–95.

Bonar, D. B., 1978. Morphogenesis at metamorphosis in opisthobranch molluscs. Pages 177–196 in F. S. Chia and M. E. Rice, Eds., *Settlement and Metamorphosis of Marine Invertebrate Larvae*. Elsevier, New York.

Cameron, R. A., and R. T. Hinegardner, 1974. Initiation of metamorphosis in laboratory cultured sea urchins. *Biol. Bull.*, **146**: 335–342.

Cameron, R. A., and R. T. Hinegardner, 1978. Early events in sea urchin metamorphosis, description and analysis. *J. Morphol.*, **157**: 21–32.

Chia, F. S., and B. J. Crawford, 1973. Some observations on gametogenesis, larval development and substrate selection of the sea pen *Ptilosarcus gurneyi*. *Mar. Biol.*, **23**: 73–82.

Chia, F. S., and B. J. Crawford, 1977. Comparative fine structural studies of planulae and primary polyps of identical age of the sea pen *Ptilosarcus gurneyi*. *J. Morphol.*, **151**: 131–157.

Cormier, M. J., K. Hori, and J. M. Anderson, 1974. Bioluminescence in coelenterates. *Biochim. Biophys. Acta*, **346**: 137–164.

Cormier, M. J., J. Lee, and J. E. Wampler, 1975. Bioluminescence: Recent advances. *Annu. Rev. Biochem.*, **44**: 255–272.

Gohar, H. A. F., 1940a. Studies on the Xeniidae of the Red Sea. *Publ. Mar. Biol. Stn. al Gharadqa*, **2**: 25–118.

Gohar, H. A. F., 1940b. The development of some Xeniidae (Alcyonaria). *Publ. Mar. Biol. Stn. al Ghardaqa*, **3**: 27–78.

Kastendiek, J., 1976. Behavior of the sea pansy *Renilla köllikeri* Pfeffer (Coelenterata: Pennatulacea) and its influence on the distribution and biological interactions of the species. *Biol. Bull.*, **151**: 518–537.

Mackie, G. O., 1965. Conduction in the nerve-free epithelia of siphonophores. *Am. Zool.*, **5**: 439–453.

Mackie, G. O., 1975. Neurobiology of *Stomotoca*. II. Pacemakers and conduction pathways. *J. Neurobiol.*, **6**: 357–378.

Mackie, G. O., 1976. Propagated spikes and secretion in a coelenterate glandular epithelium. *J. Gen. Physiol.*, **68**: 313–325.

Mackie, G. O., L. M. Passano, 1968. Epithelial conduction in hydromedusae. *J. Gen. Physiol.*, **52**: 600–621.

Matthews, A., 1916. The development of *Alcyonium digitatum* with some notes on the early colony formation. *Q. J. Microsc. Sci.*, **62**: 43–94.

Morin, J. G., 1974. Coelenterate bioluminescence. Pages 397–438 in L. Muscatine and H. M. Lenhoff, Eds., *Coelenterate Biology*. Academic Press, New York.

Müller, W. A., 1973. Metomorphose-Induktion bei Planulalarven. I. Der bakterielle Induktor. *Wilhelm Roux' Arch. Entwicklungsmech Org.*, **173**: 107–121.

Nicol, J. A. C., 1955a. Observations on luminescence in *Renilla* (Pennatulacea). *J. Exp. Biol.*, **32**: 299–320.

Nicol, J. A. C., 1955b. Nervous regulation of luminescence in the sea pansy *Renilla köllikeri*. *J. Exp. Biol.*, **32**: 619–635.

Pantin, C. F. A., 1935. The nerve net of the Actinozoa. I. Facilitation. *J. Exp. Biol.*, **12**: 119–138.

Parker, G. H., 1920. Activities of colonial animals. II. Neuromuscular movements and phosphorescence in *Renilla*. *J. Exp. Zool.*, **31**: 475–513.

Satterlie, R. A., P. A. V. Anderson, and J. F. Case, 1976. Morphology and electrophysiology

of the through-conducting systems in pennatulid coelenterates. Pages 619–627 in G. O. Mackie, Ed., *Coelenterate Ecology and Behavior.* Plenum Press, New York.

SPENCER, A. N., 1971. Myoid conduction in the siphonophore *Nanomia bijuga. Nature,* **233**: 490–491.

SPENCER, A. N., 1974. Non-nervous conduction in invertebrates and embryos. *Am. Zool.,* **14**: 917–929

SPENCER, A. N., 1975. Behavior and electrical activity in the hydrozoan *Proboscidactyla flavicirrata* (Brandt). II. The medusa. *Biol. Bull.,* **149**: 236–250.

SPENCER, A. N., 1978. Neurobiology of *Polyorchis.* I. Function of effector systems. *J. Neurobiol.,* **9**: 143–157.

WILSON, E. B., 1883. The development of *Renilla. Philos. Trans. R. Soc. Lond. B Biol. Sci.,* **174**: 723–815.

Reference: *Biol. Bull.* **157**: 524–535. (December, 1979)

ON FEEDING MECHANISMS AND CLEARANCE RATES OF MOLLUSCAN VELIGERS

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The teeth of a mammal or the mouthparts of a copepod can tell a knowledgeable biologist much about that animal's feeding habits. Deductions based on the structure of ciliary bands can be at least as useful. This study is part of a larger effort to relate quantitative aspects of ciliary feeding to the morphology of ciliary feeders. Much more extensive biogeographic or taxonomic comparisons of developmental adaptations can be made when feeding capacities of larvae can be predicted from the length of cilia and the lengths of their ciliated bands.

Molluscan veliger larvae possess a lobed velum which produces both feeding and locomotory currents. Those veligers which feed on suspended particles concentrate these particles between two opposed bands of cilia which line the velar edge (Fretter, 1967; Strathmann, Jahn, and Fonseca, 1972; Thompson, 1959; Werner, 1955). The preoral band consists of long compound cilia which produce the swimming and feeding currents. The postoral band consists of shorter cilia which beat towards the preoral band. The combination of the two bands captures and retains particles. Between these bands is a food groove with small cilia which transport particles towards the mouth.

Veligers are tiny feeding machines which convert small eggs into larger juveniles. Parents which produce small eggs can produce large numbers of offspring, but there are costs that limit larval success as larval size is reduced. A reduced feeding capacity is one cost of a decreased larval size. This can take the form of a reduction in the clearance rate (volume of water cleared of particles per unit time) or restriction to a smaller range of particle sizes. In molluscan veliger larvae the clearance rate is likely to be limited by both the length of the velar edge and the length of the preoral cilia (Strathmann, *et al.*, 1972). The size of the particles captured is probably limited by the length of the preoral cilia.

Here we are testing the hypothesis that longer preoral cilia contribute to higher clearance rates. The test consists of comparative observations with high speed microcinephotography of movements of the preoral cilia, the postoral cilia and particles captured at the velar edge. Clearance rates are calculated from these measurements. We used three species whose veligers have different lengths of preoral cilia: the oyster *Crassostrea gigas,* the nudibranch *Tritonia diomedea* and the mud snail *Nassarius obsoletus.* Movements of cilia and particles, but no particle captures, were also observed for a veliger of an unidentified species of prosobranch gastropod. Our cinefilms of feeding larvae also have extended previous interpretations of the veliger feeding mechanism.

Methods

Oyster (*Crassostrea gigas*) gametes were obtained by placing adults in sea water at 30° C until spawning occurred (3 to 4 hrs). The eggs were fertilized at ambient sea water temperatures (12 to 16° C). Larvae of *T. diomedea* and *N. obsoletus* were obtained from eggs laid in laboratory aquaria. The unidentified prosobranch veliger was taken from the plankton in Friday Harbor. All larvae were reared in culture dishes in the laboratory at 12 to 16° C and were fed the green flagellate *Dunaliella tertiolecta*.

Test subjects were starved for 24 hr before being filmed to promote maximum feeding rates during filming. Coverglasses were supported by plasticene feet just low enough to impede larval swimming and high enough not to impede velar cilia in the plane of focus. The larvae were usually filmed with plane of focus perpendicular to the velar edge, as in Figure 4, for velocities of cilia and particles. In some cases larvae were filmed with plane of focus parallel to the velar edge for metachronal wavelength.

Larvae were filmed with Nomarski differential interference optics with 16 or 40× objectives, a high speed cinecamera at 100 or 200 frames per second, and continuous light. A timing light exposed the margin of the film every 0.01 seconds and is accurate to ±1% according to its manufacturer, the Redlake Corporation. Temperatures were maintained at 12° to 13° C with a Cloney cooling stage (Cloney, Schaadt, and Durdeen, 1970) except for some sequences of *C. gigas* filmed at 20° to 22° C.

Particles used for feeding observations were 2-μm plastic spheres and the flagellates *Dunaliella tertiolecta* and *Monochrysis lutheri* (5 to 10-μm). We observed only one capture of a plastic sphere so the data on captures apply to the flagellates. High concentrations of particles were used to insure some captures in the plane of focus during high speed filming. Since particles are rapidly removed from suspension by the larvae and also settle in the confined space on a slide, particle concentrations could not be accurately determined. Therefore no cell counts were made.

Tracings of the films were made frame by frame for cilium and particle paths. All velocities were calculated from the tracings. The fraction of particles captured was calculated using all particles passing within reach of the preoral cilia from the beginning to the end of several filmed sequences.

Table I

Species	Egg Diameter (μm)	Shell length at start of feeding (μm)	Preoral cilium length (μm)
Crassostrea gigas	45–50	90*	30
Tritonia diomedea	90	145*	40
Nassarius obsoletus	165*	270*	70

* Data from Costello, Davidson, Eggers, Fox, and Henley (1957), Kempf & Willows (1977), Quayle (1969), Scheltema (1967).

Results

We assume that the veligers are actively feeding whenever numerous particles are passing around the food groove to the mouth. When this occurs both the preoral and postoral bands are beating. Occasionally both bands would cease beating but we did not observe the preoral band beating while the postoral band stopped. *Crassostrea gigas, Tritonia diomedea,* and *Nassarius obsoletus* are consistent with our impression that the size of veligers and the length of preoral cilia increase with egg size (Table I).

Velocities of cilia and particles

Among species, the velocities of the tips of the preoral cilia in the effective stroke increase with the length of cilium (Fig. 1). It is difficult to see the bases of cilia in many filmed sequences, so radius of arc was estimated by projecting the straight section of a cilium in successive frames back to a point of intersection. For each species the mean estimated radius of arc is greater than the lengths of those cilia which could be measured accurately. The velocities are taken from the maximum movement observed between two successive frames during the effective stroke.

Angular velocities are more convenient for comparisons among species and calculation of clearance rates. The angular velocities plotted in Figure 2 are calculated by dividing the tip velocity by the radius of arc. For cilia which are held

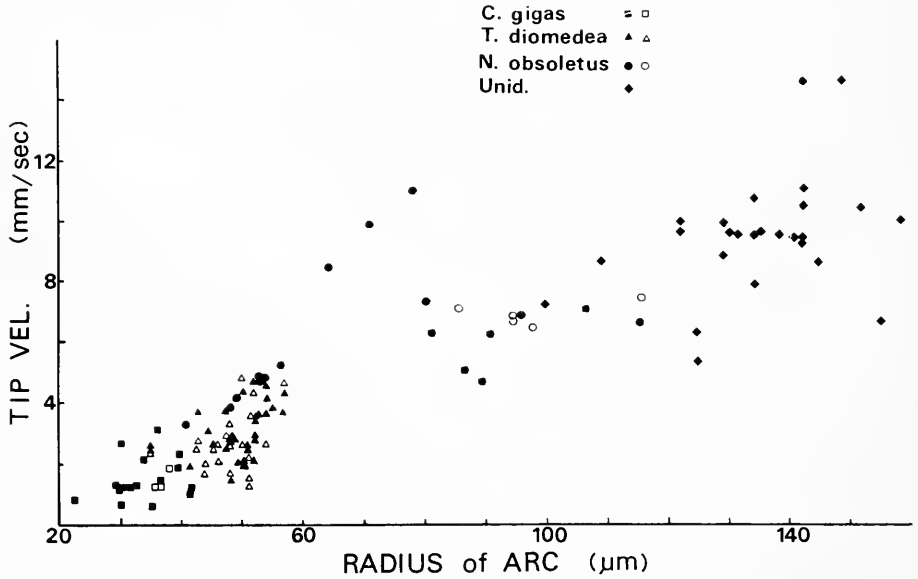

Figure 1. Maximum observed tip velocities of preoral cilia during their effective strokes versus radius of arc (estimate of cilium length) for veligers of *Crassostrea gigas, Tritonia diomedea, Nassarius obsoletus* and an unidentified prosobranch. Open circles are effective strokes associated with particle captures; solid circles with no capture.

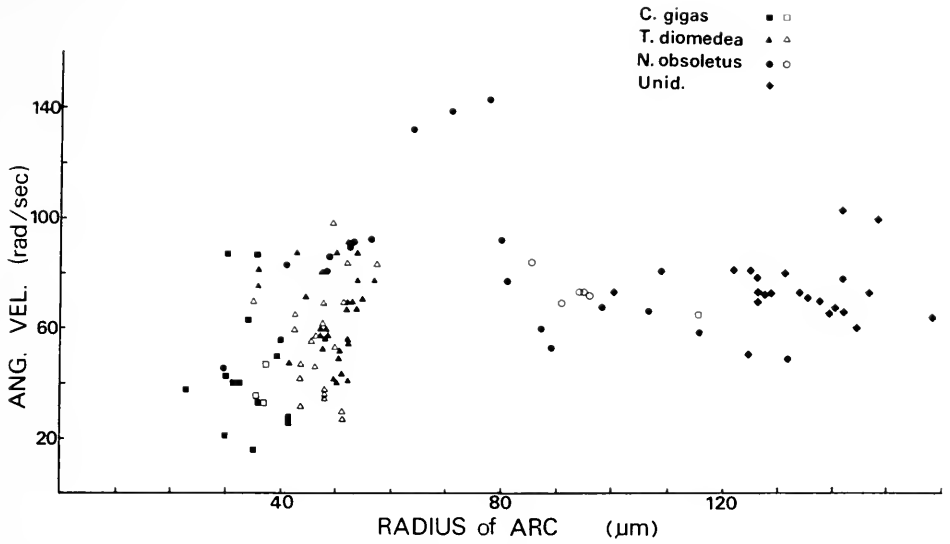

FIGURE 2. Maximum observed angular velocities of preoral cilia during their effective strokes (same larvae as in Figure 1). Open circles are effective strokes associated with particle captures; solid circles with no capture.

straight during the effective stroke, the angular velocity is constant over almost the entire length of the cilium. Angular velocity may increase with cilium length (see below) but the trend is less striking than the increase of tip velocity with the length of the cilium (Fig. 1).

One-way analysis of variance of the angular velocities of the four species indicates significant differences among the species (Table II). A multiple comparison among species indicates that the species fall into three significantly different groups: *C. gigas* with the lowest mean angular velocity, *T. diomedea* in

TABLE II

Comparisons among means for species. Ho: Means equal; reject if $P < 0.001$

Species	Mean angular velocity (radians/sec)	(n)	Mean ratio of velocities of cilium and particle (C/P)	Range of (C/P)	(n)
Crassostrea gigas	32	(19)	1.6	1.1–2.1	(6)
Tritonia diomedea	54	(51)	2.0	1.1–3.0	(36)
Nassarius obsoletus	70	(25)	1.5	1.0–2.4	(20)
Unidentified	63	(24)	1.5	1.1–1.9	(21)
	Reject Ho (one way Anova) $P < 0.001$		Reject Ho (Kruskal-Wallis test) $P < 0.001$		

Groups by Student-Newman Keuls multiple comparison (reject Ho if $P < 0.01$): significantly different groups are (1) *C. gigas*, (2) *T. diomedea*, and (3) *N. obsoletus* and unidentified veliger.

TABLE III

Two sample rank tests for C/P (ratio velocities of cilium and particle) and W (angular velocity of cilium) Ho: Groups same; reject Ho if P < 0.05. Ho accepted for all tests (Mann-Whitney U, two tailed).

Species	Comparison	(n_1, n_2)	*P*
Crassostrea gigas	W at 12° C and 20° C	(24, 23)	>0.20
	W for captures and misses	(3, 3)	>0.20
	C/P for captures and misses	(3, 3)	>0.20
Tritonia diomedea	W for captures and misses	(19, 17)	>0.20
	C/P for captures and misses	(19, 17)	>0.20
Nassarius obsoletus	W for captures and misses	(5, 16)	>0.20
	C/P for captures and misses	(5, 16)	>0.05
	C/P for algae and 2 μm spheres	(12, 9)	>0.20
Unidentified	C/P for algae and 2 μm spheres	(16, 5)	>0.20

the middle, and *N. obsoletus* and the unidentified veliger with the highest mean angular velocities (Table II). Preoral cilium length increases along with the angular velocity in these three groups. Thus among species angular velocities of preoral cilia may increase with the lengths of preoral cilia, but more species must be examined to confirm this trend. The observed differences in angular velocities may be associated with the species or the taxonomic order rather than the lengths of the preoral cilia. Also, the mean angular velocity of *N. obsoletus* is greater than for the unidentified prosobranch veliger which has much longer preoral cilia. The implications of the anomalously lower angular velocity in the unidentified veliger are not obvious because this veliger was not observed to catch particles. It is nevertheless clear from these data that the small veligers are not compensating for the shorter length of preoral cilia with increased angular velocities.

Temperature could affect the rate of beat of cilia but we did not observe this. The mean angular velocity of the preoral cilia of *C. gigas* at about 20° C is only slightly greater than at 12 to 13° C, and the difference is not significant (Table III).

The preoral cilia in their effective strokes move one to three times faster than the nearby particles. We could not demonstrate that the ratio of cilium velocity to particle velocity (C/P) aries with the angular velocity of the cilium or the type of particle (Tables III, IV). In *T. diomedea* particle velocities increase and then decrease with the distance from the base of preoral cilium (Fig. 3). The velocities were measured for particles near the middle of the effective stroke of the preoral cilia. Since velocity of the cilium increases from the base to the tip, the ratio of cilium to particle velocity should increase near the tip, but we cannot demonstrate this (Table IV, C/P versus D). Because we cannot show the existence of these possible confounding factors, we have lumped all observations on C/P for each species in a test for differences among species. Differences in C/P among species are highly significant (Table II), but the cause of the difference is not clear. The species with a substantially different ratio of cilium velocity to particle velocity is *T. diomedea*, with a mean ratio of 2 as opposed to about 1.5 for

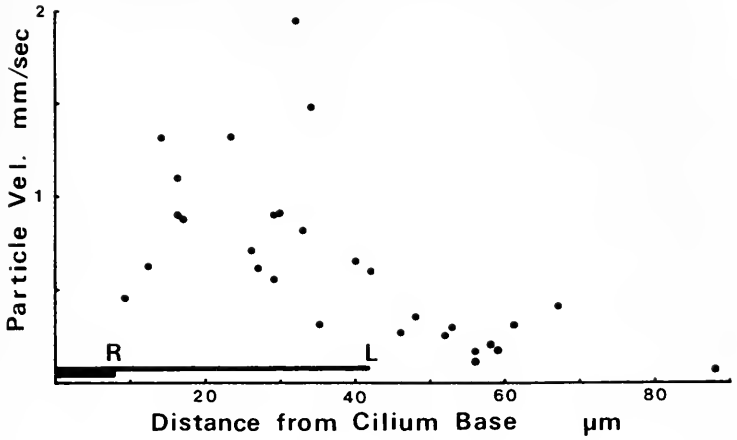

FIGURE 3. Particle velocity versus distance from base of preoral cilium for veliger of *Tritonia diomedea*. L = length of preoral cilium. R = portion blocked by recovery stroke.

the others. The preoral cilia of *T. diomedea* are of intermediate length. We cannot show that the ratio of cilium velocity to particle velocity changes in a regular way with cilium length, cilium speed, or food particle. Therefore, longer preoral cilia, which have angular velocities greater than or equal to those of shorter cilia, move more particles and presumably more water past the preoral band of cilia.

Clearance rate

The volume of water moving through the preoral band can be estimated from the above data. Our estimate combines the angular velocity (W), the ratio of cilium velocity to particle velocity (C/P), the length of the preoral cilium (L), and a correction for the part of the current blocked by the recovery stroke (R). Our reasoning is as follows. The pie-shaped area within an arc of one radian with

TABLE IV

Regression lines for C/P (ratio of velocities of cilium and particles) against W (angular velocity) or D (distance toward base from tip of cilium) Ho: slope equals 0; reject Ho if $P < 0.05$ (t-test, two tailed).

Species	Comparison	(n)	P
Crassostrea gigas	C/P versus W	(6)	> 0.50
	C/P versus D	(6)	$0.10 > P > 0.05$
Tritonia diomedea	C/P versus W	(36)	> 0.50
	C/P versus D	(36)	> 0.50
Nassarius obsoletus	C/P versus W	(21)	$0.50 > P > 0.20$
	C/P versus D	(21)	$0.10 > P > 0.05$
Unidentified	C/P versus W	(21)	$0.50 > P > 0.20$
	C/P versus D	(21)	$0.50 > P > 0.20$

TABLE V

Species	L preoral cilium length (μm)	R recovery stroke correction (μm)	V = $(L^2 - R^2)WP/2C$ volume through preoral band (μm³/sec μm)	F fraction caught out of n particles	(n)	FV clearance rates (μm³/sec μm)
Crassostrea gigas	30	9	8,200	0.44	(13)	3,600
Tritonia diomedea	40	9	21,000	0.24	(36)	4,900
Nassarius obsoletus	70	9	110,000	0.15	(23)	17,000
Unidentified	100	9	210,000	—	—	—

radius equal to L is $L^2/2$. Subtracting the smaller pie-shaped area blocked by the recovery stroke gives $(L^2\text{-}R^2)/2$. We assume that particle velocity equals water velocity, so this pie-shaped area times the angular velocity times a unit length of the velar edge divided by the ratio of cilium to particle velocities gives the volume of water passing a unit length of the preoral band per unit time as $(L^2\text{-}R^2)WP/2C$. This volume increases with the length of the preoral cilia (Table V).

This estimate includes only the water out to the tips of the cilia and so does not include all the water moved past the velar edge. Three sources of bias may enter this estimate of water flow. First, our measured radius of arc exceeds the length of the cilia, so estimates of angular velocity may be low. Second, we assume a constant ratio of cilium velocity to particle velocity along the cilium, whereas this ratio may increase near the tip. Third, some cilia may have been pushing particles faster than the water. The first underestimates water flow; the second and third overestimate it. For the unidentified prosobranch veliger there is a fourth source of bias. Tips of its preoral cilia are often still bent in the recovery stroke as the base begins the effective stroke, so the effective length of the preoral cilium is less than indicated. The value of R is about the same for all species, as diagrammed in Figure 4, but could not be measured to the nearest μm. Also, R varies around the arc of the effective stroke. Despite these qualifications, the calculation gives an indication of the effectiveness of the cilia in moving water.

To convert this volume per time to an estimate of clearance rate, it is necessary to multiply by the fraction of passing particles which are actually captured. Particles further from the base of the cilia are less likely to be captured, but there is no sharply defined point beyond which no particles are captured and within which all are captured. In Figure 4 velar edges of three species are diagrammed to the same scale. The dashed lines indicate approximate paths of some particles, although the paths vary considerably even at the same distance from the base of the cilia. The films show no captures beyond the outer dashed line. Almost all the particles within the inner dashed line are captured. Particles also tend to travel across the arc described by a cilium. An effective length of cilium is therefore not readily defined.

To obtain the fraction of passing particles which are captured, we recorded the total number of particles passing the preoral band in a filmed sequence of an actively feeding veliger for each species and noted the fraction captured (F, Table V). The sample of captures is small but the sample confirms our impression that the longer preoral cilia are less efficient at removing particles of this size from suspen-

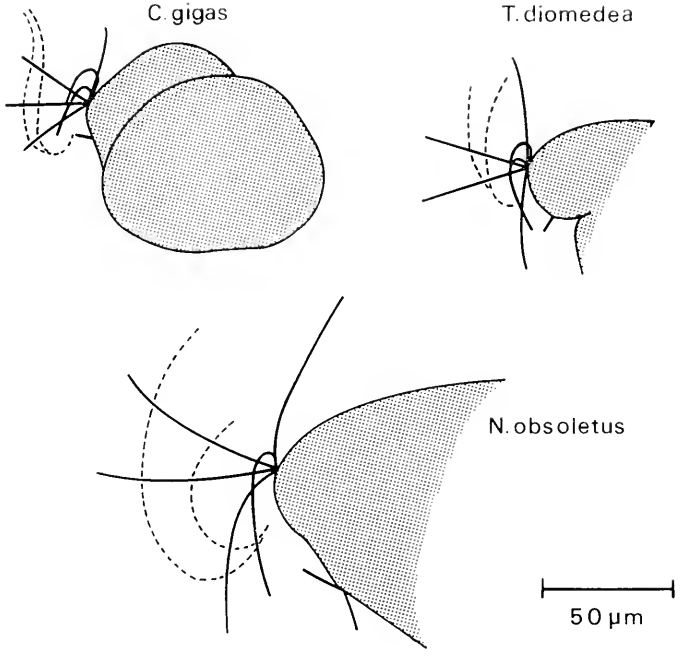

FIGURE 4. Optical section across the velar edge diagrammed for three species to same scale. Preoral and postoral cilia indicated but food groove cilia omitted. Preoral cilium shown in several positions. Dashed lines mark two particle paths (see text).

sion. The clearance rate is estimated by multiplying the volume passing the cilia by the fraction of particles cleared. Though longer cilia are less efficient, there is a definite gain in rate of clearance per unit length of ciliated band with longer preoral cilia.

Mechanisms of concentrating particles

If particles are to be concentrated, they must be moved relative to the water. We have no means of observing water movement in this study, but the observed motion of cilia and particle gives some information on the mechanism by which particles are concentrated. Several combinations of events could occur during concentration: the particles could move faster or slower than the water by either adhering to the cilia or being sieved by two adjacent cilia in their effective strokes. Our observations indicate that more than one mechanism of concentration could be operating.

The simplest hypothesis for concentration is that preoral cilia push adhering particles faster than the water during the effective stroke. In many captures, a preoral cilium overtakes a particle and then moves along next to it. This motion is consistent with the hypothesis that the cilium is pushing an adhering particle faster than the water. Captures occur in which no cilia are observed to touch the particles, but a capturing cilium may have been out of focus.

Particles reverse their direction and move from posterior to anterior into the food groove beneath the preoral cilia in the recovery stroke (Fig. 4). It seems unlikely that the particles are concentrated when they are held or moved against a posterior current here, but we cannot rule out the possibility.

Particles might also be concentrated by a sieve formed by adjacent cilia in their effective strokes. In four captures by the *Tritonia diomedea* veligers two cilia in their effective strokes were separated by an angle of less than 11°. The pair of cilia move next to a particle during part of the effective stroke and could be pushing it faster than the water. Since the gap between the cilia is less than the diameter of the particle, the pair of cilia may be forming a sieve. Because the depth of focus is small, these pairs of cilia may be immediate neighbors. Captures of this sort were not observed with veligers of *Crassostrea gigas* and *Nassarius obsoletus*.

Pairs of cilia separated by a small angle are not visible in most capture sequences. Adjacent preoral cilia are often separated by a large angle in their effective strokes. In such cases the metachronal wavelength rather than the distance between neighboring cilia must set the pore size of the sieve. The preoral cilia of *Nassarius obsoletus* were filmed in a plane perpendicular to the plane of the effective stroke as well as parallel to it. The gap between preoral cilia in the same position of the effective stroke exceeded 20 μm in most instances, whereas these veligers capture particles of a diameter less than 10 μm. For veligers of this size the sieve formed by the metachronal wave is only effective for rather large particles, if it is effective at all.

Another hypothesis is that particles are retained by a sieve composed of both the preoral and postoral cilia in the latter part of their effective strokes. Under this hypothesis water in the current generated by the effective strokes would be squeezed out past the cilia of both bands at then end of the effective strokes when the tips of the opposing cilia are close together. We observed a postoral cilium tip meeting a preoral cilium tip in only one of the frames examined. Observed closest approach between preoral and postoral cilium tips in *N. obsoletus* is about 15 μm, which is about twice the diameter of the captured particles. The actual distances may be somewhat less, however, because the closest approach can fall in the interval between frames of the film.

The films therefore indicate that the spacing of cilia does not set the minimum size for captured particles and that other mechanisms must be operating either in addition to such sieving or instead of it. The hypothesis of weak adhesion of cilia and particles is consistent with observed movements during captures; the lower frequency of capture of particles nearer cilium tips where differences in velocities are greatest; and the lower efficiency of capture with longer preoral cilia of equal or higher angular velocities. However, direct evidence for adhesion is still lacking.

The role of the postoral cilia in capturing particles is not clear. Occasionally particles enter the food groove past the postoral band but most captured particles do not follow this route. Yet Strathmann *et al.* (1972) found that captures by a serpulid trochophore ceased when the postoral band ceased beating but the preoral band continued beating. Possibly the postoral band is necessary for retaining particles but does not aid in concentrating them. Captured particles reverse their direction of motion where the preoral cilia end their effective stroke. The captured

particles then move anteriorly into the food groove beneath the preoral cilia in their recovery stroke. The recovery strokes of the preoral cilia may be insufficient by themselves to carry the particles into the food groove and keep them there. The current from the postoral cilia may help retain particles during capture and subsequent transport toward the mouth.

Discussion

Our studies of three species of veliger larvae suggest that shorter preoral cilia clear particles from the water current more efficiently than longer preoral cilia but produce lower clearance rates per unit length of velar edge. This lower clearance rate is the result of both shorter cilia and lower angular velocities. However, larvae with longer preoral cilia may capture small particles less efficiently than large particles. Our observations with small flagellates may therefore underestimate the advantages associated with longer preoral cilia. We are not aware of any studies comparing length of preoral cilia to egg size in species of veligers, but our impression, from past casual observations and the three species reared from eggs in this study, is that veligers from larger eggs tend to have longer preoral cilia when they begin feeding. Our tentative conclusion is that veligers from smaller eggs have both a shorter velar edge and a lower clearance per unit length of velar edge, so their maximum clearance rates are lower.

The techniques of this study could be extended to more species and a greater range of sizes of food particles. A broader comparison could establish quantitative relationships between easily measured traits (cilium length and length of velar edge) and feeding capabilities (maximum clearance rate and efficiency of clearance of particles of different sizes). This would permit comparisons of feeding capabilities of larvae in numerous species which would test hypotheses on costs associated with small egg size and geographic shifts in developmental strategies.

Strathmann *et al.* (1972), using Harris' (1961) argument, speculate that with preoral cilia, which are each composed of a bundle of numerous individual cilia, cilium length could vary independently of cilium velocity. There seems to be no physical necessity for higher angular velocities with longer preoral cilia. Observations on more species are therefore needed to establish the trend found with the species studied here.

Rubenstein and Koehl (1977) have categorized the mechanisms by which suspension feeders concentrate food particles. Our observations of veligers indicate direct interception of a particle by a fiber, with the preoral cilium as the fiber. Our best guess from these limited observations is that preoral cilia overtake the particles in the latter part of the effective stroke and weakly adhere to them, pushing them faster than the water. Possibly two preoral cilia sometimes act together as a sieve. The postoral cilia probably help retain particles but do not necessarily play an important role in concentrating particles.

The veligers in this study were maintained at comfortable temperatures during filming, but they were confined on a microscope slide, exposed to bright light, and fed unnaturally high concentrations of particles. They could have reduced their rate of clearance by some means which was not detected. Bayne (1976) calculates a clearance rate of 10,000 to 15,000 μm^3/sec per μm of velar edge for the veliconcha

of *Mytilus edulis*. This value is based on measurement of the velum and on clearance rates calculated from rates of ingestion by unconfined veligers in a known concentration of *Isochrysis galbana*. Bayne's values fall between those determined here for *T. diomedea* and *N. obsoletus*. The shell length of 250-μm for the *M. edulis* veliconcha falls between those of early stage *T. diomedea* and *N. obsoletus*, so Bayne's values appear to be in close argeement with our estimates, although the length of preoral cilia of *M. edulis* veligers at this stage is not given. In the worst case, if the preoral cilia of the *M. edulis* veliconcha are about the same as in the early stage *C. gigas* veligers, then our estimates of clearance rates could be one third to one half the maximum rate found by Bayne. In either case, Bayne's result indicates that our values are not far from maximum clearance rates for unconfined veligers.

Strathmann (1971) calculated clearance rates of 5000 to 10,000 μm^3/sec per μm of ciliated band for echinoderm larvae feeding on *Amphidinium carteri*. This flagellate is somewhat larger than *Monochrysis lutheri*. The cilia of these larvae are shorter than the preoral cilia of *C. gigas* veligers, and the feeding mechanism is different, but the rates are in rough agreement with those determined here. As in Bayne's study, the estimate for the echinoderm larvae is based on ingestion rates of unconfined larvae.

Ratios of preoral cilium velocity to particle velocity in veligers are similar to ratios reported by Sleigh and Aiello (1972), who found ratios of about 1.6, 3.3, and 4.0 near the cilium tips of *Pleurobrachia pileus* comb plates, *Mytilus edulis* gill lateral cilia, and *Stentor polymorphus* membranelles. The plot of particle velocity against distance from cilium base for the veliger of *T. diomedea* is similar to the plots of Sleigh and Aiello for *Stentor* and *Pleurobrachia*, except that the velocities decrease more abruptly beyond the tips of the *T. diomedea* preoral cilia.

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Summary

1. Beat of preoral cilia and particle paths were filmed for veligers of *Crassostrea gigas, Tritonia diomedea, Nassarius obsoletus* and an unidentified prosobranch. Particle captures were filmed for the three identified species.

2. Clearance rates per unit length of velar edge are estimated from the equation $(L^2\text{-}R^2)WPF/2C$, where L is cilium length, R a correction for recovery stroke, W angular velocity, C/P the ratio of velocities of cilium and particle, and F the fraction of particles captured. The clearance rates are in rough agreement with Bayne's values for veligers of *Mytilus edulis*.

3. In the three identified species, longer preoral cilia clear particles at a higher rate but with less efficiency. Since veligers from larger eggs generally have both longer preoral cilia and a longer velar edge, a larger egg generally produces a veliger with a higher maximum clearance rate when the veliger begins to feed.

4. Angular velocities increase with cilium length in the three identified species of veligers but the larger unidentified species did not continue this trend.

5. Preoral cilia in their effective strokes move 1 to 3 times faster than particles travelling in about the same arc with a mean of about 1.5 times the speed of the particles. In mid effective stroke, the ratio of velocities of cilia and particles is not significantly different for captured and non-captured particles, nor does the ratio vary significantly with angular velocity of cilium. The ratio does vary significantly among species.

6. Particles passing closer to the base of the preoral cilia are more likely to be captured.

7. We hypothesize that suspended particles are concentrated when they are overtaken by preoral cilia in their effective stroke, weakly adhere to the preoral cilia, and are pushed faster than the water. Capture is completed when particles are drawn into the food groove, probably by the action of the recovery stroke of preoral cilia, the current from postoral cilia, or both.

LITERATURE CITED

Bayne, B. L., 1976. The biology of mussel larvae. Pages 81–120 in B. L. Bayne, Ed., *Marine Mussels: their Ecology and Physiology*, Cambridge University Press, Cambridge, London.

Cloney, R. A., J. Schaadt, and J. V. Durdeen, 1970. Thermoelectric cooling stage for the compound microscope. *Acta Zool (Stockh.)*, **51**: 95–98.

Costello, D. P., M. E. Davidson, A. Eggers, M. H. Fox, and C. Henley, 1957. *Methods for Obtaining and Handling Marine Eggs and Embryos*. Marine Biological Laboratory, Woods Hole, Massachusetts, 247 pp.

Fretter, V., 1967. The prosobranch veliger. *Proc. Malacol. Soc. Lond.*, **37**: 357–366.

Harris, J. E., 1961. The mechanics of ciliary movement. Pages 22–36 in J. A. Ramsey, V. B. Wigglesworth, Eds., *The Cell and the Organism*. Cambridge University Press, Cambridge, England.

Kempf, S. C., and A. O. D. Willows, 1977. Laboratory culture of the nudibranch *Tritonia diomedea* Bergh (Tritoniidae: Opisthobranchia) and some aspects of its behavioral development. *J. Exp. Mar. Biol. Ecol.*, **30**: 261–276.

Quayle, D. B., 1969. Pacific oyster culture in British Columbia. *Bull. Fish. Res. Bd. Can.* **169**: 1–192.

Rubenstein, D. I., and M. A. R. Koehl, 1977. The mechanisms of filter feeding: some theoretical considerations. *Am. Nat.*, **111**: 981–994.

Scheltema, R. S., 1967. The relationship of temperature to larval development of *Nassarius obsoletus* (Gastropoda). *Biol. Bull.*, **132**: 253–265.

Sleigh, M. A., and E. Aiello, 1972. The movement of water by cilia. *Acta Protozool.*, **11**: 265–278.

Strathmann, R. R., 1971. The feeding behavior of planktotrophic echinoderm larvae: mechanisms, regulation, and rates of suspension feeding. *J. Exp. Mar. Biol. Ecol.*, **6**: 109–160.

Strathmann, R. R., T. L. Jahn, and J. R. C. Fonseca, 1972. Suspension feeding by marine invertebrate larvae: clearance of particles by ciliated bands of a rotifer, pluteus, and trochophore. *Biol. Bull.*, **142**: 508–519.

Thompson, T. E., 1959. Feeding in nudibranch larvae. *J. Mar. Biol. Assoc. U.K.*, **38**: 239–248.

Werner, B., 1955. Über die Anatomie, die Entwicklung und Biologie des Veligers und der Veliconcha von *Crepidula fornicata* L. (Gastropoda Prosobranchia). *Helgoländ. Wiss. Meeresunters.*, **5**: 169–217.

Reference: *Biol. Bull.* 157: 536–547. (December, 1979)

IRREVERSIBLE NONGENETIC TEMPERATURE ADAPTATION OF OXYGEN UPTAKE IN CLONES OF THE SEA ANEMONE *HALIPLANELLA LUCIAE* (VERRILL)

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For many years physiological differences have been observed among latitudinally separated populations of the same species (Bullock, 1955; Prosser, 1955; Vernberg, 1962). These differences have been attributed to a variety of phenotypic adaptations and also to genetic variation.

One of the earliest examples of physiological variation was the difference in acutely measured bell pulsation rates of the jellyfish *Aurelia aurita* collected from Nova Scotia and Florida (Mayer, 1914), the direction of which agreed with Krogh's (1916) prediction of increased rates in cold-adapted animals compared to warm-adapted animals, when the rates are measured at a common temperature. More recent studies of oxygen uptake as well as motor activity in cnidarians confirmed the existence of distinct responses in latitudinally separated populations within a species (Sassaman and Mangum, 1970; Mangum, Oakes, and Shick, 1972; Shick, 1976).

In many examples of intraspecific variation, latitudinal differences disappear with acclimation to common conditions, indicating their phenotypic origin. In several studies of latitudinally separated populations however, the differences persist despite prolonged exposure to common conditions, which has been interpreted alternatively as genetic divergence and "irreversible nongenetic adaption" (Kinne, 1962). There are numerous examples of variation which is highly likely to have a genetic basis, *e.g.* interspecific variation (Vernberg, 1962). The possibility of nongenetic but irreversible adaptation within species is less certain.

Evidence of this phenomenon in *Drosophila subobscura* was presented by Smith (1956), who induced variation in heat tolerance among groups of flies raised at different temperatures. The differences, which did not disappear completely upon acclimation to a common temperature, were adaptive in character; flies developing at the higher temperature survived longer at an upper lethal limit. However, selection, operating during development, could conceivably explain these results. Kinne (1962) reported that fish developed from eggs which had been transferred to a different salinity 3 to 6 hr after spawning exhibit lower food conversion efficiencies relative to controls developed from eggs which remained in the spawning salinity. He does not, however, compare food conversion efficiencies of fish acclimated to a common salinity which had developed at different salinities. It is not clear, therefore, whether the observed differences in food conversion efficiencies are reversible upon acclimation to common conditions. Bradley (1978) showed that copepods raised at 20° C are more tolerant of high temperatures than those raised at 10° C. However, these results could have arisen from selection during rearing or to a thermal acclimation that requires more than two days' exposure to a common

temperature to disappear. Schneider (1968) concluded that differences in acclimated oxygen uptake rates among populations of the crab *Rhithropanopeus harrisi* can be reversed only in part by breeding the crabs at a common temperature, but the full report of these data has not appeared.

The mode of reproduction in the actinian *Haliplanella luciae* (Verrill) offers an interesting approach to the question of nongenetic adaptation which is irreversible within a period of prolonged acclimation to common conditions. This species reproduces largely by asexual mechanisms, longitudinal fission being the most common (Shick and Lamb, 1977). The fission process in *Haliplanella luciae* and the effects of temperature and other environmental factors on it have been studied in detail by several authors (Torey and Mery, 1904; Davis, 1919; Uchida, 1932; Miyawaki, 1952; Minasian, 1976). Longitudinal fission can be induced in the laboratory by storing the animal at 10° C or below for 6 to 8 weeks, followed by raising the temperature above 10° C (Sassaman, personal communication). This treatment apparently simulates increasing water temperatures during spring. After fission the progeny regenerate tissues to close the body wall torn in the process. Thus isogenic clones of animals can be raised under different environmental conditions and later compared, virtually eliminating the possibility of genetic variability among the experimental animals.

In the present study, three generations of isogenic anemones were reared at two temperatures, and their rates of oxygen uptake compared after acclimation to common thermal conditions. A few observations were also made on the electrophoretic banding patterns of five enzymes and on the dimensions of the gas exchange surface.

Materials and Methods

Collection, maintenance of animals and experimental design

Specimens of *Haliplanella luciae* (Verrill) were collected from the York River estuary (15 to 21‰) at the mouth of Indian Field Creek, Virginia in January 1977 (10° C). Included in this sample were animals of Uchida's (1932) color types 1 and 3, indicating genetic heterogeneity in the population. Assuming all animals to be genetically unique, single individuals were placed in 30-ml plastic beakers filled with York River estuary water. One half of the animals were placed at 28° C and the remainder stored at 18° C. After approximately 2 weeks the animals used at both temperatures had undergone fission, each original individual producing two or more progeny (clonemates). When regeneration was complete, the animals were transferred to 5° C for 6 to 8 weeks. At the end of this period, the clonemates were separated and allowed to undergo fission and subsequent formation of new tissue at different developmental temperatures: one member of each clone was randomly chosen and placed at 18° C and its clonemate was placed at 28° C. After fission and regeneration, the second generation clonemates were separated, stored at 5° C for 6 to 8 weeks and then returned to their developmental temperature, where a third generation was produced. Thus there are four groups of animals according to developmental temperature and the number of generations produced at a given temperature: two consist of animals taken from the field that

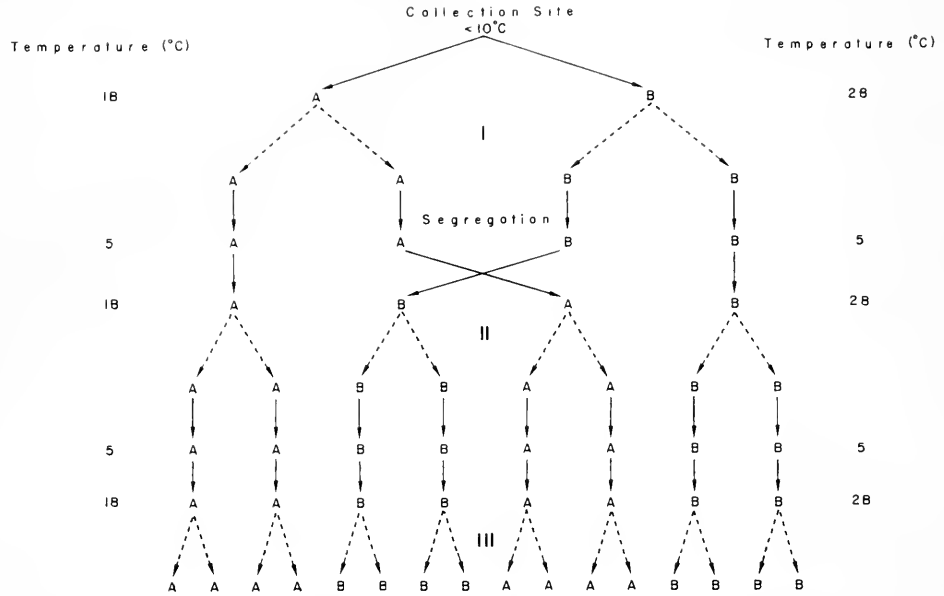

FIGURE 1. Cloning of *H. luciae* at 18° and 28° C. The example shows two animals (A and B) assumed to be genetically unique. Solid arrows indicate transfer from one temperature to another; dotted arrows indicate fission and regeneration. Roman numerals indicate generation.

divided and formed new tissue three times at either 18° or 28° C; one consists of animals that divided first at 18° C and a second and third time at 28° C, and one of the animals that divided a second and third time at 18° C. Because of the difference in the number of fissions at each developmental temperature (Fig. 1), a control was necessary to permit pooling the data for all clones at a particular developmental temperature. The oxygen uptake rates for animals which divided twice at a developmental temperature were compared with those for animals which had divided three times at that developmental temperature. The important aspect of the experimental design is, however, that each individual has at least one clonemate treated in the same way but given the alternative thermal regime (Fig. 1), and that each of the four experimental groups contains members of the same clones, in the same proportions. Thus the rearing procedures cannot have permitted selection.

Development and growth occurred at the specified temperature in York River estuary water (15 to 21‰, with members of all groups given the same salinity regime). At least 4 days prior to the measurement of oxygen uptake, the salinity was brought to 18‰. Animals were fed an excess of freshly hatched *Artemia* nauplii every other day.

Oxygen uptake measurements

Oxygen uptake ($\dot{V}_{O_2}$) of the third generation clonemates was measured at the developmental temperature (TD), the same as the acclimation temperature

(TA). These data are designated TD = 18° C = TA and TD = 28° C = TA. Other, but isogenic, individuals were transferred to the alternative temperature, held there for 2 to 4 weeks and oxygen uptake measured. These data are designated TD = 18° C, TA = 28° C, and TD = 28° C, TA = 18° C. All measurements were made in millipore filtered water at 18‰ salinity by adjusting the concentration of natural York River estuary water (15 to 21‰) with distilled water or commercial sea salt (Dayno Corp). Animals were starved for 60 to 84 hr before an experiment, and transferred to the respirometry chambers the night before the measurement to permit attachment to the wall of the chamber. Oxygen depletion was measured with a Yellow Springs Instrument Co. Model 53 Biological Oxygen Monitor, using a high sensitivity (0.0005 in) teflon membrane and a 1 × 10 mm stirring bar. The animals were permitted to adjust to stirring for 1 hr before sealing the chamber and the data were discarded when the tentacles were retracted before completion of the measurement. All data in the P_{O_2} interval 110 to 159 mm Hg were analyzed. For each experiment a measurement of the oxygen uptake of the electrode was made using an empty chamber, and this value was subtracted from the experimental rate.

At the end of an experiment animals were stimulated to expel fluid from the gastrovascular cavity, removed from the chambers, lightly blotted and weighed to the nearest tenth of a milligram (Cahn Electrobalance, Model G).

Electrophoresis

Animals were starved for one week and then frozen whole, either singly or in groups from the same clone and treatment group, in two volumes of buffer: 0.05 M tris (titrated to pH 8 with concentrated HCl) and 0.001 M EDTA to which NADP (4 mg/liter) was added. Freezing took place quickly in a bath of dry ice and acetone, and the samples were then stored at −70° C until homogenized. Samples were thawed, homogenized and centrifuged. When necessary the supernatants were diluted as much as 50% with distilled water to obtain 25 μl which were loaded into slots of horizontal starch gels (13% w/v; Sigma starch).

Gel and electrode buffers for glucose-6-phosphate dehydrogenase (G-6-PDH, E.C. 1.1.1.49) and isocitrate dehydrogenase (IDH, E.C. 1.1.1.42) were modified from Markert and Faulhaber (1965): 0.9 M tris, 0.5 M boric acid, 0.02 M EDTA diluted 1:20 for the gel buffer and 1:6 for the electrodes; in addition, 30 mg and 10 mg NADP were added to the gel and to the cathodal buffer tray, respectively. The gel and electrode buffers for malate dehydrogenase (MDH, E.C. 1.1.1.37) were those described by Nichols and Ruddle (1973). The discontinuous LiOH buffer system of Selander, Hunt, and Yang (1969) was used for phosphoglucose isomerase (PGI, E.C. 5.3.1.9) and hexokinase (HK, E.C. 2.7.1.1.). The period of electrophoresis was 3 to 4 hr. After electrophoresis at 350 volts, gels were sliced and stained with slight modifications of the procedures described by Brewer (1970) and Shaw and Prasad (1970). The gels were preserved overnight in 50% ethyl alcohol, blotted and stored in plastic wrap until photographed.

Gas exchange surface area

Animals from the TD = 18° C = TA and the TD = 28° C = TA treatment groups were anesthetized with $MgCl_2$. Tentacles were counted and the external

surface areas of the body wall and the tentacles were approximated from measurements made at 10 to 25×, using formulas for the frustum of a cone and for a cylinder, respectively.

Statistical analyses

Mean V_{O_2} values were computed by determining the rate during each time interval of the continuous record of the change in oxygen concentration. Thus, unless specifically stated otherwise, the N value given in the results represents the sum of all data obtained from the members of an experimental group, which are treated as a homogenous population.

The comparison was complicated, however, by the different body sizes of the experimental groups (see below). Two alternative procedures were used to eliminate differences due to body size from the conclusions. First, a regression coefficient of −0.43 was used to correct rates to a common weight of 1.6 mg, a representative value, by covariance analysis. This coefficient was determined by performing a regression analysis on data from the TD = 18° C = TA treatment group ($n = 14$ animals, $r = 0.75$, $P < 0.001$). This relationship is homogenous, showing no evidence of the discontinuity observed by Shick, Brown, Dolliver, and Kayar (1978). Second, the data were grouped by weight classes established from an analysis of frequency distributions, and the comparison made within the classes.

Although the experimental design permits analysis of the data as paired observations, the available procedures for computing Student's t require an equal number of observations in each member of the pair, a condition which was not met. The element of genetic homogeneity was maintained in the experimental design by keeping virtually constant the percent of the total number of observations made on each clone under each combination of developmental and experimental temperatures.

Results

Effect of developmental temperature on body size

At 28° C fission occurred more rapidly and more frequently than at 18° C, resulting in more numerous but smaller progeny (see below). As suggested earlier by a number of investigators (Ray, 1960; Mangum, 1963; Shick, 1972) this finding suggests that the often observed phenomenon of a larger body size in the colder regions of a species range results from latitudinal differences in developmental temperature.

The frequency distribution of weight in each experimental group (Fig. 2) indicates, in general, that body size of the animals is more widely distributed in the 18° C developmental group than in the 28° C developmental group. Almost 73% of the animals in the TD = 28° C = TA treatment group weigh 1.1 mg or less, and 91% of the animals in the TD = 28° C, TA = 18° C experimental group weigh 2.0 mg or less. In contrast, only 29% of the animals in the TD = 18° C = TA group and 17% of those in the TD = 18° C, TA = 28° C treatment group weigh 1.1 mg or less. In these groups, reared at low temperature, greater frequencies of animals in the higher weight classes are also evident (Fig. 2). A

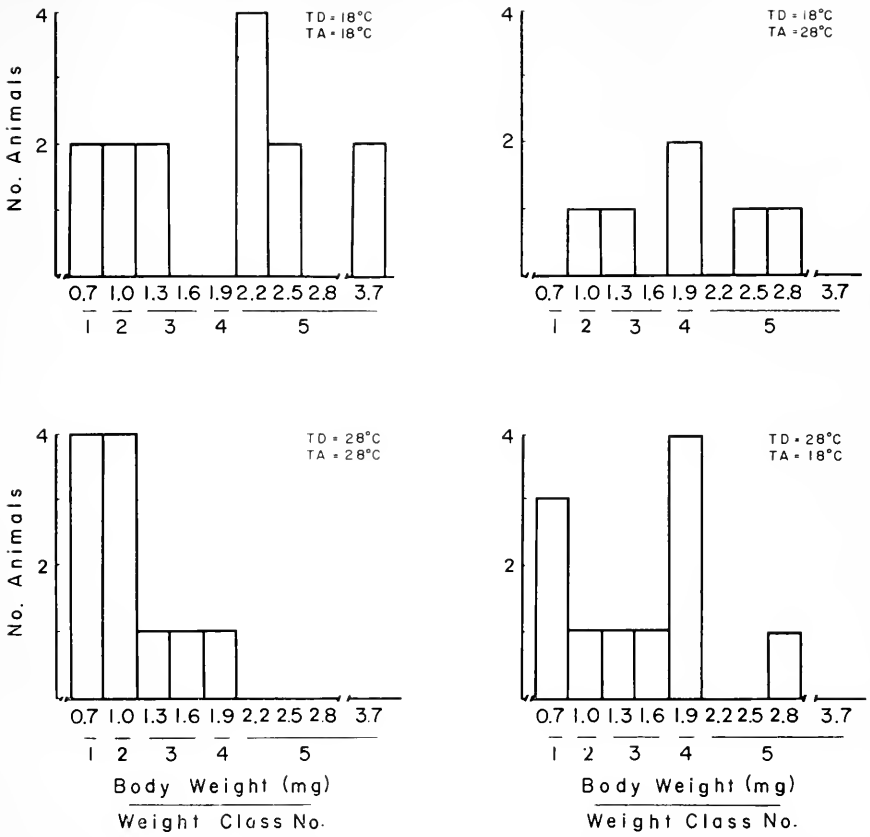

FIGURE 2. Frequency distribution of body weight in treatment groups, designated by developmental (TD) and acclimation (TA) temperature.

comparison of the frequency distributions by weight class of the two developmental temperature groups emphasizes the trends (Fig. 3). The mean body weight of the TD = 28° C = TA and TD = 28° C, TA = 18° C animals is 1.4 mg; the mean weight of the two TD = 18° C groups is 2.0 mg. The median weight class of the two TD = 28° C groups is 0.6 to 0.8 mg, and the corresponding figure for the TD = 18° C groups is 2.1 to 2.3 mg.

Oxygen uptake

Three clones of animals which first divided at 18° C and one clone of animals which first divided at 28° C were used to measure O_2 uptake; all clones produced third generation animals at both developmental temperatures. No significant differences were found between the control groups ($P > 0.85$) indicating that V_{O_2} in clones that have produced two generations at a particular developmental temperature is the same as in clones that have produced three generations at that developmental temperature.

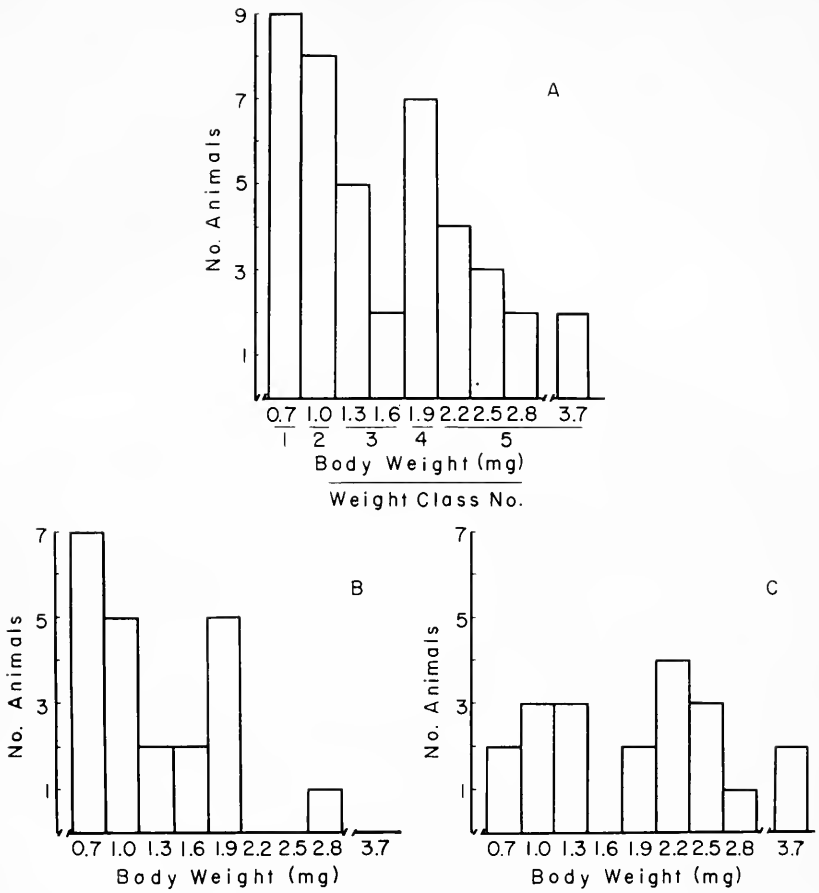

FIGURE 3. Frequency distribution of body weight in (A) all animals used in the oxygen uptake experiment, (B) TD = 28° C developmental groups only, and (C) TD = 18° C developmental groups only.

The mean weight corrected oxygen uptake rates of the two TD = 18° C groups are significantly higher ($P < 0.01$) than those of the two TD = 28° C groups at both acclimation temperatures (Table I). At the test temperature of 18° C the rates in the TD = 18° C groups are 18% higher than those of the TD = 28° C groups, and at 28° C the rates are 19% higher. However, using the raw data for the smaller animals produced at 28° C and the larger ones produced at 18° C, the difference diminished to 4% at the test temperature of 18° C and it is actually reversed at 28° C (−8%). Thus a confounding influence of developmental temperature, its effect on body size, may obscure the direct effect on the acclimated rate of oxygen uptake.

The frequency distribution by weight of all animals used in the oxygen uptake experiments is shown in Figure 3. When the rates are compared by weight class, significant differences between the TD = 18° C and the TD = 28° C groups are

TABLE I

A. *Oxygen uptake ($\mu l/g \cdot hr$) in isogenic specimens of* Haliplanella luciae *reared for two to three generations at different temperatures. Mean ± s.e. (N). Probability values from one-way analyses of variance comparing developmental temperature groups at each test (= acclimation) temperature.*

Test temperature (°C)	Body wt. (mg)	Developmental temperature (°C)		P
		18	28	
18	1.9 ± 0.2	243.7 ± 12.0 (111)	235.0 ± 16.2 (72)	n.s.
28	1.3 ± 0.1	487.1 ± 24.1 (36)	524.7 ± 21.6 (54)	n.s.

B. *Oxygen uptake ($\mu l/g \cdot hr$) corrected to a common body weight (1.6 mg) by covariance. Data analysis as above.*

Test temperature (°C)	Developmental temperature (°C)				P
	18	Q_{10}	28	Q_{10}	
18	257.0 ± 9.4 (111)	1.99	218.7 ± 13.9 (72)	1.96	$P < 0.001$
28	510.3 ± 22.6 (36)		427.8 ± 14.5 (54)		0.0018

found in one of the four possible comparisons at 18° C and in two of three at 28° C (Table II). The trend of higher rates in the TD = 18° C groups is uniformly consistent, but the probability levels rise due to the smaller number of observations (N).

TABLE II

Weight corrected oxygen uptake rates ($\mu l/g \cdot hr$), Mean ± s.e. (N). Probability (P) values from one-way analyses of variance comparing the developmental temperature group rates at each experimental (= acclimation) temperature for each weight class.

Experimental temperature (°C)	Weight class		Developmental temperature (°C)		P
	No.	Range (mg)	18	28	
18	1	(0.6–0.8)	270.5 ± 46.2 (10)	194.5 ± 33.2 (19)	n.s.
	2	(0.9–1.1)	269.8 ± 38.0 (11)	146.6 ± 41.4 (7)	0.0499
	3	(1.2–1.7)	218.6 ± 22.7 (23)	159.1 ± 21.8 (16)	n.s.
	4	(1.8–2.0)	—	288.5 ± 18.7 (24)	—
	5	(2.1–3.7)	254.7 ± 11.2 (67)	258.8 ± 4.1 (6)	n.s.
28	1	(0.6–0.8)	—	444.0 ± 22.8 (20)	—
	2	(0.9–1.1)	413.3 ± 38.1 (6)	417.4 ± 27.3 (20)	n.s.
	3	(1.2–1.7)	568.9 ± 13.8 (5)	408.3 ± 37.7 (9)	0.003*
	4	(1.8–2.0)	608.9 ± 34.3 (13)	439.9 ± 24.1 (5)	0.01
	5	(2.1–3.7)	427.4 ± 31.6 (12)	—	—

* Bartlett's Test indicates non-homogenous variances for the two developmental groups of this weight class. A Separate Variance Estimate t-test was used to make this comparison.

TABLE III

Gas exchange surface area (cm^2/g wet wt.) in each developmental temperature group of Haliplanella luciae. *N = Number of animals. Weight classes as in Table II.*

Developmental temperature (°C)	Weight class	N	No. tentacles	Tentacle surface area	
				cm^2/g	% Total body surface area
18	1	2	13	5.6	33.9
	2	2	16	5.0	57.5
	3	3	20	7.6	42.7
28	1	2	18	19.2	73.5
	2	4	30	14.7	72.9
	3	3	29	18.6	78.7

Q_{10} values for both developmental temperature groups (Table II) are similar, and they also approximate the values reported previously over the same temperature range (Sassaman and Mangum, 1970). Thus, no evidence of a change in temperature sensitivity with developmental temperature is seen. No overall pattern of temperature sensitivity is apparent in the data grouped by weight class, thus supporting previous conclusions that it is not correlated with body size (Sassaman and Mangum, 1970). Nor is there a trend in Q_{10} with developmental temperature.

Gas exchange surface

A possible explanation of the irreversible difference between cold and warm-reared anemones is a change in gas exchange surface area. This hypothesis would be supported by an increase in tentacular surface, the primary site of oxygen uptake in epifaunal anemones (Sassaman and Mangum, 1972; Shick *et al.*, 1978) in the TD = 18° C groups. In fact, measurements of gas exchange surface (Table III) indicate the opposite relationship, suggesting that the difference would be even larger if the gas exchange surface had remained the same.

Surface area is not correlated with body size. Pooling data for all weight classes at each developmental temperature, anemones in the TD = 28° C groups have more tentacles and a significantly larger (Mann-Whitney U-Test) total surface area available for gas exchange than anemones in the TD = 18° C groups. Not only are the tentacle numbers greater in the TD = 28° C groups, but the average surface area per tentacle is greater as well. Moreover, the percent of the total body surface area contributed by the tentacles always exceeds 73 in the TD = 28° C groups, compared to a maximum of 57.5% in the TD = 18° C animals (weight class 2).

Electrophoresis

The enzymes examined showed no qualitative differences in banding pattern either among the separate clones of one treatment group or among clonemates of different treatment groups. The results for all of the enzymes tested are similar

and they suggest that the population of *H. luciae* at Indian Field Creek exhibits no variation at these loci. Of the five enzyme systems examined, only IDH was clearly polymorphic, the banding pattern suggesting fixed homozygosity at two loci. However, no qualitative differences in banding pattern were detected, either between clones or within clones. Since these loci differ in latitudinally separated populations and within populations of *H. luciae* (Shick and Lamb, 1977), the uniform pattern could reflect conservative selection pressures on these loci for this particular population. Regardless, there is no evidence that the different alleles were either induced or repressed by the formation of tissues at different temperatures.

Discussion

Differences in aerobic metabolism that cannot be reversed by 2 to 4 weeks acclimation to common conditions clearly result from reproduction, regeneration and the formation of new tissues at different temperatures, when the effects of other variables are eliminated from the data. The direction of the change is generally compensatory, resulting in higher metabolic rates in the animals produced at low temperatures. The magnitude of the change, however, is not great enough to override opposing effects of developmental temperature on body size and gas exchange surface area. Oxygen uptake rates go up at low developmental temperature but down with increasing body size and decreasing tentacular surface, and body size increases and tentacular surface area decreases at low developmental temperature. The net outcome of the concomitant and counteracting trends is little or no difference between the two groups, when body size and gas exchange surface are retained as variables.

The question remains of the origin of the metabolic difference in animals of the same size. One alternative is a direct but irreversible effect of developmental temperature on the metabolic machinery within the cell. The five enzyme systems examined were selected in part for their variability in the species (and in other anemones; Manwell and Baker, 1970), and in part for their relation to aerobic metabolism. Hexokinase, phosphoglucose isomerase and isocitrate dehydrogenase vary in different populations of *H. luciae* (Shick and Lamb, 1977); isocitrate dehydrogenase and malate dehydrogenase catalyze reactions in the tricarboxylic acid cycle and glucose-6-phosphate dehydrogenase regulates a branchpoint of the pentose phosphate pathway. No qualitative changes with developmental temperature or acclimation temperature were found for any of the five enzymes tested. Quantitative data, on various enzyme systems, however, are not available. Robert and Gray (1972) have shown an increase in the specific activity of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of the blue crab *Callinectes sapidus* during cold exposure, accompanied by no qualitative electrophoretic changes. A thorough test of this possibility, which we regard as a promising hypothesis, would entail an exhaustive investigation of polymorphic enzyme systems that influence the rate of oxidative reactions, directly or indirectly.

The only obvious alternative explanation would be an irreversible effect of developmental temperature on the gas exchange system. Since fluid movements on both sides of the tentacles are generated by cilia, an irreversible difference in oxygen convection would seem to be highly unlikely. An irreversible change in gas

exchange surface was detected, but its effect on oxygen uptake should be the opposite of that observed. Thus a systemic explanation seems highly unlikely.

Regardless of its basis, the present findings clearly demonstrate the reality of irreversible physiological adaptation, and they raise the possibility that the compensatory responses found earlier in geographically separate populations of the species may result from developmental as well as acclimation temperature.

We are grateful to R. J. Hoffmann for the use of his facilities and expertise in performing the electrophoreses, and to D. Reed for his help with data analysis.

Summary

1. Isogenic clones of *H. luciae* were raised at each of two developmental temperatures, 18° and 28° C. Despite prolonged acclimation to common thermal conditions, oxygen uptake rates differ according to the temperature of reproduction, regeneration and development.

2. The effects of developmental temperature, however, are masked by body size differences. Only when this variable is eliminated can the underlying effect of developmental temperature be detected.

3. The irreversible change is not due to an increase in the gas exchange surface area at the primary site of O_2 uptake, the tentacles.

4. No qualitative changes in banding patterns for five enzymes (HK, PGI, IDH, MDH, G-6-PDH) were found.

Literature Cited

Bradley, B. P., 1978. Increase in range of temperature tolerance by acclimation in the copepod *Eurytemora affinis*. *Biol. Bull.*, **154**: 177–187.

Brewer, G. J., 1970. *An introduction to isozyme techniques*. Academic Press, New York, 186 pp.

Bullock, T. H., 1955. Compensation for temperature in the metabolism and activity of poikilotherms. *Biol. Rev. Camb. Philos. Soc.*, **30**: 31–342.

Davis, D. W., 1919. Asexual multiplication and regeneration in *Sagartia luciae* Verrill. *J. Exp. Zool.*, **28**: 161–263.

Kinne, O., 1962. Irreversible nongenetic adaptation. *Comp. Biochem. Physiol.*, **5**: 265–282.

Krogh, A., 1916. *The respiratory exchange of animals and men*. Longmans Green, London, 173 pp.

Mangum, C. P., 1963. Studies on speciation in maldanid polychaetes of the North American Atlantic Coast III. Intraspecific and interspecific divergence in oxygen consumption. *Comp. Biochem. Physiol.*, **10**: 335–349.

Mangum, C. P., M. J. Oakes, and J. M. Shick, 1972. Rate temperature responses in scyphozoan medusae and polyps. *Mar. Biol.*, **15**: 298–303.

Manwell, C., and C. M. A. Baker, 1970. *Molecular biology and the origin of species: heterosis, protein polymorphism, and animal breeding*. University of Washington Press, Seattle, Washington. 394 pp.

Markert, C. L., and I. Faulhaber, 1965. Lactate dehydrogenase patterns of fish. *J. Exp. Zool.*, **159**: 319–332.

Mayer, A. G., 1914. The effects of temperature upon tropical marine animals. *Papers of the Tortugas Lab.*, **6**: 1–24. Publications of the Carnegie Institution, 183.

Minasian, L. L., 1976. Characteristics of asexual reproduction in the sea anemone, *Haliplanella luciae* (Verrill), reared in the laboratory. Pages 289–298 in G. O. Mackie, Ed., *Coelenterate Ecology and Behavior*. Plenum Press, New York.

MIYAWAKI, M., 1952. Temperature as a factor influencing upon the fission of the orange-striped sea anemone, *Diadumene luciae*. *J. Fac. Sci. Hokkaido Univ., Ser. VI Zool.*, **11**: 77–80.

NICHOLS, E. A., AND F. H. RUDDLE, 1973. A review of enzyme polymorphism, linkage and electrophoretic conditions for mouse and somatic cell hybrids in starch gels. *J. Histochem. Cytochem.*, **21**: 1066–1081.

PROSSER, C. L., 1955. Physiological variation in animals. *Biol. Rev. Camb. Philos. Soc.*, **30**: 229–262.

RAY, C., 1960. The application of Bergmann's and Allen's rules to the poikilotherms. *J. Morphol.*, **106**: 85–108.

ROBERT, M., AND I. GRAY, 1972. Enzymatic mechanisms during temperature acclimation of the blue crab, *Callinectes sapidus*—I. Oxygen consumption and activity of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. *Comp. Biochem. Physiol.*, **42B**: 377–387.

SASSAMAN, C., AND C. P. MANGUM, 1970. Patterns of temperature adaptation in North American Atlantic coastal actinians. *Mar. Biol.*, **7**: 123–130.

SASSAMAN, C., AND C. P. MANGUM, 1972. Adaptations to environmental oxygen levels in infaunal and epifaunal sea anemones. *Biol. Bull.*, **143**: 657–678.

SCHNEIDER, D. E., 1968. Temperature adaptations in latitudinally separated populations of the crab *Rhithropanopeus harrisii*. *Am. Zool.*, **8**: 772.

SELANDER, R. K., W. G. HUNT, AND S. Y. YANG, 1969. Protein polymorphism and genic heterozygosity in two European subspecies of the house mouse. *Evolution*, **23**: 379–390.

SHAW, C. R., AND R. PRASAD, 1970. Starch gel electrophoresis of enzymes—a compilation of recipes. *Biochem. Genet.*, **4**: 297–320.

SHICK, J. M., 1972. Temperature sensitivity of oxygen consumption of latitudinally separated *Urosalpinx cinerea* (Prosobranchia: Muricidae) populations. *Mar. Biol.*, **13**: 276–283.

SHICK, J. M., 1976. Ecological physiology and genetics of the colonizing actinian *Haliplanella luciae*. Pages 137–146 in G. O. Mackie, Ed., *Coelenterate Ecology and Behavior*. Plenum Press, New York.

SHICK, J. M., AND A. N. LAMB, 1977. Asexual reproduction and genetic population structure in the colonizing sea anemone *Haliplanella luciae*. *Biol. Bull.*, **153**: 604–617.

SHICK, J. M., W. I. BROWN, E. G. DOLLIVER, AND S. R. KAYAR, 1978. Oxygen uptake in sea anemones: Effects of expansion, contraction and exposure to air, and the limitations of diffusion. *Physiol. Zool.*, **52**: 50–62.

SMITH, J. M., 1956. Acclimatization to high temperatures in inbred and outbred *Drosophila subobscura*. *J. Genet.*, **54**: 497–505.

TOREY, H. B., AND J. R. MERY, 1904. Regeneration and non-sexual reproduction in *Sagartia davisi*. *Zoology* **1(6)**: 211–226. University of California Publications.

UCHIDA, T., 1932. Occurrence in Japan of *Diadumene luciae*, a remarkable actinian of rapid dispersal. *J. Fac. Sci. Hokkaido Univ., Ser. VI Zool.*, **2**: 69–85.

VERNBERG, F. J., 1962. Comparative physiology: Latitudinal effects on physiological properties of animal populations. *Ann. Rev. Physiol.*, **24**: 517–546.

INDEX

A

B

C

D

E

F

G

H

M

N

O

P

R

S

T

U

V

W

Y

Z

Continued from Cover Two

of biological journal titles is that published each year by BIOLOGICAL ABSTRACTS (BIOSIS List of Serials; most recent issue, 1976). Foreign authors, and others who are accustomed to use THE WORLD LIST OF SCIENTIFIC PERIODICALS, may find a booklet published by the Biological Council of the U.K. (obtainable from the Institute of Biology, 41 Queen's Gate, London, S.W.7, England, U.K. at £0.65 or $1.75) useful, since it sets out the WORLD LIST abbreviations for most biological journals with notes of the USASI abbreviations where these differ. CHEMICAL ABSTRACTS publishes quarterly supplements of additional abbreviations. The following points of reference style for THE BIOLOGICAL BULLETIN differ from USASI (or modified WORLD LIST) usage:

A. Journal abbreviations, and book titles, all underlined (for *italics*)

B. All components of abbreviations with initial capitals (not as European usage in WORLD LIST *e.g. J. Cell. Comp. Physiol.* NOT *J. cell. comp. Physiol.*)

C. *All* abbreviated components must be followed by a period, whole word components *must not* (not strictly as USASI usage, *i.e. J. Cancer Res.*)

D. Space between all components (*e.g. J. Cell. Comp. Physiol.* not *J.Cell.Comp.Physiol.*)

E. We strongly recommend that more unusual words in journal titles be spelled out in full, rather than employing lengthy, peculiar "abbreviations" or new abbreviations invented by the author. For example, use *Rit Vísindafjélags Islendinga* without abbreviation. Even in more familiar languages, *Z. Vererbungslehre* is preferred to *Z. VerebLehre* (WORLD LIST) or *Z. Vererbungsl.* (USASI). *Accurate and complete communication of the reference is more important than minor savings in printing costs.*

F. All single word journal titles in full (*e.g. Veliger, Ecology, Brain*).

G. The order of abbreviated components should be the same as the word order of the complete title (*i.e. Proc.* and *Trans.* placed where they appear, not transposed as in some BIOLOGICAL ABSTRACTS listings).

H. Spell out *London, Tokyo, Paris, Edinburgh, Lisbon, etc.* where part of journal title.

I. Series letters *etc.* immediately before volume number.

J. A few well-known international journals in their preferred forms rather than WORLD LIST or USASI usage (*e.g. Nature, Science, Evolution* NOT *Nature, Lond.; Science, N.Y.; Evolution, Lancaster, Pa.*)

K. The correct abbreviation for THE BIOLOGICAL BULLETIN is *Biol. Bull.*

5. *Figures.* The dimensions of the printed page, 5 by 7⅜ inches, should be kept in mind in preparing figures for publication. Illustrations should be large enough so that all details will be clear after appropriate reduction. Explanatory matter should be included separately in legends as far as possible, although the axes should always be numbered and identified on the illustration itself. Figures should be prepared for reproduction as either line-cuts or halftones; no other methods will be used. Figures to be reproduced as line-cuts should be drawn in black ink on white paper, good quality tracing cloth or plastic, or blue-lined coordinate paper; those to be reproduced as halftones should be mounted on board, and both designating numbers or letters and scale-bars should be affixed directly on the figures. We recommend that halftones submitted to us be mounted prints made at about 1½ times the linear dimensions of the final printing desired (the actual best reductions are achieved from copy in the range from 1¼ to 2 times the linear dimensions). As regards line-blocks, originals can be designed for even greater reductions but are best in the range 1½ to 3 times. *All* figures should be numbered in consecutive order, with no distinction between text and plate-figures. The author's name should appear on the reverse side of all figures, and the inked originals for line-blocks must be submitted for block-making.

6. *Mailing.* Manuscripts should be packed flat. All illustrations larger than 8½ by 11 inches must be accompanied by photographic reproductions or tracings that may be folded to page size.

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CONTENTS