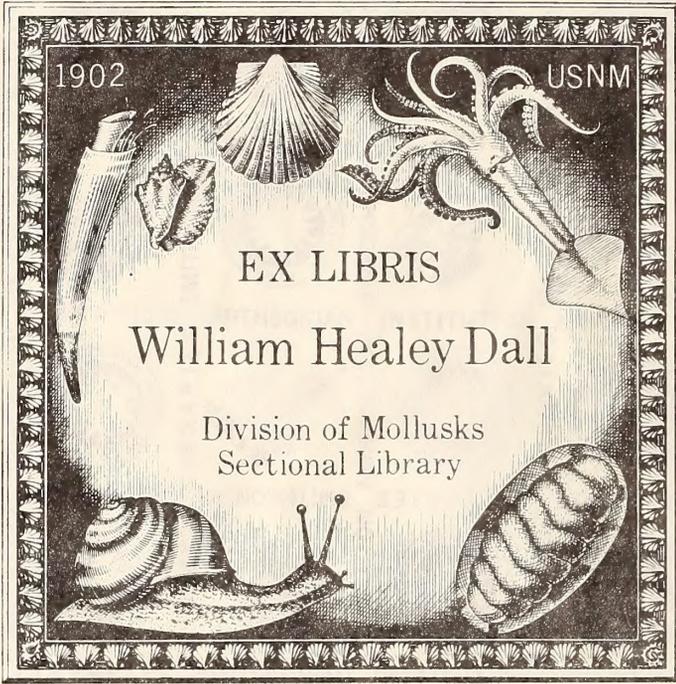


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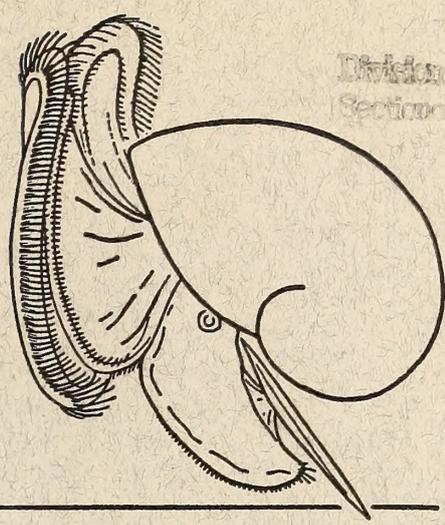
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VOLUME 11

JULY 15, 1968

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## The Biology of *Acmaea*

EDITED BY

DONALD P. ABBOTT

DAVID EPEL

JOHN H. PHILLIPS

ISABELLA A. ABBOTT

AND

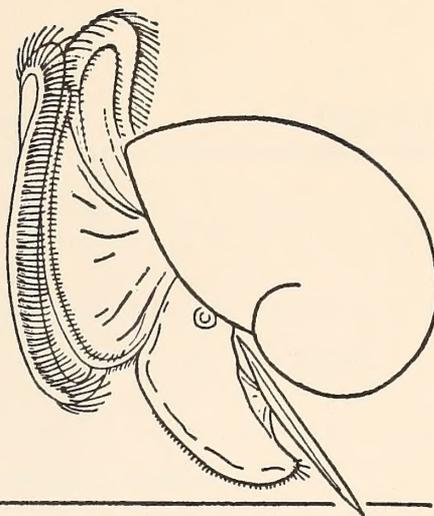
RUDOLF STOHLER





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## TABLE OF CONTENTS

Undergraduate Research and the Biology of <i>Acmaea</i> DONALD P. ABBOTT, DAVID EPEL, JOHN H. PHILLIPS, & ISABELLA A. ABBOTT . . . . .	1
The Activity and Food of the File Limpet, <i>Acmaea limatula</i> (7 Text figures; 1 Table) CHARLES MCKENDREE EATON . . . . .	5
The Activity Pattern and Food Habits of the Limpet <i>Acmaea pelta</i> (Plate 1; 5 Text figures; 1 Table) PETER C. CRAIG . . . . .	13
The Effects of Light and Tide on Movements of the Limpet <i>Acmaea scutum</i> (4 Text figures; 1 Table) DON A. ROGERS . . . . .	20
Light Responses in the Limpet <i>Acmaea limatula</i> (3 Text figures; 3 Tables) TOM L. ROSS . . . . .	25
Orientation and Movement of the Limpet <i>Acmaea digitalis</i> on Vertical Rock Surfaces (18 Text figures; 4 Tables) ALAN C. MILLER . . . . .	30
The Clustering Behavior of <i>Acmaea digitalis</i> (4 Text figures; 1 Table) CAROL SPENCER MILLARD . . . . .	45
Studies of Homing Behavior in the Limpet <i>Acmaea scabra</i> (4 Tables) WILLIAM F. JESSEE . . . . .	52
Occurrence and Behavior of <i>Hyalé grandicornis</i> , A Gammarid Amphipod Commensal in the Genus <i>Acmaea</i> (Plates 2 and 3; 4 Text figures) SAMUEL E. JOHNSON II . . . . .	56
Factors Affecting the Attraction of <i>Acmaea asmi</i> to <i>Tegula funebris</i> (1 Text figure) LANI LEE ALLEMAN . . . . .	61
Shell Damage and Repair in Five Members of the Genus <i>Acmaea</i> (Plate 4; 2 Tables) P. TODD BULKLEY . . . . .	64

[continued next page]

## TABLE OF CONTENTS [continued]

Some Observations of Predation on <i>Acmaea</i> Species by the Crab <i>Pachygrapsus crassipes</i> (Plate 5)	
DEXTER CHAPIN . . . . .	67
A Study of Morphological Variation in the Limpet <i>Acmaea pelta</i> (Plate 6; 1 Text figure; 2 Tables)	
ALAN JOBE . . . . .	69
Anatomical and Oxygen Electrode Studies of Respiratory Surfaces and Respiration in <i>Acmaea</i> (Plate 7; 6 Text figures; 1 Table)	
ROGER S. KINGSTON . . . . .	73
Manometric Measurements of Respiratory Activity in <i>Acmaea digitalis</i> and <i>Acmaea scabra</i> (3 Text figures; 2 Tables)	
SIMEON BALDWIN . . . . .	79
A Comparative Study of Lethal Temperatures in the Limpets <i>Acmaea scabra</i> and <i>Acmaea digitalis</i> (4 Text figures; 1 Table)	
DANE D. HARDIN . . . . .	83
Studies on the Jaw, Digestive System, and Coelomic Derivatives in Representatives of the Genus <i>Acmaea</i> (13 Text figures; 1 Table)	
CATHERINE GENE WALKER . . . . .	89
A Comparison of Carbohydrate Digestion Capabilities in Four Species of <i>Acmaea</i> (2 Tables)	
WILLIAM J. BEPPU . . . . .	98
Metabolic Activity and Glycogen Stores of Two Distinct Populations of <i>Acmaea scabra</i> (2 Tables)	
T. JEFFERY WHITE . . . . .	102
Laminarinase and Hexokinase Activity during Embryonic Development of <i>Acmaea scutum</i> (2 Text figures; 1 Table)	
ANDREW V. MUCHMORE . . . . .	105
Nitrogen Excretory Products in the Limpet <i>Acmaea</i> (1 Text figure; 2 Tables)	
WILLIAM H. BARIBAULT . . . . .	109



## Undergraduate Research and the Biology of *Acmaea*

BY

DONALD P. ABBOTT, DAVID EPEL, JOHN H. PHILLIPS, AND ISABELLA A. ABBOTT

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THE PAPERS WHICH FOLLOW, on aspects of the biology of *Acmaea*, are the outcome of original research conducted by undergraduate students at Hopkins Marine Station in the period April through mid-June, 1966. The students were enrolled full time in a special class (Biology 175h, Problems in Marine Biology) which was scheduled all day five days a week, and was designed specifically to give undergraduate students the chance to do original research in a situation where the opportunities were maximal and the outside distractions minimal.

Most undergraduate science courses are geared to the concept of science as organized knowledge. Lectures, readings, laboratory exercises, and discussions are arranged to give students a grasp of related principles, ideas, and facts. Examinations are designed to test knowledge, and the prizes go to students who best demonstrate a mastery of the facts and a clear understanding of concept and principle.

With this emphasis, it is quite possible for a student to major in a science and graduate without much of an idea of how organized knowledge comes into being. Since many science majors are considering becoming practitioners in the field of their major, it is important that somewhere along the line as undergraduates they gain an understanding of science as a process, and with it get some feeling of what it is like to be a scientist.

It has been the experience of a good many scientist-teachers that the most effective way to do this is to have the student tackle a research problem of his own — a problem which has not been solved before. It need not be a big problem, but it must represent such a real and strong challenge to the student that it calls forth his best efforts in response. Where the student responds strongly he gains a much deeper and more immediate insight into the nature of scientists and scientific endeavor than he could get through lectures or readings.

The impact of a successful undergraduate venture in research may be profound. ANNE ROE (1952, 1953), generalizing from her study of 64 eminent scientists, has observed that the "average" member of the group she

studied decided on a career as a scientist during his junior or senior year in college. "What decided him (almost invariably) was a college project in which he had occasion to do some independent research — to find things out for himself. Once he discovered the pleasures of this kind of work, he never turned back" (ROE, 1952, p. 22). ROE's finding that undergraduate research experience was so frequently the most important factor in the final decision to become a scientist is, as she noted, a fact of real importance for educational practice, and has influenced our own handling of the present course.

The general philosophy and organization of the course have been outlined earlier (ABBOTT, BLINKS & PHILLIPS, 1964), and have undergone no fundamental change. The program usually begins with a brief (three-day) introduction to the biota and physical environment of the rocky shore, where the students, working in teams of four, learn the commonest species and plot distributions of their populations along selected shoreline profiles. This is followed by two and a half weeks of intensive investigation of one species (or a group of related species) from the varied viewpoints of anatomy, development, behavior, physiology, ecology, and biochemistry.

The work is guided by a faculty of three or four biologists. Trained in diverse branches of the field, the faculty try nonetheless to treat their own approaches as different ways of looking at the same biological whole, and emphasize that in the living animal under study there is no separation of anatomy, physiology, biochemistry, and behavior. Laboratory and field studies are interspersed, and we try to avoid any dichotomy between observation and experiment. Pertinent facts, concepts, and special methods are introduced, not as things having importance in their own right, but as tools for the investigator, to be used as needed.

Some of the work during this exploratory phase is done by individuals working alone, but more often the students work in pairs or in selected teams of four. Some observations require that the class be divided into shifts for round-the-clock studies of activity patterns during

diel and tidal cycles. Along with these firsthand studies we try to make available the journal articles describing all of the previous biological studies made on the species and its close relatives.

At the end of the third week of class we take stock of our position in open class discussion. We usually find that we have gained a great deal of information about the animal or animals chosen for study; much of it is original and heretofore unknown, but much of it is also relatively incomplete and tentative. We have found out some things we can do and some things we can't — at least with the tools at hand. And we have gathered a host of intriguing leads and stockpiled many unanswered questions and unsolved problems of a sort suitable for investigation by individual students.

The students chose their own individual problems with the advice and consent of the faculty. Many students have already tentatively chosen problems, arising from their own discoveries in laboratory or field, prior to this time. When problems have been agreed upon, basic questions tentatively framed, and a start made on research, the class meets again as a whole, and each student reports to the group on what he is trying to do and how he will try to do it.

During the remaining seven weeks class members work on individual schedules; students are expected to put in at least an eight-hour day, and the upper limits are set only by enthusiasm and endurance. The laboratory is made available day and night, though a faculty member is not continuously present. Each student works under the general supervision of one or more faculty members who must be kept informed in detail as to what is being done, how it is being done, and what the results are. The faculty advise and criticize; they stay out of the way when not really needed, and try to stimulate student originality and initiative as much as possible.

With limited time available the students are under pressure not only to work to full capacity but also to select the best leads, plan observations and experiments carefully, and profit quickly from mistakes. Each student is encouraged to aim at completing a piece of work which, if successful, might be published as a scientific paper. Fulfillment of this objective is not paramount, and in all events we try hard to avoid situations where the student serves merely as a technician carrying out the instructions of a faculty member.

As the weeks go by the findings of one student begin to throw light on problems under study by others. Students are strongly encouraged to share findings at frequent intervals, and to keep up with what others are doing. The faculty tries to keep up, too, and fosters interchange by bringing people together, arranging small dis-

cussion groups, and spreading significant findings by word of mouth.

In every research class so far, and with organisms as different as barnacles and snails, it has been our experience that excitement in the study increases as information accumulates. A spectacular breakthrough in one project generates increased efforts on the part of other investigators, and a class esprit de corps develops which is hard to match in conventional courses.

In the final week of the course, sessions are held which are patterned after the programs of presented papers at regular scientific meetings. Each student is allowed 30 minutes to present his results to the class and visitors. Figures, tables, and graphs are projected with an opaque projector, and each presentation is followed by open discussion of the paper. Since the students are now in fact real authorities on the species studied, discussions are usually far better than those at open scientific meetings and more closely resemble discussions at small meetings or seminars where attendance is limited to those whose research lies in the immediate field.

Each student is also required to submit a written report of his work, composed and illustrated as if it were to be submitted to a regular scientific journal for publication and designed to meet both the requirements of the AIBS Style Manual and the format standards of a particular appropriate journal. The faculty subject these papers to stern editorial scrutiny and criticism, and papers are rewritten as many times as necessary to bring them up to reasonable professional standards of organization, brevity, and clarity. Where the final results justify it, we encourage the student to submit the paper for publication under his own name.

The gastropod family Acmaeidae provides exceptionally favorable material for biological research. At least 10 species occur in the intertidal region along the central California coast in sufficient numbers to make possible extensive work on individual species and also comparative studies. The taxonomy of the group is reasonably well known (see GRANT, 1937; A. R. G. TEST, 1946; and McLEAN, 1966), and the efforts of previous workers on the group have provided a fair stock of background information. Considering only the acmaeids of the west coast of the United States, studies have been made from the standpoints of **shell form** (e. g., THOMPSON, 1912; SHOTWELL, 1950a), **anatomy** (e. g., FISHER, 1904), **functional anatomy** (e. g., YONGE, 1962), **body composition** (e. g., STOHLER, 1930), **ecology** (e. g., GRANT, 1937; TEST, 1945; SHOTWELL, 1950b; HAVEN, 1964a, 1964b; EIKENBERRY & WICKIZER, 1964; GLYNN, 1965; FRANK, 1965b), **physiology** (e. g., SEGAL, 1956, 1961, 1962; SEGAL & DEHNEL, 1962; WEBBER, 1966), **behavior** (e. g.,

VILLEE & GROODY, 1940; HEWATT, 1940; F. H. TEST, 1945; BULLOCK, 1953; ABBOTT, 1956; FEDER, 1963; MARGOLIN, 1964; GALBRAITH, 1965), and **growth and reproduction** (e. g., FRITCHMAN, 1961a, b, c, 1962; FRANK, 1965a, b; SEAPY, 1966). It is perhaps significant that approximately half of the studies cited above were carried out by undergraduate or graduate students, and it is safe to say that many a zoologist who has gone on to other things cut his research teeth, so to speak, on limpets.

Notwithstanding what has been done so far, the opportunities for further work are wide open; indeed, it was the feeling of both faculty and students at the completion of the present studies that work on the biology of the Acmaeidae is in its infancy. We hope these contributions will help stimulate others to work with these attractive animals.

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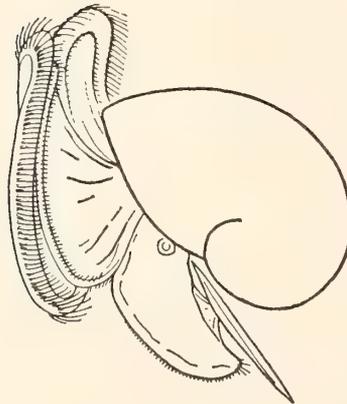
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# The Activity and Food of the File Limpet *Acmaea limatula*

BY

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(7 Text figures; 1 Table)

## INTRODUCTION

*Acmaea limatula* CARPENTER, 1864, HAS BEEN studied from the standpoint of taxonomy, distribution, and habitat (TEST, 1946), response of the heart rate to changes in temperature (SEGAL, 1962), osmotic behavior (SEGAL & DEHNEL, 1962), and reproduction and growth (SEAPY, 1966). Published accounts of other aspects of its biology, however, are lacking. It has been the purpose of the present study to investigate the activity pattern and feeding habits of this species.

## GENERAL ACTIVITY IN RELATION TO PHASE OF TIDE

To determine the general activity pattern of *Acmaea limatula*, field observations on a population of 13 limpets were carried out over a period of 45 hours from May 2 to May 4, 1966. The population was located at Mussel Point, Pacific Grove, California, on a flat, vertical granite face which was totally exposed and covered by water twice a day.

Coordinates were drawn on the surface of the rock with a lacquer paint, and the animals were marked with colored paints to distinguish each individual. The position and orientation of each animal were then observed and recorded at intervals of 1½ hours. At the end of the 45-hour observation period, a series of positions for each animal had been determined, the distance between successive positions representing the net minimum displacement between observation periods. Figure 4 a, b, c shows displacement tracks for 3 individuals. In order to observe the movements of the limpets during periods of high tide and rough water, it was necessary to wear a wet suit with 2 weight belts and to use underwater writing material (x-ray film with the emulsion removed and the surface roughened with sandpaper proved very satisfactory for pencil notes).

As can be seen from Figure 1, individuals of *Acmaea limatula* move actively only during periods when they are either splashed by water or submerged. The time of day or night at which the period of wetting occurs does not appear to influence the amount of activity. The movement during periods of complete exposure (0130 to 0600, May 3) approaches zero. During each period of rising tide a large amount of activity was observed, but the animals do not begin moving immediately upon being splashed, and some remain inactive for a time after they are first submerged (0600 to 0730, May 3; 1730 to 1900, May 3; and 0730 to 0900, May 4). Following the incoming tide, the activity characteristically drops during periods of high water, to be followed again by a sharp increase as the tide is falling. These periods of high tide are often accompanied by great surge, and many of the animals stop moving altogether, clamping down on the surface of the rock. Although the movement again increases as the tide recedes, it is interesting to note that in all cases, except for the period between 2230 to 2400, May 2, the activity does not reach the level observed during incoming tides.

## VERTICAL MOVEMENT IN RELATION TO PHASE OF TIDE AND TIME OF DAY

The vertical displacement of the population of 13 limpets with reference to tide and time of day is shown in Figure 2. It is clear that during periods of nocturnal rising tides, *Acmaea limatula* shows a definite upward displacement, but during the daytime hours (0730 to 1350, May 3; and 0900 to 1500, May 4), the displacement is characteristically in a downward direction, whether the tide is rising or falling. This correlates nicely with the studies on light sensitivity in various *Acmaea* species made by ROSS (1968); *A. limatula* was the only species studied showing a strong negative response to light. It should also be noted that the tendency to move downward during the daytime, in terms of either the number of individuals

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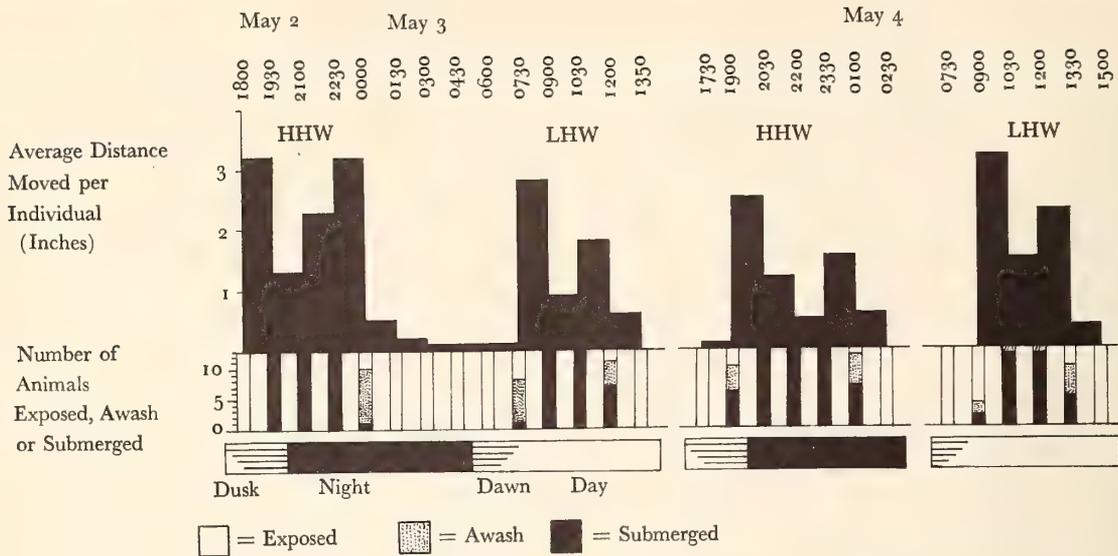


Figure 1

Movement of *Acmaea limatula* in relation to phases of the tide, May 2 to 4, 1966. Time of day is shown at the top. Distance moved represents average net displacement for 13 limpets. Since

the limpets occupied different vertical positions on the rock, the number exposed, awash, and submerged at each observation time is indicated. HHW = higher high water; LHW = lower low water.

moving or the total displacement, is proportionally greater during the receding tides than on the incoming tides. Again, although nocturnal receding tides tend to produce random movement (5 individuals moved up, 4 down in each case) the displacement upward is proportionally less than that for the rising nighttime tides.

ROGERS (1968), in his study of *Acmaea scutum*, found that this species conforms to the more typical vertical displacement pattern shown by many intertidal invertebrates (WIESER, 1952; GLYNN, 1965, fig. 30 and pp. 53 - 55). *Acmaea scutum* moves up with rising tides, and down with falling tides, no matter whether the period of submersion comes during the day or night, though upward movement during the day is less than that at night.

A series of experiments was done in the laboratory in order to substantiate *Acmaea limatula*'s partial non-conformity to the general rule for the vertical movements of transient intertidal invertebrates. In all 4 experiments 8 individuals were subjected to simulated rising tides for periods of  $1\frac{1}{2}$  hours; only the amount of light was varied. In each case, fresh organisms were collected from the field at low tide, except for the first daylight experiment in which the same individuals were used as in the preceding darkroom experiment. The specimens were placed in a line in the middle of a roughened 16-inch piece of marble, 4 facing upward, 4 downward. After the activity of the animals had ceased, the marble slab was placed in

an aquarium with the water level coming 2 inches below the line of limpets. The water was then adjusted so as to rise 2 inches every 15 minutes and compressed air was bubbled into the water to create simulated waves and splash. The first 2 experiments were run in the darkroom, the last 2 outside in the early afternoon sunlight.

These experiments (Figure 3) confirmed *Acmaea limatula*'s general tendency to move downward during daylight incoming tides and upward during nighttime incoming tides. It should be mentioned here that the graphs denoting distance of vertical displacement for the experiments run in the daylight are not entirely accurate, for in both experiments 2 individuals moved off the piece of marble onto the bottom of the aquarium during the first half hour. Their vertical downward movement was then listed as a minus 8 inches in both experiments, even though the downward displacement might have been considerably greater had the marble slab been longer.

### HOMING TENDENCY

Homing, as used in this paper, is the consistent returning to exactly the same location with the same orientation. Figures 4a and 4b illustrate the non-homing behavior shown by 12 of the 13 limpets in the population followed in the field. These typical displacement tracks show that the animals spent each consecutive exposed period in a new location.

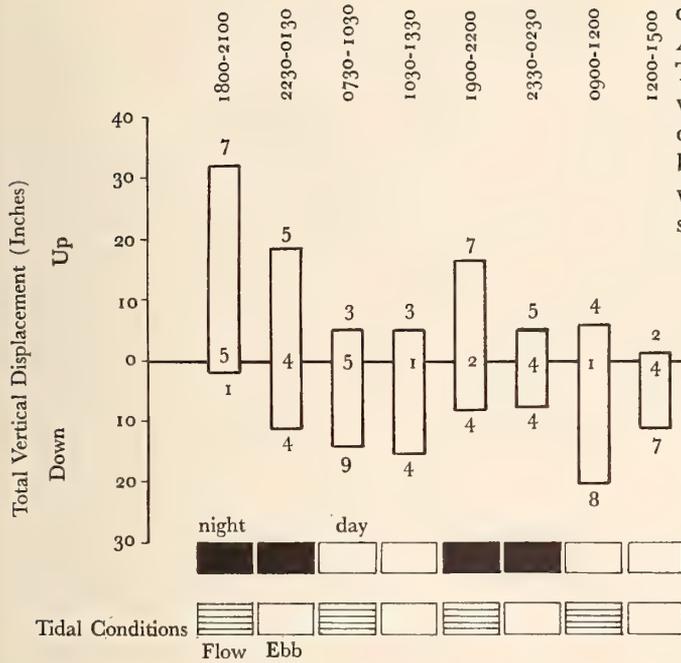


Figure 2

Vertical movement of *Acmaea limatula* on a vertical rock face at selected phases of tide and time of day, May 2 to 4, 1966. Only the vertical components of movement are represented. Each vertical bar shows total net displacement upward or downward for 13 individuals over a 3-hour period when conditions on the rock surface changed from total exposure to total submersion on a rising tide, or from total submersion to total exposure on a falling tide. Numbers above and below each vertical bar show the numbers of animals whose net displacement during the period was upward and downward, respectively; numbers on the zero line indicate the numbers of animals remaining stationary, or showing only horizontal movement.

Only one individual in the population homed consistently for the entire 45 hour period (Figure 4c). Interestingly enough, the homing limpet not only returned to its home site as the tide went out, but also during periods of high tide accompanied by surge. This type of behavior was also observed in homing *Acmaea limatula* studied in other locations. They would leave their home sites as the tide was rising, return when the tide was high and then either remain at home or leave again as the tide was falling. This behavior helps to emphasize the characteristic drop in activity during periods of high tide as shown in Figure 1.

Although only one of 13 animals in the population whose activity was closely followed, homed consistently for a period of 4 low tides, the percentage of homing

animals may be higher for the total population. In another field observation made on Mussel Point, 9 of 15 *Acmaea limatula* homed consistently for a 48-hour period. These limpets were located on a horizontal rather than a vertical surface, and although the number of animals observed is small, the findings suggest that homing may be more prevalent among populations of *A. limatula* which must cope with desiccation accompanied by direct sunlight.

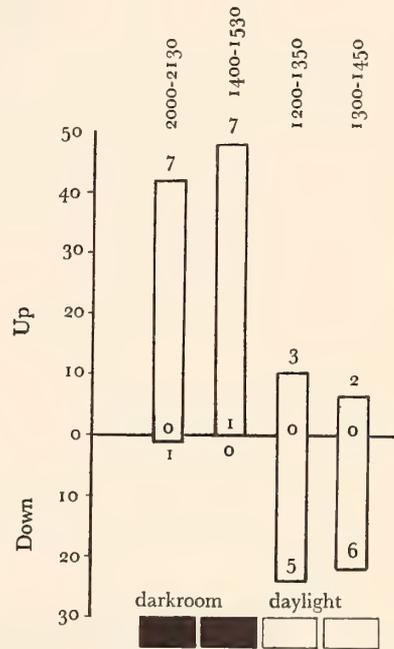
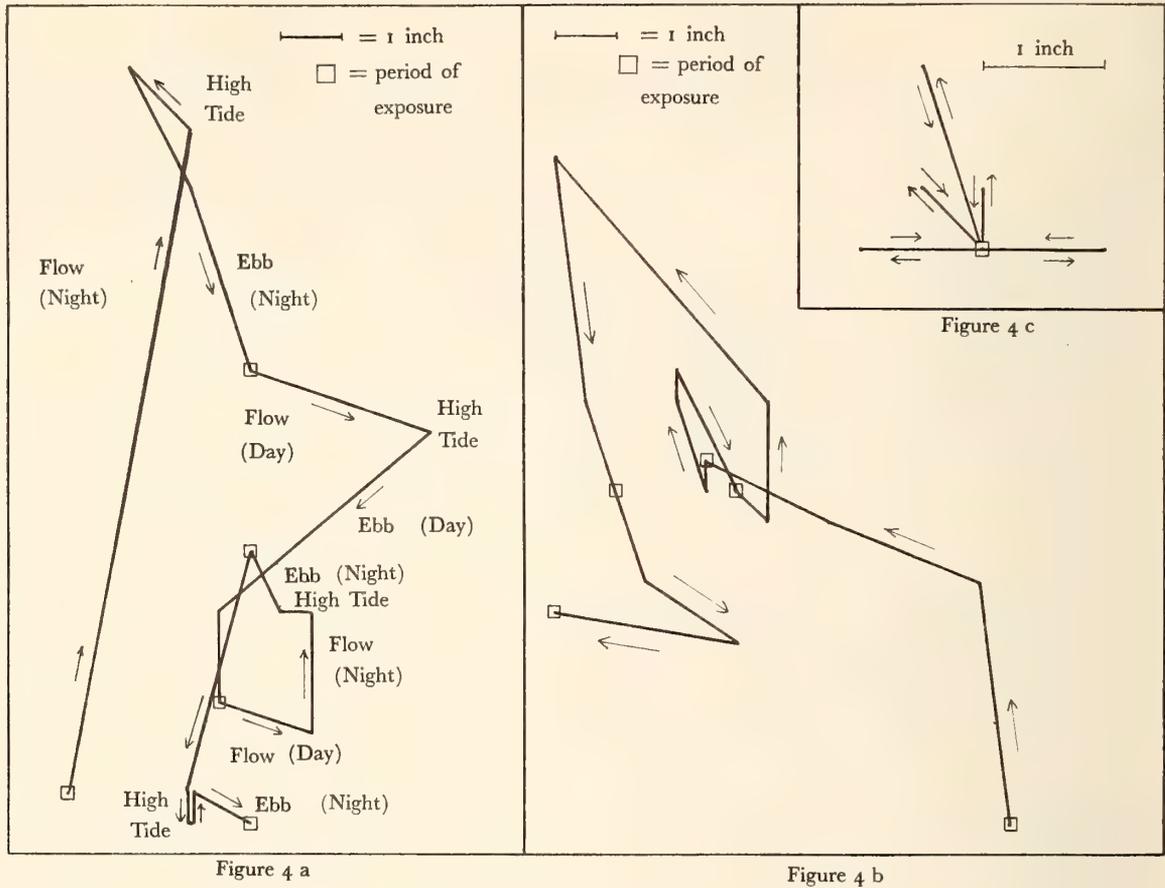


Figure 3

Vertical movement in the laboratory under simulated conditions of incoming tides.

### DISTRIBUTION IN RELATION TO ALGAE

The algae present seem to play a significant role in the distribution of *Acmaea limatula*. A good example of this can be cited from the population whose movements are shown in Figure 1. The surface of the vertical face on which the 13 limpets were located was occupied mainly by diatoms and microscopic green and blue-green algae on the left-hand upper side of the rock, and by encrusting red algae, mainly *Hildenbrandia* and some *Peyssonelia*, on the lower right-hand side. These two regions occupied approximately equal areas, and both areas bore occasional small clumps of *Endocladia* or



Typical displacement tracks for 3 individuals of *Acmaea limatula*. Observations were made at intervals of  $1\frac{1}{2}$  hours over a period of

45 hours, May 2 to 4, 1966. Limpets did not always move between successive observation periods, even when submerged or awash.

*Gigartina*. Though *Hildenbrandia* and *Peyssonelia* dominated only about half of the rock surface (Figure 5), the 13 limpets observed spent a combined total of 500 hours in this lower right-hand region. During the 45-hour observation period, only 2 limpets ventured into the region bearing mainly green and blue-green algae and diatoms, where they spent a total of 85 hours.

Although individual *Acmaea limatula* can be found in widely differing regions within the intertidal of Mussel Point, the larger populations (up to 37 per square yard) are invariably located in regions where *Hildenbrandia* or *Peyssonelia* or both are abundant. In order to demonstrate this relationship between limpets and algae, a 3.4 square foot rock surface was chosen containing 14 *A. limatula*, a fairly abundant crop of the red encrusting alga *Hildenbrandia*, and a wide variety of other algal growths. A clear plastic sheet was placed over the region and the areas occupied by the various algae were outlined with wax marking pencils. The locations of the 14

*A. limatula* were noted. The drawing was traced onto a large piece of graph paper and the different regions were cut out and weighed. By comparing these weights to the weight of 1 square inch of graph paper, it was possible to get a fairly accurate determination of the areas occupied by the various algae present. Figure 6 shows what more qualitative observations confirm, that, at least during low tide, *A. limatula* is often found in direct association with the red encrusting algae. This, of course, is only an instantaneous glance at the distribution of *A. limatula*, but it seems pertinent when viewed in conjunction with Figure 5 and the gut content analyses below.

Although on Mussel Point large populations of *Acmaea limatula* were found only on the red encrusting algae *Hildenbrandia* and *Peyssonelia*, this may not necessarily apply to other regions along the Pacific Coast. It was noted, for example, that on Point Lobos, just south of Carmel, California, fairly large populations of *A. limatula* seemed to thrive on low horizontal expanses of sand-

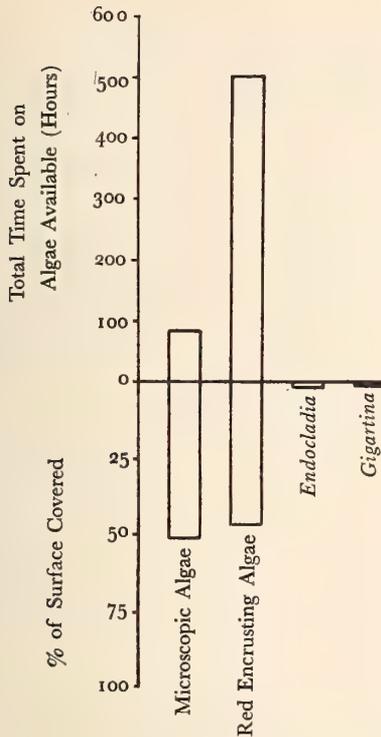


Figure 5

Distribution of 13 *Acmaea limatula* in relation to algae available, for a period of 45 hours, May 2 to 4, 1966.

stone covered primarily with microscopic and encrusting coralline algae. Extensive low, horizontal rock surfaces are not found on Mussel Point, but as can be seen from the gut content analyses below, *A. limatula* does eat microscopic algal films and encrusting coralline algae when they are available.

#### RELATIONSHIP OF FOOD AVAILABLE TO FOOD EATEN

To correlate the availability of various algae with the foods actually eaten by *Acmaea limatula*, a series of gut content analyses were performed on animals from differing areas. Eight regions were chosen which showed differences either in the algal species present, or in the relative abundance of the species. Five limpets were collected from each region after making a rough assessment of the relative amounts of different algae available to the animals. The stomach contents of these 40 animals were observed microscopically, and with the kind help of Dr.

Isabella Abbott the algal fragments present were identified and an estimate of the relative abundance of different species was made.

Figure 7a to 7h and Table 1 indicate that the main source of food for *Acmaea limatula* consists of the microscopic algae and of the encrusting forms, *Hildenbran-*

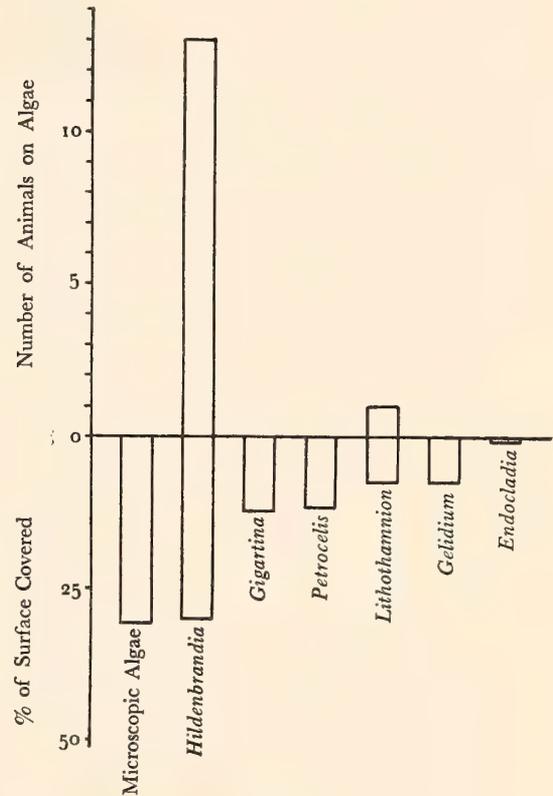
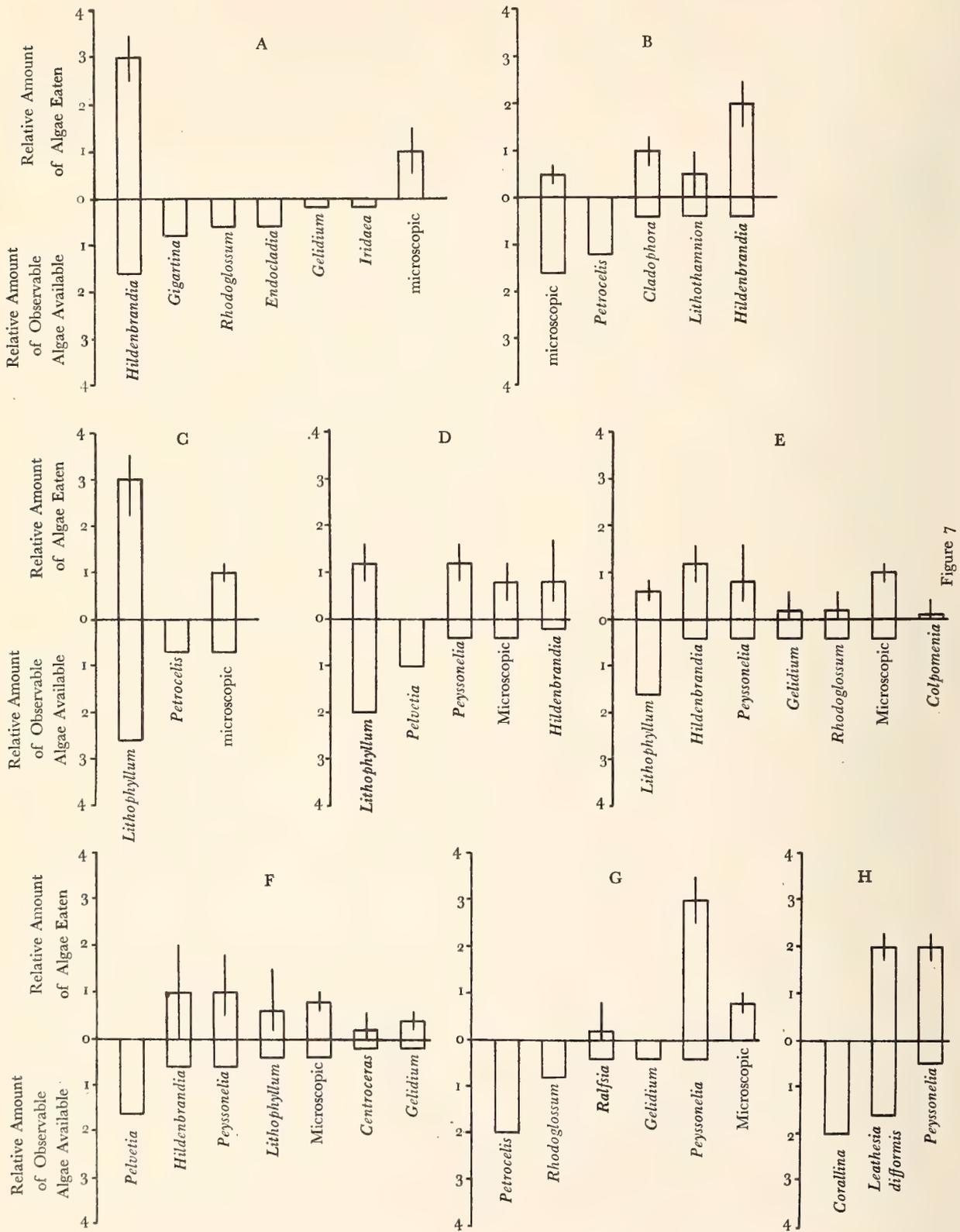


Figure 6

Distribution of 14 *Acmaea limatula* at low tide in relation to algae available within a 3.4 square foot area, mid-May, 1966.

*dia*, *Peyssonelia*, *Lithophyllum*, and *Lithothamnion*. Strangely enough, *A. limatula* seems to ignore the encrusting red alga *Petrocelis* (Figure 7g) even though it grows in relative abundance within the intertidal region inhabited by this limpet.

The non-encrusting algae are rarely ingested except for tiny individuals of larger species, or species which form a short fuzz growing close to the rock surface (Table 1, III; Figure 7b, 7e, 7f, 7h). In such cases the thalli found were very small and capable of being swallowed whole. Only once was a fragment of a large, non-encrusting alga (not an entire thallus) found within the stomach, although many such algae were available to the individ-



Relationship of food available to food eaten in 8 different areas on Mussel Point, May, 1966. The lines at the ends of the upper vertical bars show the range of variation encountered in the 5 limpets examined in each region.

Table 1

Frequency of Occurrence of Algae  
Found in Gut of *Acmaea limatula*

Type of Algae	Number of Animals with Algae in Gut
I. Microscopic Algae (small greens, blue-greens, and diatoms)	40
II. Encrusting Algae	
1. <i>Hildenbrandia occidentalis</i>	25
2. <i>Peyssonelia pacifica</i>	25
3. <i>Lithophyllum</i> sp.	15
4. <i>Lithothamnion</i> sp.	10
5. <i>Ralfsia pacifica</i>	1
III. Low, Turf-forming Algae (up to 3 mm high)	
1. <i>Gelidium coulteri</i>	10
2. <i>Cladophora trichotoma</i>	5
3. <i>Leathesia difformis</i>	5
IV. Other Algae	
1. <i>Centroceras clavulatum</i>	1
2. <i>Colpomenia peregrina</i>	1
3. <i>Rhodoglossum affine</i>	1
4. Fragment of a large brown alga	1

uals analyzed. The item found ingested was a section of a large brown alga which could not be found in the surrounding area.

Figure 7h provides an excellent example of *Acmaea limatula*'s tendency to avoid the larger non-encrusting algae as food sources. Although this limpet eats the encrusting coralline algae *Lithophyllum* and *Lithothamnion*, the non-encrusting form *Corallina* was completely ignored here, even though it dominated the region.

#### FOOD NICHE SPECIALIZATION IN *Acmaea limatula* AND *Acmaea pelta*

*Acmaea limatula* and the very eurytopic limpet *A. pelta* may often be found occupying the same general areas and habitats in the intermediate and low intertidal zones on Mussel Point. In order to substantiate the presence of overlapping populations, Peter Craig and the author mapped species distribution in 3 transects, each 3 feet wide, extending from the low to the high intertidal zones. Populations of the two species overlap broadly, and several places were discovered where both *A. limatula* and *A. pelta* were abundant (see also CRAIG, 1968).

With such an overlap in distribution on the part of two species in the same genus, one might expect consid-

erable competition, especially for food since both species are herbivores and scrapers. Interestingly enough, little competition for food actually exists. The two species live side by side, *A. pelta* eating the larger, non-encrusting algal forms (CRAIG, 1968) and *A. limatula* ingesting primarily the encrusting red and coralline algae.

#### SUMMARY

1. Individuals of *Acmaea limatula* move actively only during periods when they are either splashed by water or submerged. The time of day or night at which the period of wetting occurs does not appear to influence the amount of activity.
2. Animals do not begin moving immediately upon being splashed, and some remain inactive for a time after they are first submerged. Activity reaches a high level as the tide is rising, characteristically drops during periods of high tide, and shows some increase again as the tide recedes.
3. With rising tides at night, a definite upward movement occurs. During the daytime hours, the displacement is characteristically in a downward direction during both rising and falling tides. The tendency to move downward during the daytime is proportionally greater during the receding tides than on the incoming tides.
4. Only one of the 13 limpets in the population studied in detail homed consistently for the entire 45-hour observation period. However, on a large horizontal rock, 9 of 15 homed consistently for a 48-hour observation period.
5. Where the red encrusting algae *Hildenbrandia* and *Peyssonelia* are present, *Acmaea limatula* spends most of its time on these.
6. The main foods of *Acmaea limatula* are microscopic algae and the encrusting red and coralline algae *Hildenbrandia*, *Peyssonelia*, *Lithophyllum*, and *Lithothamnion*. The encrusting red alga *Petrocelis* is ignored, even though it is relatively abundant in the region inhabited by the limpet. Non-encrusting forms are not eaten unless they are very short, growing close to the rock surface.
7. Although *Acmaea limatula* and *A. pelta* may often be found occupying the same general areas and habitats in the intermediate, and low intertidal zones of Mussel Point, there is little interspecific competition for food. *Acmaea pelta* eats the larger, non-encrusting algae, while *A. limatula* ingests primarily the encrusting red and coralline algae.

## ACKNOWLEDGMENTS

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# The Activity Pattern and Food Habits of the Limpet *Acmaea pelta*

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(Plate 1; 5 Text figures; 1 Table)

*Acmaea pelta* ESCHSCHOLTZ, 1833, IS ABUNDANT in the rocky intertidal zone along the California coast. Described as the most eurytopic member of the genus *Acmaea* by TEST (1945), *A. pelta* ranges in its intertidal habitat from the higher *Endocladia* to the lower *Egregia* association at Mussel Point, Pacific Grove, California. Studies of its biology to date have been concerned with its general ecology (TEST, 1945), the effects of grazing on diatom populations (CASTENHOLZ, 1961), the reproductive cycle (FRITCHMAN, 1961, 1962), and its ecological role in the *Endocladia* zone (GLYNN, 1965). The present study was conducted to provide more information on the behavior and foods of *A. pelta*.

## FIELD STUDIES

In order to determine activity patterns of *Acmaea pelta*, 16 limpets were individually marked and observed at hourly intervals during high tides over a continuous period of 4 days. Later, another 10 limpets were observed at 30 minute or hourly intervals for a 24 hour period. A small mark was painted on the substrate at each end of each limpet to indicate the animal's original position; at each successive observation, measurement of the distance and angle of the limpet in relation to this point gave its new position. General movement, feeding activity, and degree of tidal exposure were noted at each observation. The degree of tidal exposure was indicated using the descriptive terms of GLYNN (1965): exposed - periods when the animals were exposed to air without wetting by waves or splash; awash - when the animals were wetted by the sea, but not for more than 50% of the time; submerged - when the animals were wetted more than 50% of the time by splash or were continually immersed.

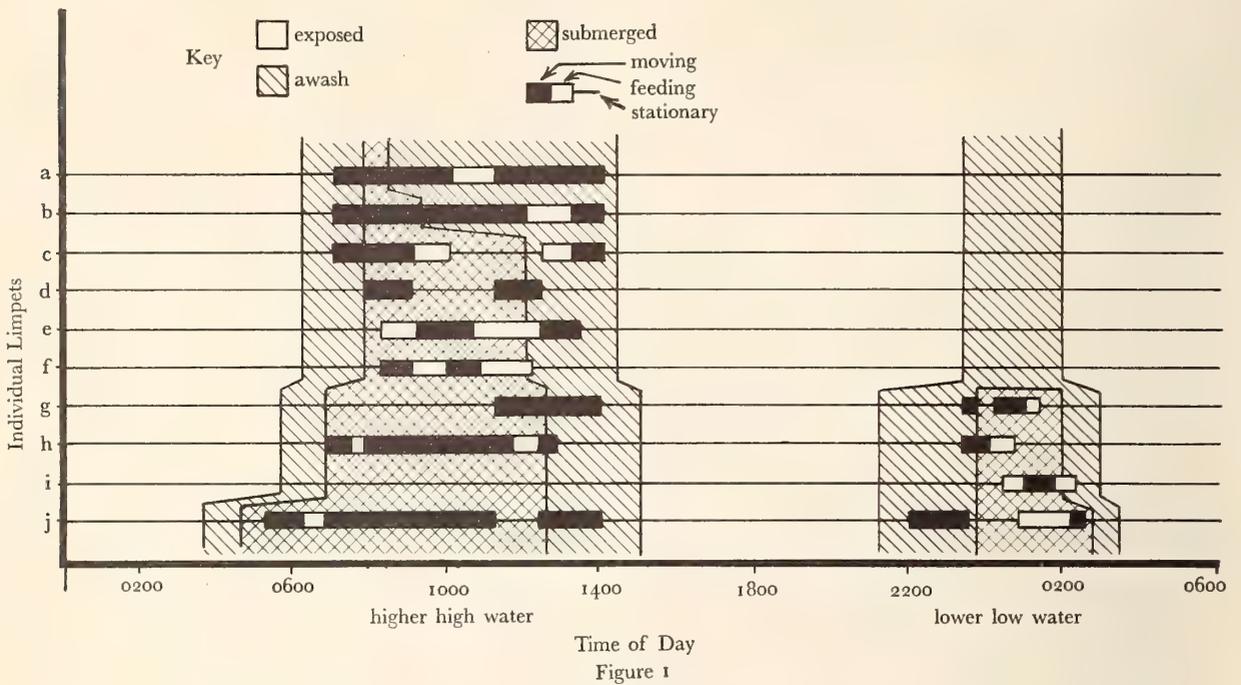
The behavioral criteria used to determine the occurrence of feeding in the field were based on observations of *Acmaea pelta* under laboratory conditions, and are described later.

General movement of the population is shown in Figure 1. Periods of movement in *Acmaea pelta* show a consistent relationship with the tidal cycle. The limpets remain stationary when out of water. Movement usually does not occur until the end of the period when they are awash on an incoming tide. While only 3 of the limpets depicted in Figure 1 moved during the initial awash period of the higher high water, all moved during the early part of the period of submersion. During the following period of lower high water (Figure 1), those animals too high on the rock to be submerged did not move at all, although they were awash for an extensive period. In general, *A. pelta* do not move until they are submerged, though after being submerged, some were observed to move upward on the rock into the zone still awash, climbing at a rate equal to that of the incoming tide. *Acmaea pelta* usually do not remain active for the high tide period and in several instances have been observed not to move at all (e. g. limpet "i", Figure 1). Such stationary individuals usually move during the following high tide.

Exact paths of movement were not plotted, but net displacement on the rocks between successive observation periods was noted for each limpet. This provides a measure of minimum distance moved. The amount of movement and the area covered varies considerably with individuals; one limpet moved no more than 2 inches during any high tide period over a span of 4 days, another covered a distance of 6 feet during a single high tide period.

Figure 2 shows examples of the types of paths taken by different individuals during a 4 day observation period.

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Movement and feeding activity of 10 *Acmaea pelta* observed at 30 minute or hourly intervals over a period of 24 hours, May 4 to 5, 1966. Limpets are indicated in order of their vertical positions was due to downward movement on the rock of these animals.

on the rock surface; individuals lower on the rock were awash and submerged for longer periods. The extended time during which the two highest limpets were awash on the receding higher high water

Path A shows rather random movement with the limpet coming to rest at a different spot at each exposure period. At some time during the 4 day study, 11 of the 16 limpets exhibited movement patterns like those of path B; they returned at least once to exactly the same location previously occupied. Homing, i. e., returning to exactly the same spot and here adopting the same orientation (Figure 2, path C), was noted in 4 of the limpets. One individual homed after moving 3 feet away from its spot. One limpet was observed to move into a spot and assume the same orientation as another limpet which had previously occupied that spot. Path D represents a type often observed. The majority of movement repeatedly occurs in a particular area, and the limpet returns to the same small area (approximate diameter 1 inch) with each exposure period but does not necessarily settle in exactly the same spot with the same orientation.

VERTICAL MOVEMENT

To test whether there was a general trend of vertical movement on the rock surface in relation to tidal exposure, the vertical component of movement of 10 limpets was observed at hourly intervals. The results reveal a specific pattern (Figure 3). When the tide rises at night a net upward displacement occurs, followed by a net downward displacement as the tide recedes. Similar movement patterns have been noted in *Acmaea limatula* (EATON, 1968) and *A. scutum* (ROGERS, 1968). Comparable data are not available for movements during the day.

FEEDING ACTIVITY

Studies were made to determine the frequency and duration of feeding periods, and the relation of feeding to

Explanation of Plate 1

A: Radula pattern of *Acmaea pelta* produced by a limpet feeding on a glass plate covered with a film of microscopic green algae and diatoms.

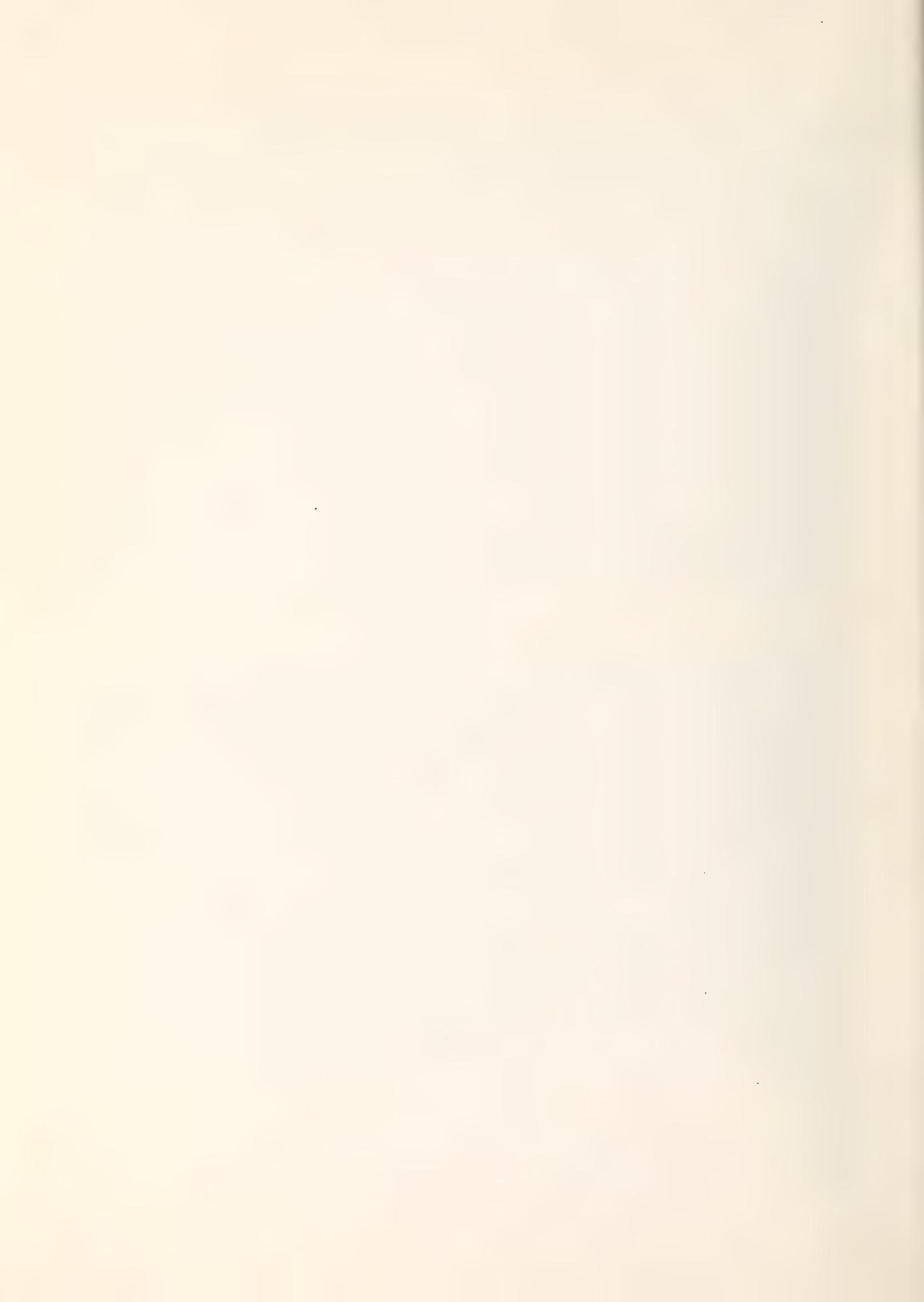
B: Portion of a blade of *Iridaea* taken in the field. An *Acmaea pelta* was found on top of the radular marks, and *Iridaea* was almost exclusively present in its stomach.



Figure 1



Figure 2



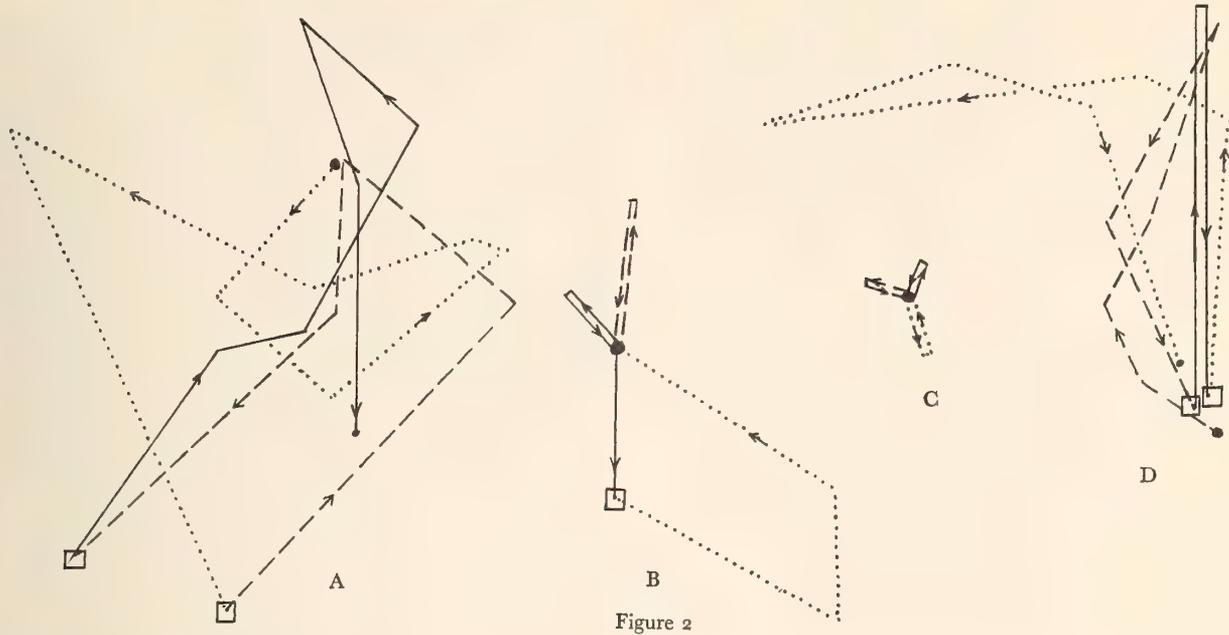


Figure 2

Representative tracks of 4 individual *Acmaea pelta* on a vertical rock surface over a period of 3 days, May 26 to 28, 1966. For each track, each type of line represents movement during one complete tidal cycle, and the limpets were exposed only at lower low water. Points enclosed by small squares are the places where the limpets remained stationary during periods of low water. Consistent homing behavior is indicated by the track in Figure 2 C.

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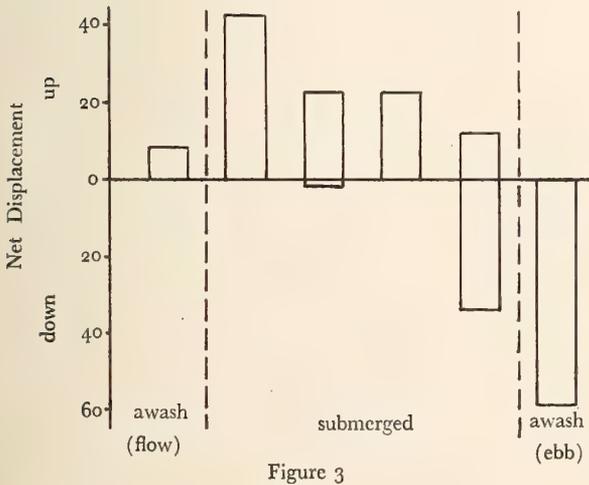


Figure 3

Net vertical displacement of the 10 limpets in Figure 1 at successive intervals during one nighttime higher high water, May 4 to 5, 1966. Each bar represents approximately the movement during one hour. Differences due to different vertical positions of the limpets have been compensated for by independently calculating the movement each animal underwent during the phases of tide indicated.

tidal exposure. *Acmaea pelta* placed in aquaria or on glass plates covered with an algal film, and arranged so that radular movements could be seen in ventral view, revealed a characteristic feeding behavior. The head sways from side to side, completing a cycle in 1 to 2 minutes. This can be detected in a dorsal view, even in the field, by watching the cephalic tentacles. The mouth, if visible, is flattened and spread over the substrate. Forward locomotion is relatively slow, in one case about 1 cm in 5 minutes. Feeding behavior in the field and laboratory, as recorded by patterns scraped by the radula on larger algae or on alga-covered glass plates, corresponds with the above description. In such patterns, produced by radular action as the limpet's head moves from side to side, each individual rasp of the radula is visible (Plate 1, Figure B). At greater magnification the marks of individual radular teeth can be seen (Plate 1, Figure A). This pattern of feeding provides a moderately efficient coverage of the surface.

It was often difficult to tell whether animals were feeding or not under field conditions, but some information on the feeding activity of *Acmaea pelta* was obtained. Of the limpets shown in Figure 1, 3 were not observed to feed during the period of higher high water, and 6

showed neither feeding nor other movement at lower high water. While feeding may have occurred between observations, some limpets taken from the field as the tide receded had no food in their stomachs. It appears that limpets do not necessarily feed during every tidal cycle; for much of the period of activity the animals move about without showing clear evidence of feeding. The statement by TEST (1945, p. 397) that *Acmaea pelta* "feeds at any and all times, regardless of whether the tide is in or out," is not supported by the present study.

### FOODS OF *Acmaea pelta*

Since *Acmaea pelta* is a very eurytopic organism, the question arises whether it is able to feed on a wide variety of plant material or feeds on a few forms which are widely distributed in the intertidal region. Published accounts (TEST, 1945; FRITCHMAN, 1961) indicate that *A. pelta* eats a variety of algae, both microscopic and macroscopic, but quantitative information is lacking. A study was therefore made to determine the foods available to *A. pelta* and the foods actually eaten, and to assess evidences of food selection.

*Acmaea pelta* are most abundant in mid to upper intertidal regions that can often be characterized by the presence of *Endocladia*, *Pelvetia*, *Egregia*, or *Postelsia* (Figure 4). Four areas were chosen, each a region where a

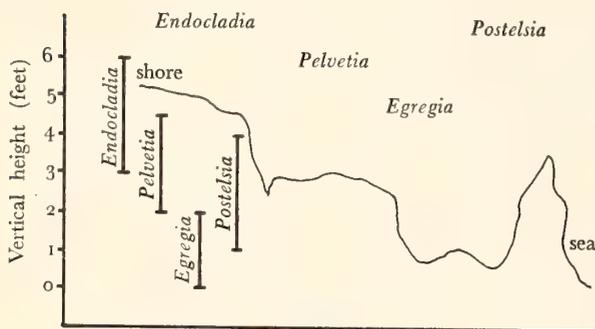


Figure 4

Horizontal and vertical distribution on a diagrammatic transect of a rocky shore, showing the main zones where *Acmaea pelta* occurs. Each zone is characterized by a predominating species of alga.

Vertical ranges of the algae are based on SMITH (1944).

different one of the above algae predominated. Since any alga present might represent a possible food source for *A. pelta*, an attempt was made to estimate the relative quantities of the different macroscopic algae present (Figure 5). Estimates are crude, for it is difficult to compare the

availability of an alga with a thallus many feet long with the availability of encrusting forms. The abundance of microscopic algae was not determined. These forms, consisting of diatoms and unicellular and filamentous green and blue-green algae, occurred on otherwise bare rock surfaces and as epiphytes on many of the larger algae in all 4 areas. Very small juvenile individuals of larger algae were included with the larger algae.

Twenty five *Acmaea pelta* whose guts retained food materials in recognizable states were collected from each of the 4 areas. A portion of the material from the stomach of each animal was microscopically examined. Identifications of algae in both field environment and stomachs were made with the kind help of Dr. Isabella A. Abbott of the Hopkins Marine Station. An attempt was made to assess the relative amount of each alga present to within 10%. Materials which could not be identified in microscopic examination are listed as "unidentified debris." The values obtained from the analyses of the stomach contents of the 25 *A. pelta* from each area were then averaged (Figure 5).

The *Endocladia* region, between 6.0 and 3.0 feet above mean lower low water (SMITH, 1944; a somewhat greater range is indicated by GLYNN, 1965), is characterized by macroscopic red algae. In this region *Acmaea pelta* ingested a variety of algae (Figure 5 A). Macroscopic algae (55%) were present in twice the volume of microscopic algae (26%). The macroscopic algae present in the gut are not minute, immature plants but consist of small fragments of much larger specimens. Field observation suggests that these are probably obtained from plants which are growing in small crevices and which have been grazed repeatedly, and from the holdfasts of larger plants growing on the open rock surface.

The brown alga *Pelvetia* characterizes a zone between 4.5 and 2.0 feet above mean lower low water (SMITH, 1944). This plant is the primary food of *Acmaea pelta* here (Figure 5 B). Most limpets in this region are found in the moist area under the blades of the *Pelvetia*, and except for a few small individuals they are not often seen on the blades themselves. They apparently feed on the holdfast where limpets are often observed during a period of submersion. *Pelvetia* appeared unusually macerated in the stomach of these limpets and is thought to constitute a large portion of the 33% of unidentified debris.

The *Egregia* region occupies the 2.0 to 0.0 foot level in the intertidal zone (SMITH, 1944). Here, too, macroscopic plants form the major part (62%) of the diet of *Acmaea pelta* (Figure 5, C). As in the *Pelvetia* region, one brown alga predominates in the environment and in the gut contents. Limpets are often found directly on the holdfast

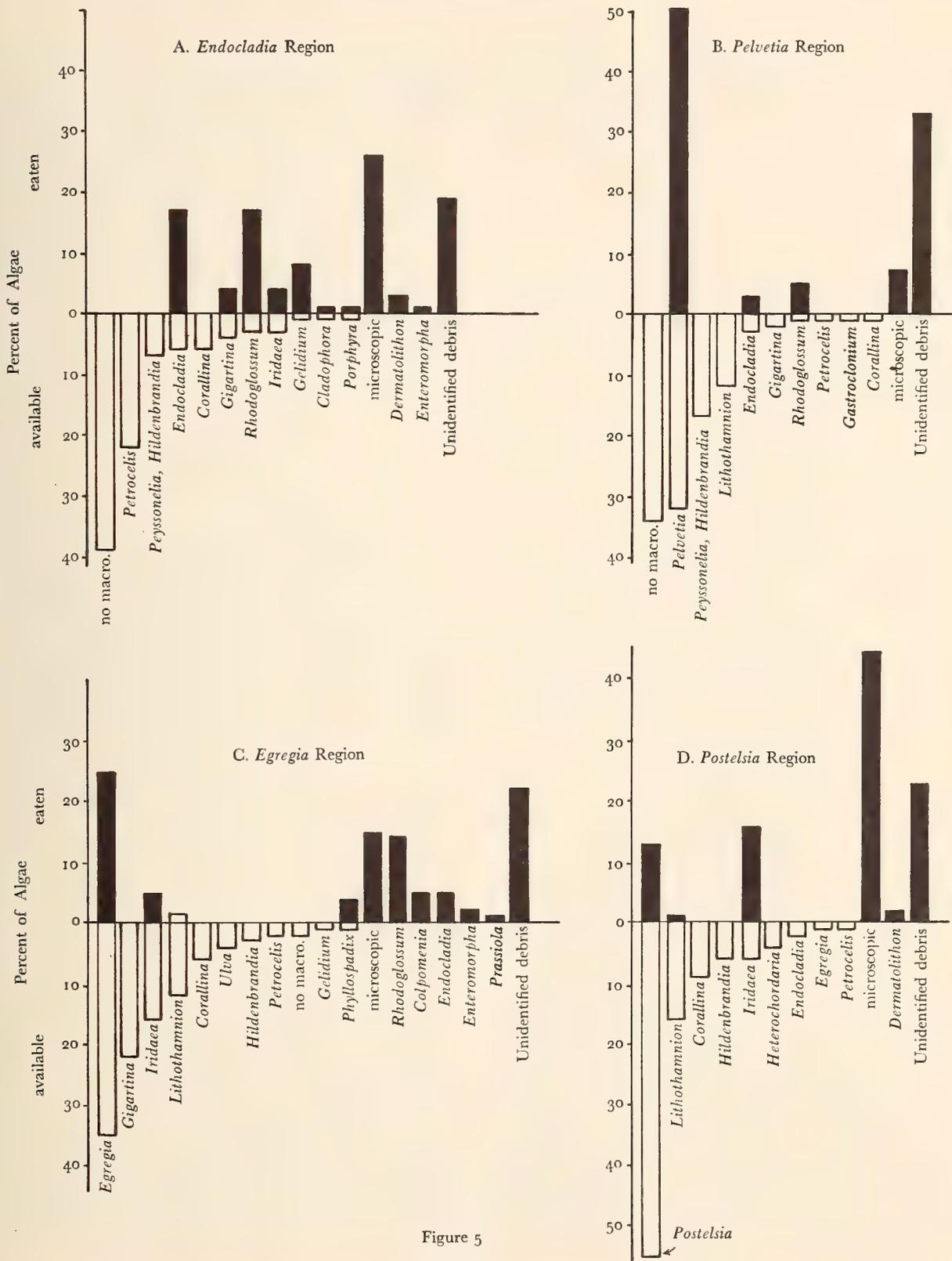


Figure 5

The foods available and the foods eaten in 4 regions, each characterized by a predominating species of alga, where *Acmatea pelta* is abundant. "No Macro." refers to areas devoid of visible macro-

scopic algae. These areas may contribute a portion to the microscopic algae eaten; other microscopic algae occur as epiphytes on larger plants.

Table 1

Algae Eaten by *Acmaea pelta*  
(and the per cent of the 25 limpets per region in which each alga was found)

	Regions:				
	<i>Endocladia</i> 84%	<i>Pelvetia</i> 100%	<i>Egregia</i> 100%	<i>Postelsia</i> 56%	Total 85%
I. Macroscopic Plants					
A. Green Algae					
1. <i>Prasiola meridionalis</i> SETCHELL & GARDNER, 1920			12	8	5
2. <i>Enteromorpha intestinalis</i> (LINNAEUS) LINK, 1820	8		8		4
3. <i>Ulva</i> sp.	-	-	4	-	1
4. <i>Cladophora trichotoma</i>	4	-	-	-	1
B. Red Algae					
1. <i>Rhodoglossum affine</i> (HARVEY) KYLIN, 1928	44	28	48	-	30
2. <i>Endocladia muricata</i> (POST. & RUPR.) J. G. AGARDH, 1847	48	20	20	-	22
3. <i>Iridaea</i> sp.	36	12	16	24	22
4. <i>Gelidium</i> sp.	32				8
5. <i>Lithothamnion</i> sp.	8	-	12	4	6
6. <i>Gigartina</i> sp.	20	-	-	-	5
7. <i>Dermatolithon dispar</i> (FOSLIE) FOSLIE, 1909	8	-	-	8	4
8. <i>Porphyra perforata</i> J. G. AGARDH, 1883	8	-	-	4	3
9. <i>Peyssonelia pacifica</i> KYLIN, 1925	-	4	-	-	1
C. Brown Algae					
1. <i>Pelvetia fastigiata</i> (J. G. AGARDH) DE TONI, 1895	-	92	-	-	23
2. <i>Egregia menziesii</i> (TURNER) ARESCHOU, 1876	-	-	52	-	13
3. <i>Postelsia palmaeformis</i> RUPRECHT, 1852	-	-	-	32	8
4. <i>Colpomenia peregrina</i> (SAUVAGEAU) HAMEL, 1931-39			16		4
5. <i>Heterochordaria abietina</i> (RUPR.) SETCH. & GARD. 1924	-	4	-	-	1
D. Flowering Plants					
1. <i>Phyllospadix scouleri</i> HOOKER,	-	-	8	-	2
II. Microscopic Algae					
1. unicellular green algae, diatoms, blue-green algae	80	24	64	84	63
2. <i>Dermocarpa</i>	8	4	-	-	3
3. <i>Goniotrichum</i> sp.			4	4	2
4. <i>Entophysalis deusta</i> (MENECH.) DROUET & DAILY, 1948	8	-	-	-	2
5. <i>Ectocarpus</i> sp.	8	-	8	-	4
6. <i>Hapalospongidion gelatinosum</i> SAUNDERS, 1899	-	4	-	-	1
7. <i>Pylaiella gardneri</i> COLLINS, 1898	-	-	-	12	3

and stipes of *Egregia* plants, and scars apparently caused by extensive feeding are frequently found beneath them.

This tendency of *Acmaea pelta* to ingest more macroscopic than microscopic algae is reversed in the exposed and surf-swept *Postelsia* region (4.0 to 1.0 foot intertidal level; SMITH, 1944). Over half the limpets collected in this region were taken from *Postelsia* stipes, but *Postelsia* is not the major food found in their stomachs. They apparently feed mainly on diatoms and other epiphytic microscopic algae growing on *Postelsia* (Figure 5 D).

Limpets not directly on *Postelsia* also ingested quantities of *Iridaea*. Microscopic algae constitute 45% of the volume of stomach contents and macroscopic algae only 32% in this zone.

Comparisons of plant foods available and stomach contents from the 4 regions show that *Acmaea pelta* does not feed at random but ingests significantly large quantities of macroscopic algae. However, all the major phyla of marine plants are represented in the diet. JOBE (1968), in a study of the digestive enzymes of *A. pelta*, found

amylase activity marked, and that of fucoidinase and alginase somewhat less. The variety of foods eaten, shown in Table 1, may be an important factor influencing the ability of *A. pelta* to live in a wide range of intertidal conditions.

Where different species of the same genus occupy the same general area and habitat, and are more or less sympatric, the extent to which they compete for various requirements is always of interest. *Acmaea pelta* is often found in company with other species of *Acmaea*, especially *A. limatula*. In a study of the foods of *A. limatula*, EATON (1968) found that *A. limatula* ingests primarily the red encrusting algae, *Hildenbrandia*, *Peyssonelia*, *Lithothamnion*, and *Lithophyllum*. In contrast, *A. pelta* eats very little of these species. The dietary studies suggest that in situations where both species occur, there is relatively little competition for food.

### SUMMARY

1. Most movement and feeding of *Acmaea pelta* occurs while the animals are submerged and while they are being splashed during tidal ebb. At night, the population shows a net upward displacement when the tide rises and a net downward displacement as the tide recedes. All but 1 of 16 limpets observed over a 4-day period returned at least once to exactly the same location previously occupied, but only 4 of them consistently homed.
2. Feeding is not continuous during periods of activity, and apparently animals do not feed during every tidal cycle.
3. *Acmaea pelta* ingests a wide variety of algae, both microscopic and macroscopic. The most common macroscopic plants eaten are the red algae *Rhodoglossum affine*, *Endocladia muricata*, and *Iridaea* sp., and the brown algae *Pelvetia fastigiata* and *Egregia menziesii*.
4. *Acmaea pelta* and *A. limatula* often occur in close proximity. Dietary studies suggest that in such situations there is relatively little competition for food.

### ACKNOWLEDGMENTS

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# The Effects of Light and Tide on Movements of the Limpet *Acmaea scutum*

BY

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(4 Text figures; 1 Table)

THE LIMPET, *Acmaea scutum* ESCHSCHOLTZ, 1833, inhabits the midtide zone on rocky shores along the California coast. TEST (1945) has noted some aspects of the ecology of this species, but detailed information is lacking on its activity pattern. The present study was undertaken to determine the movements of the *Acmaea scutum* population and the effects of tide and light on these movements.

## FIELD STUDY

### METHODS AND MATERIALS

Except where noted, all studies were carried out on the rocky shores of Mussel Point, Pacific Grove, California. Two vertical rock surfaces, located on the leeward side of Mussel Point, were chosen for the first set of field studies. Both surfaces faced west and both were exposed to considerable surf. To facilitate the measurement of limpet movement, narrow horizontal lines were painted on both rock surfaces approximately one foot above the highest *Acmaea scutum*. One inch divisions were marked along this line. The *A. scutum* were individually marked by dots of paint on their shells. Marking was done at low tide on a warm day, and the animals were not removed from the rock. The horizontal positions of the marked limpets were indicated with reference to the divisions on the horizontal line; vertical positions were measured with a yard stick. Further painted lines on the rock were avoided because it was felt they might affect limpet movements.

On April 27 and 28, 1966, the positions of 19 *Acmaea scutum* were recorded every hour or every two hours for a 24-hour period. On May 3 and 4, 1966, the positions of 11 other *A. scutum* were recorded hourly for a 24-hour period. The light conditions and the level of the tide were

recorded with each observation. At each observation it was noted whether the limpets were submerged by the sea, awash, or exposed to the air. The term "awash" covers all conditions between the first dampening of the animals by splash to complete submersion.

The positions recorded from the field observations were plotted to scale on graph paper. The resulting points for each limpet were connected with straight lines to provide a track indicating minimum net displacement in successive intervals of time. Preliminary observations showed that the marked *Acmaea scutum* were underwater about 16 hours a day. A wet suit, snorkel, mask, and underwater light were used to observe the limpets when submerged. During the period of higher high water on both days the heavy surf conditions made observations impossible.

### INDIVIDUAL TRACKS

Of the 30 *Acmaea scutum* marked in the 2 areas, 6 were either washed away or had their paint marks removed by the water. All of the remaining 24 *A. scutum* moved during the 24-hour observation periods. Earlier observations had indicated that *A. scutum* moves very little when out of water. For this reason the intervals between successive observations were greater when the animals were out of the water. Figure 1 shows the trails of 2 *A. scutum* as plotted from positions recorded every hour or every 2 hours. In Table 1 I have tried to show the percentage error in my tracking method. Without continuous observations one cannot be certain of the exact path the limpet followed to reach the new point. Table 1 shows that there is an apparent decrease of 15% in the trail length if one records positions every 2 hours rather than once every hour. Even with hourly readings the distances represented by the recorded tracks are probably less than the actual distances moved, but the limpets move so

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Table 1

Differences in Trail Length Recorded When Positions of Limpets Were Measured Every Hour vs. Every Two Hours

Individual <i>Acmaea scutum</i>	Distance Tracked (Inches)	
	Observed every hour	Observed every two hours
1	28	23
2	35	29
3	76	65
4	53	42
5	23	20
6	16	16
7	30	23
8	25	24
Total:	286	242
	$\frac{242}{286} = 85\%$	

slowly that the error is probably small. The trails of the 2 limpets shown in Figure 1 are characteristic of the trails of all the marked *A. scutum*. The trails demonstrate that individuals cross one another's tracks during periods of activity. Figure 2 shows that between successive periods of lower low water *A. scutum* moves an average of 40 inches with extremes of 16 and 76 inches.

- ⊙ Start of observation period
- \* End of observation period

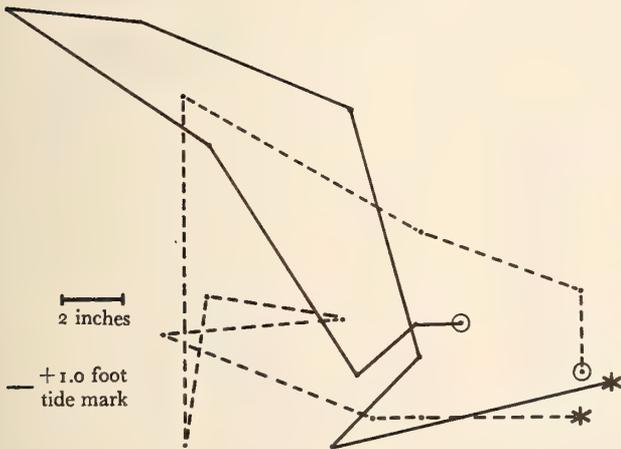


Figure 1

Trails of two *Acmaea scutum* on a vertical rock surface during periods of awash and submersion from 1500, April 27, 1966 to 0900, April 28, 1966. Each point represents the position of the limpet at the time of observation.

While plotting the trails of the 24 limpets it was noticed that at low tide they returned to the vicinity of the spot they had occupied the previous low tide. The average net displacement of the 24 limpets from one low tide to the next was 5.5 inches. The largest net displacement for any one of the 24 limpets was 14 inches. Only one of the 24 limpets returned to exactly the same spot it had occupied the previous low tide, and here it assumed its original orientation. Subsequent observations at Cypress Point, Pebble Beach, California, confirmed the general observa-

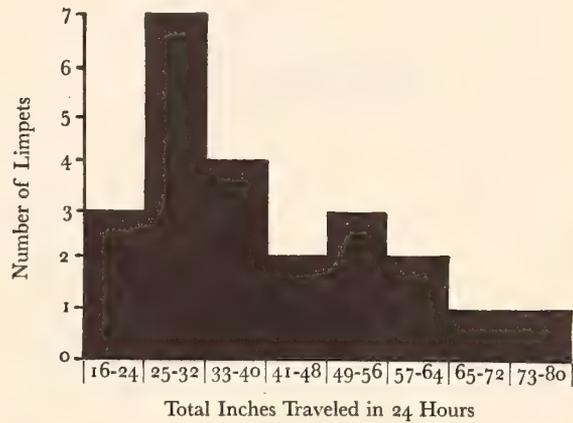


Figure 2

Total distance moved by 24 *Acmaea scutum* on a vertical rock surface during a 24 hour period.

tion that individuals tend to return to a spot near that occupied at the previous low tide. Combining the net displacement figures with the data in Figure 2 shows that *Acmaea scutum* moves an average of 40 inches between low tides,, but the average net displacement is 5.5 inches.

### MOVEMENTS IN RELATION TO TIDE AND LIGHT

In an attempt to find a relationship between tide and light and the movements of *Acmaea scutum*, the total hourly movement and the vertical vector of the hourly movement were compared at different phases of the tide and under different light conditions. The total hourly movement is the distance between the positions occupied by a limpet before and after a one hour interval. The vertical vector of the hourly movement is the vertical separation of the limpet's position before and after a one hour interval. The relation of total and vertical movement to light is represented in Figure 3 for the 2

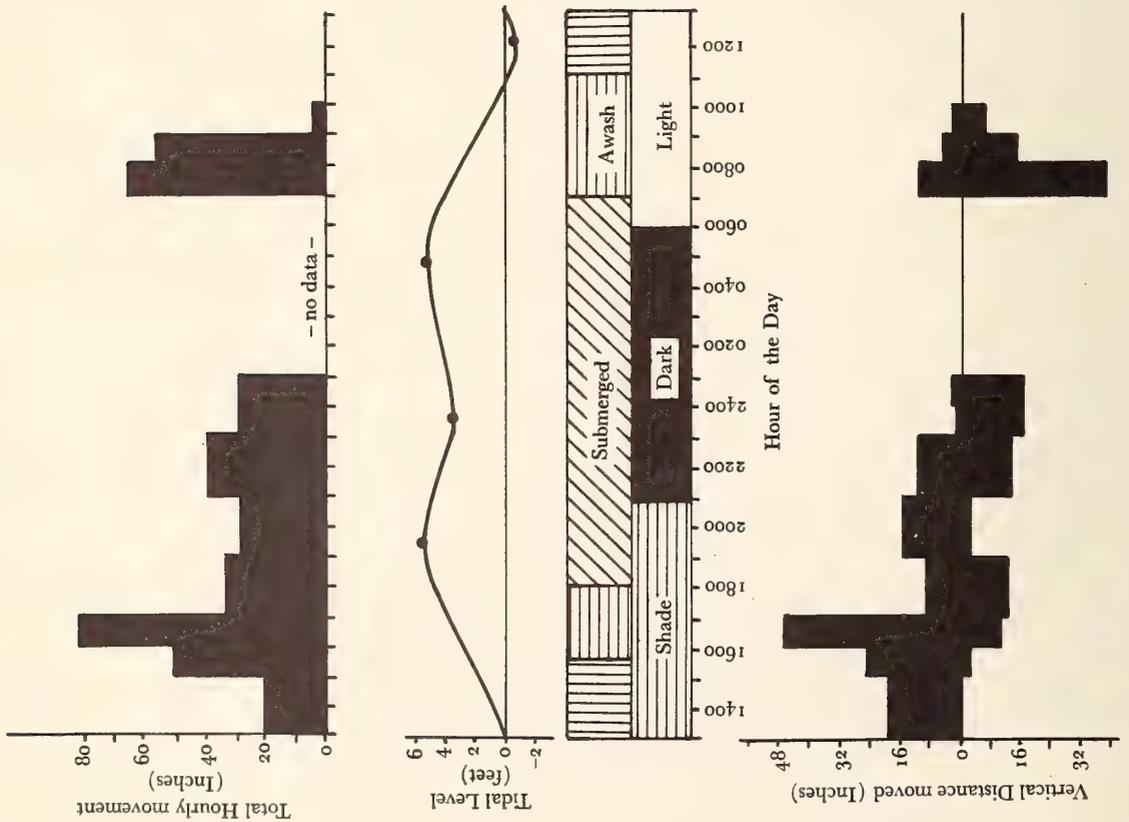
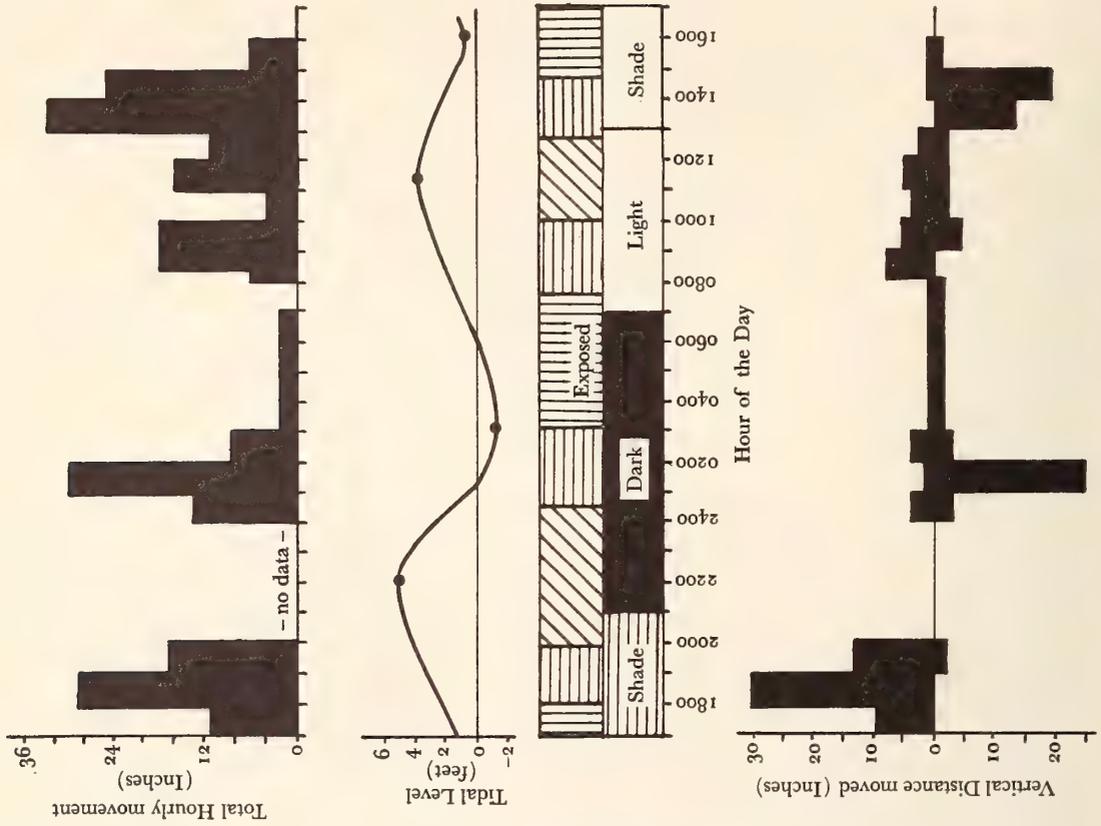


Figure 3

(← on facing page)

Comparison of light and tide conditions with total movement and the vertical component of total movement. Figure 3 A refers to data collected April 27 to 28, 1966, from the movements of 16 limpets. Figure 3 B refers to data collected May 3 to 4, 1966, from the movements of 8 limpets. Time is represented on the horizontal axes of both graphs. The vertical axes of the upper graphs, showing total hourly movement, represent the total distance moved during the hour for all of the limpets. The vertical axes of the lower graphs, showing the vertical component of movement, represent the upward or downward displacement each hour of all the limpets from the position they had occupied at the beginning of the hour period.

observation areas. The *A. scutum* represented in Figure 3 A were uncovered by the tide only once during the 24-hour watch. The *A. scutum* represented in Figure 3 B were uncovered twice during the 24-hour watch.

Figure 3 shows that *Acmaea scutum* moves most when it is awash. Each peak on the graphs showing total movement corresponds to a period when the animals were awash. *Acmaea scutum* does not move when out of the water and in sunlight, but the animal may move a short distance during the day when out of the water in the shade. When completely submerged, *A. scutum* moves sporadically, and the effects of light and darkness on movement under these conditions appear negligible.

The graphs showing the vertical component of movement (Figure 3) demonstrate that *Acmaea scutum* moves upward when washed by the incoming tide and downward when washed by the outgoing tide. The light conditions during the period of wash appear to affect the distance *A. scutum* moves. In Figure 3 B, when the incoming wash was accompanied by sunlight, both the total and the vertical movements were much less than when the incoming tide was accompanied by shade. The effects of light and darkness on the vertical movements of submerged animals are difficult to evaluate from these field studies.

### LABORATORY STUDIES

To test the correlation of *Acmaea scutum* movements with the conditions of tide and light as found in the field, several laboratory experiments were made. Preliminary studies showed that when submerged in laboratory aquaria *A. scutum* generally moves upward. It was decided to test the effects of light and turbulence on this movement. Turbulence was chosen as a variable, in addition to light, because the field studies had indicated that

*A. scutum* moves most when awash. A 20 inch by 7 inch glass plate was placed vertically in an 18 inch deep cylinder (diameter 8 inches). Light was provided by a 100 watt bulb placed directly above or directly below the glass cylinder. In all of the experiments air was bubbled through the water, but where turbulence was desired, 2 bubblers were placed in the cylinder and the compressed air flow was increased to the point where the surface of the water was vigorously agitated. In each ex-

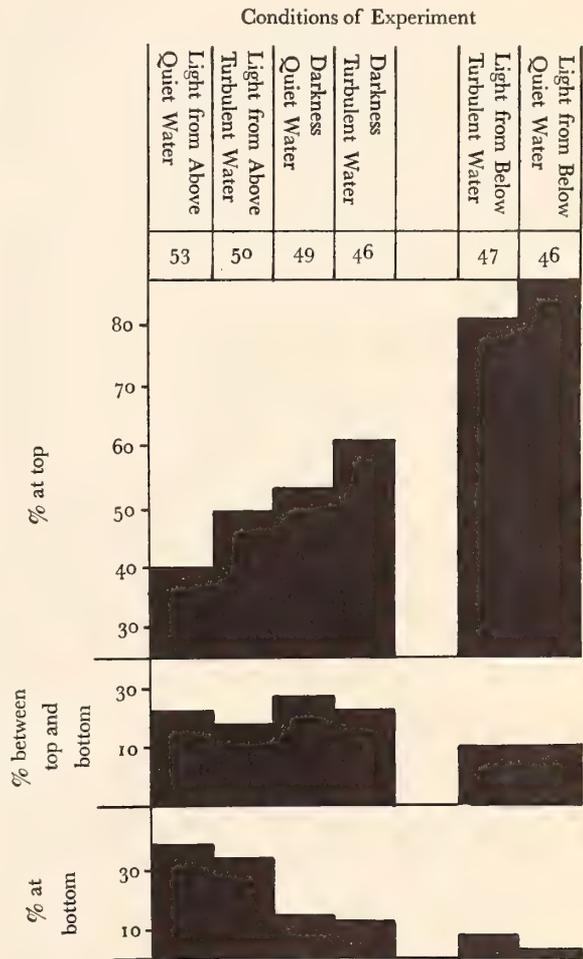


Figure 4

Results of laboratory experiments. Each experiment lasted 2 hours. The top graph shows the percentage of limpets that were at the surface after the 2 hour period. The middle graph shows the percentage of limpets that were between the bottom of the glass plate and the surface after the 2 hour period. The lower graph shows the percentage of limpets that were still at the bottom of the glass plate after the 2 hour period.

periment 12 to 16 *A. scutum* were placed on each side of the glass plate about 1 inch from the bottom. Each experiment was run twice; a total of approximately 50 limpets were subjected to each combination of variables for a period of 2 hours.

The results of the experiments are shown in Figure 4. The first 4 bars of the graph represent the combinations of variables most closely approximating those in the field. In the trial with light above the cylinder and calm water the fewest limpets reached the surface. This result corresponds well with the field observation that *Acmaea scutum* move upward only a short distance when they are awash on a rising tide during the day. Of the first 4 experiments, the greatest number of limpets reached the surface under conditions of darkness and turbulence. This result correlates well with the field observation that *A. scutum* move upward most rapidly when they are awash on a rising tide at night.

Preliminary studies showed that, when submerged, *Acmaea scutum* exhibits a negative geotaxis, and moves upward. Ross (1968) found that submerged *A. scutum* also exhibit a negative phototaxis. In the first 4 experiments it appears that these two responses are operating. When animals were submerged in the light, the negative responses to light and gravity opposed each other, and only 40% of the limpets reached the surface in the 2-hour period. To test this interpretation the light was placed beneath the cylinder in the last 2 experiments. Under these circumstances the negative taxes to gravity and light reinforced one another and more than 80% of the limpets moved to the surface in the 2 hour period.

### SUMMARY

1. The effects of light and tide on the movements of the limpet *Acmaea scutum* were studied in the field and in the laboratory.
2. *Acmaea scutum* moves most when subject to the turbulence of the tidal wash during tidal ebb and flow,

but continues to move when submerged at high water.

3. *Acmaea scutum* moves upward with the incoming wash during tidal flow and downward with the outgoing wash during tidal ebb.
4. The movements of *Acmaea scutum* during periods of wash appear to be dependent on the light conditions; vertical and total movements are greater at night than during the day.
5. In the field situation *Acmaea scutum* moved an average of 40 inches between 2 successive periods of lower low water but returned to within an average distance of 5.5 inches from the starting point.
6. In the laboratory *Acmaea scutum* moves upward when submerged; the rate is slowest when animals are illuminated from above, higher under condition of darkness, and still higher when the animals are illuminated from below. These results generally confirm results obtained in the field, and are interpreted in terms of a negative geotaxis (stronger) and a negative phototaxis (weaker) in limpets awash or submerged.

### ACKNOWLEDGMENTS

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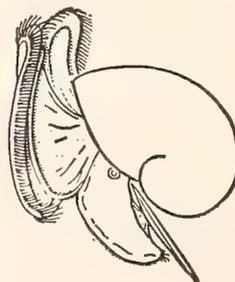
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395 - 405



# Light Responses in the Limpet *Acmaea limatula*

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(3 Text figures; 3 Tables)

PRELIMINARY OBSERVATIONS of the phototactic responses of the limpet *Acmaea limatula* CARPENTER, 1864, led to the studies of spectral sensitivity reported in this paper. Mean response times of dark-adapted animals to light of different colors and intensities were determined, and attempts made to anatomically localize the photoreceptors. The results suggest the possibility of at least two photoreceptors and several visual pigments in this organisms.

## METHODS AND MATERIALS

The animals used were all collected at Mussel Point, Pacific Grove, California, in the intertidal zone between +2 and +6 ft. The observations of responses were made in a plastic-lined, water-tight wooden trough, which could be continuously supplied with fresh sea water. At each end of the trough, a 150 watt tungsten lamp, enclosed in a sheet metal box, supplied the illumination. The boxes, painted flat black to reduce reflection, had  $\frac{3}{4}$ " holes drilled in the face at the height of the lamp filaments. Each light, connected through a rheostat, could be independently operated for illumination from one, or both ends of the trough.

Various areas of the spectrum were isolated by Corning glass filters, nos. 554, 401, and 244, corresponding respectively to blue, green, and red. Maximum transmittance with the blue filter was at 425 m $\mu$ , decreasing to 10% transmittance at 500 m $\mu$ , and cutting off completely above 540 m $\mu$ . The green filter transmitted maximally at 520 m $\mu$ , with no transmittance below 460 m $\mu$  or above 600 m $\mu$ . The red filter transmitted all wavelengths greater than 600 m $\mu$ . A standard infrared absorbing filter, composed of acidic 0.5% CuSO<sub>4</sub> in distilled water, was also used. Light intensity was varied by adjustment of a rheostat.

In experiments with white light the animals were placed 32 cm from the light source and the intensity at

each rheostat voltage setting determined with a thermopile. Where color and intensity were varied, this thermopile reading was used to approximate equal intensities of the light at each color at any given voltage. This was accomplished by varying the distance of the animals from the light source, as indicated by the thermopile readings. The difference in distance between the placement of animals in white light and the placement in any other color never exceeded 10 cm (in blue), and in some cases was as little as 2 cm (in red). Because of the insensitivity of the galvanometer, accurate measurements were not possible below 60 volts. The equalizations of intensity are, therefore, only approximate.

Data were obtained on response (yes or no), response time, and response time as a function of color and intensity. The response time given is from initial illumination until a definitive turning response (described below) was ascertained. All animals used in the experiments were allowed to dark adapt for a minimum of one hour.

## RESULTS AND DISCUSSION

In the initial work it was necessary to determine a definitive response of the limpet to illumination, such as a turning response (FRAENKEL & GUNN, 1940), or to changing illumination, such as in the clam *Mya* (HECHT, 1919). Also it was necessary to determine whether the genus *Acmaea* would exhibit sufficiently consistent responses on which to base a study. Ten animals each of the species *Acmaea pelta* ESCHSCHOLTZ, 1833, *A. scabra* (GOULD, 1846), *A. scutum* ESCHSCHOLTZ, 1833, *A. digitalis* ESCHSCHOLTZ, 1833, *A. limatula* CARPENTER, 1864, and *A. asmi* MIDDENDORFF, 1849, were collected and tested for light responses.

The general response to light, when exhibited in any species, was found to be a combination of backward movement, sideways movement, and a 180° reversal of orientation. During illumination the animal shows a characteristic extension of pallial and cephalic tentacles, while the shell is kept fairly close to the substrate.

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Quantitative results (Table 1) show that both the percentage of animals responding to light, and the speed of this response, is greater in *Acmaea limatula* than in

Table 1

Responses of Six Species of *Acmaea* to White Light.  
Animals 32 cm distant from light source (rheostat at 110 volts)

Species	Number of Animals	Number of Responses	Mean response time (seconds)	Standard deviation (seconds)
<i>Acmaea scabra</i>	10	4	173	22
<i>Acmaea pelta</i>	10	4	140	35
<i>Acmaea scutum</i>	10	4	101	56
<i>Acmaea digitalis</i>	10	2	201	18
<i>Acmaea asmi</i>	10	0	-	-
<i>Acmaea limatula</i>	10	9	84	21

the other species. This species, therefore, was chosen for continued study. It was decided that the reversal response, if exhibited within 4 minutes after illumination, would be scored as the definitive response. Responses after 4 minutes were scored as negative.

Fifty-one *Acmaea limatula* were collected and tested, of which 50 showed positive responses with a mean response time of 76 seconds and a standard deviation of 26 seconds. The responses of these animals to illumination from the opposite direction were also tested. In only 7 out of 50 cases did the animal stop in its initial turning response when the illumination was changed from one end of the trough to the other. After an extended time those animals which did not initially respond to the second light would migrate away from it. These observations suggest some process of bleaching or adaptation to illumination. All the following studies were done with the 50 animals which exhibited the initial positive responses, regardless of the response to the second illumination.

A control experiment was then done to determine the animal's natural response to being removed from an aquarium and being placed, in total darkness, into the testing apparatus. Of 25 animals tested in the dark, only 2 showed behavior which might have been construed as a positive response had the animal been illuminated. The above findings show that the results obtained in all experiments are not significantly affected by the animal's natural response to being moved, but depend only on illumination.

In the next experiment 10 animals were placed in a dry trough to ascertain the effect on response time and

percentage of responses. Six of the 10 animals showed responses with a mean time of 112 seconds and standard deviation of 36. This experiment shows that although the animals do respond to light when out of water, there is a significant decrease in both rate and number of responding animals.

In the next experiment correlations between size and response time were examined. Thirty animals were chosen; 10 each in size ranges less than 9 mm, 9 to 16 mm, and greater than 16 mm. All 3 groups showed at least 9 out of 10 responses, with respective times and standard deviations of  $71 \pm 22$ ,  $68 \pm 14$ , and  $77 \pm 21$  seconds. These results suggest no correlation between response to light and size of the animal.

Spectral sensitivity of the response was then investigated, using the various color filters. The results of this experiment, shown in Table 2, indicate equal sensitivity in the visible spectrum at the maximum intensity of the lamp.

Table 2

Responses of *Acmaea limatula*, Normal and Without Eye Spots, to Light of Varying Spectra.  
Intensity is about equal in all colors.

Filter	Animals tested	Number of responses	Mean response time (seconds)	Standard deviation (seconds)
Normal Animals				
Infrared				
absorbing	10	10	57	8
Blue	10	10	51	12
Green	10	10	52	10
Red	10	9	61	31
Animals Without Eyespots				
Infrared				
absorbing	5	4	51	9
Blue	5	4	79	17
Green	5	1	56	0
Red	5	1	59	0

In the next experiment, attempts were made to localize the photoreceptors of *Acmaea*. The presumptive receptors are the eyespots, which in *Acmaea limatula* are greenish in color and are located at the base of the cephalic tentacles on the back of the head. Several attempts were made to remove the eye spots by cauterization with a hot needle. The 5 out of 18 animals which recovered completely had lost their cephalic tentacles as a result of the operation,

Table 3

Responses of Normal *Acmaea limatula* to Light of Different Colors and Intensities, and Responses of Eyeless Animals to Varying Intensities of Blue Light in Seconds.

The numbers indicate mean response time in seconds, standard deviation, and (in parentheses) the number of responses versus the number of animals tested.

Relative Intensity (Volts <sup>1</sup> )	Response Time (Seconds) to Different Colors				
	white	Normal blue	green	red	Eyeless blue
100	47 ± 9 (5, 5)	82 ± 13 (5, 5)	83 ± 26 (5, 5)	75 ± 25 (5, 5)	81 ± 12 (4, 5)
90	88 ± 17 (5, 5)	57 ± 13 (5, 5)	80 ± 11 (5, 5)	57 ± 23 (5, 5)	115 ± 27 (4, 5)
80	124 ± 18 (5, 5)	76 ± 25 (5, 5)	107 ± 26 (5, 5)	119 ± 31 (5, 5)	138 ± 20 (2, 5)
70	127 ± 22 (4, 5)	141 ± 33 (4, 5)	124 ± 20 (4, 5)	115 ± 24 (4, 5)	124 ± 4 (2, 5)
60	156 ± 31 (5, 5)	79 ± 16 (5, 5)	153 ± 12 (3, 5)	51 ± 11 (5, 5)	0 ± 0 (0, 5)
50	114 ± 24 (4, 5)	68 ± 18 (5, 5)	240 ± 0 (1, 5)	51 ± 16 (5, 5)	-
40	41 ± 9 (5, 5)	67 ± 17 (5, 5)	0 ± 0	95 ± 22 (5, 5)	-
30	121 ± 15 (3, 5)	130 ± 31 (3, 5)	-	154 ± 12 (2, 5)	-

<sup>1</sup> (rheostat setting in volts)

and hence no indications of eyespots were apparent. These 5 animals were then tested for sensitivity to light of various spectra and intensity. The second part of Table 2 shows the eyeless animals react significantly only to white and blue light at maximum intensity.

The last experiment run in this study determined the effect of varying intensity of white, blue, green, and red

light on the responses of normal and eyeless *Acmaea limatula*. The results of this experiment are shown in Table 3 and Figures 1, 2, and 3. In normal animals, the response time in white, blue, and red light is biphasic, whereas in eyeless animals responses are only seen at high intensities of blue and white light.

The results of the last two experiments suggest the

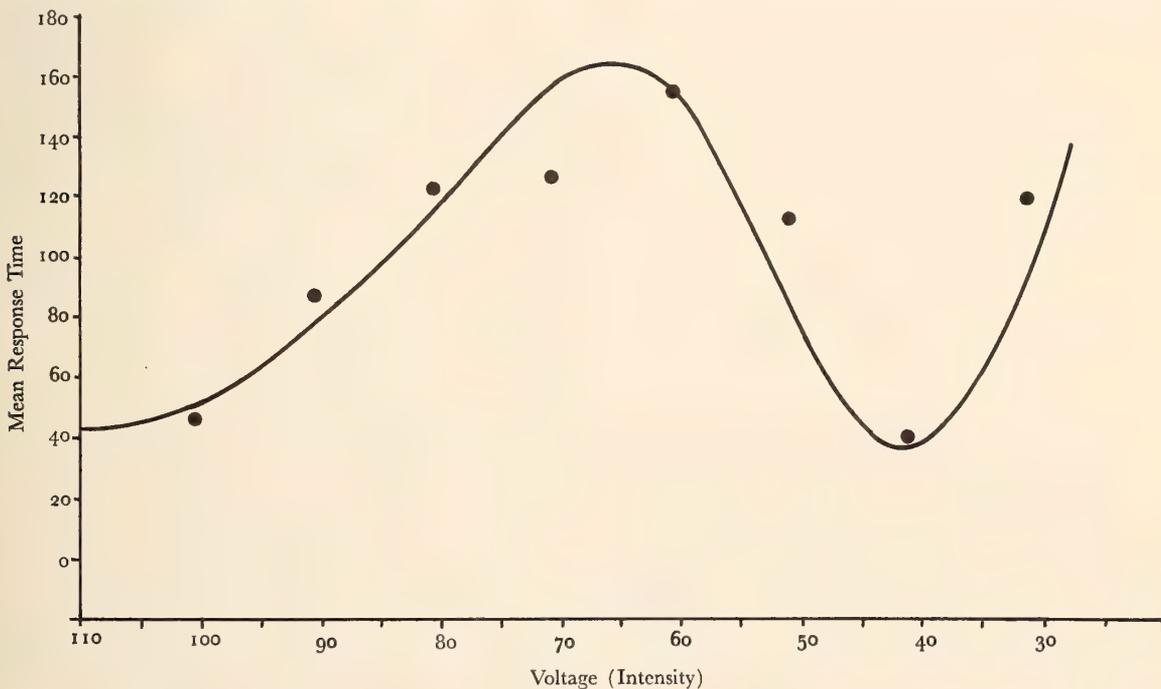


Figure 1

Responses of *Acmaea limatula* to varying intensities of white light.

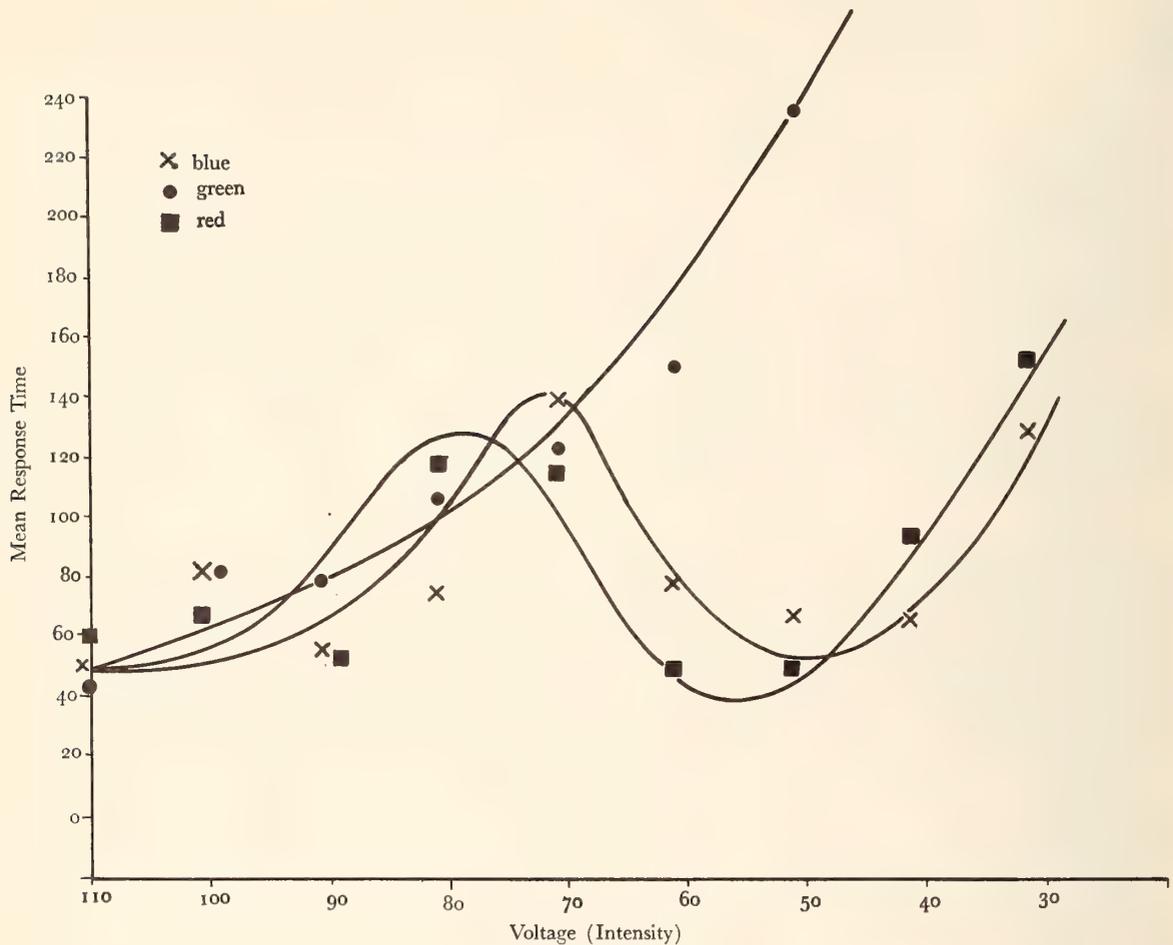


Figure 2

Responses of *Acmaea limatula* to blue, green, and red light of varying intensities.

presence of at least two pigments in *Acmaea limatula*, with the eyespots probably most important at the lower intensities. The greenish color of the eyespot might account for the fact that the animal exhibits poor responses to green light at lower intensities. The site of the other photoreceptor is completely undetermined. Two possible candidates are either the pallial tentacles, which normally are extended from under the shell edge, or, in *A. limatula*, the heavily pigmented side of the foot. Either of these possibilities would entail an amazing nerve network to determine the source of light since both the pallial tentacles and the foot would be symmetrical sites for the

photoreceptors as opposed to the normal condition of directional asymmetry of photoreceptors.

## SUMMARY

Light responses in 6 species of the genus *Acmaea* (*A. scabra*, *A. digitalis*, *A. scutum*, *A. pelta*, *A. asmi*, *A. limatula*) were investigated. *Acmaea limatula* showed the strongest responses to light. In all cases, responses when exhibited, were of a negative phototactic nature. Experiments varying color and intensity indicate the eyespots

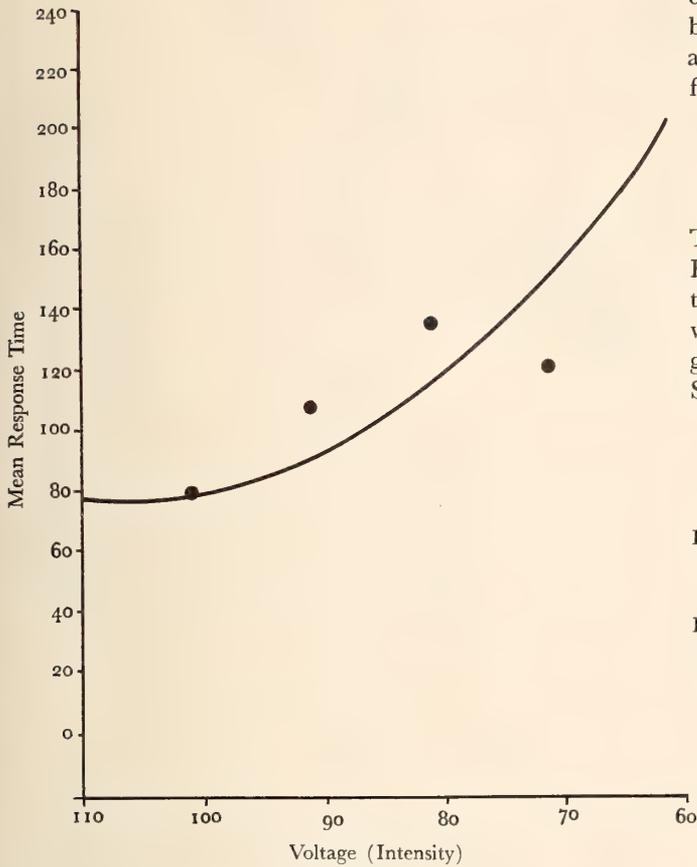


Figure 3

Responses of *Acmaea limatula*, without eyespots, to blue light of varying intensities.

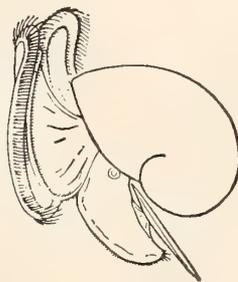
of *A. limatula* are important as photoreceptors in colors blue, green, and red at high and low intensities. There is at least one other photoreceptor and pigment, which is functional only in blue light at higher intensities.

#### ACKNOWLEDGMENT

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# Orientation and Movement of the Limpet *Acmaea digitalis* on Vertical Rock Surfaces

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(18 Text figures; 4 Tables)

AT MUSSEL POINT, Pacific Grove, California, *Acmaea digitalis* ESCHSCHOLTZ, 1833, is abundant on granite rocks above the *Endocladia-Balanus* zone (see GLYNN, 1965). Preliminary observations showed that these limpets tend to orient with their heads facing downward on vertical or nearly vertical rocks during periods of low water. No previous studies on orientation in *A. digitalis* have been found. However, in studies on ciliary currents of *Lottia gigantea* SOWERBY, 1843 by ABBOTT (1956), and the ecology of *Acmaea dorsuosa* GOULD, 1859 by ABE (1931), both authors found that the majority of these limpets were oriented head downward at low tide. Studies of the movements of *A. digitalis*, which might help explain this orienting behavior, are lacking. Previous observations of movement in this species have been related mainly to such matters as homing (VILLEE & GROODY, 1940; GALBRAITH, 1965), or to shifts in the distribution of populations over extended periods of time (FRANK, 1965).

In the present study an attempt was made to determine the orientation and movements of *Acmaea digitalis* on vertical rocks at various stages of the tidal cycle and to gain some insight into the factors influencing them.

## FIELD STUDIES ON ORIENTATION

An initial field study was carried out to determine the orientation tendency of *Acmaea digitalis* on vertical surfaces at low tide. *Acmaea digitalis* were observed on one large vertical rock, and the orientation of 136 limpets was recorded in terms of the position on a clock toward which the head of each was pointing. Animals with their anterior ends straight up were recorded at 12 o'clock, those with heads straight down at 6 o'clock, etc. The

results, shown in Figure 1, reveal a clear tendency, at low tide, to orient with the head downward from 4 to 7 o'clock. Later observations on other populations, including those in movement studies, confirm this tendency (Table 1). Young *Acmaea scabra* (GOULD, 1848) on

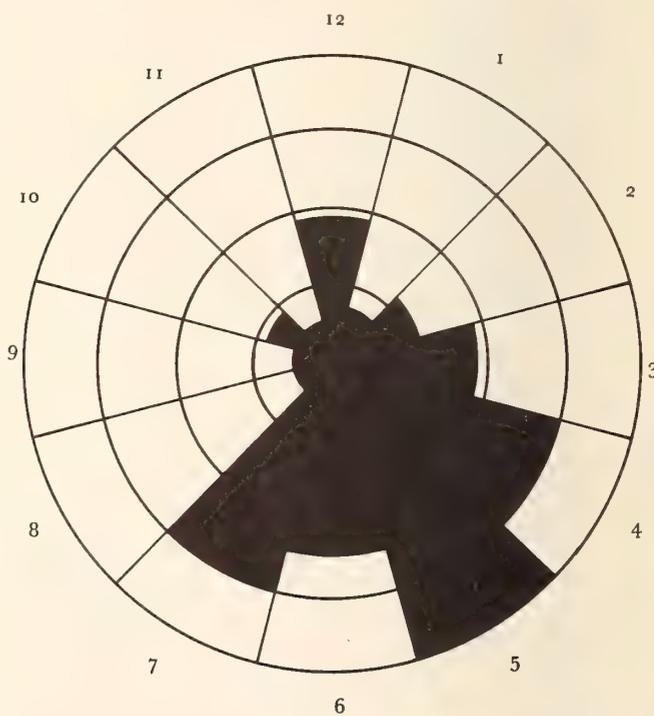


Figure 1

Orientation of 136 *Acmaea digitalis* at low tide on a vertical rock surface. The per cent of the population with heads pointed in each clock direction, from 1 to 12, are: 3, 6, 9, 15, 20, 13, 15, 2, 2, 4, 2, and 9.

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Table 1

Orientation shown by a limpet population during the period from lower low water to higher low water on three successive days, May 3 to 5, 1966.

Rock Surface	Per cent of Limpets (N = 152) Oriented in Each Clock Direction												Time
	1	2	3	4	5	6	7	8	9	10	11	12	
May 3	Dry	1	7	7	10	24	8	16	8	4	5	1	0430
	Damp	2	7	5	10	27	5	17	9	7	4	4	0730
	Splash	5	9	5	6	22	7	4	10	7	12	7	1030
	Damp	3	9	4	8	26	7	10	14	4	7	6	1330
	Dry	3	8	3	10	24	7	9	16	4	7	7	1600
May 4	Dry	3	14	2	10	25	5	12	8	5	5	8	0530
	Dry	3	14	2	10	25	5	12	8	5	5	8	0830
	Splash	3	9	5	14	17	8	10	14	2	8	6	1130
	Damp	2	15	1	13	24	5	12	9	3	6	6	1445
	Dry	2	14	2	13	23	5	12	10	3	6	7	1630
May 5	Damp	4	9	5	11	25	6	8	16	3	7	5	0300
	Damp	4	10	5	11	25	6	7	16	5	7	3	0545
	Damp	4	10	5	11	25	6	8	16	5	7	3	0830
	Splash	4	8	6	13	24	5	8	14	6	6	4	1230
	Damp	4	10	5	14	22	4	9	12	6	7	4	1730
	Dry	4	10	5	14	22	5	9	12	6	7	4	1900

vertical surfaces appear to show a similar tendency, though detailed observations are lacking.

Upon finding an orientation tendency at low tide, the next step was an investigation of orientation during a tidal cycle. All the following field observations on orientation were made on a vertical granite rock face (see Table 4, rock 1). On this rock surface 152 *Acmaea digitalis* were individually marked. The shell of each limpet was painted with a patch of yellow paint upon which an identifying number was marked in India ink. Each limpet's orientation was recorded approximately every 3 hours during lower low water (LLW), lower high water (LHW), higher low water (HLW), and the intervening mid-tides, on 3 successive days. No readings were taken at higher high water (HHW) due to heavy surf.

The results, shown in Table 1, clearly indicate that changes in orientation take place during the tidal cycle. The net change in orientation for each individual between successive observations was then computed, assuming that rotation in each case was accomplished by turning the minimum number of clock units necessary to account for the change (Figure 2). During the first tidal cycle the population showed more rotation upward with a rising tide and more rotation downward with a

receding tide, but this was not repeated in the next two tidal cycles.

The general activity of the population, as measured by the sum of the up and down rotation, and by the per cent of the population rotating (Figure 2), shows a peak associated with each high tide. The decrease in the height of these peaks of activity from one recorded high tide to the next is associated with a slight decrease in the heights of successive LHWs, and a resultant decrease in both the number of animals being effectively wetted and the duration of the period of splash. Also, during the first recorded LHW a fog bank blocked the sunlight, while on later LHWs direct sunlight dried the rock surface sooner. The results shown in Figure 2 suggest that activity of the population is proportional to the degree of wetting of the limpet population and the rock surface at high tide.

Since at some LHWs the whole population was effectively wetted and at others only the lower limpets received significant splash, for subsequent high tide studies the population on the rock face was divided into groups; a high group ranging from about 14 to 18 feet above mean LLW, and a low group located 9 to 12 feet above mean LLW. Hourly observations were made during a HHW

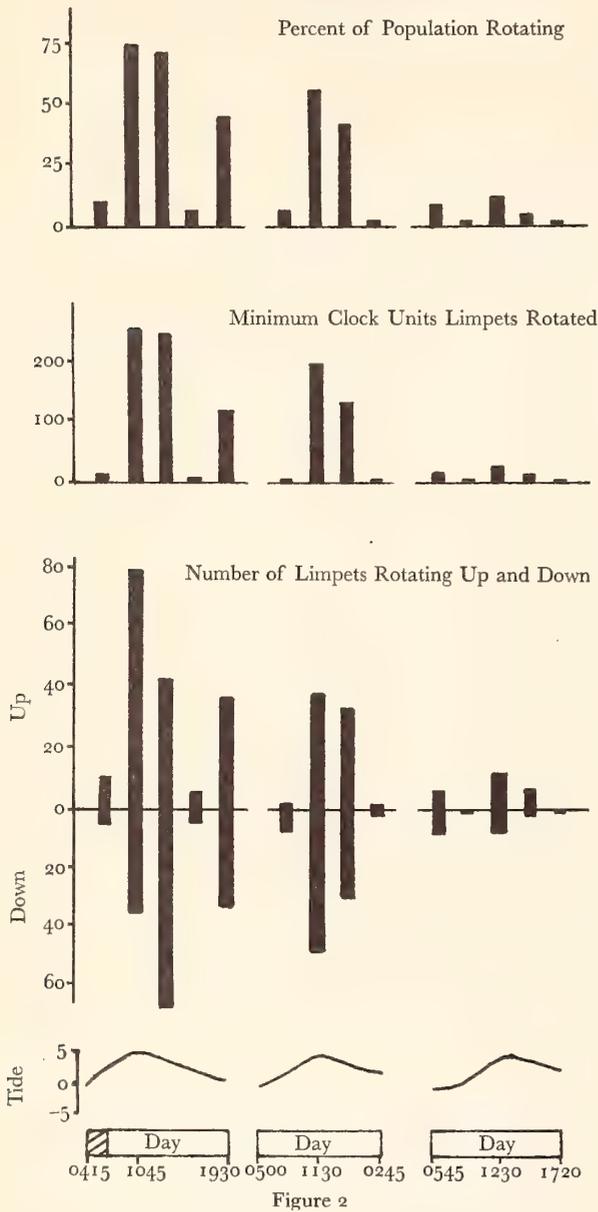


Figure 2

Change in orientation in the limpet population shown in Table 1, during the period from lower low water to higher low water on three successive days, May 3 to 5, 1966.

and a LHW period. The results are shown in Table 2 and Figure 3. The amount of rotation and the per cent of the population rotating are clearly related to the amount of splash received. The graphs in Figure 3 perhaps explain the differences in rotational activity shown

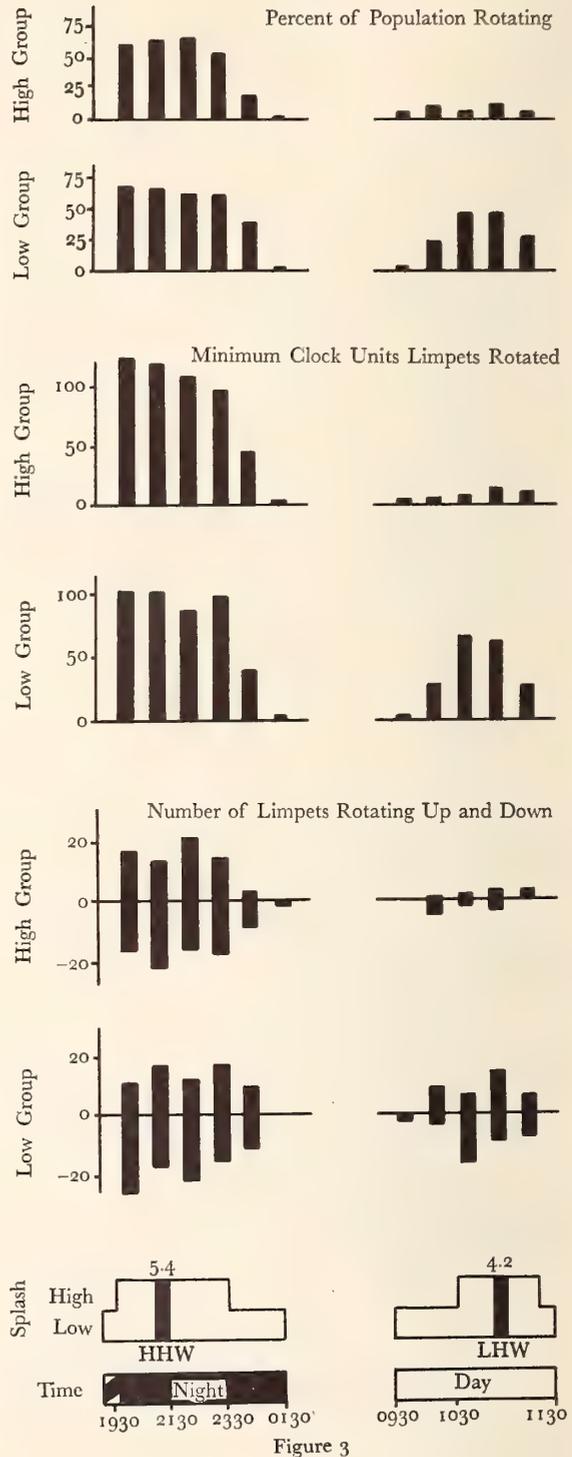


Figure 3

Table 2

Per cent of limpets oriented in each clock direction, during higher high water and lower high water, May 16 to 17, 1966. Condition on the rock surface: S = splash; W = damp; D = dry.

Per cents of Limpets Oriented in Each Clock Direction

Clock Direction	High Limpet Population N = 55							Time Condition of Rock Surface	High Limpet Population N = 56															
	1930		2030		2130		2230		2330		0030		0130		0830	0930	1030	1130	1230	1330				
	S	S	S	S	S	W	W		D	D	W	S	S	S										
1	5	4	2	5	0	0	0		0	0	0	2	0	2										
2	13	11	13	5	9	13	13		12	12	11	11	11	12										
3	4	11	5	5	5	4	4		7	7	11	5	7	9										
4	20	16	16	25	25	33	31		14	12	11	12	11	9										
5	16	16	14	5	7	13	14		25	27	25	25	30	30										
6	4	0	5	5	5	5	5		14	14	18	18	14	9										
7	9	5	7	5	7	7	7		11	11	9	9	9	12										
8	4	9	11	14	13	5	5		9	9	9	9	11	25										
9	4	2	5	4	4	5	5		2	2	4	4	4	4										
10	11	9	9	7	13	7	7		2	2	0	0	0	0										
11	4	13	5	11	11	7	7		4	4	4	5	4	4										
12	7	4	0	5	0	0	0		0	0	0	0	0	0										
	Low Limpet Population N = 50							Condition of Rock Surface	Low Limpet Population N = 48															
	S	S	S	S	S	S	W		D	W	S	S	S	S										
1	8	2	2	4	8	6	6		4	4	4	2	4	4										
2	12	18	8	10	6	10	10		12	12	12	8	8	6										
3	6	0	4	2	2	4	4		4	2	6	4	2	0										
4	10	10	12	14	14	12	12		8	10	10	15	17	12										
5	8	6	14	14	22	30	28		35	35	33	31	23	27										
6	6	2	0	6	4	4	6		6	8	4	12	8	8										
7	4	18	20	18	8	8	8		2	0	2	12	12	8										
8	6	10	12	6	8	10	10		8	8	10	4	6	15										
9	10	4	6	14	6	4	4		4	4	4	0	8	6										
10	10	14	8	4	12	6	6		10	10	8	6	8	8										
11	8	8	8	6	6	2	2		4	4	2	2	0	2										
12	3	2	6	2	4	4	4		0	0	1	2	2	2										
	Higher High Water								Lower High Water															

Figure 3  
(← on facing page)

Quantitative measures of changes in orientation of limpets high (14 to 18 feet) and low (9 to 12 feet) on a vertical rock face, May 16 to 17, 1966, based on data in Table 2. Duration of the period of splash for groups of limpets high and low on the rock face is shown just above the scale showing time of day. Time and height of high water are indicated by the black bars on the splash diagram and the numbers just above them.

by the limpets during the 3 successive tidal cycles portrayed in Figure 2. In Figure 3, in the low group of limpets at the high tide at 2000 hours, about 30 animals were rotating; as a group they rotated a minimum of 105 clock units. Similar high amounts of rotation occurred all during the splash period. These results, combined with the results on direction of rotation presented in Figure 2, show that orientation and direction of rotation tend to be random during the time the rock is thoroughly wetted. This tendency toward random orient-

ation was also apparent from watching the limpet movements during periods of splash.

For the limpet population to shift from a primarily head downward orientation at low tide to a more random orientation on the rocks during high tide requires that

Table 3

Mean orientations (clock directions) and standard deviations for left- and right-facing limpets during HHW and LHW, May 16 to 17, 1966, computed from Table 2.

	Limpets Oriented			
	Left		Right	
	Mean	Standard Deviation	Mean	Standard Deviation
<b>High Limpet Population</b> N = 55				
1930	9.05	±1.82	4.06	±2.41
2030	9.50	±1.59	3.79	±1.93
2130	8.88	±1.46	3.73	±1.36
2230	9.19	±1.72	3.90	±2.04
2330	9.04	±1.56	3.82	±1.12
0030	8.90	±1.63	3.86	±1.12
0130	8.90	±1.63	3.89	±1.14
<b>Low Limpet Population</b> N = 50				
1930	9.52	±1.68	4.18	±3.20
2030	8.82	±1.64	3.41	±1.60
2130	9.18	±2.81	4.45	±2.74
2230	8.50	±1.60	3.75	±1.50
2330	9.00	±1.63	4.07	±2.18
0030	8.53	±1.62	4.12	±2.03
0130	8.53	±1.62	4.15	±2.03
<b>High Limpet Population</b> N = 56				
0830	7.74	±1.56	4.11	±1.31
0930	7.74	±1.56	4.11	±1.31
1030	7.63	±1.54	4.16	±1.06
1130	7.80	±1.67	4.17	±1.40
1230	7.74	±1.49	4.24	±1.28
1330	7.76	±1.56	4.08	±1.40
<b>Low Limpet Population</b> N = 48				
0830	9.00	±1.46	4.03	±1.47
0930	8.93	±1.58	4.06	±1.46
1030	8.71	±1.39	4.15	±1.97
1130	7.80	±1.59	4.48	±1.85
1230	8.10	±2.05	4.28	±2.03
1330	8.29	±1.38	4.48	±2.05

some limpets rotate upward. To check whether there was a tendency for them to turn upward toward the right (counter-clockwise) or upward toward the left (clockwise), the data from Table 2 were used as follows. The limpet population was divided into left-oriented animals (limpets facing clock directions 7 to 11) and right-oriented animals (limpets facing clock directions 1 to 5). The limpets facing 6 o'clock and 12 o'clock were divided equally between right and left facing groups. The mean direction of orientation and standard deviation were calculated for both left and right facing groups for each period of observation shown in Table 2. The results are given in Table 3. Some tendency to turn upward at the beginning of the observation period and downward toward the end is indicated, but there is no clearcut preference for either a clockwise or counter-clockwise rotation. Standard deviations are largest and show most variation during periods when the rock was splashed, reflecting the rather random orientation of the high and low *Acmaea digitalis* populations during the 2 tidal cycles. Rotational movement was greatest during HHW, and greatest for the low group of limpets which received the most splash. As the water level dropped and splash declined, activity of the population decreased and the per cent of limpets with heads oriented downward and toward the right, in the directions of 4 to 6 o'clock, increased.

While it is established that on vertical rock surfaces *Acmaea digitalis* populations tend to orient with heads directed downward and to the right during periods of low tide, neither the selective advantage nor the causes of this behavior are entirely clear. The owl limpet *Lottia gigantea* orients much as does *A. digitalis* at low tide, and ABBOTT (1956) has suggested that with the head down, water run-off coming down the rock from wave splash would help clean the nuchal cavity of waste materials. He also points out that water trapped in the mantle groove as the tide recedes would drain toward the anterior end, thus keeping the head and ctenidium wet while the limpet is exposed at low tide. ABBOTT also suggests that an orientation with the head down and to the right might be slightly more advantageous than with the head down and to the left, since in the former case water running down the rock would tend to reinforce the existing ciliary currents in the nuchal cavity.

In *Acmaea*, as in *Lottia*, the anal opening lies on the right side of the nuchal cavity, so water run-off on the rock could easily help remove the waste materials from a limpet oriented toward 3 to 5 o'clock. Orientation toward 7 to 11 o'clock may present more of a problem since the feces would have to travel a greater distance through the mantle cavity to get out. However, according to YONGE

(1962), and my own observations, the ctenidium is flexible enough to be moved from one side of the mantle cavity to the other, carrying with it fecal material. This could transfer the fecal material to the opposite side where it could be washed out if the limpet were oriented to the left on the rock face. With reference to ABBOTT's suggestion about retaining water around the anterior parts at low tide, it was noticed that when the first splash covered *A. digitalis* facing downward on a dry, vertical surface, most animals raised their shells posteriorly and extended the mantle for less than a minute, permitting water running down the rock to flow under the shell toward the head. This activity occurred on successive splashes and slowly decreased; then the limpet usually moved. Animals facing upward on the rock sometimes

raised the fronts of their shells at the first signs of water run-off, permitting water to run about the head.

Almost nothing is known of the factors acting as immediate causes of particular responses and behavior patterns in *Acmaea digitalis*. Although limpets clamp down more tightly on rock surfaces in reaction to light from a flashlight at night and to a shadow cast in the day, the present studies, showing that downward orientation can occur at night as well as during the day, suggest light is not a primary factor here. Laboratory experiments involving geotropism have not been carried out. HEWATT (1940), in tests with *Acmaea scabra*, found that geotropism did not seem to be a factor in homing ability. There is certainly good correlation between an increasing exposure to splash and an increase in activity, the latter resulting in a more random orientation on the rocks. Likewise, the decline of splash during a receding tide is correlated with increasing downward orientation.

FIELD STUDIES ON MOVEMENT

Observations made during the orientation studies suggested that changes in orientation during the period of wetting might be associated mainly with vertical movements. To determine whether this was the case, 25 *Acmaea digitalis* on vertical granite rocks were watched during night high tides and 25 during day high tides. Rocks 1, 2, 3, and 4 were used in these observations (see Table 4). A spot of paint was placed on the rock surface

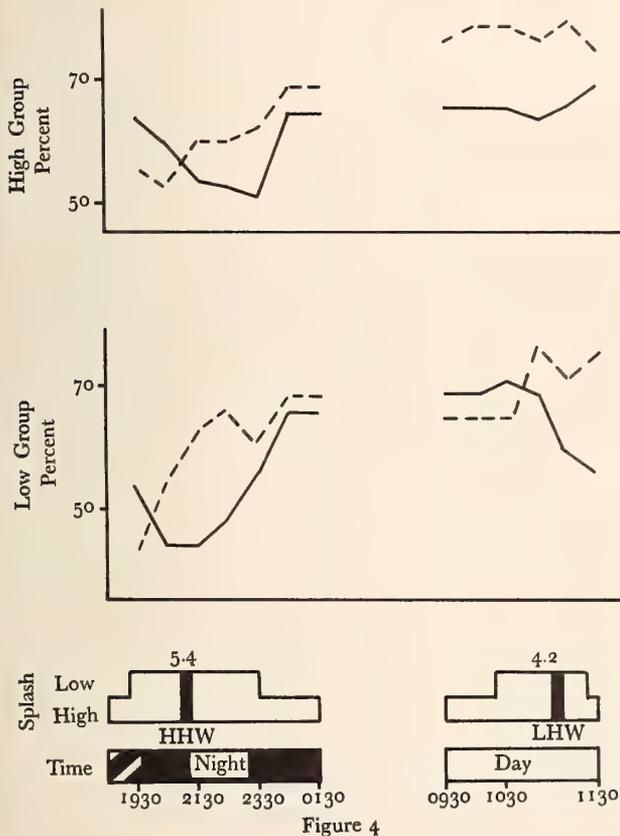


Figure 4

Tendency of limpets to orient with heads downward and to the right during HHW and LHW, May 16 to 17, 1966. Dotted lines show percent of limpet population oriented with the head downward (4 to 8 o'clock, plus half the individuals headed towards 3 o'clock and 9 o'clock). Solid lines show percent of limpet population oriented with the head to the right (1 to 5 o'clock, plus half the individuals headed toward 12 o'clock and 6 o'clock). Time and splash diagrams are the same as those in Figure 2.

Table 4

Description of rocks used in orientation and movement studies. Widths and heights give the size of the vertical surface available for limpet movement. Secondary waves occurred after the main force of the waves was broken by outer rocks. The height of the lowest portion of each rock above tidal datum is given in column 6.

Rock	Rock Face Direction	Width	Height	Wave Exposure	Height Above Tidal Datum
1	N	305 cm	671 cm	direct	9 ft
2	NW	91 cm	91 cm	secondary	3 ft
3	N	122 cm	152 cm	secondary	3.3 ft
4	NE	213 cm	69 cm	secondary	4 ft
5	N	43 cm	81 cm	secondary	3 ft
6	N	274 cm	91 cm	secondary	3.5 ft
7	NW	274 cm	122 cm	direct	4.5 ft
8	N	122 cm	122 cm	direct	4.5 ft
9	W	610 cm	305 cm	direct	3 ft
10	NNE	152 cm	137 cm	direct	3 ft

in front of each limpet's head and also on the peak of its shell; both spots of paint were marked with matching numbers in India ink. During the watches the vertical and horizontal distances between the anterior portion of each limpet and its spot of paint on the rock were recorded every 10 minutes.

Two of the 50 individual movement paths are shown in Figure 5. The movement paths showed that moving

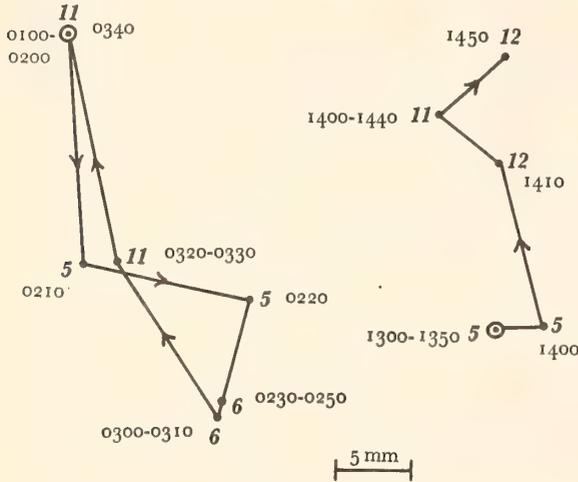


Figure 5

Typical movement paths for *Acmaea digitalis* during high tide. The clock orientation of the limpet (**bold face figures**) and the time at each particular position are given. The starting position is indicated by the double circle. The limpet whose path is shown on the left returned to its original home site.

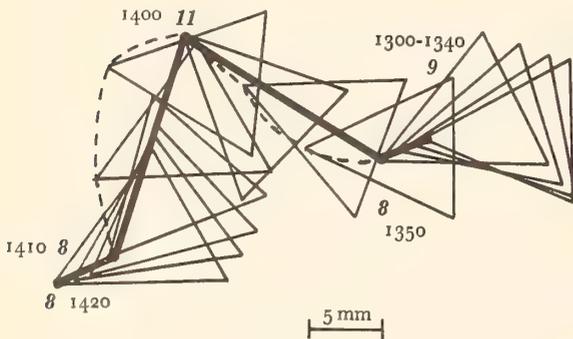


Figure 6

Time lapse diagram of a moving *Acmaea digitalis* showing the relationship between movement and change in orientation. Outlines show displacement at intervals of 3.33 minutes. Time and orientation are as given in Figure 5. The dotted line shows the actual movement of the head of the limpet (the apex of the triangle). The solid line shows the movement path and the orientation that would have been recorded had the observations been made every 10 minutes as in Figure 5.

animals were almost always oriented with the anterior end of the shell pointed in the direction of movement, but a change in orientation sometimes occurred with relatively little forward movement (Figure 6). Data on movement and rotation of limpets, collected on 9 different watches, are presented in Figures 7 and 8; data are grouped for each 30 minute interval for each watch.

The complex relationships between the various variables shown in Figures 7 and 8 are best considered in relation to specific questions. These are presented below, and pertinent data are summarized in Figures 9 through 14.

First, is there a direct relationship between amount of movement and amount of rotation? A scatter diagram (Figure 9) of the data presented in Figures 7 and 8 indicates that there is a general relationship, but the correlation between movement and rotation is not a strong one.

Second, is there initial movement upward (i.e. movement in the direction of 10, 11, 12, 1, or 2 o'clock within the first 30 minutes of motion) by the limpet population in either day or night? Such an upward movement might help explain a tendency to rotate upward as movement commences on a rising tide. In order to supplement the data from Figures 7 and 8, another similar watch was undertaken on rock 3 (Table 4), except the movements of the population were recorded for only the first hour of the wetting period during a LHW and the following HHW. The data from these observations, presented in Figure 10, show that 50% or more of the limpets do show an initial upward movement. No significant difference between day and night is demonstrated.

Third, is there a final movement downward, corresponding with the tendency to orient head down at low tide? The final individual movements and orientations for the 50 limpets depicted in Figures 7 and 8 are presented in Figure 11. Most limpets exhibit a final movement downward associated with a final head down orientation. Of those whose final movement was upward, some only changed direction from, say, 6 to 4 o'clock. While this is an upward movement, the final orientation is still down, explaining why fewer limpets had a terminal head up orientation than exhibited final movement upward.

Fourth, does the per cent of limpets which moved during a given high tide increase with increasing duration of the period of wetting? Data from the 9 watches in Figures 7 and 8 are plotted in Figure 12; they show that the percent of animals moving does tend to increase as the period of wetting gets longer. The longer wetting periods occurred at night, so one cannot adequately compare the results obtained during night and day.

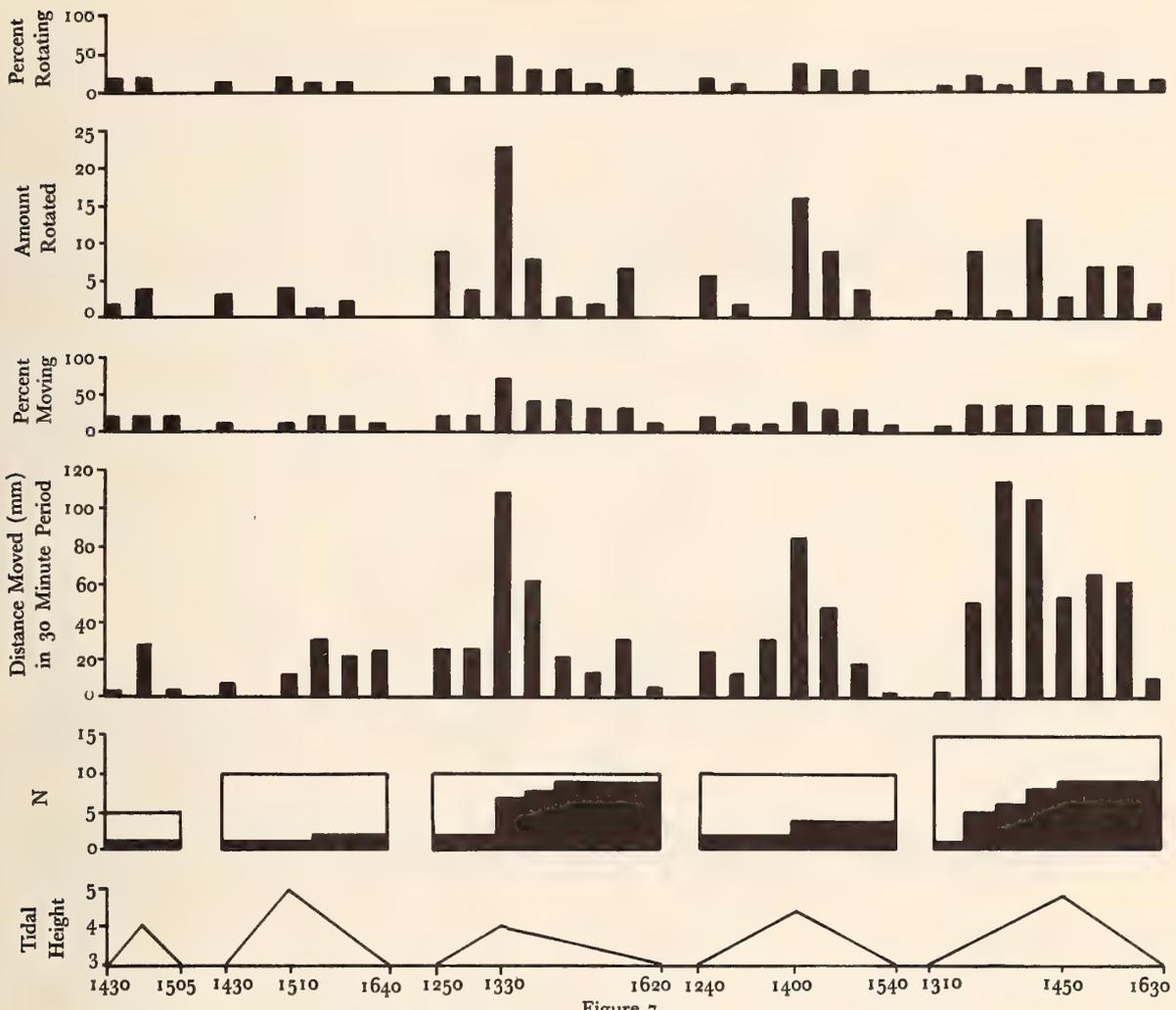


Figure 7

The relation between the per cent of the population which changed their positions, the number of clock units rotated by all individuals, the per cent of the population which rotated, and the distance moved by all individuals together in successive 30 minute periods, during 9 different high tides. Height of the population boxes (N) shows the total number of limpets watched in each conditions of splash. Results obtained during the day.

tidal period; the darkened area in each box shows a cumulative total of the number of these limpets which have moved at all at each successive period during the watch. The triangular tidal diagrams show only the time and height of the tidal peak and the points of initial and final splash. For each tide all limpets were at approximately the same level, and were subjected to the same conditions of splash. Results obtained during the day.

Fifth, are there relationships between the amount of time spent moving, the distance moved, the duration of wetting and the time of day? Data for individual limpet movements appear in Figure 13. The results show that the distances moved and time spent moving tend to increase as the wetting periods get longer. Distance moved is roughly proportional to time spent moving. As the animals were generally wetted for longer periods at night, it is again difficult to contrast the day and night results.

Six, do limpets tend to move more rapidly, on the average, during high tides at night than during high tides in daylight hours? The average rates of movement for all of the individual limpets used to obtain data for Figures 7 and 8 are summarized in Figure 14. The average rates for day and night differ by only 0.1 mm per minute. In a "t test" applied to these results,  $t=1.31$  with a probability of 0.10 to 0.20 that the null hypothesis that there is no difference between day and night rate of

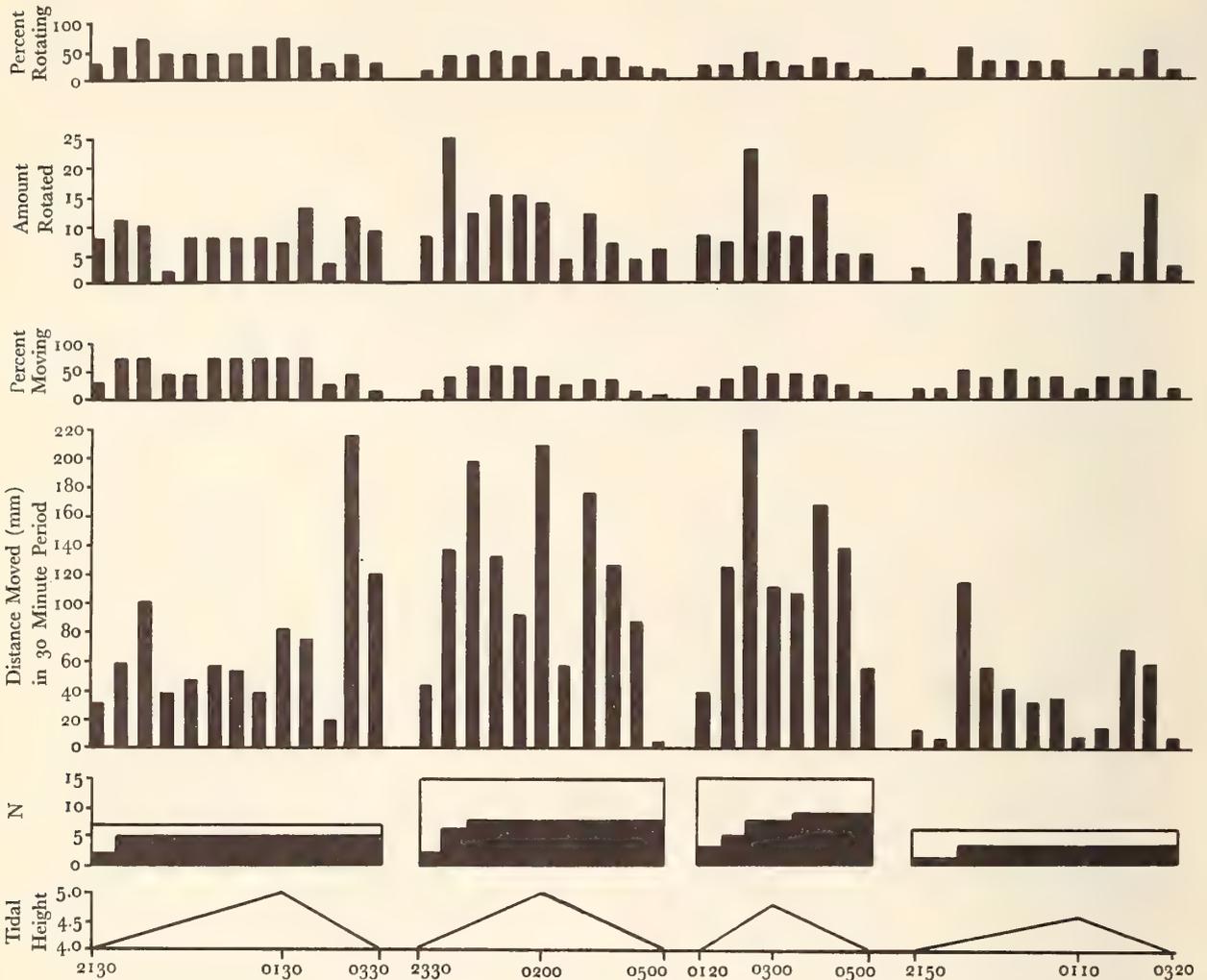


Figure 8

The relation between the per cent of the population which changed their positions, the number of clock units rotated by all individuals, the per cent of the population which rotated, and the distance moved by all individuals together in successive 30 minute periods, during 9 different high tides. Height of the population boxes (N) shows the total number of limpets watched in each conditions of splash. Results obtained during the night.

tidal period; the darkened area in each box shows a cumulative total of the number of these limpets which have moved at all at each successive period during the watch. The triangular tidal diagrams show only the time and height of the tidal peak and the points of initial and final splash. For each tide all limpets were at approximately the same level, and were subjected to the same

locomotion should be rejected. Therefore, the difference is not statistically significant. The highest rate encountered at any time in these studies occurred during an uncompleted daytime watch when one limpet moved 13 mm per minute during a 5-minute period. However, rates of locomotion well above average (i. e., 5 to 8 mm

per minute) for short periods on the part of individual limpets were more commonly observed at night than during the day.

It was noticed in some watches that a majority of the population of limpets moving showed a net vertical displacement either upward or downward. FRANK (1964)

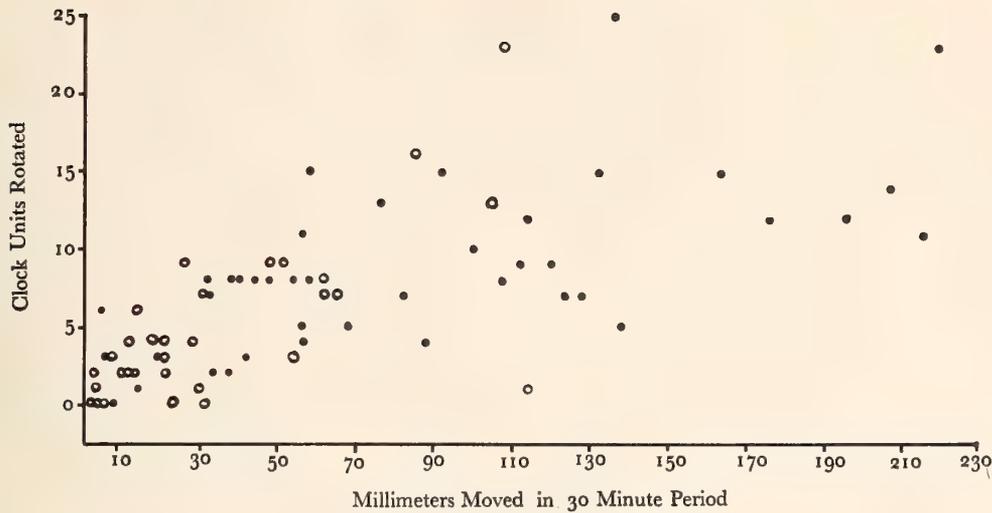


Figure 9

Scatter diagram of the clock units rotated and millimeters moved in each 30 minute period for the limpets shown in Figure 7 (circles) and Figure 8 (solid dots).

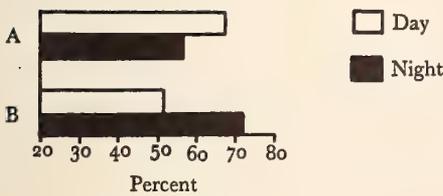


Figure 10

The per cent of the limpets which showed an initial upward rotation during two high tide studies. N = 25 for group B (calculated from data used in figures 7 and 8). In group A (supplementary data; see text), N = 21 for the night and N = 13 for the day.

found that vertical movements of his *Acmaea digitalis* were more common than horizontal ones. Two hypotheses which might account for this behavior are: (1) local limpet populations may show a net upward displacement during HHW, and a net downward displacement during LHW; (2) the net displacement of the population upward or downward during a high tide may differ according to whether it occurs during the day or night. Sixty *A. digitalis* on vertical rocks 1, 6, 7, 8, 9, and 10 were individually marked and their positions recorded before and after each period of high water for several successive days. The per cent known to have moved to a new position (homing limpets and non-moving limpets had the

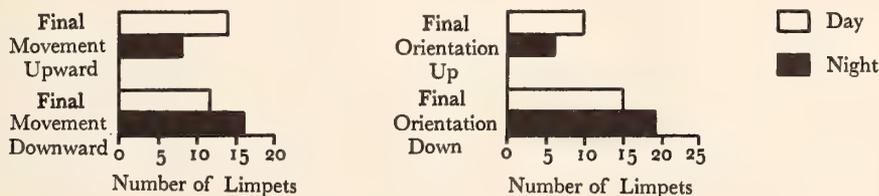


Figure 11

Terminal movement and orientation of *Acmaea digitalis* exposed on receding tides. N = 25 for both day and night observations.

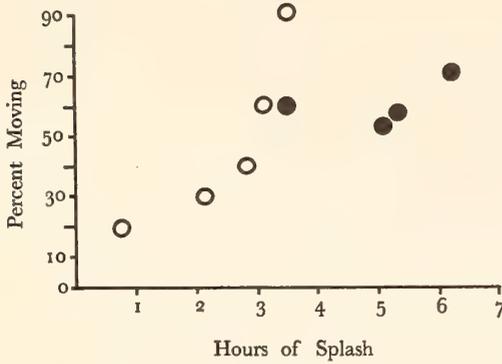


Figure 12

The per cent of the population that moved during a high tide versus the duration of wetting. Circles give results for daytime high tides, solid dots give those for high tides at night.

same positions) and the per cent showing a net upward displacement at each LHW and HHW period are recorded in Figures 15 and 16. The results clearly show that the per cent of limpets moving and showing an upward displacement consistently increases during HHW and decreases during LHW. Two factors which might be responsible for this behavior, duration of the period of wetting and time of the high tide, are considered below.

Is there a correlation between duration of the period of wetting and the net upward (or downward) displacement of the population at high tide? Actual duration of splash was not measured, but the periods of HHW and LHW were arranged in order of decreasing height of the tide (as actually measured on the tide gauge operated by the U. S. Naval Postgraduate School, about 1 mile from the observation site). The per cent of the population showing net vertical displacement upward and downward (Figure 17, top), and the amount of vertical

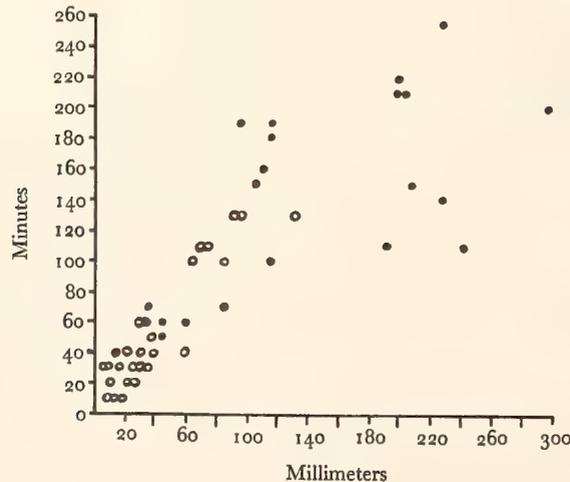
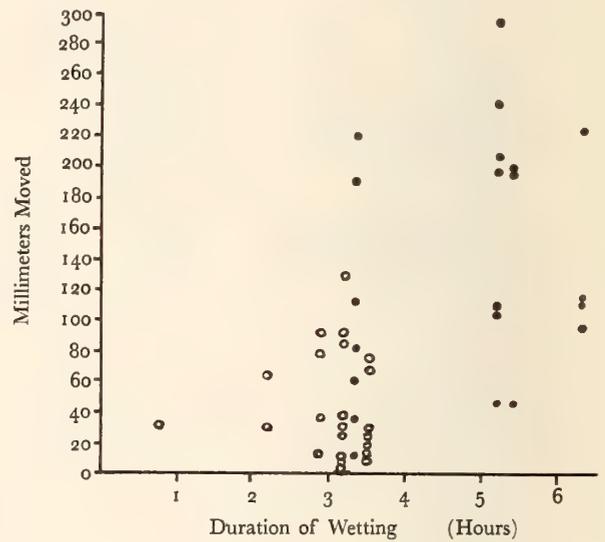
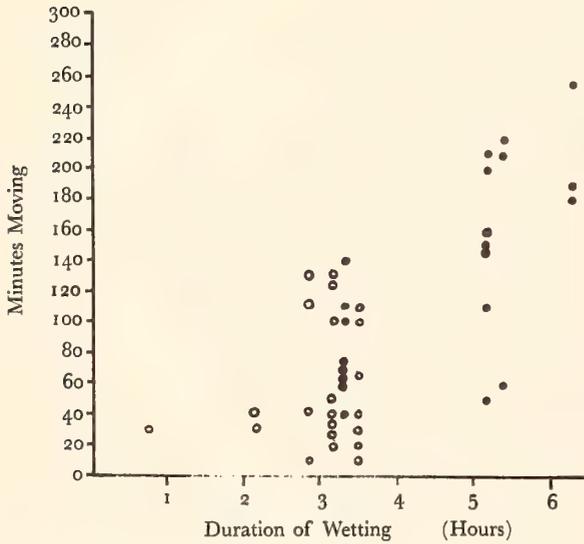


Figure 13

Scatter diagrams showing distance moved and time spent moving versus duration of wetting, and time spent moving versus distance moved. Circles give daytime rates, dots give rates at night.

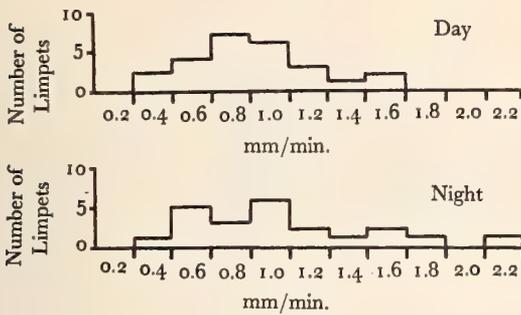


Figure 14

Rates of movement of *Acmaea digitalis* during day and night high tides. N = 25 for the day; N = 22 for the night. Average rate for the day is 0.9 mm per minute; average rate for the night is 1.0 mm per minute.

displacement upward and downward (Figure 17, bottom) were calculated for each high tide. While the method used to establish relative duration of wetting periods did not take into account variations due to differences in turbulence, the results show that, in general, the higher the height of high water, the larger the per cent of the population exhibiting a net upward displacement. The amount of vertical displacement upward also tends to be greater during the longer periods of wetting and lower during the more brief wetting periods. The tendency of the population to respond as it does, moving up or down depending on the height of the tide, appears to provide a mechanism by which the population is maintained at a favorable level on the rocks.

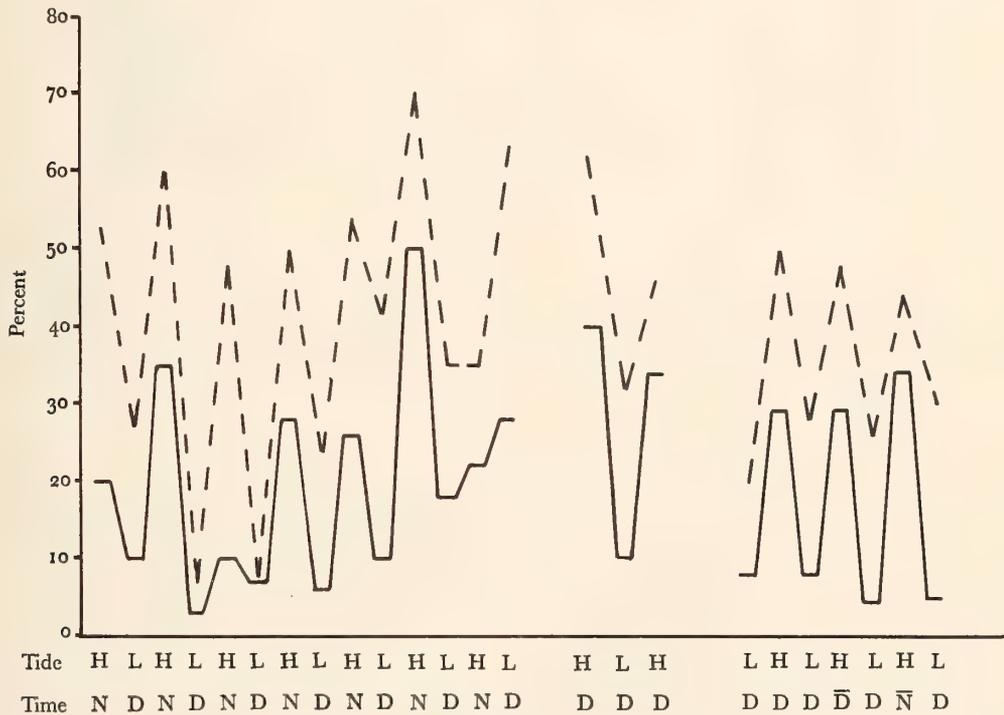


Figure 15

Vertical displacement observations on a population of 40 to 60 limpets for the periods: July 30 - 31; August 1 - 5; 9 - 10; 23 - 26, 1966. The broken line shows the per cent of the population known to have moved at each high tide. The solid line indicates the

per cent of the population which showed a net vertical displacement upward (i. e., the per cent of the moving limpets whose terminal positions were higher than their starting positions). H represents HHW; L represents LHW; N is night; D is day;  $\bar{D}$  and  $\bar{N}$  represent dawn and dusk.

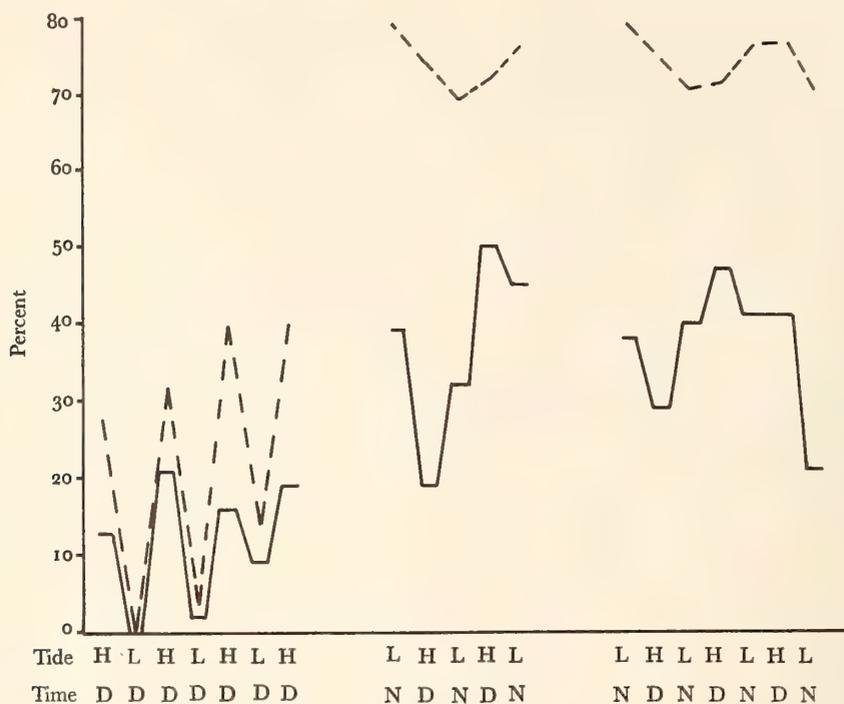


Figure 16

Vertical displacement observations on a population of 40 to 60 limpets for the periods: September 5 - 8; 17 - 19; October 13 - 17, 1966. The broken line shows the per cent of the population known to have moved at each high tide. The solid line indicates the

per cent of the population which showed a net vertical displacement upward (i. e., the per cent of the moving limpets whose terminal positions were higher than their starting positions). H represents HHW; L represents LHW; N is night; D is day.

Conspicuous upward vertical displacements may occur during periods of unusually heavy surf. One such instance occurred on rock 9 (see Table 4) between LHW on September 18 and LHW on September 19; during a storm, 9 of 10 limpets observed moved up on the rock an average of 33 cm (range = 4 to 78 cm). These observations are similar to those of FRANK (1965) who found that a population of *Acmaea digitalis* on the Oregon coast moved higher on the rocks during the winter, an action apparently related to heavy surf caused by winter storms. However, in the present case the marked upward movement was observed to occur in less than 24 hours.

Is there any difference in vertical displacement tendencies during high tides at night as opposed to those during the day? Pertinent data from the population shown in Figures 15 and 16 have been summarized in Figure 18. The tendency to move upward during HHW and downward at LHW is much more marked when the high water occurs during the day. The differences in

behavior at LHW and HHW would probably have been more marked had it not been for two circumstances. First, rough surf occurred during the last two watches in Figure 16, for both HHW and LHW, causing longer than usual periods of wetting and also causing the population to move upward on the rock. Second, the height of LHW in the last watch was about 5 feet, and that of HHW only about 6 feet, so the periods of wetting were of nearly the same duration for both high tides. It was only during LHW periods with a maximum height of 4.4 to 3.0 feet (Figure 17) that a tendency toward a downward displacement with little upward displacement became marked.

Approximately 25% of the limpets observed in the course of the present study showed a tendency to home, i. e., return to a specific position and orientation on the rock. GALBRAITH (1965) reported finding homing *Acmaea digitalis* on relatively smooth rocks bearing ridges and numerous small depressions. MILLARD (1968) likewise found some *A. digitalis* homing on granite rocks at

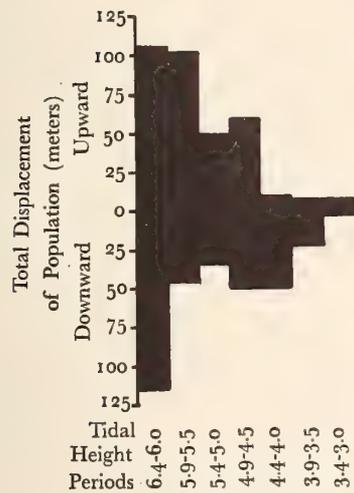
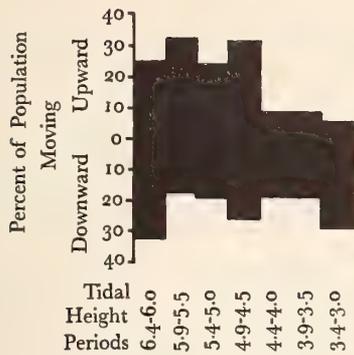


Figure 17

Net displacement upward or downward on the rock face during high tide for the limpets whose movements are recorded in Figures 15 and 16. This figure shows per cent of population moving up or down (top), and total net displacement up or down (bottom) in relation to height of tide. The data from the last watch in Figure 16 were not used as the correct tidal heights are not known.

Pacific Grove. VILLEE & GROODY (1940) also noted this tendency but preferred not to call it homing behavior. FRANK (1964, 1965), in Oregon, reported no evidence of strict homing but found statistical evidence for a home range.

SUMMARY

1. At low tide *Acmaea digitalis* on dry vertical rock surfaces remain in place and tend to orient with the head pointed downward and to the right (facing toward 3 to 6 o'clock).

- When wetted by a rising tide, many limpets start to move; they tend to turn upward, either to the right or left; orientation of the population becomes more random as movements increase and movement either up or down on the rock face may occur.
- On a receding tide, as splash lessens, limpets on vertical surfaces show decreased activity and again tend to orient facing downward and to the right.
- In general, the longer the period of wetting at high water, the larger the percentage of the population which moves, the longer the period of movement, the greater the amount of body rotation, and the greater the distance moved by the population.

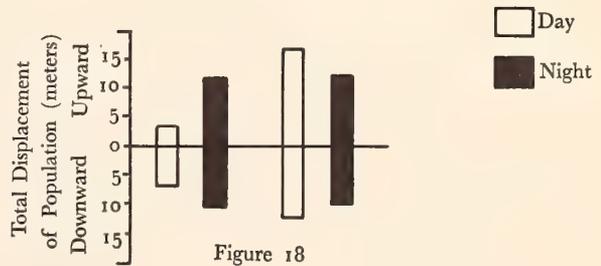
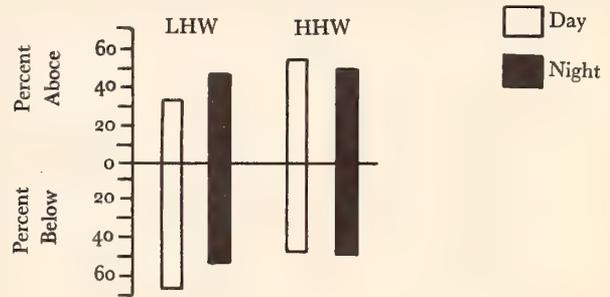


Figure 18

Net displacement upward or downward on the rock face during high tide for the limpets whose movements are recorded in Figures 15 and 16. This figure shows per cent of population moving up or down (top), and total displacement up or down (bottom), in relation to time of day for all LHW and HHW periods.

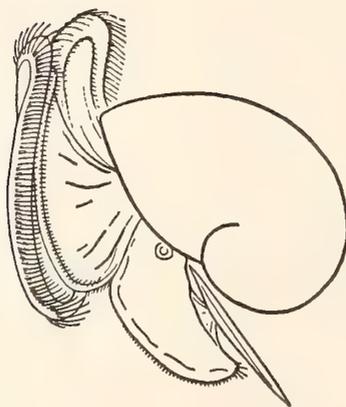
- The limpet population tends to move upward on the rocks at higher high water, and downward at periods of lower high water. The tendency is more marked for high water periods occurring during daylight hours. With rough surf, the limpet population as a whole moves upward on vertical rock faces, and comes back down during ensuing periods of calm weather.
- Approximately 25% of the *Acmaea digitalis* observed showed homing behavior.

## ACKNOWLEDGMENTS

I would like to express my thanks to Dr. Donald P. Abbott who helped and advised me throughout this study. Thanks are also due to Galen Hilgard and Raymond Markel for advice and help. The research was supported by Grant GY806 from the Undergraduate Research Participation Program of the National Science Foundation.

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## The Clustering Behavior of *Acmaea digitalis*

BY

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(4 Text figures; 1 Table)

THE LIMPET *Acmaea digitalis* ESCHSCHOLTZ, 1833 is a common inhabitant of the high intertidal rocky shores of the North American Pacific coast. Populations of *A. digitalis* are not randomly distributed but are often clustered into groups in limited areas with comparatively few solitary limpets on the surrounding regions. No specific studies of clustering behavior have been made on *A. digitalis*, although FRANK (1966) mentions the occurrence of aggregations in depressions and other irregularities in the rock. ABE (1932) studied the behavior of clusters of the limpet *A. dorsuosa* GOULD, 1859 in Japan. ABE's study, however, covered a period of two years and his results showed mainly seasonal variations.

The present study, conducted at Hopkins Marine Station on Mussel Point, Pacific Grove, California was carried out to provide a better picture of the clustering behavior of *Acmaea digitalis*. On April 25, 1966, 14 clusters were placed under observation. Eleven of these persisted only two to three days and only three clusters remained intact with regard to the area occupied and members present for a period of a month. This paper documents the activity of the largest of the three remaining clusters.

For present purposes a cluster is defined as an aggregation of 3 or more animals, each within 1 cm of its nearest neighbor. Animals farther than 1 cm from the closest member of a cluster were not included in the cluster. The clusters studied were located on the east face of a large island of granite rock some 80 feet offshore. Waves broke on the opposite side of the island, rolled around and surged below the limpets in a narrow channel, splashing them thoroughly at high water. The particular cluster studied was located 6ft above 0.0 water level. No macroscopic algae were present in the area and the microscopic algae seemed evenly distributed. The rock surface was fairly smooth with a fold or crack rising up the center. The upper end of this fold, where the cluster

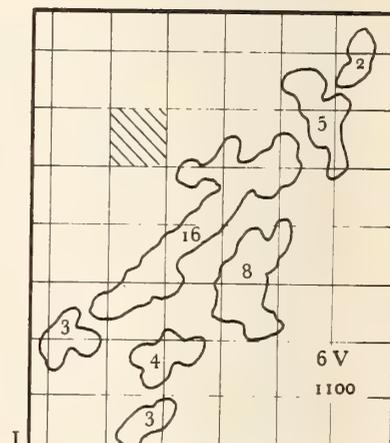
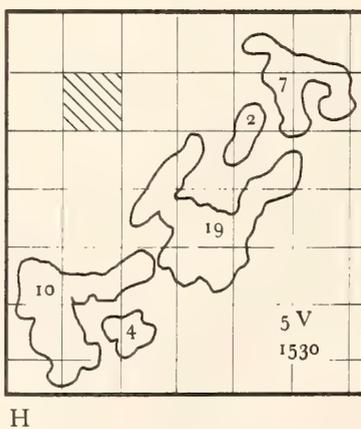
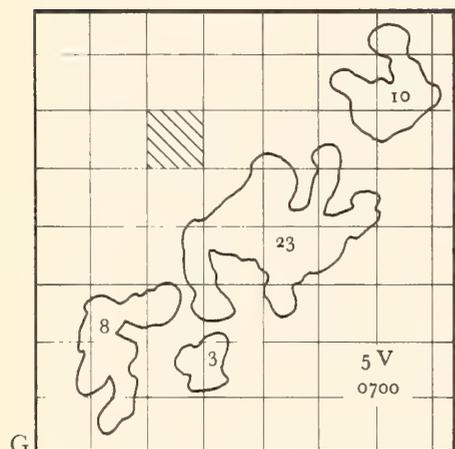
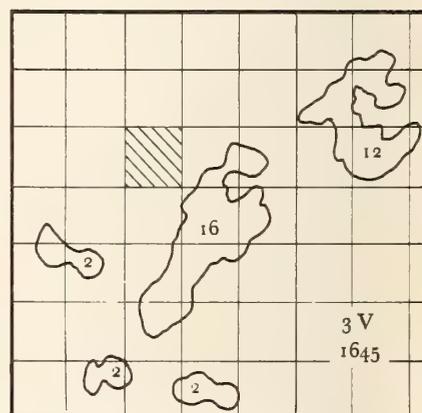
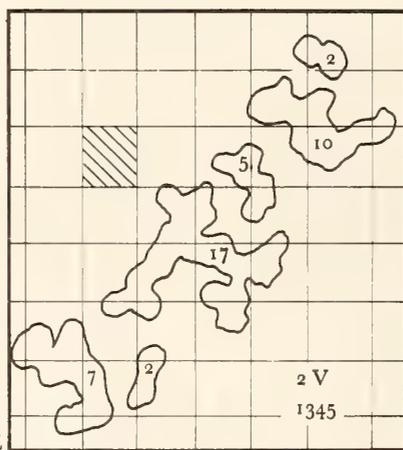
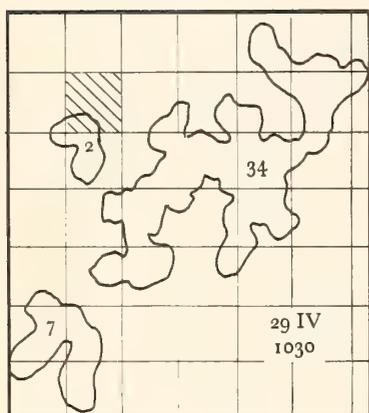
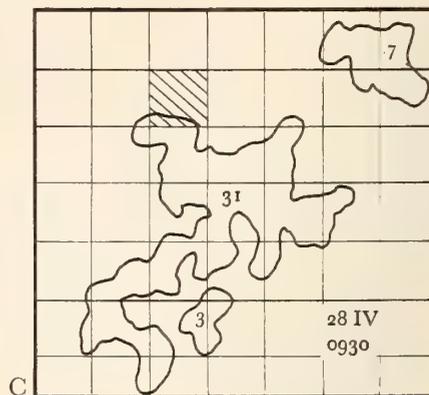
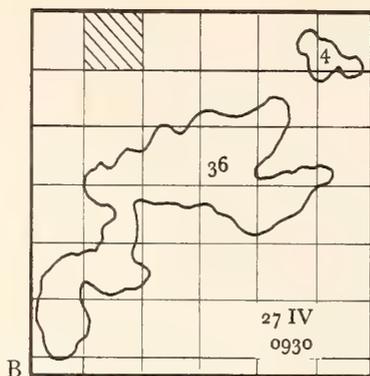
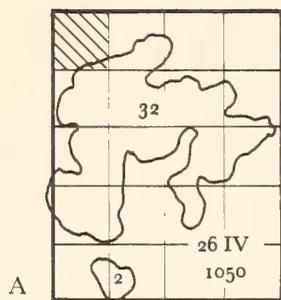
studied was located, presented a shallow depression on the rock face which became shaded between 1:30 and 3:00 P. M. The location offered some protection from the prevailing winds.

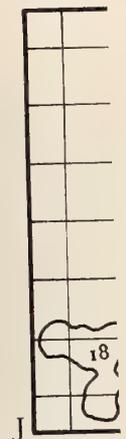
To facilitate the plotting of cluster position on the rock a grid of red dots was painted at  $1\frac{1}{2}$ " intervals on the rock surface. Within this grid the positions of individuals could be designated with good accuracy. The limpets present on April 25, 1966 were marked with India ink to distinguish them from each other and from the original members. The limpets were observed daily at low water for a week and their positions mapped at each observation. Thereafter, they were observed at intervals of 1 to 7 days. Several attempts were made to chart the movements of the limpets at rising and falling tides. For these observations pencil notes were made on x-ray film with the emulsion removed and surface roughened, facilitating writing underwater. The water invariably became so rough that plotting the movements of the cluster throughout an entire tidal cycle was impossible.

General observation of the cluster showed that the limpets move when they are being wetted by the splash of incoming waves on a rising or falling tide. Within a half hour after they have first been splashed, the majority of the limpets begins to move upward and laterally on the rock. In this reaction, corroborated by MILLER (1968), the lower limpets begin to move before the higher ones; the low animals are more frequently and heavily splashed. Once movement has started the animals crawl upwards and laterally for distances of 2 to 6 ft. Thus at high water the cluster is dispersed. The animals remain more or less active while the tide is in. As the water level recedes again, they tend to regroup themselves into a cluster, in approximately the position occupied by the previous cluster.

The first question posed was: does the cluster occupy the same amount of space and the same area on the rock consistently? To answer this question the daily positions and areas occupied by the cluster at low water were

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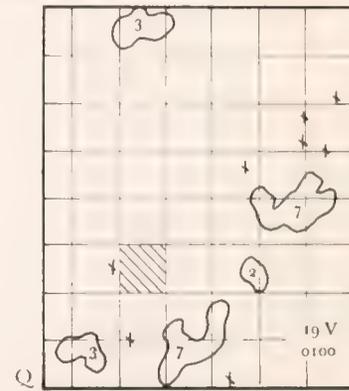
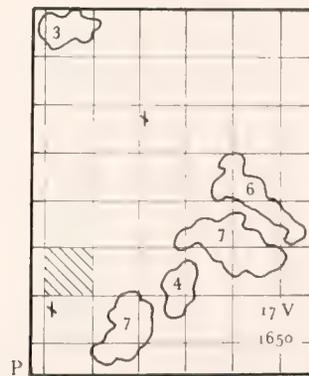
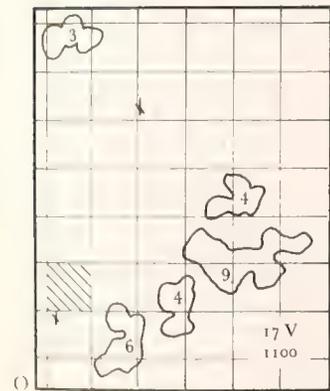
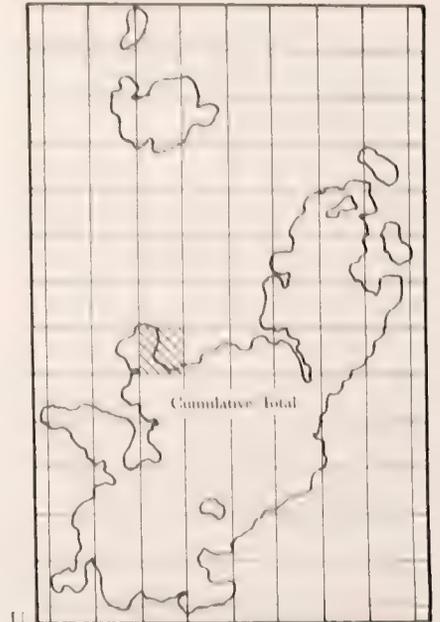
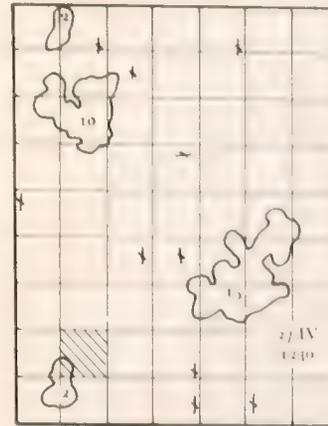
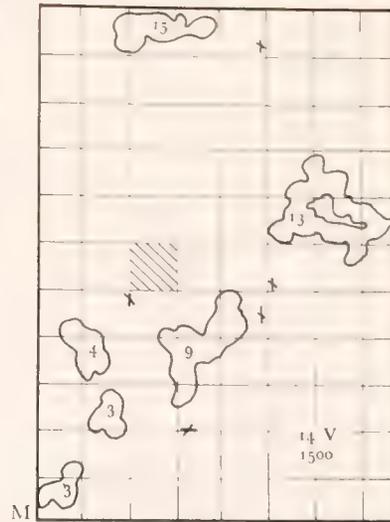
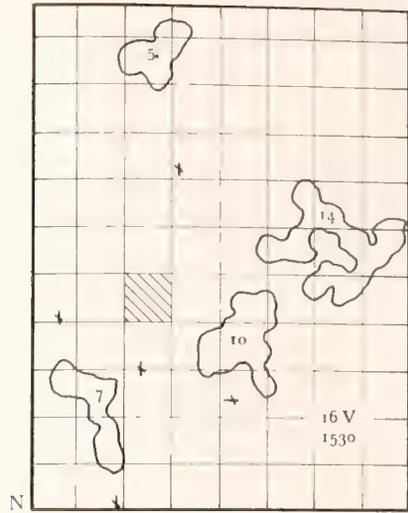
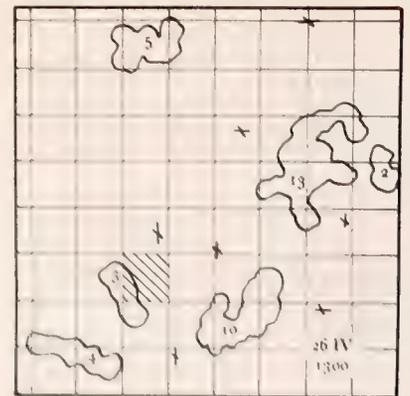
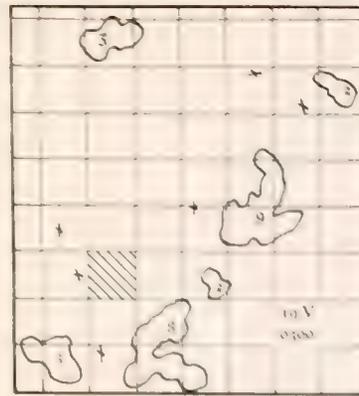
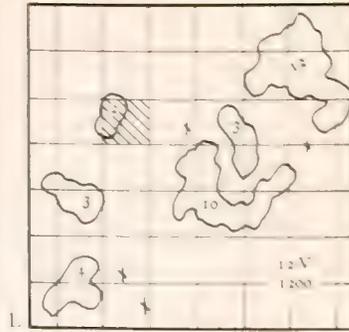
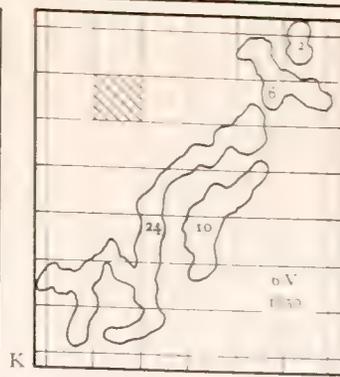
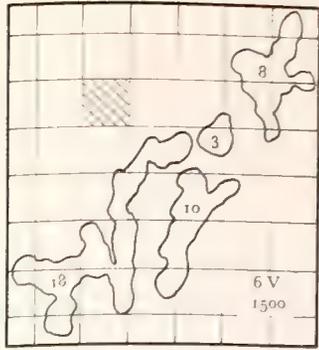


Figure 1  
(see pages 46 and 47)

A to U: Daily changes in size, shape, and number of individuals in a cluster of *Acmaea digitalis* on a vertical rock face, April 26 to May 27, 1966. Outlined clusters contain limpets 1 cm or less from nearest neighbors. The number on the cluster shows the number of limpets in it. Day and month are shown in arabic and roman numerals respectively; hours of observation are given in the lower right hand corner of each figure. Lines on the grid were 1½ inches apart on the rock. Figure 1 U shows the cumulative total of all the area occupied by the cluster during the month of the study.

compared for successive days (see Figures 1 and 2). It can be seen that the cluster shifted its position from day to day. The area occupied averaged 15 square inches and varied from 8 to 20 square inches. The cumulative total area of rock surface occupied by the cluster during the month of observation was 77 square inches (see Figure 1, u). There was always some overlap between the positions occupied by the cluster on successive days. This

overlap ranged from 37% to 75% of the total area occupied at the time of observation (see Table 1). New area, not previously inhabited during the period of observation, was added as the cluster changed daily in its shape and location on the rock.

Another question to be answered concerns cluster membership (Figures 3 and 4). The membership of the cluster varied from day to day as animals entered the cluster, remained associated with it for a period, and then either left the area or remained nearby but not actually in the cluster. Later some of these limpets rejoined the cluster. Thus, it could be observed that the turnover and variation in cluster membership was something occurring in a relatively constant group. Only a few unmarked limpets entered the cluster area after the first week, during which 11 newcomers joined the cluster. In the month that followed, fewer than 10 unmarked limpets joined. Two animals were observed to wander 1 to 4 ft from the cluster area. The closer of the two returned after 5 days; the other did not return.

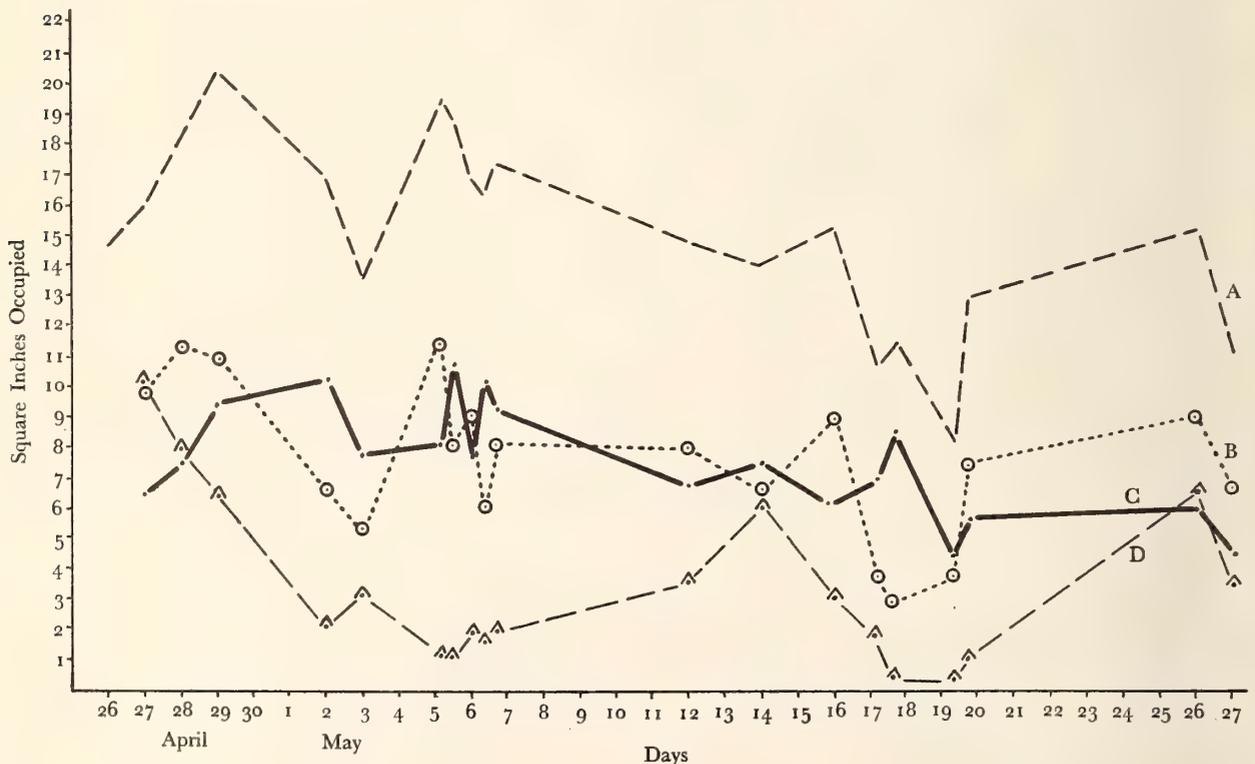


Figure 2

Area occupied by the cluster on successive days, based on the data in Figure 1. Line A represents the total area covered by the cluster at any given observation time. Line B represents the area occupied by the cluster during a given observation which was not occupied

at the previous observation. Line C represents the overlap of the cluster at any given reading with the area occupied at the previous observation. Line D represents the area which has not been occupied previously by the cluster at any time during the present study.

Table 1

Comparison of Area Occupied by the Cluster with Number of Animals in the Cluster and the Percentage of the Area Occupied Which Overlaps that Occupied at the Previous Observation.

Date	Hour	Area occupied by the cluster in square inches	Number of animals	Percentage overlap with previous day
April 1966				
26:	1050	14.75	34	-
27:	0930	16	40	40%
28:	0930	18.75	41	37%
29:	1030	20.25	43	46%
May 1966				
2:	1345	16.75	43	61%
3:	1645	13.5	34	57%
5:	0700	19.5	44	42%
5:	1530	18.7	42	57%
6:	1100	16.75	41	46%
6:	1500	16.25	39	64%
6:	1850	17.25	42	54%
12:	1200	14.75	34	46%
14:	1500	14	37	53%
16:	1530	15.25	36	41%
17:	1100	10.25	26	65%
17:	1700	11.5	27	75%
19:	0100	8.25	22	56%
19:	0300	13	27	43%
26:	1300	15.25	37	41%
27:	1300	11.25	29	40%

Observations of *Acmaea digitalis* in several different locations around the Monterey Peninsula indicate that, in general, clusters are found in more protected areas. They tend to occur in spots which receive more shade than the surrounding rock face due to a slight depression in the rock barely noticeable when the full sun is on the whole rock face. On the rock island where most of this study was carried out, between 1:30 and 3:00 P.M. the cluster areas would be outlined by a shade pattern which covered them but left the rest of the rock in full sunlight. Clustering also tends to occur on surfaces at right angles to the sea which receive some surge and splash from waves but do not bear the full brunt of the breakers. In areas directly exposed to wave action, *Acmaea digi-*

*talis* have not been observed to cluster, although their populations may be very dense. Here they remain randomly dispersed, although small, temporary clusters of 3 to 6 animals may aggregate in a crack or depression in the rock.

The factors stimulating clustering are not fully known. Animals cluster when the tide is out and disperse when the tide is in, at night as well as during the day; thus light does not seem to be a direct and immediate causal factor. Tide level and wave action appear to play an important role. The limpets recluster when they are still being splashed on an ebbing tide and the area is still totally wet, as though the frequency of splash and the amount of water passing over them were being measured. The fact that the animals recluster when the rock is wet and may do so at night seems to indicate that clustering is not similar to the phenomenon of aggregation in sow bugs which keep moving until they end up in the dampest and shadiest spot available (ALLEE, 1926). It is also difficult to look at clustering as a purely random occurrence because it is seen that limpets return to a fairly stable cluster area.

Some observations made suggest that the height of the sea at high water and the degree of wave action may influence the height on the rocks where clustering occurs. Toward the end of the month that the cluster shown in Figure 1 was observed, the weather grew progressively more stormy, and wave action increased. During this period the cluster rose in its position on the rock (Figure 1), gradually occupying a higher area. Also, the cluster underwent progressive fragmentation with time. This behavior recalls the finding of ABE (1932) that clustering is seasonal, and that the clusters of *Acmaea dorsuosa* break up shortly after the winter storms begin.

The means by which animals are able to find their ways back to the cluster area are not clear. There is a possibility that mucous trails are involved. Another animal which occurs slightly above *Acmaea digitalis* in the intertidal region, *Littorina planaxis* PHILIPPI, 1847, has been proven to follow mucous trails left on rocks by members of its own or other species (MRYAMOTO, 1964; PETERS, 1964). Whatever the mechanism, it may be similar to that involved in "homing" in limpets. During the month that the clusters were observed 4 of the animals were seen to return to exactly the same location and orientation at successive low tides for a period of 2 or 3 days. They then changed resting spots and repeated

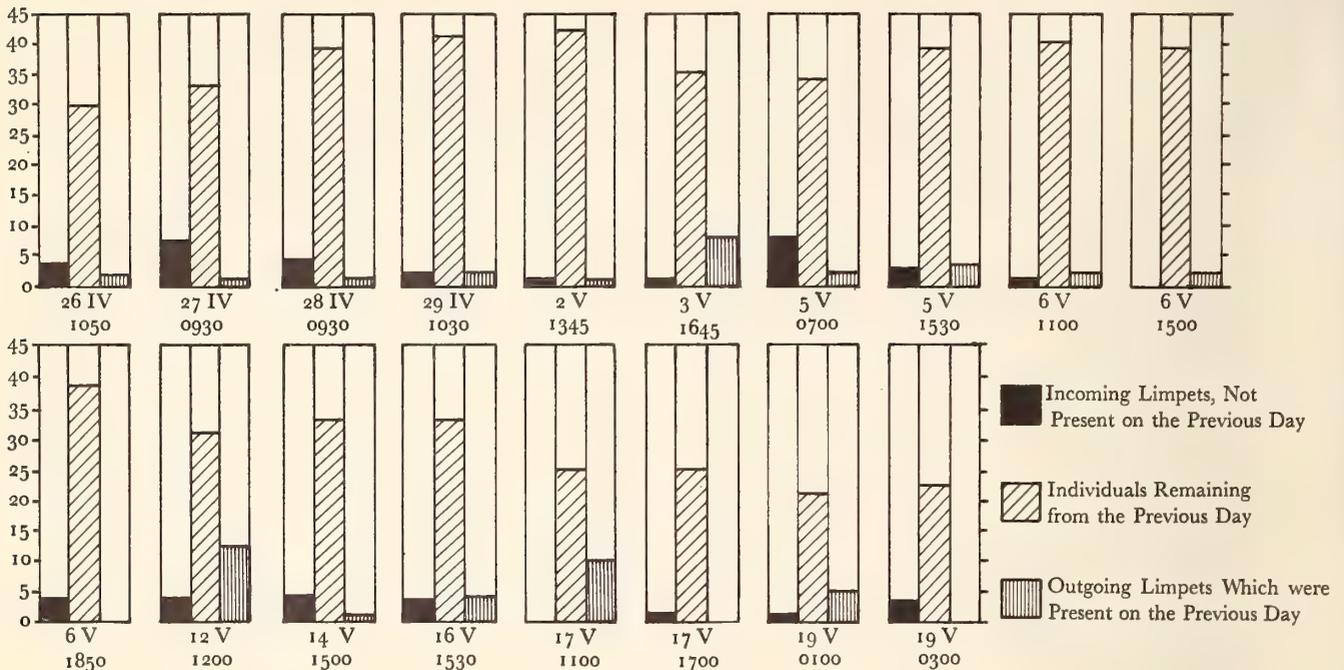


Figure 3

Turnover rate of limpets in cluster membership between successive observations. Dates and times are the same as those in Figure 1.

this behavior for several more days. Homing in some *A. digitalis* was also noticed by MILLER (1968).

SUMMARY

*Acmaea digitalis* populations often cluster on intertidal rocks at low tide. Clustering occurs in areas which receive some shade and some protection from direct wave action. Animals disperse on a rising tide. Reclustering occurs during tidal ebb while rocks are still wet, and takes place both day and night.

A cluster varying in size from 22 to 44 individuals occupied an average of 15 square inches (range 8 to 20 square inches) each day over a 32 day period. Daily shifts in cluster position occurred; total cumulative area occupied was 77 square inches.

Only 17 members of the original cluster remained after 32 days while a total of 22 new members entered.

ACKNOWLEDGMENTS

I would especially like to thank Dr. Donald P. Abbott of Hopkins Marine Station for his assistance and guidance during this study. I also wish to thank Galen Hilgard and Raymond Markel who helped me obtain equipment, and Tudy Balesteri for keeping an eye on me when field work was necessary at high tide and access to my offshore rock was difficult. My sincere appreciation goes to Susan E. Fitzpatrick for the lettering on the original text figures. This work was made possible by Grant GY806 from the Undergraduate Research Participation Program of the National Science Foundation.

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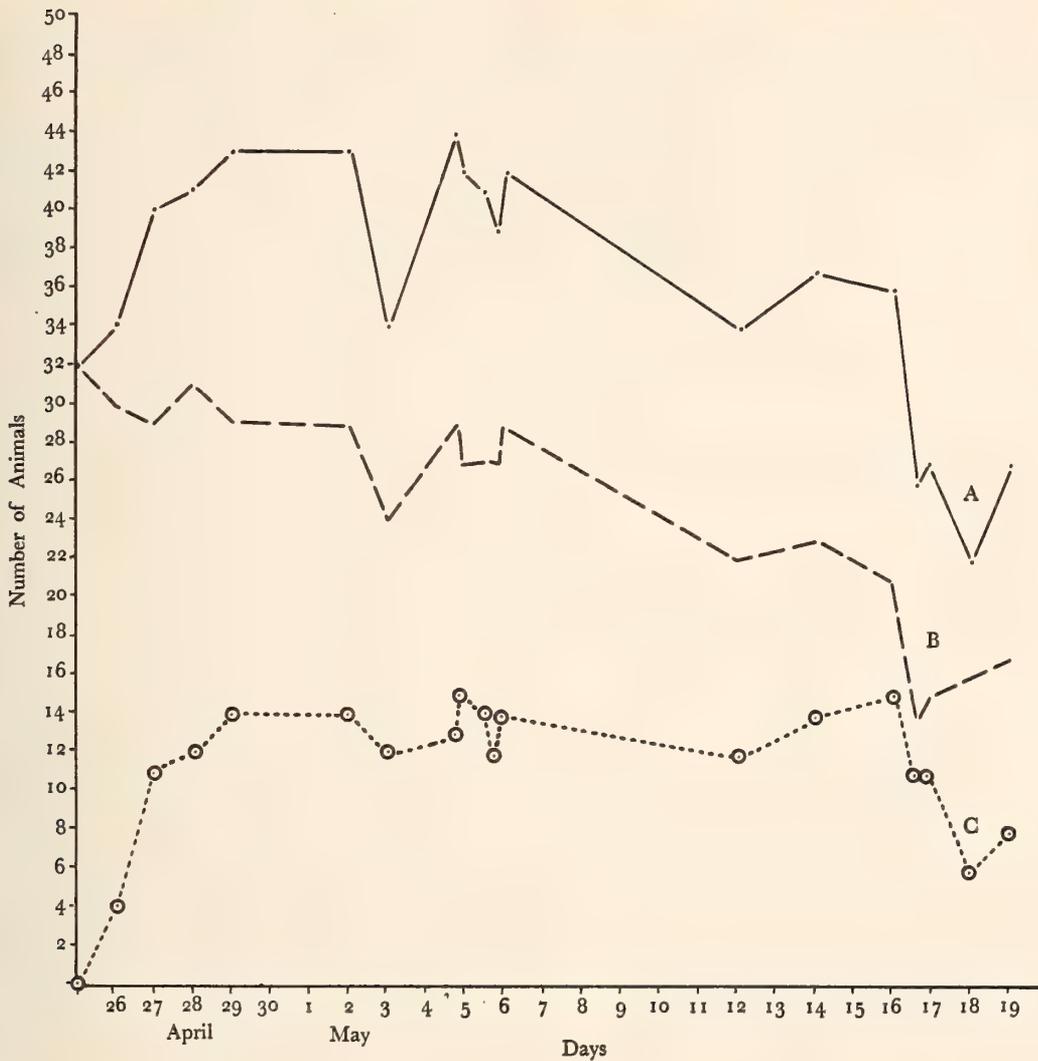


Figure 4

Composition of the cluster at each observation period. Line A represents the total number of limpets present in the cluster during each observation. Line B represents the number of limpets present

which were members of the cluster when the study began; some individuals left the cluster for a few days and later rejoined. Line C represents limpets present which were not charter members but joined the cluster after the study began.

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# Studies of Homing Behavior in the Limpet *Acmaea scabra*

BY

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(4 Tables)

THE TENDENCY OF MEMBERS of the species *Acmaea scabra* (GOULD, 1846) to return repeatedly to a specific location on their rock substratum, i. e. homing, has been reported from time to time.

The first such study on the Pacific species of *Acmaea*, by WELLS (1917), gives the impression that homing is a highly individual trait, even among members of the single species *A. scabra*. However, the tendency to home was apparently not studied in more than a few representatives of each species, and the frequency of homing behavior was not evaluated.

Two attempts at such an evaluation were subsequently reported (HEWATT, 1940; VILLEE & GROODY, 1940). HEWATT's study of 31 *Acmaea scabra* on granite rocks revealed homing behavior in all 31 individuals. On the other hand, VILLEE & GROODY concluded from their study of 86 members of this species on a sandstone substratum, that there was no evidence for homing behavior. This latter study, however, was restricted to observations made principally at low tide, a time when there is little or no activity in the limpet population. All animals that had not been observed away from their homesite during the period of study were ignored.

In a more recent quantitative study by BRANT (1950), 298 marked *Acmaea scabra* were observed over a period of 24 days. Homing behavior was reported in 98.7% of this test population. Preliminary observations by the author confirmed these findings.

The principal object of the present study was to investigate the mechanism involved in homing behavior. In addition, two other aspects of homing which have received little investigation are included in this study. These are the homing behavior of animals from high and low elevations in the intertidal, and the behavior of different size classes of limpets.

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## METHODS AND RESULTS

The observations and experiments described below were made at Pescadero Point on the Monterey Peninsula, California during April and May, 1966.

The first series of experiments performed was designed to detect any differences in the homing ability of high and low populations, and in that of large and small animals. One hundred individuals of intermediate size, between 10 and 15 mm in length, were measured, and the animal and its homesite marked with red nail polish. Fifty of these animals were on a horizontal granite surface with a median elevation of +2.4ft; this population will be referred to as the "low" group. The other 50 animals, hereafter called the "high" group, were on a gently sloping rock with a median elevation of +7.1ft. After waiting 3 days to insure that the position marked was indeed the animal's home, a procedure that was followed in all subsequent experiments, 30 in each group were displaced randomly 3 to 4 cm from their homesites. The other 20 in each area served as controls. The animals were observed after 24 hours, and as shown in Table 1,

Table 1

Homing Behavior of *Acmaea scabra* Populations at High and Low Intertidal Locations. Observations Made 24 Hours After Displacement of Experimental Animals.

	Total	Home	Not Home	Missing
Low Animals Displaced	30	25	2	3
Low Control Animals	20	19	0	1
High Animals Displaced	30	27	1	2
High Control Animals	20	20	0	0

no significant difference was seen in the homing ability of the high and low groups.

A similar experiment was then carried out in an area of intermediate elevation, +3.9ft, to compare the homing behavior of large animals, greater than 15 mm in length, with that of small ones between 6 and 10 mm long. Here, too, no significant differences were observed, as can be seen from Table 2. However, a less complete study suggested that animals smaller than 6 mm usually do not home.

Table 2

Homing Behavior of Large (greater than 15 mm in length) and Small (6 to 10 mm in length) Individuals of *Acmaea scabra*. Observations Made 24 Hours After Displacement of Experimental Animals.

	Total	Home	Not Home	Missing
Large Animals Displaced	20	19	0	1
Large Control Animals	15	15	0	0
Small Animals Displaced	40	36	2	2
Small Control Animals	30	27	2	1

Experiments in the laboratory, utilizing glass plates over which limpets had been allowed to move, revealed no residual mucus in the form of trails as determined by the India ink test of PETERS (1964). Nonetheless, field experiments were performed to investigate the possibility that mucus or some other chemical substance on the surface of the rock was the agent active in governing homing behavior.

In the first type of experiment, a stiff fiber brush was used to scrub a 1½" strip around the homesites of 20 previously marked animals. The areas surrounding 15 other animals, which served as controls, were left unscrubbed. All animals were then displaced 4 to 5 cm from their homes to a position outside this strip. Their locations after 24 hours were noted, and are presented in Table 3. A variation of this experiment was also carried out in which the home itself was included in the area scrubbed. This was to eliminate any possible attractant on the home, as well as "trails" leading to the home. As shown in Table 3, neither of these treatments completely destroyed the ability to home.

A second group of experiments was performed to remove chemical substances. All animals in a one foot square area were removed and the rock painted with a

Table 3

Homing Behavior of *Acmaea scabra* Following Scrubbing of Home or Surroundings or Both. Observations Made 24 Hours After Displacement of Experimental and Control Groups.

	Total	Home	Not Home	Missing
Surroundings Scrubbed	20	10	6	4
No Scrubbing	10	10	0	0
Home and Surroundings Scrubbed	30	23	3	4
No Scrubbing	20	16	0	4

solution of 32% NaOH. The surface was then rinsed repeatedly with copious quantities of seawater until the pH of the wash water was near neutrality. The animals were then replaced within 4 cm of their homes. Five animals outside the treated area were also displaced a similar distance and utilized as controls. At the end of the customary 24 hour waiting period, 8 of the 10 animals in the experimental group had returned to their homes.

Having established that the removal of chemical substances on the rock had no noticeable effect on the homing ability, experiments were begun in which the topography of the rock surface surrounding the home was altered. A geologist's pick and a chisel were used to create a strip 1" wide around the homes of 25 animals. The topography of the rock in this strip was totally changed. The animals were then replaced 4 cm from their homes, and outside the chiseled area. Fifteen other animals were similarly displaced, but the topography of their surroundings was left unaltered. This latter group served as a control. Within 24 hours only 4 of the 25 experimental animals had homed. Seventeen of the 25 were not in their homes, the remaining 4 having disappeared.

Some additional evidence suggesting the involvement of topography in the homing mechanism resulted from an experiment performed with a small group of animals at China Point. Seven animals were marked and displaced the customary 4 to 5 cm from their homesites. A hammer and chisel were then used to create a strip about 1" wide between the animal and its home. As the tide came in, the animals were observed to move to the edge of the chiseled area, then travel along the edge of this area until they reached its end. At this point they resumed their travel toward the homesite, 6 of the 7 returning by the next low tide. This reaction to territory made unfamiliar by alteration suggests that recognition of some element of topography may be involved in the homing mechanism.

## DISCUSSION

If populations at the high and low levels are studied, using animals of comparable size, there is no significant difference in the homing ability of the two groups. Nor is there any significant difference in the homing ability of animals of different sizes at the same intertidal level. These observations confirm earlier ones by HAVEN (1966). However, it was also observed that animals of less than 5 to 6 mm in length usually do not home. Such animals are found mainly in the lower intertidal. The inclusion of these extremely small animals in earlier studies of homing behavior may account for some of the discrepancies in the findings of this study as compared to these earlier works.

The results of the experiments designed to elucidate the mechanism of homing suggest that it is related to the perception of the topography of the rock surface in the vicinity of the limpet's homesite. The fact that the animals would not cross an area of unfamiliar topography, but went directly home upon reaching familiar territory points to a "memory" of the convolutions of the rock surface. It is, however, possible that some other factor in the environment which was not investigated is responsible, at least in part, for homing. Light or its polarization pattern, for instance, could be such a factor. The probability that the mechanism is indeed involved with topography is in good agreement with the tentative results reported by GALBRAITH (1965) on the mechanism of homing in *Acmaea digitalis* ESCHSCHOLTZ, 1833 and *Lottia gigantea* SOWERBY, 1843.

The high percentage of animals which homed after treatment of the rock with sodium hydroxide argues against the possible use of some chemical agent as a homing guide, as the concentrated alkali would be expected to dissolve or denature any substances deposited by the limpets. While other external factors, such as polarized light or water currents, have not been ruled out, it is doubted that the animal possesses the sensory equipment to utilize such highly variable sources for orientation. Work should, however, be carried out to examine these possibilities. The implications of a homing mechanism based on a knowledge of topography, in an animal such as the limpet, demand additional investigation.

## ADDENDUM

Two additional series of experiments were completed later in an effort to determine what portion of the sensory apparatus is involved in homing behavior.

The first experiments, designed to determine the role of the cephalic tentacles in homing, were performed at Pebble Beach, San Mateo County, California in January and February, 1967. In these experiments 60 animals were marked in the customary manner and left for 3 days to insure that the position marked was the home spot. Then a small typewritten number was affixed to the shell of each animal, and the same number placed adjacent to its home using Duco cement. The animals were then brought into the laboratory, where all were anesthetized in a solution of magnesium chloride isotonic with seawater. Both cephalic tentacles were then excised from 40 animals, distal to the eye spot. The remaining animals were uninjured and were utilized as controls. All animals were then placed in a salt water aquarium for a period of 4 to 5 days to allow recuperation of the operated animals.

The animals were then returned to the beach during a period of low tide, and placed within 3 to 4 cm of their own homesites. The locations of the animals at the end of 24 hours, or two high tides, were observed and are summarized in Table 4. Of those animals which could be located at the end of the 24 hour period, 29% of the experimental group had homed, as compared to 75% of the controls.

Table 4

Homing Behavior of *Acmaea scabra* Following Excision of Both Cephalic Tentacles or Bilateral Destruction of the Eyespots. Observations Made 24 Hours After Replacement of Experimental and Control Groups.

	Total	Home	Not Home	Missing
Cephalic Tentacles Excised	40	9	22	9
Controls	20	12	4	4
Eyespots Destroyed	40	28	4	8
Controls	20	11	4	5

The second series of experiments was performed at Moss Beach, San Mateo County, during April and May, 1967, and was designed to determine whether or not the eyespots are utilized in homing. Sixty animals were marked and individually numbered as before. After the usual waiting period the limpets were brought to the laboratory, where all were anesthetized with the magnesium chloride solution. A dissecting needle which had been heated to redness was then employed to cauterize both eyespots on 40 of the animals, the remainder being utilized as controls. After a 4 to 6 day period of recovery

in the aquarium all the animals were returned to the field and replaced within 3 to 4 cm of their homes as before. The positions of these animals were observed at the end of 24 hours, and are also summarized in Table 4.

The eyespot is apparently not utilized in homing. Examination of the 4 experimental animals which failed to home indicated that in these, substantial damage had been done to the cephalic tentacles. In those animals in which no damage was observed, there was 100% homing.

The loss of the cephalic tentacles appears to have a statistically significant effect on homing. This observation is, however, open to question. It is a distinct possibility that the decrease in the instance of homing was a result of trauma or other factors. The fact that some 29% of the experimental group returned to their homes despite the loss of the tentacles seems to substantiate this possibility; the presence of alternative systems which may be utilized in homing is another possible explanation.

### SUMMARY

1. Homing behavior of *Acmaea scabra* was studied in the field with respect to the intertidal height of the population, the size of the individuals, and the mechanism involved in homing movements.
2. No significant difference was found in the homing ability of populations in the high intertidal region as compared to those at lower levels.
3. The size of the animals was found to be unimportant in determining their ability to home, except that extremely small animals, less than 5 to 6 mm in length, were usually found to be non-homing.
4. The possibility that chemical substances on the rock surface are the agents of homing behavior appears highly unlikely.
5. Although a few other environmental factors were not experimentally eliminated, the evidence presented indicates that the topography of the rock surface is utilized in homing by *Acmaea scabra*.
6. (From Addendum) Excision of the cephalic tentacles significantly reduced the tendency to home, while

destruction of both eyespots had practically no effect on the incidence of homing.

### ACKNOWLEDGMENTS

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# Occurrence and Behavior of *Hyale grandicornis*, A Gammarid Amphipod Commensal in the Genus *Acmaea*

BY

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(Plates 2 and 3; 4 Text figures)

IN THE COURSE of preliminary studies of the genus *Acmaea* at Hopkins Marine Station, Pacific Grove, California, mottled grey-green amphipods were frequently encountered under the shell of *A. digitalis* ESCHSCHOLTZ, 1833, *A. limatula* CARPENTER, 1864, *A. pelta* ESCHSCHOLTZ, 1833, *A. scabra* (GOULD, 1846), *A. scutum* ESCHSCHOLTZ, 1833, and *Lottia gigantea* SOWERBY, 1843. Dr. J. Laurens Barnard of the Smithsonian Institution has identified the amphipods as immature specimens of *Hyale grandicornis* (KRØYER, 1845) (Plate 2, Figure 1). No mature amphipods have been found in association with any of the above limpets. Dr. Barnard (personal communication) states that he found mature specimens on cobbles and with *Ulva* in Carmel Bay, California. An examination of the algae *Endocladia*, *Gigartina*, *Ulva*, and *Iridaea* growing in areas adjacent to the *Acmaea* populations yielded no amphipods resembling those found with *Acmaea*, although an unidentified species of *Hyale*, mentioned by GLYNN (1965), does occur here and has been found in the present study. This species

differs from immature *H. grandicornis* in the pattern of its dorsal markings and in having brown rather than black, silver-spotted eyes. The *H. grandicornis* found under *Acmaea* species, averaging 2 to 3 mm in length (the range is 1 to 6 mm) is much smaller than this unidentified *Hyale*, which has an average length of 6 mm.

*Hyale grandicornis* occurs under individuals of *Acmaea* species in many different localities along the coast of the Monterey Peninsula. Population studies on this amphipod were carried out at Pescadero Point, on the open coast just north of the northern edge of Carmel Bay, California, from 25 April to 30 May, 1966. The intense wave action in this area, the presence of vertical granite surfaces ranging to 30 feet above the level of mean lower low water, and the varying conditions of exposure and protection afforded by large boulders and sheltered pools provide a variety of different habitats. The sites selected for study (Plate 2, Figures 2 and 3; Text figure 1) were not exposed to direct wave action, being situated either obliquely to the line of waves or on the shore side of large boulders. The sites chosen were divided up into zones (Plate 2, Figures 2 to 4) based on both plant and animal

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## Explanation of Plate 2

Figure 1: An immature *Hyale grandicornis*, showing dorsal and lateral markings. The scale represents 1 mm.

Figure 2: Zones A, C, and D. Pescadero Point, Carmel Bay, California. Taken on 23 May 1966 at 10:00 A. M. Arrow points to marker representing an elevation of +5.2 feet. The scale represents 2 feet.

Figure 3: Zone B. Pescadero Point, Carmel Bay, California. Taken on 23 May 1966 at 10:00 A. M. The scale represents 2 feet.

Figure 4: Zones A, D, and E. Pescadero Point, Carmel Bay, California. Taken on 23 May 1966 at 10:00 A. M. The scale represents 2 feet.



Figure 1



Figure 3



Figure 2

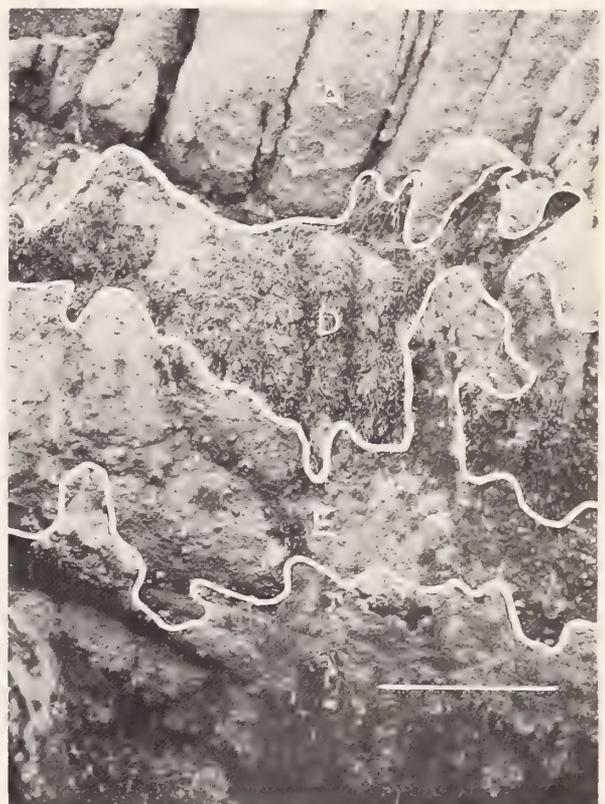
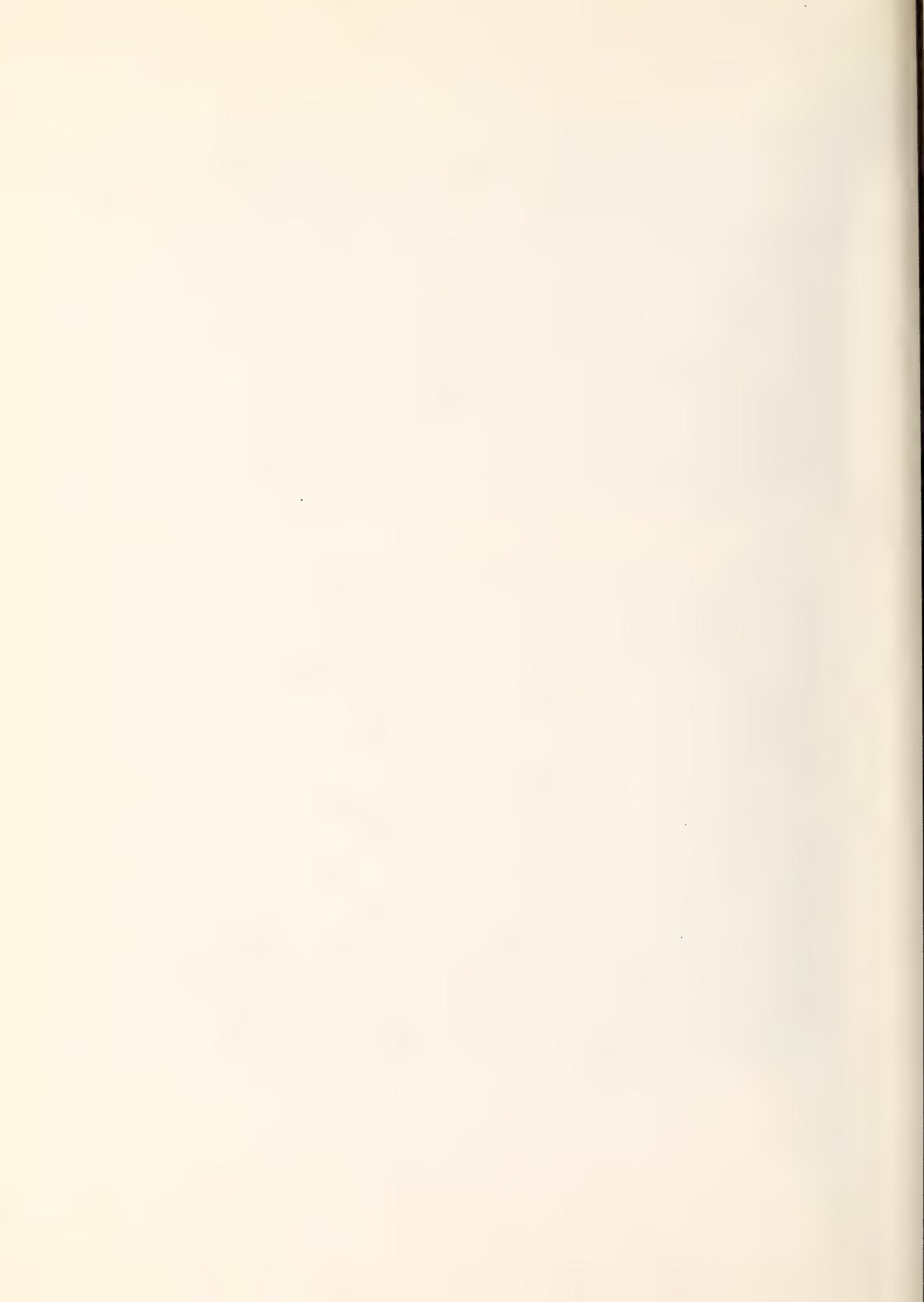


Figure 4



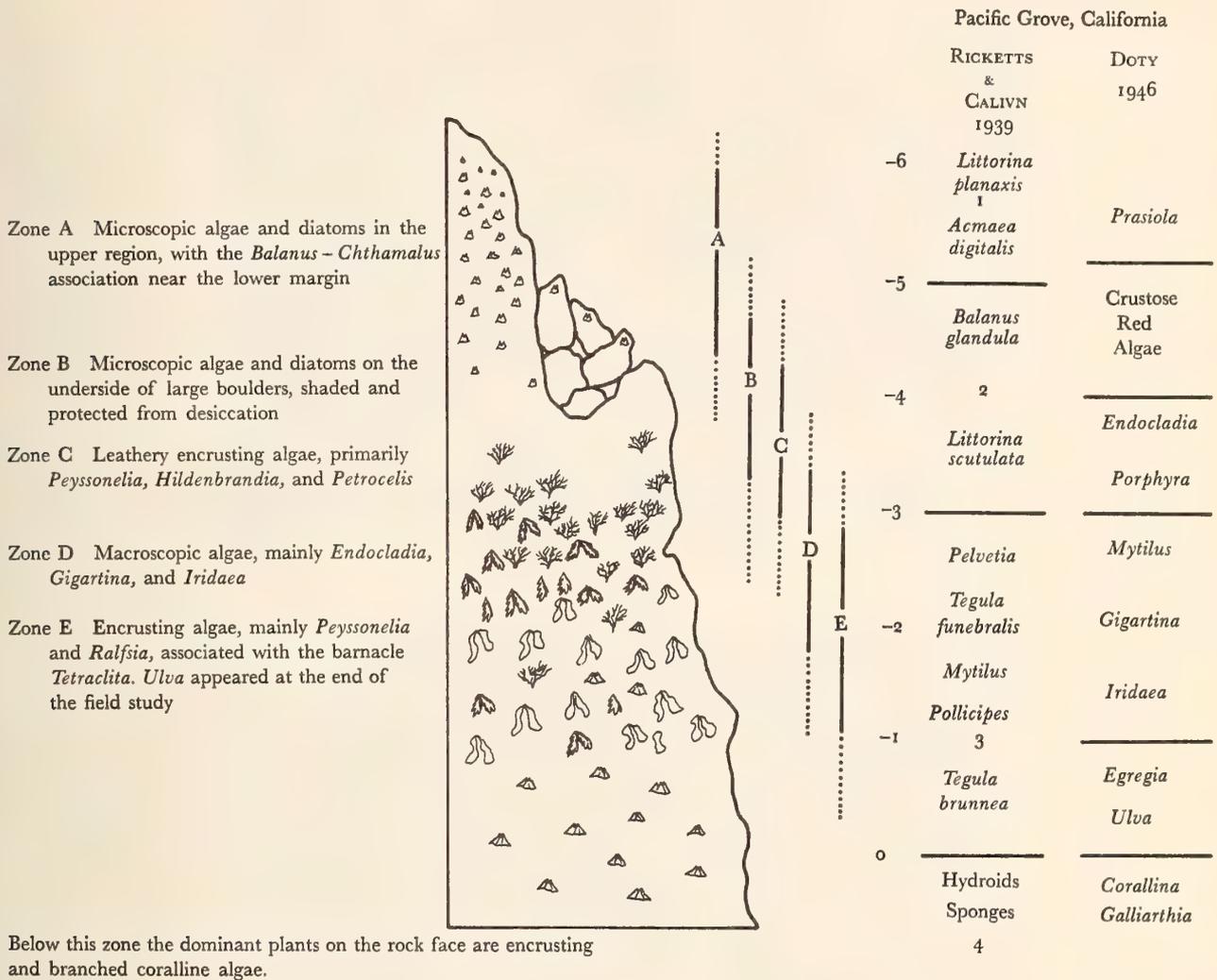


Figure 1

Description and Vertical Position of Zones at Pescadero Point, and Correlation with the Zones of RICKETTS & CALVIN (1939) and DOTY (1946). - 25 April to 30 May 1966.

indices, and following natural groupings of organisms on the rocks. Correlation of these zones with those of RICKETTS & CALVIN (1939) and DOTY (1946) is shown in Text figure 1. Intertidal elevations were determined by measurement from a United States Geological Survey bench mark on Pescadero Point and were checked against the theoretical tidal heights as determined from the United States Coast and Geodetic Survey Tide Tables for the Pacific Coast, using the time and height corrections for Monterey.

The population densities of the 5 species of *Acmaea* studied were determined by dividing the surface of the sampling sites into quadrats of 400 cm<sup>2</sup>, and recording numbers of each *Acmaea* species present. The total area of each zone studied in the sampling sites ranged from 2½ to 3 m<sup>2</sup>. After determining the distribution and numbers of *Acmaea* species, the distribution and frequency of occurrence of *Hyale grandicornis* was determined by sampling of *Acmaea* species in the individual zones. Attempts were made to collect representative numbers

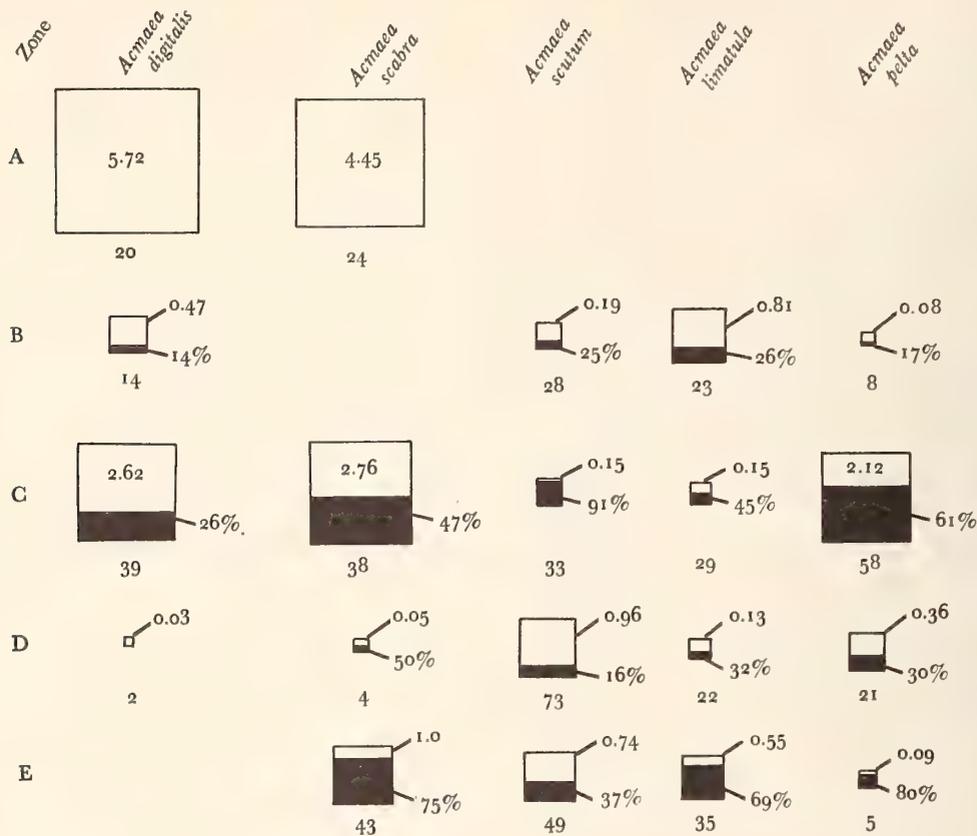


Figure 2

Distribution of *Acmaea* Species and Immature *Hyale grandicornis* at Pescadero Point, California, 25 April to 30 May 1966. Figures inside or to the right of the white squares indicate the average number of *Acmaea* per 400 cm<sup>2</sup>. The size of the square is proportional to this number. The black area within the squares is proportional to the percent of *Acmaea* hosting amphipods; all percentage figures shown refer to the percent of *Acmaea* bearing one or more amphipods. The numbers below the boxes show the total number of limpets of each species examined from each zone.

of the 5 species studied from each zone but certain species in some areas were extremely scarce or non-existent. Extreme care was needed in collecting, for the amphipods often jump away when the limpet is lifted from the substrate. Each limpet collected was then identified, and its shell length measured. The amphipods present with each limpet were counted and sorted into 3 size groups (0.5 to 2 mm; 2+ to 4 mm; 4+ to 6 mm).

Text figure 2 shows the population density of each species of *Acmaea* for each zone, and the percentage of each *Acmaea* species which served as hosts to *Hyale grandicornis*. In Zone A, no amphipods occur even though suitable hosts are present. The *Acmaea* species of Zone B, slightly lower in the intertidal but overlapping Zone

A, bear a small population of amphipods. The large boulders characterizing this zone shade and protect it from desiccation, keeping it moister than Zone A. From this point down to the lowest populations of *Acmaea* examined, the population of amphipods increases, with the exception of Zone D. Zone D is characterized by the macroscopic algae *Endocladia*, *Gigartina*, and *Iridaea*, while Zones C and E are distinguished primarily by the encrusting algae *Hildenbrandia*, *Peyssonelia*, *Petrocelis*, and *Ralfsia*. *Ulva* was just beginning to grow in Zone E when field studies were discontinued.

The present studies do not indicate any clear preference on the part of the amphipods for any particular species of *Acmaea*. Moreover, for those limpets which did house

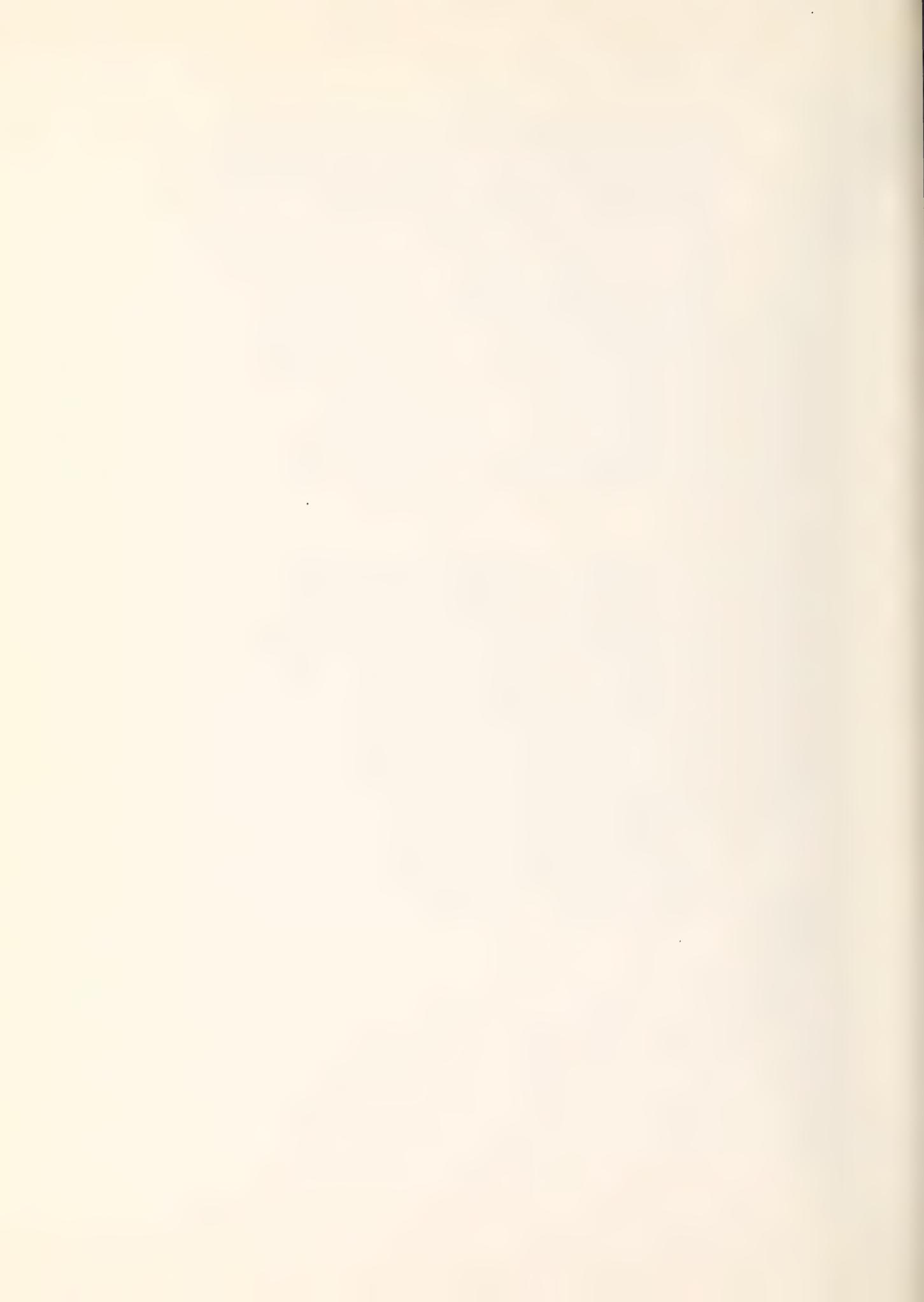


Figure 5 a



Figure 5 b

Amphipod orientation and feeding position in *Acmaea scabra*.  
The scale represents 5 mm.



amphipods, there appears to be no clear correlation between the shell length of the limpet and the number of amphipods present (Text figure 3). However, for limpets

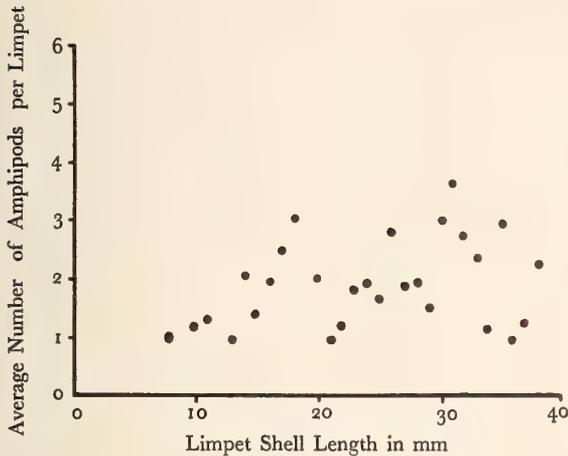


Figure 3

Correlation Between Limpet Shell Length and the Number of Amphipods Housed, for those Limpets which bore Amphipods.

with a shell length of 8 mm or more there does appear to be a slight positive correlation between shell length and the percent of the population bearing amphipods (Text figure 4). Limpets less than 8 mm long did not accommodate amphipods.

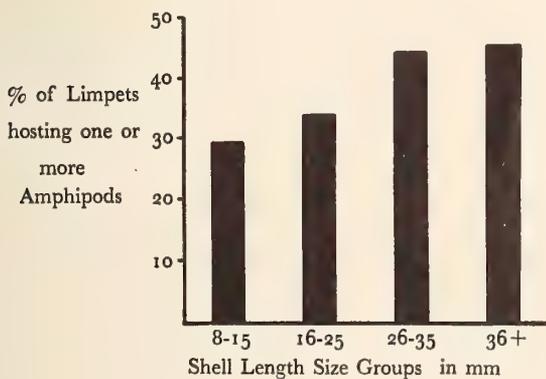


Figure 4

Correlation Between Limpet Shell Length and the Incidence of Amphipods.

## NATURAL HISTORY

Field examinations carried out at Pescadero Point and at Mussel Point during day and night and at high and low tide periods indicate that the amphipods do not leave the limpet at any time unless the latter is removed from the substratum. These observations are supported by the absence of free-living immature *Hyale grandicornis* in areas adjacent to the limpet populations.

The position of the amphipods under the shell was determined from laboratory observations using aquaria in which field conditions were approximated. During the day the amphipods are found behind the head in the nuchal cavity or deep in the groove between the mantle fold and the foot. Sometimes an amphipod may be observed at the edge of the mantle fold, but this behavior is rare in the daytime. In conditions of near darkness and splash or submergence they are found lying on their sides, in contact with the pallial tentacles of the limpet at the mantle margin (Plate 3). In this position the amphipods groom themselves, and from it they also feed, reaching around the edge of the shell or moving completely out onto its dorsal surface and scraping up the algae growing there. Gut contents were unidentifiable, but the material on the shell is composed of numerous varieties of diatoms and several types of blue-green algae, primarily *Enteromorpha*. Occasionally small growths of *Ulva* are found on the shells. The amphipods seem never to leave the limpet, but seek cover under the shell when disturbed. If an amphipod is trapped outside and is unable to crawl under the host's shell again, it either presses itself closely against the edge of the shell and remains there, or moves away to a nearby limpet. Other types of shelter, either in field or laboratory, appear to be ignored. When forced to swim, *Hyale grandicornis* moves very rapidly at first, but quickly slows and appears to seek shelter. If repeatedly disturbed, it soon ceases all movement.

It is of particular interest that Dr. Barnard found mature specimens of *Hyale grandicornis* in *Ulva*, and that the present study has revealed only juvenile individuals in the mantle groove and nuchal cavity of *Acmaea*. *Ulva* occurs mainly in the warmer months in the region studied, and at the end of the present study it was just beginning to grow. Perhaps the immature forms of *H. grandicornis* migrate to the *Ulva* as they attain sexual maturity and as the alga appears each summer, and possibly the juvenile amphipods survive the winter and spring under *Acmaea* shells.

The author plans to continue research on the problem.

## SUMMARY

1. Immature specimens of *Hyale grandicornis* (KRØYER, 1845) are found in the nuchal cavity and pallial groove in 5 species of *Acmaea* and in *Lottia gigantea*.
2. The percentage of limpets hosting immature *Hyale grandicornis* increases with decreasing height in the intertidal region.
3. The amphipod shows no clear preference for particular limpet host species.
4. No amphipods were found in any limpets less than 8 mm in shell length. For limpets above this size there is a slight correlation between shell length and the percent of limpets bearing amphipods. However, for those limpets which housed amphipods, there was no correlation between shell length and number of amphipods borne by each limpet.
5. Immature *Hyale grandicornis* remain in contact with their limpet hosts under all prevailing conditions of tide and light. They appear to feed on algae growing on the surface of the limpet shells.
6. No mature *Hyale grandicornis* have been found in association with any limpets.

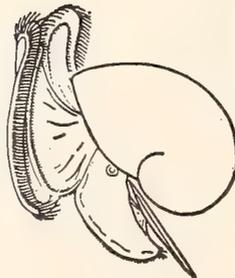
## ACKNOWLEDGMENTS

This work was made possible by Grant GY806 from the Undergraduate Research Participation Program of the National Science Foundation. Dr. J. Laurens Barnard of

the Smithsonian Institution identified the amphipods as *Hyale grandicornis*. The author is very grateful for his help and suggestions. Appreciation is also expressed for pertinent literature supplied by Dr. Peter W. Glynn of the Institute of Marine Biology, University of Puerto Rico. Discussions with David A. Egloff of Hopkins Marine Station proved helpful in suggesting approaches to field and taxonomic problems. Special thanks must go to Dr. Donald P. Abbott of Hopkins Marine Station, Stanford University for his time and effort spent in discussing the project with the author, and particularly for his criticism and editorial comments on this paper. The Monterey Foundation authorized the use of Pescadero Point for field studies.

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# Factors Affecting the Attraction of *Acmaea asmi* to *Tegula funebris*

BY

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(1 Text figure)

## INTRODUCTION

*Acmaea asmi* (MIDDENDORFF, 1849) lives almost exclusively as a commensal on the shell of *Tegula funebris* (A. ADAMS, 1854). Previous workers found this association to be quite specific, *A. asmi* preferring *T. funebris* to *T. brunnea* (PHILIPPI, 1848). TEST (1945) suggested that *T. funebris* released a chemical attractant. The source of this attractant was considered to be the shell by RADFORD (1959), whereas EIKENBERRY & WICKIZER (1964) concluded that both animal and shell were necessary. The following study attempts to settle these differences and to provide further information on behavioral and chemical aspects of this association.

## MATERIALS AND METHODS

Most organisms used in this study were collected in intertidal areas near Hopkins Marine Station, except for *Tegula brunnea* which was collected in the Pebble Beach area, Carmel Bay, California. All experiments were run over night in pyrex dishes kept at 12° to 13°C in a darkroom.

Preliminary experiments showed that if no choice were offered, *Acmaea asmi* would climb onto any shells tested. Therefore, to measure relative preferences, the limpets were allowed to choose between two different substrates. In the test 5 limpets were placed in about 2 cm of water in the center of a 24 cm pyrex pie plate. Ten test shells were placed equidistantly around the periphery of

the plate. There were 5 of each type to be compared, and they were placed alternately in the circle.

To permit washing of the *Tegula* shells with various solvents, the operculum was sealed with canning wax (Parowax). The animals survived this sealing treatment, and most importantly, were apparently unaffected by such solvents as alcohol or distilled water.

In all experiments the dishes were then placed overnight in a darkroom at 12° to 13° C, and the number of limpets on the test shells determined the following morning.

## RESULTS AND DISCUSSION

RADFORD (1959) found that the shells of *Tegula funebris* were not preferred by *Acmaea asmi* if the shells were boiled in alcohol for 15 minutes. The results shown in Figure 1a indicate that simple room-temperature washing in ethanol for 1 hour also removes any attractant on the shell. In this experiment the limpets were given a choice between parowax-sealed shells washed in alcohol and control parowax-sealed shells. In 5 trials (25 limpets) the control shells attracted 90% of the limpets, while the alcohol-washed shells attracted 10%.

The attractant is also partially removed by distilled water. If sealed shells containing *Tegula funebris* are washed for 2 hours in distilled water and compared with normal, sealed shells, only 25% are found on the washed shells versus 65% on the control shells (Figure 1b). (Where the total percentage does not equal 100% the difference represents those animals not found on any shell.) However, distilled water washing is not as effective as alcohol washing. As seen in Figure 1c, 60% of the

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animals preferred the water-washed shells as compared to only 15% on the alcohol-washed shells.

Although *Acmaea asmi* is rarely found on *Tegula funebris* shells inhabited by *Pagurus* spp., the limpets do not discriminate between these two types of shells. Thus, using the usual test system, equal numbers of limpets

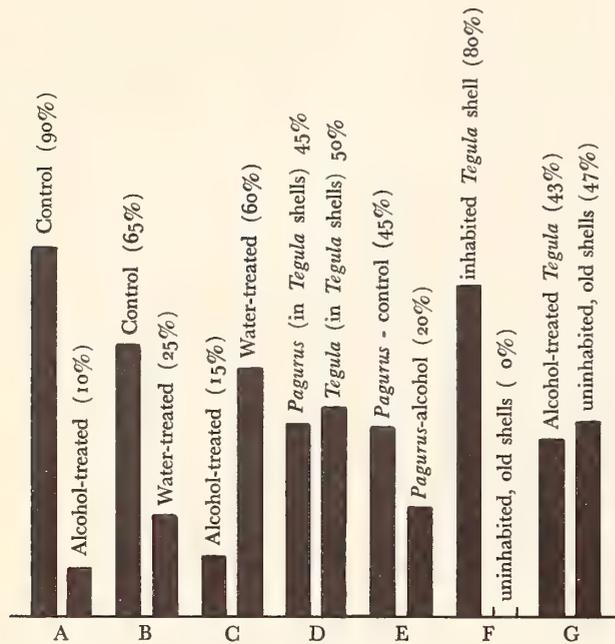


Figure 1

Preference of *Acmaea asmi* for various substrates.

Twenty-five *Acmaea asmi* were tested in each experiment. Each test (A to G) represents the percentages of animals found on the indicated substrate (percentages of those not responding are not shown).

were found on sealed *T. funebris* shells containing *Pagurus* spp. as on parowax-sealed *T. funebris* shells containing its normal host (Figure 1d). Similar to normal shells, the attraction of *Pagurus*-inhabited shells is lost when treated with alcohol (Figure 1e).

A final preference test series was made with old uninhabited *Tegula* shells found on the beach. Figure 1f shows that *Acmaea asmi* prefers normal uninhabited shells to these old shells. However, as shown in Figure 1g, treatment of normal shells with alcohol renders them as unattractive as the old shells.

The above experiments suggest that the "attractant" is completely removed or destroyed by alcohol, and partially removed or destroyed by distilled water. Furthermore, it is found on *Tegula* shells inhabited by either *T. funebris*

or *Pagurus* spp., but is not present on uninhabited shells found on the beach. The "attractant," then, could be an alga or bacterial film associated with the shell, which is removed or destroyed by alcohol or distilled water. However, the major algal epiphyte found on *T. funebris* is also found on the shells of *T. brunnea* and *Acanthina spirata* (BLAINVILLE, 1832) (EIKENBERRY & WICKIZER, 1964), which are not the normal hosts for *Acmaea asmi*.

## BEHAVIORAL OBSERVATIONS

The above results on preference are consistent with a diffusible "attractant" emanating from the *Tegula* shell. Behavioral observations, however, indicate that the preference might be made at the tactile rather than the olfactory or chemosensory level.

Continuous observations were made of the selection process in a test situation where normal, sealed *Tegula funebris* shells were alternated with alcohol-treated ones.

As soon as the *Acmaea asmi* were placed in the center of the *Tegula funebris* circle, they extended their tentacles and began to feel the substrate. These tentacles are thin and almost as long as the shell when fully extended. They are moved in a tapping manner from side to side as the animal crawls in a seemingly random fashion across the bowl. When a specimen touched another *A. asmi* with its tentacles, it felt the shell and then climbed immediately onto it.

If a limpet crawled between two *Tegula funebris* shells, one alcohol-treated and the other not, it would tap each shell with its tentacles and then, in every case observed (11), climb onto the untreated shell. If, however, the *Acmaea asmi* encountered only one shell, whether alcohol-treated or not, it would generally climb on. Once on a shell, the *A. asmi* continued to move and sometimes changed shells. Out of 25 animals tested, 9 were at one time or another on alcohol-treated shells. After 8 hours, when the experiment was concluded, only 2 limpets were still on these shells.

From these latter observations, it seems that *Acmaea asmi* is not reacting to some diffusible chemical attractant from the shell, but rather testing the substrates with its tentacles. It will crawl on the first curved surface encountered, but if other choices are available, will eventually end up on the preferred substrate. It is interesting to note that TEST (1945) stated that the diffusible attractant was sensed at 7 mm and RADFORD (1959) felt the range to be 10 mm. If an *A. asmi* is placed this close to a *Tegula funebris*, it can generally touch it with its tentacles and would, therefore, react to its presence.

## SUMMARY

The behavioral basis of the association between *Acmaea asmi* and *Tegula funebris* has been investigated. The observations indicate that *A. asmi* is not attracted by a diffusible substance, but senses its substrate through contact with its tentacles. The critical substance(s) on the shell is (are) easily destroyed or dissolved by ethanol and slightly removed by distilled water.

These factors are also present on *Pagurus*-inhabited *Tegula* shells, but are not found on uninhabited shells found on the beach.

## ACKNOWLEDGMENTS

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National Science Foundation. I also wish to thank Dr. David Epel, Dr. Donald P. Abbott, and Mr. Roger Kingston for offering helpful advice.

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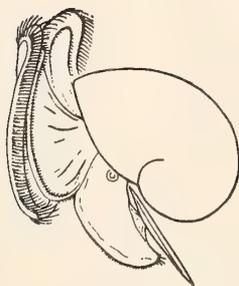
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## Shell Damage and Repair in Five Members of the Genus *Acmaea*

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(Plate 4; 2 Tables)

SHELLS OF THE COMMON LIMPETS of the genus *Acmaea* found in the rocky intertidal of the California coast often show evidence of having sustained and repaired extensive damage. Studies of shell damage and repair were made on *Acmaea scabra* (GOULD, 1846), *A. digitalis* ESCHSCHOLTZ, 1833, *A. pelta* ESCHSCHOLTZ, 1833, *A. limatula* CARPENTER, 1864, and *A. scutum* ESCHSCHOLTZ, 1833.

PEPPARD (1964) studied growth and repair in the shells of *Tegula funebris* (A. ADAMS, 1854) and studies of normal growth over a 3 year period were made of *Acmaea digitalis*, *A. pelta* and *A. paradigitalis* FRITCHMAN, 1960 by FRANK (1965). SEAPY (1966) reports on the growth of *A. limatula* over a period of one year. FRETTER & GRAHAM (1962) discuss the general growth in prosobranch mollusks and include a chapter on the shell. No previous work, however, has been done on repair in the 5 species of *Acmaea* under consideration here.

Specimens of each of the 5 species showing evidence of shell damage were collected from Pescadero Point, Monterey County, and Mussel Point, Pacific Grove, California on May 24 to 26, 1966. All levels of the intertidal area were covered, and both protected areas and regions exposed to very heavy surf were included in the survey. One hundred animals showing evidence of having repaired shell damage were collected; all but two of these occurred where the wave action was heavy and where debris such as rocks and large shells were tossed and shifted about by the waves at high tide. Even in the latter areas animals with damaged shells constituted a very small minority of the total population.

Of several hundred *Acmaea digitalis* examined only 3 were found to be damaged significantly. *Acmaea scabra*

and *A. pelta* populations showed the same small proportion of damaged shells, although in the areas studied *A. pelta* was not as abundant as the other species. Many more damaged shells of *A. scutum* and *A. limatula* were found, but they comprised less than 10% of the observed populations of these species. Often several *A. scutum* or *A. limatula* with damaged shells were found in the same small area. Such groups of injured animals were not found in the other species.

The numbers of damaged animals collected and the types of damage are shown in Table 1. As can be seen, injury to the edge of the shell, with pieces of the margin chipped off, is the most common (Plate 4, F). Repair of the margin can be recognized readily not only by the newness of the repaired portion but by the ridge which is produced where the new shell material meets the old.

Table 1

Frequency and Types of Natural Shell Damage in 100 Limpets

Species	Number collected	Damage to edge	Apex crushed	Cracks	<i>Didymella</i> erosion	<i>Balanus</i> damage	Small hole
<i>Acmaea scutum</i>	60	53	1	4	0	0	2
<i>Acmaea pelta</i>	3	2	0	0	0	0	1
<i>Acmaea digitalis</i>	3	0	0	0	2	1	0
<i>Acmaea scabra</i>	2	0	0	0	1	0	1
<i>Acmaea limatula</i>	32	26	3	2	1	0	0

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In many cases pieces up to 20% of the width of the shell have been broken off and replaced. Often the entire margin of the shell has been broken off and a ring of newer shell material can be seen clearly all around the original shell. In *Acmaea limatula* this new shell material has the same characteristic file-like appearance as the original shell.

In 4 of the animals collected a portion of the top of the shell near the apex had been crushed (Plate 4, A). Pieces of the shell had been pushed inward, pressing down the viscera. Repair had been achieved by the laying down of new nacreous layers below these pieces of shell, cementing together the crushed fragments of the top of the old shell and partially embedding them to form a solid unit. Any holes left by missing pieces of shell had been covered over on the inside by new shell material (Plate 4, B). Where the shell had been cracked severely from margin to apex, new nacreous material had been laid down over much of the interior of the shell, binding the pieces into a solid unit. In some cases quite extensive damage of this sort has been repaired (Plate 4, C). Four of the shells each contained a small hole from  $1\frac{1}{2}$  to  $3\frac{1}{2}$  mm in diameter. Although the cause of these holes is unknown, they appear too irregular in outline to be the work of predatory boring snails. The holes had been covered over on the interior with new nacreous material.

Two of the shells of *Acmaea digitalis*, one of *A. limatula* and one of *A. scabra* had been very severely eroded by a fungus growing in the shell (Plate 4, D). The erosion was heaviest at the apex and extended down the sides to varying degrees. The surface of these shells was much softer than that of normal shells and had a spongy appearance when viewed under the dissecting microscope. BONAR (1936) reported finding an ascomycete, *Didymella conchae*, in the shells of *Acmaea*, and noted that among *A. digitalis* uninfected shells are actually rare. Examination of populations of *A. digitalis* bore this out, but only in unusual cases was the damage particularly noticeable. One *A. digitalis* shell was found with one large and several small barnacles, *Balanus glandula* DARWIN, 1854, living on it. There were deep pits in the shell, not caused by *Didymella conchae*, which were clearly formerly inhabited by barnacles and may actually have been eroded by them (Plate 4, E).

In conjunction with observations of natural damage, laboratory studies of repair of artificially induced damage were undertaken. Three animals of each species were chosen with normal, undamaged shells. Slots were drilled in the shell with a high speed dental drill. All the slots were of uniform width, approximately  $1\frac{1}{2}$  mm, and extended varying distances from the margin towards the apex. In no case did the slot extend up to the attachment

of the shell muscle. Care was taken not to damage the mantle. The animals were placed on rocks in aquaria with constantly circulating seawater and aeration and kept submerged throughout the experimental period (May 16 to May 23). They were removed each day, placed on a glass slide, and the extent to which they had repaired the slots observed under a compound microscope and measured with an ocular micrometer. The results are presented in Table 2.

Table 2

Repair of Artificially Damaged Limpet Shells

Species	Shell length (mm)	Depth of injury (mm)	Average daily repair (mm)
<i>Acmaea scutum</i>	25	2	0.075
	25	4	0.047
	28	8	0.000
<i>Acmaea pelta</i>	22	2	0.05
	24	5	0.07
	28	10	0.000
<i>Acmaea digitalis</i>	14	1	0.001
	14	3	0.000
	14	5	0.000
<i>Acmaea scabra</i>	20	2	0.001
	19	4	0.000
	20	6	0.000
<i>Acmaea limatula</i>	21	2	0.023
	21	4	0.025
	23	8	0.000

In each case where repair took place, it began with the laying down of a thin transparent layer at the interior end of the slot. This layer was gradually extended towards the margin of the shell and thickened from beneath. Often this new shell broke off. After about 4 days the new shell became opaque as it continued to thicken. The mantle, whose margin usually conforms exactly to the margin of the intact shell, did not contract locally to a shape conforming to the margin of the slot until after about 2 days. In no case did repair begin until this had taken place. In those animals with deeper slots (see Table 2) the mantle margin was unable to contract enough to conform to the margin of the slot and no repair took place.

The high incidence of evidences of natural shell damage and repair in *Acmaea limatula* and *A. scutum* is probably the result of 3 factors. First, both species inhabit the lower areas of the intertidal (RICKETTS & CALVIN, 1952)

where wave action is heaviest, and where loose rocks and debris which might be pounded against their shells are more prevalent. Secondly their shells are both flatter and thinner than those of the limpets of the higher intertidal areas, thus presenting a larger and weaker surface. Finally, both species show a much more rapid rate of repair than either *A. scabra* or *A. digitalis*. Thus an *A. scutum* or *A. limatula* with a damaged shell would be more likely to be able to repair any damage to its shell before a predator could take advantage of the weakened shell. Limited observations on shell growth in undamaged animals maintained in laboratory aquaria from April 27 to May 21, 1966, suggest that addition of new shell at the margin occurs more rapidly in *A. scabra* than in the other 4 species, and that new shell may be added at very uneven rates at different regions of the shell margin in this species. *Acmaea scabra* is known to inhabit a particular "scar" on the substrate to which its shell conforms exactly and to which it consistently returns (HEWATT, 1940; TEST, 1945). The rapid and uneven marginal growth it exhibits might enable animals to achieve conformity of the shell to a new spot on the rock more rapidly. However, shells of *A. scabra* with slots drilled in the shell margin showed a slow rate of repair.

### SUMMARY

Shells of *Acmaea scabra*, *A. digitalis*, *A. pelta*, *A. scutum* and *A. limatula* found in the field showed a variety of types of natural damage. Damage through chipping at the edge of the shell predominated, but shells were found in which regions near the apex had been crushed; in which the tops and sides were eroded due to an ascomycete, *Didymella conchae*; in which cracks extending from the margin to near the apex were present; in which the tops were eroded apparently due to barnacles, *Balanus glandula*, living on the shell, and in which small holes of unknown origin occurred near the apex.

Repair of such damage in all cases had been accomplished by the laying down of new nacreous material on the interior of the shell below the damaged portion.

Rates of repair of artificially damaged areas on shell

margins were much higher than rates of growth on adjacent undamaged shell margins.

### ACKNOWLEDGMENTS

This work was made possible by Grant GY806 from the Undergraduate Research Participation Program of the National Science Foundation. Help in the project was given by Dr. Donald P. Abbott, Hopkins Marine Station. The photographs in Plate 4 were taken by Mr. Samuel E. Johnson. Permission to collect in the Pescadero Point area was kindly granted by the Monterey Foundation.

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### Explanation of Plate 4

- A. Shells of *Acmaea scutum* showing crushed tops.  
B. Interior of shell shown in A. Note how new nacreous material has been laid down.  
C. Shell of *Acmaea scutum* showing severe cracking.  
D. Shell of *Acmaea digitalis* showing erosion due to a fungus in the shell.  
E. Shell of *Acmaea digitalis* with barnacles growing on it. Note deep pits.  
F. Shell of *Acmaea limatula* showing damage to edge and subsequent repair.

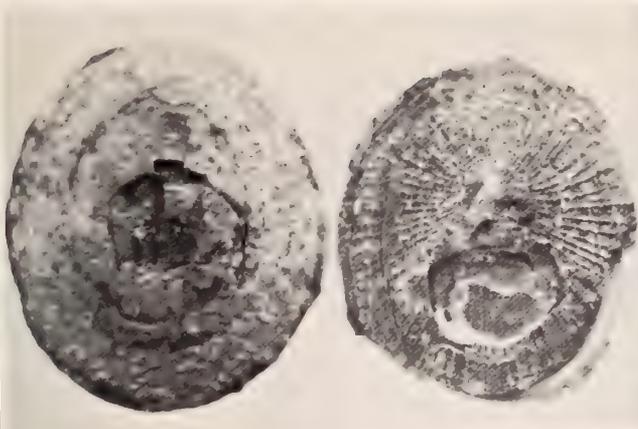


Figure 1

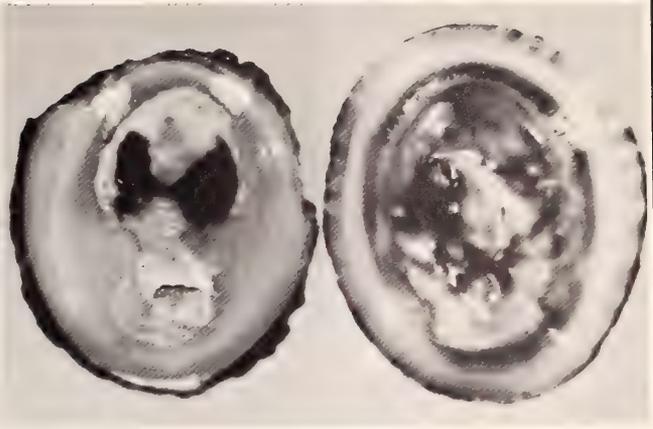


Figure 2



Figure 3



Figure 4

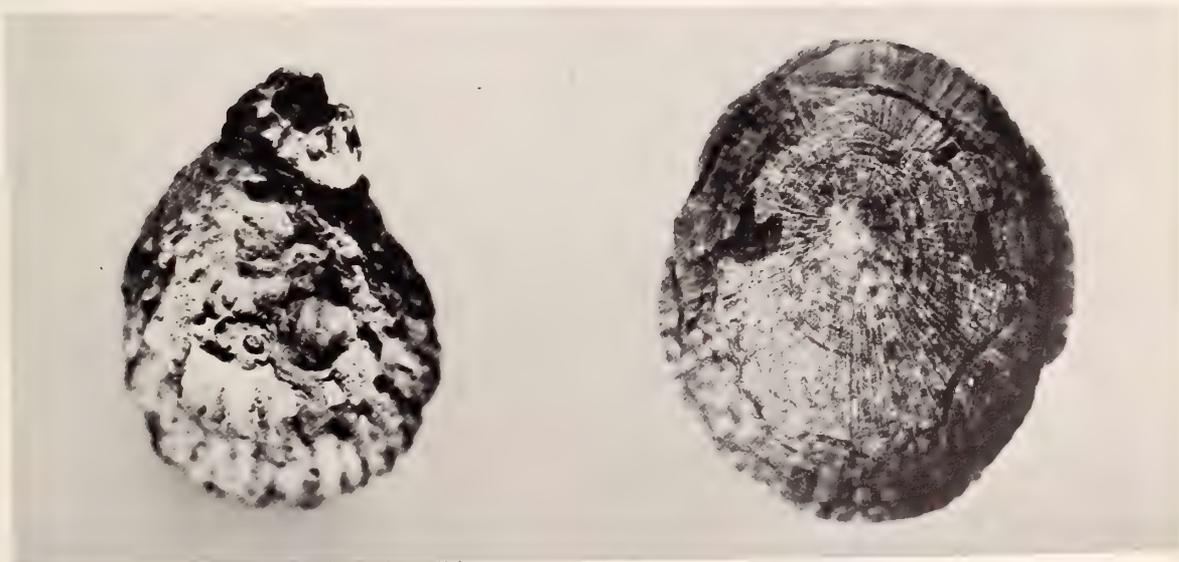


Figure 5

Figure 6



# Some Observations of Predation on *Acmaea* Species by the Crab *Pachygrapsus crassipes*

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(Plate 5)

SPECIES OF THE GENUS *Acmaea* are distributed throughout the rocky intertidal zone of the California coast in association with a variety of animals potentially predaceous on these limpets.

Predation on *Acmaea* by the fish *Gibonsia elegans* HUBBS, 1929 was observed by MITCHELL (1953). A continuation of this work by JOHNSTON (1953) indicated that *Oligocottus snyderi* GREELEY, *Gibonsia metzi* HUBBS, 1929, and *Gobiesox maeandricus* (GIRARD, 1858) were also occasionally found with limpets in their gut. FRANK (1965) has presented evidence that *Leptoplana* may be predaceous on some species of *Acmaea* on the Oregon coast. The other major predator reported in the literature is the starfish (BULLOCK, 1953; FEDER, 1959). Recorded here are observations of predation by the crab *Pachygrapsus crassipes* RANDALL, 1839, a previously unrecognized predator of these mollusks.

## MATERIALS

The following limpet species were included in the study: *Acmaea scutum* ESCHSCHOLTZ, 1833, *A. digitalis* ESCHSCHOLTZ, 1833, *A. limatula* CARPENTER, 1864, *A. pelta* ESCHSCHOLTZ, 1833, and *A. scabra* (GOULD, 1846). These animals and *Pachygrapsus crassipes* were collected between Mussel Point and Cypress Point on the coast of Monterey County, California, and studied both in the field and in the laboratory.

## RESULTS AND DISCUSSION

*Pachygrapsus crassipes* was never observed attacking *Acmaea* in the field. In the laboratory, when left undis-

turbed, the crab made sometimes as many as 5 attacks in 3 hours. While all limpets were preyed upon, crabs preferentially attacked *A. limatula*.

*Pachygrapsus crassipes* had 2 methods of attacking its prey. The first was simply to pry the limpet off the rock with its cheliped. This method was successfully used if the limpet did not have its shell clamped to the rock surface and the crab could get underneath the edge. Animals attacked in this manner showed a characteristic chipping of the edge of the shell. This method was used most often on *Acmaea scutum* and *A. digitalis*.

The second method of attack was never directly observed. However, the result of this method could be assessed by the examination of shells. Shells of limpets subjected to this form of attack had lost the peak of the shell above the muscle scar (see Plate 5). A total of 17 shells with the tops removed were taken from the aquaria containing the crabs. Such shells were never found in any other tanks. Several shells were found that had deep scratch marks on them that were possibly made by the crab's chelipeds exerting pressure on the shell. The scars were randomly oriented on the shell and were found distributed over the entire surface of the shell.

Attempts were made to mechanically duplicate the possible squeezing action of the chelipeds by means of a pair of needle-nose pliers suspended from a stand above the limpet, so that only a lateral pressure was exerted between the points. These experiments indicate that there is a fracture zone or weak area that encircles the shell just above the point of attachment of the shell muscles. This zone seems to be present in all *Acmaea* species studied, but it is most pronounced in the shells of *A. limatula* where, if a pressure of just 2 pounds was applied with the pliers, the shell might break. The maximum

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pressure needed to break an *A. limatula* shell along the shear zone was 17 pounds at the tips of the pliers.

Studies on factors influencing the shell breakage showed it is important to exert the pressure exactly on the very narrow shear zone. The shells were most easily broken if the tips of the pliers were placed on the longitudinal axis rather than the lateral axis. When the pressure was exerted below the shear zone, one *Acmaea limatula* shell withstood a pressure of 45 pounds. The same shell broke when a pressure of 12 pounds was exerted at the shear zone. If the shells were artificially broken in this manner, they did not always produce a clean circular break. The remaining shell fragments, however, could very easily be broken back to the shear zone but no farther so that a very even break could be achieved.

A second set of experiments was run in which a cheliped that had recently been removed from a crab was used to exert pressure on the shells in the same manner that the pliers were used. The results of these experiments indicated that the cheliped could puncture the shell at the fracture zone as easily as, or more easily than, the pliers although no exact pressures were recorded. The cheliped did not suffer any damage when the pressure was applied.

By having the crab squeeze a piece of balsa wood, and then duplicating the damage with pliers with about the same squeeze area, it was possible to make a very crude determination of the pressure that could be exerted by the crab. With this method, it was found that the pressures exerted by the crab exceeded 23 pounds, which is more than any pressure needed to artificially break a limpet shell at the shear zone.

Surveys of the intertidal area on Mussel Point indicate that shells from which the peaks have been removed make up about 14% of the total *Acmaea* shells cast up on the beach. Such shells were often not highly eroded and the loss of the peak did not appear to be due to erosion after the death of the limpet.

### Explanation of Plate 5

A: *Acmaea* shells broken by various means.

From left to right: (1) *Acmaea limatula* shell found in an aquarium containing only *Pachygrapsus crassipes* as a predator. (2) *Acmaea digitalis* shell artificially broken with needle-nose pliers. (3) *Acmaea limatula* shell artificially broken with needle-nose pliers. (4) *Acmaea limatula* shell found in an aquarium with *Pachygrapsus crassipes*.

### SUMMARY

The crab, *Pachygrapsus crassipes*, has not previously been recognized as an important predator of limpets. Laboratory observations and experiments suggest that the crab can remove the tops of the shells of some limpets by squeezing with the cheliped, thus making the viscera available for food. The results of a survey of the shells cast up on the beach, and the number of limpets apparently attacked by this method in the laboratory, suggest that these animals may be responsible for a significant mortality in limpet populations.

### ACKNOWLEDGMENTS

The author gratefully thanks Dr. John Phillips and Ray Markel for their patience and assistance in carrying out this study and preparing the manuscript. This work was made possible by Grant GY806 from the Undergraduate Research Participation Program of the National Science Foundation.

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B: An artificially broken *Acmaea limatula* shell.

The very smooth surface of a break in the cleavage zone is seen. The notch on the left was made by the tips of the pliers when the pressure was applied slightly below the cleavage zone.

C: A shell of *Acmaea* sp. found cast up on the beach. This illustrates the effects of the slight erosion often found in such shells.



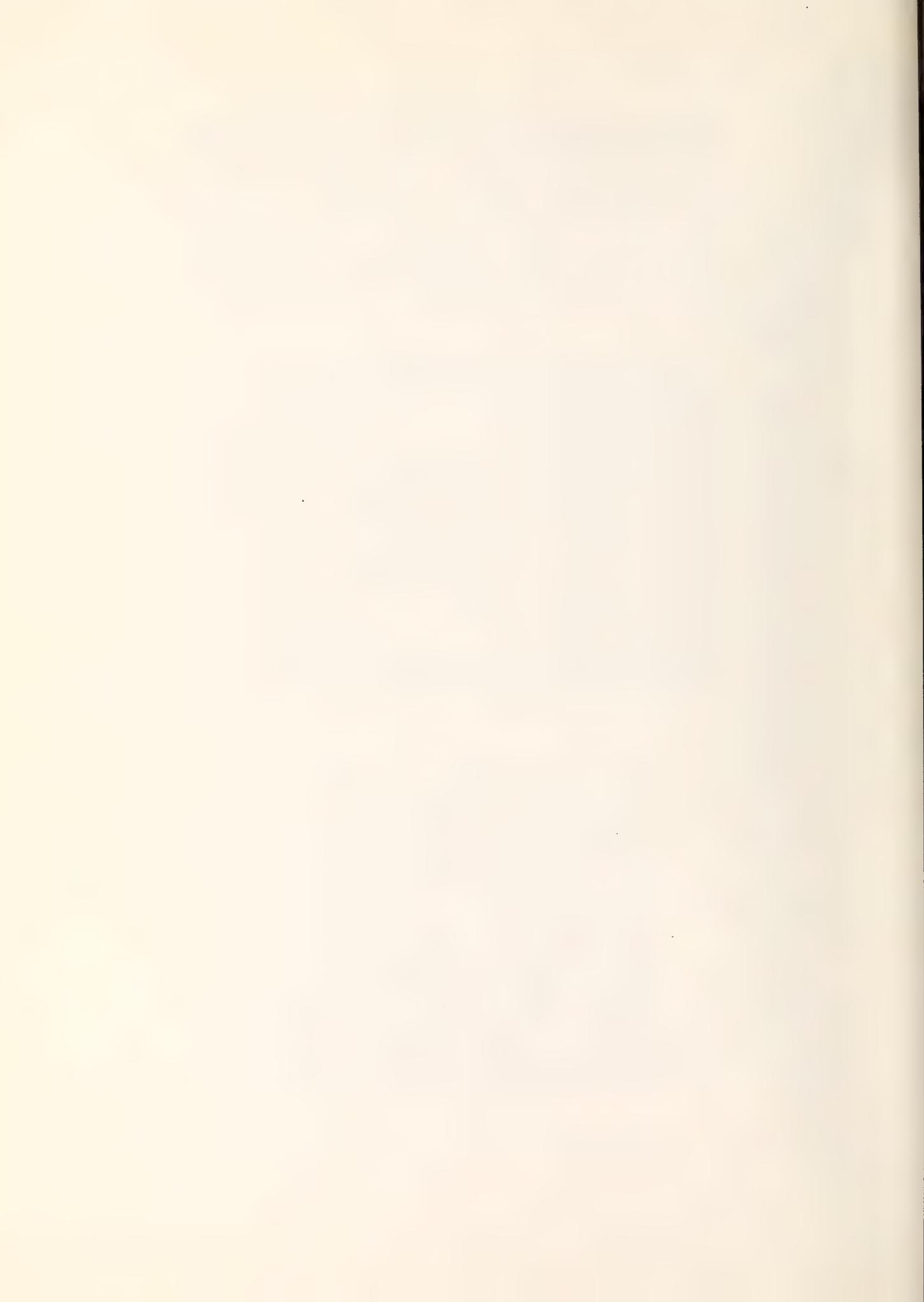
Figure 1



Figure 2



Figure 3



# A Study of Morphological Variation in the Limpet *Acmaea pelta*

BY

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(Plate 6; 1 Text figure; 2 Tables)

*Acmaea pelta* ESCHSCHOLTZ, 1833 is a low intertidal limpet that is widely distributed on the North American west coast. A great variation in shell appearance has been noted, especially when comparing large and small specimens. Preliminary observations indicated a possible correlation between habitat and morphology, which led to a study of the distribution of *A. pelta* with respect to substrate and algal association. These field studies, reported below, indicated definite relationships between morphologic types and habitat, leading to a study of digestive enzyme potential.

## FIELD STUDIES

Field observations were done at Mussel Point, Pescadero Point, and Cypress Point on the Monterey Peninsula, California. Three morphologic forms of *Acmaea pelta* were observed, differing in color, shape, and length to height ratios of the shell. These different forms of *A. pelta* were positively identified as being variations of the

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species using standard radula length and teeth determinations as well as the classification by jaw shape proposed by WALKER (1968). These various morphologic forms are described below in terms of color, shell shape, and length to height ratios as measured with a vernier caliper.

## MORPHOLOGIC FORMS

**Brown Form:** The typical large brown *Acmaea pelta* is characterized by a convex slope from the high peaked shell apex to the periphery and is often infected with the fungus common to shells of littoral animals of the Pacific coast (BONAR, 1936). These brown-colored specimens are generally over 30 mm in length, and have a length to height ratio of 2.3 as seen in Table 1. Illustrations of the brown shells are shown in Plate 6, Group A.

**Black Form (*Pelvetia*):** Two separate populations of the small black *Acmaea pelta* were observed, on the mussel beds and on and under *Pelvetia fastigiata* (J.G. AGARDH) DE TONI 1895. Those found associated with *Pelvetia* (Plate 6, Group C) had a straight to concave slope from

Table 1

Length to height ratios of the different forms of *Acmaea pelta* as related to position in the intertidal zone.

Form of Animal	Size in mm	Location	Number checked	Length to height ratio	Standard deviation
Brown <i>Acmaea pelta</i>	30	High Intertidal	24	2.32	± 0.246
Black <i>Acmaea pelta</i>	15	Under <i>Pelvetia</i>	22	2.86	± 0.270
Black <i>Acmaea pelta</i>	15	Under <i>Pelvetia</i>	20	2.93	± 0.268
Black <i>Acmaea pelta</i>	15	Mussel beds	12	2.53	± 0.295
Black <i>Acmaea pelta</i>	10 to 15	Mussel beds	27	2.89	± 0.272
Black <i>Acmaea pelta</i>	10	Mussel beds	23	3.21	± 0.209

the apex to the edge and a characteristically cleaner shell than any other *A. pelta* form. The shell shape was flatter than the brown *A. pelta*, and had a uniform length to height ratio for the entire size range encountered. This flat shell often had a rather hooked apex and a more wavy peripheral edge than the black form seen in mussel beds (see below).

**Black Form (Mussel beds):** *Acmaea pelta* was found on the mussel bed areas generally on mussels, *Mytilus californianus* CONRAD, 1837, and on the goosenecked barnacles, *Pollicipes polymerus* SOWERBY, 1833. These were generally black with length to height ratios which varied with size, as seen in Table 1. No specimens over 20 mm were found in the beds. The profile of the members of this form (Plate 6, Group D) approaches that of the large brown *A. pelta*. The shells of this form are not as clean and as shiny as those found under *Pelvetia*, and the shell periphery is smooth.

**Green Form:** The third group of *Acmaea pelta*, intermediate in size between the black *A. pelta* on *Pelvetia* and the large brown *A. pelta* form, is shown in Plate 6, Group B. This group shows a great variation in shell shape and often displays the growth ring type of change in the exterior shell. Most specimens are greenish-black in color. Some, however, are almost white and others are ribbed or checkered.

## HABITAT

A survey of the intertidal zone of the Monterey Peninsula revealed differences in the density of the *Acmaea pelta* populations.

Mussel Point and Pescadero Point had rich populations while Point Piños was sparsely populated. The reason for this uneven distribution was not investigated.

**Brown Form:** This form of *Acmaea pelta* occupies a high inshore intertidal position from +4 to above +6ft. The brown organisms were found predominantly on more barren rocks and were exposed to more sun and arid conditions than the other types. They were generally found on vertical rather than horizontal faces. The brown limpets were found in an apparently random association with encrusting algae and algal films as well as *Endocladia muricata* (POSTELS & RUPRECHT) J. G. AGARDH, 1847, high *Pelvetia fastigiata*, *Rhodoglossum affine* (HARVEY)

KYLIN, 1928, and other algae. Occasionally one was found on bare rock close to sand with no macroscopic algae evident.

**Black Form (*Pelvetia*):** At low tide 25% of this form was on the holdfast or stipe of *Pelvetia*. An obvious scar was found under these limpets, indicating that this alga may be a primary food source. The remaining 75% were attached to the rock substratum under the *Pelvetia*. The *Pelvetia* beds provided moisture and shelter from the sun. In more exposed areas, such as Pescadero Point, where *Pelvetia* is not found in large beds, the black *Acmaea pelta* were less numerous and randomly distributed. Here again they were to be found in locations offering shelter and moisture.

**Black Form (Mussel beds):** *Acmaea pelta* in the mussel beds were not associated with conspicuous algae. This form could be found exposed to the sun at low tide, as opposed to the *Pelvetia* form which was never found exposed to direct sunlight. The mussel beds provided a damp, sheltered environment that is subjected to splash except at very low tide.

**Green Form:** The middle-sized green *Acmaea pelta* had a habitat virtually identical with that of the black *Pelvetia* form. Text figure 1 shows the percent distribution of these different types of *A. pelta* as related to position and habitat in the intertidal.

## LABORATORY STUDIES

The intertidal distribution, along with CRAIG's (1968) observations on feeding habits suggested that, since the different forms were eating different algae, possibly there was a difference in digestive enzymes in the *Acmaea pelta* forms. The digestive enzyme potential of the different forms of *A. pelta* was assayed by measurement of reducing sugar released from purified polysaccharides (NELSON, 1944). The polysaccharides studied were fucoidin, laminarin, and alginic acid from brown algal sources, agar and K-carrageenin from red algae, and starch. Since some of these carbohydrates contain sulfated groups, particularly fucoidin, sodium hydroxide was substituted for barium hydroxide (NELSON, *op. cit.*) to avoid possible precipitation of the sulfated sugar fragments produced by enzymatic hydrolysis. A 10% extract of the entire digestive system including esophagus, buc-

## Explanation of Plate 6

Different varieties of *Acmaea pelta* encountered on the coast of the Monterey Peninsula. Group A: Brown form; Group B: Green form; Group C: Black form (*Pelvetia*); Group D: Black form (mussel beds).



Figure 1

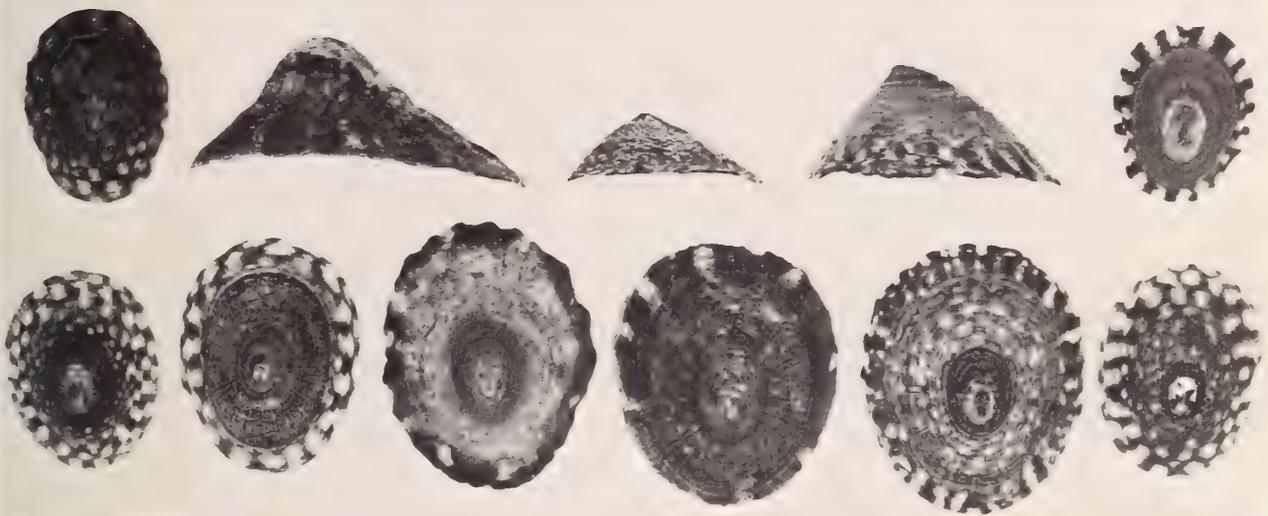


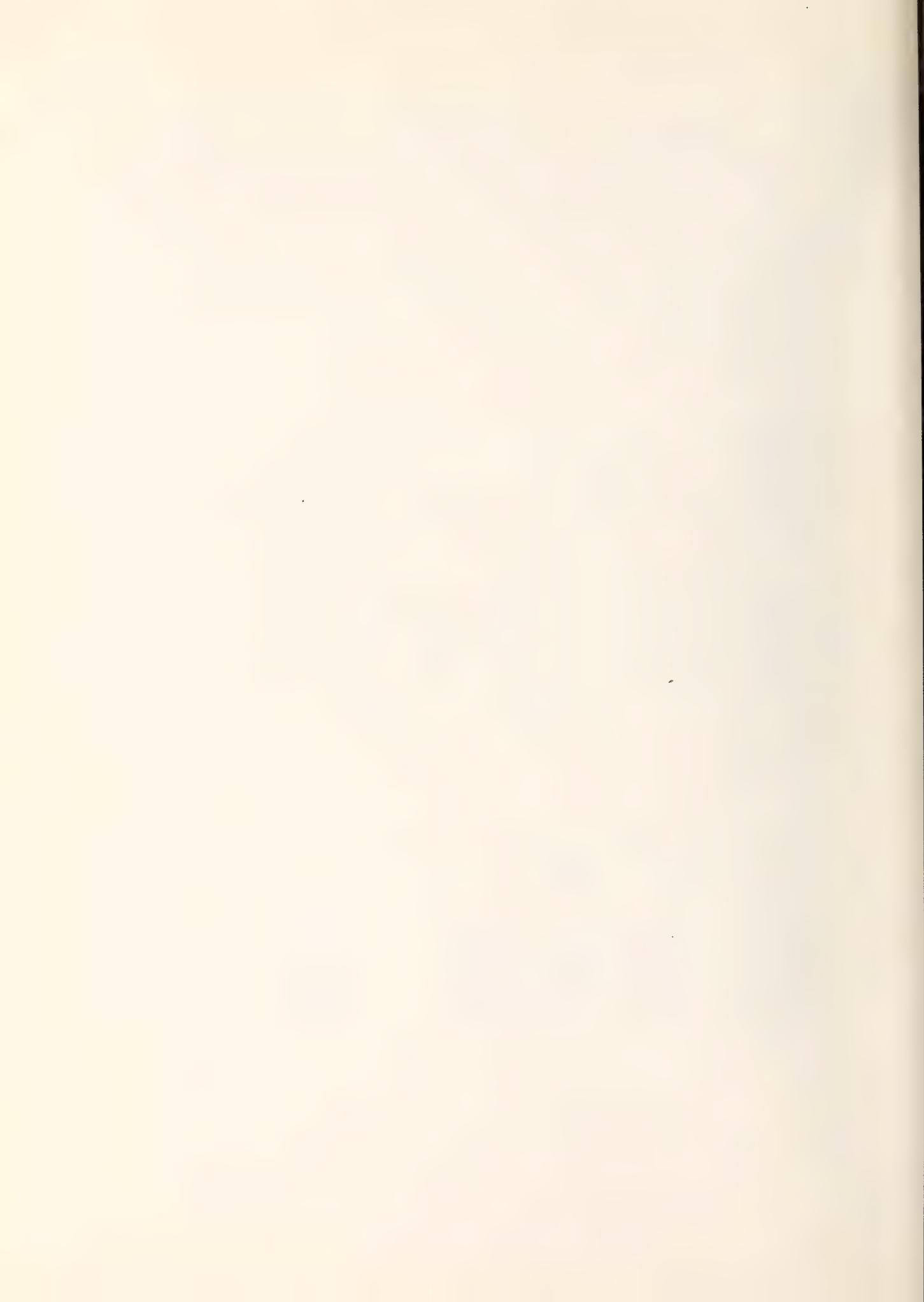
Figure 2



Figure 3



Figure 4



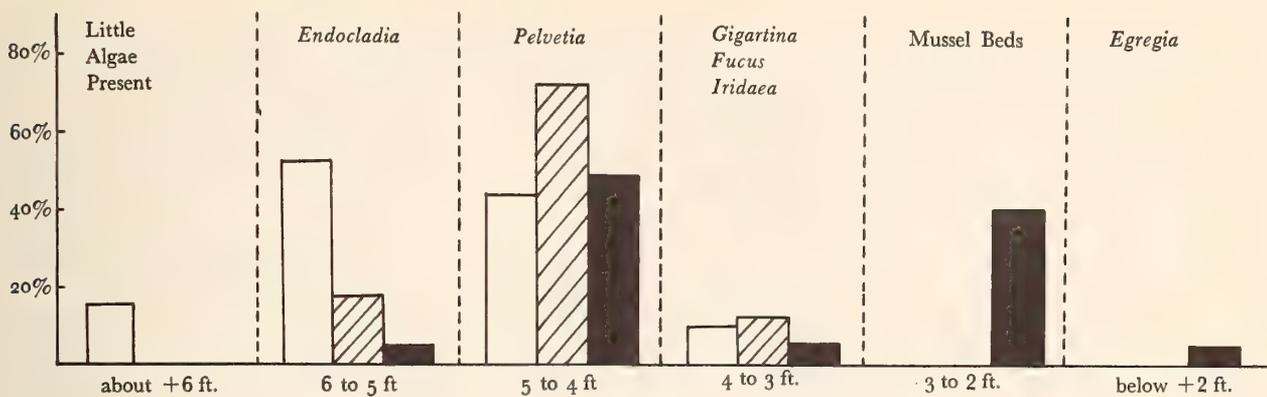


Figure 1

Percent distribution of the three morphologic types of *Acmaea pelta* (each type = 100%) as related to position and habitat in

the intertidal zone. Black *Acmaea pelta* (231 animals) shown in solid bars; brown (123 animals) in open bars; and green (168 animals) in cross hatched bars.

cal salivary glands, and hind gut was prepared from animals of the black and brown morphologic forms. The tissues were chilled on ice and homogenized in ice cold homogenizing medium. Each 1 ml of tissue was homogenized in a medium containing 1 ml of an aqueous streptomycin solution (5mg per ml) to inhibit bacterial growth, 1 ml of a saturated solution of ovomucoid isolated by the method of FREDRIGQ & DEUTSCH (1949), and 7 ml of buffer (0.2M Tris buffer at pH 7.2 and 0.2M acetate buffer at pH 5.5). Ovomucoid was used as a trypsin inhibitor because of problems encountered with proteinases in the enzyme extract. The ovomucoid increased activity approximately two-fold. One half ml of enzyme extract, one half ml of a 0.5% solution of purified polysaccharide and 1½ ml of buffer were incubated at 20° C for one hour. The amount of polysaccharide in a one ml aliquot was equivalent to approximately 200 micrograms of reducing sugar on complete hydrolysis. The colorimetric determinations were made with a Klett-Summerson photo-electric colorimeter using a green filter, and values were corrected for reducing sugar present in enzyme and substrate controls. The corrected values are expressed in terms of micrograms of glucose per hour per ml aliquot of reaction medium, and are equivalent to 0.02 ml of gut tissue.

## RESULTS

Marked amylase activity, particularly at pH 5.5, was demonstrated in extracts from large brown *Acmaea pelta* and the black form found on *Pelvetia*. Table 2 gives the quantitative results. Higher activity was observed in ex-

tracts from the brown animals. Low levels of fucoidinase and alginase were consistently demonstrable at pH 7.2. No enzymatic hydrolysis of K-carrageenin, laminarin, or agar was observed at either pH.

Table 2

Enzyme activity expressed in micrograms of reduced sugar per 0.02 ml of gut tissue. Any value less than 2 micrograms is considered inconclusive.

Polysaccharide	Black <i>Acmaea pelta</i> ( <i>Pelvetia</i> )		Brown <i>Acmaea pelta</i>	
	pH 5.5	pH 7.2	pH 5.5	pH 7.2
Starch	52µg	24µg	112µg	50µg
Agar	0	1µg	0	1µg
K-carrageenin	0	0	0	0
Laminarin	0	0	0	0
Fucoidin	3µg	4µg	0	5µg
Alginic Acid	0	8µg	0	5µg

## DISCUSSION

The field work demonstrated a difference in shell shape of the *Acmaea pelta* found in different habitats in the intertidal. It appears that shell growth and the resulting shell shape is probably a response to the substrate upon which the animal lives. The high peaked convex shell of the black variety found in the mussel beds may be an adaptation to attachment to a substrate such as is presented in the mussel beds where large flat surfaces are at a minimum. The flatter *A. pelta* under *Pelvetia* have an excess of flat but rough rock surface to grow and move

on. The wavy shell periphery is an indication of an adaptation to this environment. This seems to be a reaction to the substrate similar to but not as extreme as that of the limpet *A. scabra* whose shell grows to conform to irregularities of the home site (HEWATT, 1940). *Acmaea pelta* is characteristically a high, almost center peaked limpet. This is true when exposed and of reasonable size, as in the brown form and the larger specimens from the mussel beds. Since no large specimens were found in the mussel beds and small ones were abundant, mortality may increase with age, the larger limpets being unable to survive on this substrate. Substrate and exposure therefore appear to be large factors in determining shell morphology.

The reason for the different colors seen in *Acmaea pelta* is not clear, but a possible correlation with different algal food is plausible. The reported experiments do not seem to indicate that digestive potential is the critical factor. They also show that alginic acid and fucoidin could be utilized only slowly in comparison with utilization of starch. Although extracts prepared from brown forms displayed more activity than those obtained from black ones, this difference may be simply related to the ease of dissecting and the resulting greater purity of the extracts prepared from the larger brown limpets.

Although not related to digestive potential, the differences in color of peak and peripheral portions of shells suggest possible changes in diet during the life of the animal. Long term experiments will be required to explore this relationship.

### SUMMARY

Populations of *Acmaea pelta* ESCHSCHOLTZ, 1833 exhibit variation in shell shape and color. Field studies showed correlations of these variables with algal association, substrate, and exposure. Shell shape seems to be dependent upon substrate and exposure. Shell color may be related to diet, but preliminary investigations of gut carbohy-

drases failed to reveal any distinctive quantitative differences in the amylase, fucoidinase, and alginase activities found in the *A. pelta* forms assayed.

### ACKNOWLEDGMENTS

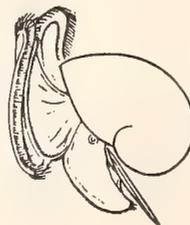
This work was made possible by Grant GY806 from the Undergraduate Research Participation Program of the National Science Foundation.

Thanks to Dr. J. Phillips, my advisor, who isolated and purified the polysaccharides, agar, K-carrageenin, laminarin, fucoidin, and alginic acid for the digestive enzyme assays.

Thanks to Miss Cathy Walker who helped in giving positive identification to the *Acmaea pelta* forms.

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# Anatomical and Oxygen Electrode Studies of Respiratory Surfaces and Respiration in *Acmaea*

BY

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(Plate 7; 6 Text figures; 1 Table)

THE LIMPETS OF THE GENUS *Acmaea* are found in abundance in the rocky intertidal zone on the shores of the Monterey Peninsula, California. Individuals of the species *Acmaea scabra* (GOULD, 1846) and *A. digitalis* ESCHSCHOLTZ, 1833, are found high in zones one and two described by RICKETTS & CALVIN (1962). Other species common to this area, *A. pelta* ESCHSCHOLTZ, 1833; *A. limatula* CARPENTER, 1864; *A. asmi* (MIDDENDORFF, 1849); and *A. scutum* ESCHSCHOLTZ, 1833, are found under water much of the time lower down in zones three and four.

These species differences in exposure suggested that *Acmaea* must be capable of differing degrees of respiration in air and water. The aim of this study was to determine the site(s) of the specialized respiratory surfaces of *Acmaea*, and to list their respiratory effectiveness when exposed to air and when submerged.

## I. ANATOMY

Initially the anatomy of the circulatory system was investigated. It was assumed that gas diffusion could occur across any body surface and that a specialized respiratory area might be one where gas exchange was facilitated by blood flow close to the external body surface.

Individuals of the species *Acmaea scutum*, *A. pelta*, and *A. digitalis* from low, middle, and high intertidal zones, respectively, were taken as samples. The principal method of study was injection of colored substances into the circulatory system.

Injections were carried out with 26-gauge hypodermic needles or fine glass needles pulled from soft glass tubing. A number of different injection fluids was tried, including latex, vital stains dissolved in alcohol or water, and

commercial inks. Best results were obtained with an aqueous colloidal suspension of carbon. The tissues remained relaxed during the carbon injections and the particles did not diffuse out of the vessels. Successful injections were made on both live, fresh animals and on animals relaxed in a solution of  $MgCl_2$  isotonic with sea water. Large areas of the circulatory system were colored by injections into the heart or visceral cavity. For study

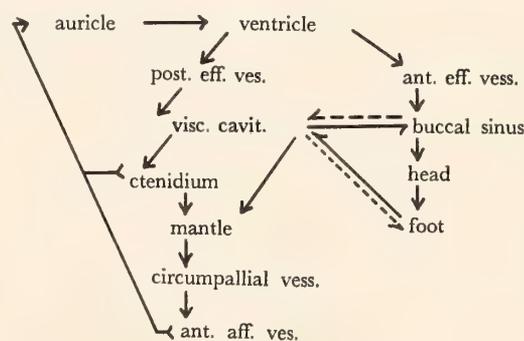


Figure 1

Circulatory system of *Acmaea*. Solid lines represent known relationships; broken lines represent supposed relationships

of localized areas, injections were made into local vessels. Blood flow direction was determined by observation of the vessels during injections of a dilute carbon suspension.

Gross blood flow, determined by the above methods, is shown in Figure 1. The results indicate two separate and distinct areas — the ctenidium and the mantle — where there is a large amount of blood flow close to the animals' external surface.

The first of these areas, the ctenidium, is generally considered the respiratory organ of *Acmaea*. Colloidal carbon injections of this organ colored the major efferent

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and afferent vessels and the capillaries of the right and left thirds of the ctenidial filaments, but not the central portions of the filaments. The filament vessels, however, were stained in their totality with vital dyes. These observations indicate that capillaries sufficiently fine to prevent the passage of colloidal carbon connect the efferent and afferent vessels.

The other area where a large amount of blood flow was found close to the surface was the ventral side of the mantle fold facing the mantle groove. Blood enters the mantle fold from the visceral cavity through vessels which pass through the shell muscle fibers. In the area between the base of the mantle groove and the circumpallial vessel these vessels anastomose. The density of interconnecting vessels in this network is extraordinary (see Plate 7, Figures 1 to 3). Near the circumpallial

vessel (Figure 2) the vessels of this network come together to form a highly branched pattern. Blood in these vessels goes to the edge of the mantle, servicing the glands and pallial tentacles, returns to the anterior afferent vessel, and thence to the heart. As in the ctenidium, there were fine capillaries in the mantle fold near the circumpallial vessel which could not be filled with suspended colloidal carbon, but which could be colored by dissolved stains.

The above observations show that the ctenidium and the mantle fold are two areas through which blood passes just before returning to the heart (Figure 1). Since, in most organisms, blood is oxygenated at the respiratory surfaces just prior to its return to the heart, these observations suggest that both the mantle fold and the ctenidium are respiratory surfaces.

The possible role of the mantle fold as a respiratory surface suggested that the ciliary mantle currents, earlier described in *Acmaea* by YONGE (1962) and believed to be cleansing currents, might also serve a respiratory role. To observe these currents, carmine particles suspended in sea water were placed in the mantle groove of overturned animals. A circular current in the mantle groove was found, moving in a plane perpendicular to the side

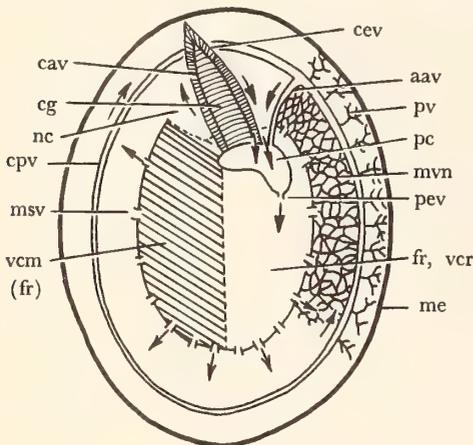


Figure 2

Ventral view of blood flow and respiratory surfaces in *Acmaea*  
 aav = anterior afferent vessel      cav = ctenidial afferent vessel  
 cev = ctenidial efferent vessel      cg = ctenidial gills  
 cpv = circumpallial vessel      fr = foot removed  
 me = mantle edge      msv = mantle supply vessel  
 mvn = mantle vessel network      nc = nuchal cavity  
 pev = posterior efferent vessel      pv = pallial vessels  
 vc = visceral cavity      vcr = visceral cavity removed

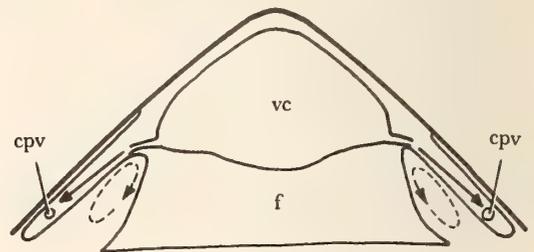


Figure 3

Respiratory ciliary current in the mantle groove.  
 Solid lines represent blood flow direction; broken lines represent ciliary current direction.  
 cpv = circumpallial vessel      f = foot      m = mantle  
 vc = visceral cavity

### Explanation of Plate 7

Figure 1: Colloidal Carbon Injection of Mantle (beginning injection)

1. glass needle
2. mantle supply vessel
3. side of foot
4. bottom of foot
5. edge of mantle

Figure 2: Colloidal Carbon Injection of Mantle (finished injection)

Respiratory vessel network is filled. Note the "standard" venation in the foot (1).

Figure 3: Roof of nuchal cavity partially injected.

Injection was made in mantle groove at left. Note network pattern of vessels (1), anterior afferent vessel (2), and head pinned back against foot (3).

Figure 4: Functional End of the Oxygen Cathode

1. recess filled with distilled water
2. glass cover
3. platinum wire

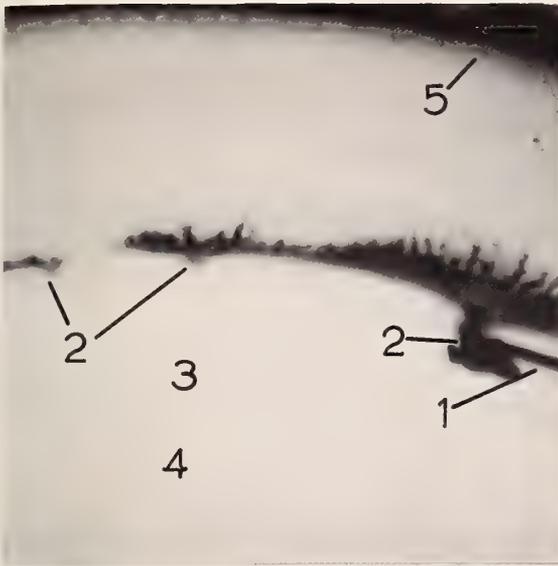


Figure 1

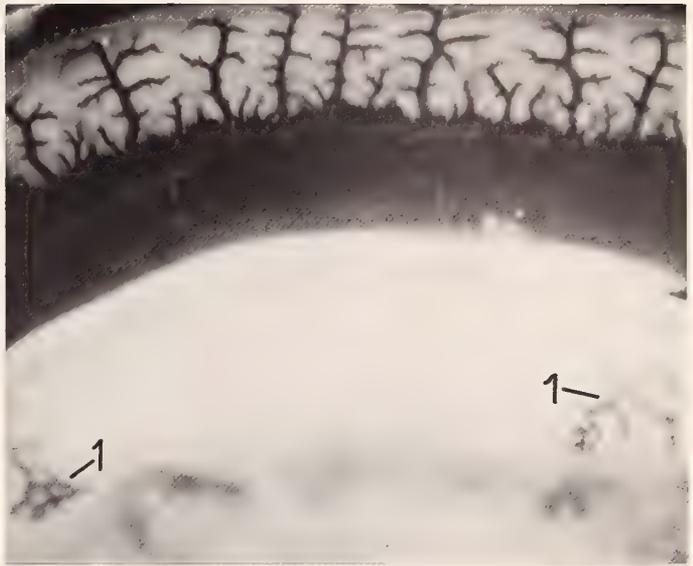


Figure 2

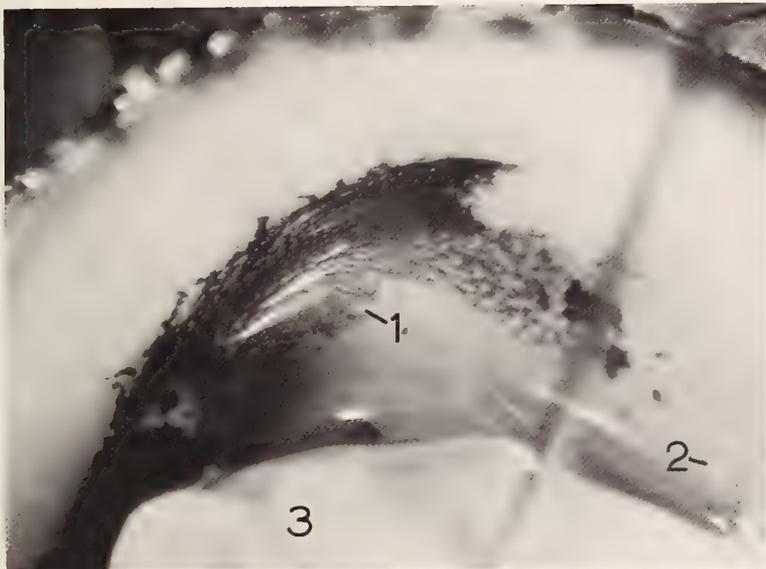


Figure 3

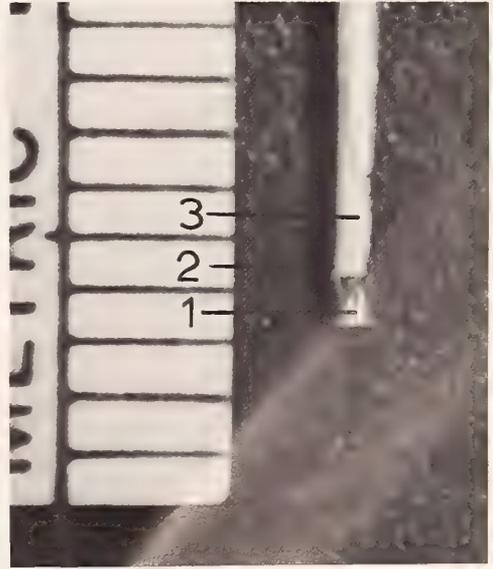


Figure 4



of the foot and to the animal's substratum. This ciliary current moved opposite to the direction of blood flow in the mantle and was a counter-current of the type often associated with respiratory surfaces (see Figure 3). A similar ciliary current was found in the nuchal cavity moving opposite to the direction of blood flow in the ctenidium. This mantle counter-current found in *Acmaea* is similar to that found in the mantle groove of *Lottia* (ABBOTT, 1956).

Because of the large amount of blood flow close to the surface, the type of venation, the position relative to the heart in terms of blood flow, and the ciliary counter-currents, both the ctenidium and the mantle are here suggested as respiratory surfaces in *Acmaea*.

## II. FIELD OBSERVATIONS

*Acmaea scabra* and *A. digitalis* from zones one and two, *A. pelta* and *A. limatula* from zone three, and *A. scutum* from zone four were observed in the field to record the behavior of the mantle and ctenidium under dry and wet conditions. The study was qualitative; observations were made of animals on dry rocks, on splashed rocks, and in quiet pools with the aid of a ten-power magnifying lens.

When an individual had been out of the water for a long enough period to have adjusted to the lack of water, the following characteristics were usually evident. The shell was pulled down onto the surface of the rock substrate (though not tightly clamped), the mantle fold was wet (as found in *Lottia* [ABBOTT, 1956]), and when the animal was disturbed it clamped down tightly with some water often exuding from the mantle groove. If the animal was taken from the rock and turned over, the wet area of the mantle fold between the foot and circum-pallial vessel was noticeably swollen, and the vessels appeared dilated and gorged with blood. The ctenidium was found to be withdrawn in the nuchal cavity, and in *Acmaea scabra* which had been dry for many hours and whose nuchal cavity was no longer filled with water, the ctenidium was hardly visible. The above characteristics were observed in members of all species but were particularly evident in *A. scabra* and *A. digitalis*.

When an *Acmaea* individual was under water, its shell was elevated one to three millimeters off the substrate, its mantle protruded somewhat beyond the edge of the shell, and a ciliary current in the nuchal cavity, demonstrated with carmine particles in a quiet pool, flowed counter to ctenidial blood flow. When an animal was overturned and compared with a member of the same species from a dry area, the wet animal's mantle appeared flatter and the mantle vessels seemed smaller in diameter.

In the laboratory, where the ventral side of submerged *Acmaea* could be observed through aquaria walls, the ctenidia of *A. scutum*, *A. pelta*, and *A. limatula* were consistently found extended and lying partially in the right mantle groove. In *A. scabra* and *A. digitalis* taken from high, dry areas and kept under water in aquaria for 48 or more hours, the ctenidia were never seen to extend more than one-half the distance from the back to the front of the nuchal cavity. They are, therefore, apparently not sufficiently long to extend to the mantle groove, and are much reduced in size compared to the ctenidia of *A. pelta*, *A. limatula*, and *A. scutum*.

These field studies indicate that the mantle is the site of increased blood flow when the animal is out of the water, whereas the ctenidium is usually the site of similar increased flow when the animal is under water. Under water, the elongated, filamentous ctenidium is a principal wetted surface. Out of water, however, the ctenidium contracts and the surface of the mantle fold is kept wet at the expense of water in the nuchal cavity, suggesting that the mantle fold is the chief respiratory site in dry environments. The laboratory and field observations show that ctenidial elongation and mantle swelling may be controlled by the water or air environment, and also suggest an evolutionary reduction in the ctenidium and a corresponding increase in mantle capacity from low to high intertidal species of *Acmaea*.

## III. POLAROGRAPHIC STUDIES

Polarographic methods were used to substantiate the respiratory functions of the ctenidium and mantle indicated in the previous studies. Electrodes of the recessed type first described by BRINK & DAVIES (1942) were used to measure the difference in the oxygen tensions in different parts of the body of *Acmaea*.

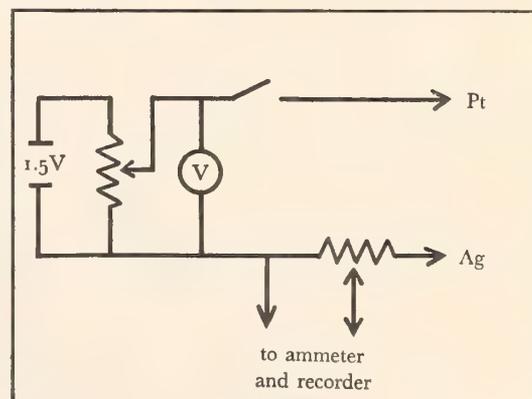


Figure 4

Circuit for oxygen electrode

Experimental apparatus consisted of several platinum cathodes, one silver-silver chloride anode, a 0.8 volt D. C. power source, a Keithley ammeter of  $10^{-9}$  ampere sensitivity, and a chart recorder. Cathodes were made by sealing 26 gauge (B&S) platinum wire in hand-pulled soft glass capillaries. The wire was first heated to white incandescence in a flame to drive off the surface-adhering gases, and the capillary then fused around it. Next the capillary was cut to extend one millimeter beyond the polished end of the platinum wire. The recess thus formed was filled with distilled water and covered with a collodion membrane to prevent the entry of proteinaceous material into the recess.

After construction each cathode was calibrated. First the interval required for equilibration of oxygen within and without the electrode's recess was determined by comparing successive readings from a constant environment. Next the optimum duration for voltage application was sought, this being the shortest interval yielding a linear amps. *vs.*  $O_2$ /relationship. By comparing the electrode output at different time intervals in sea water

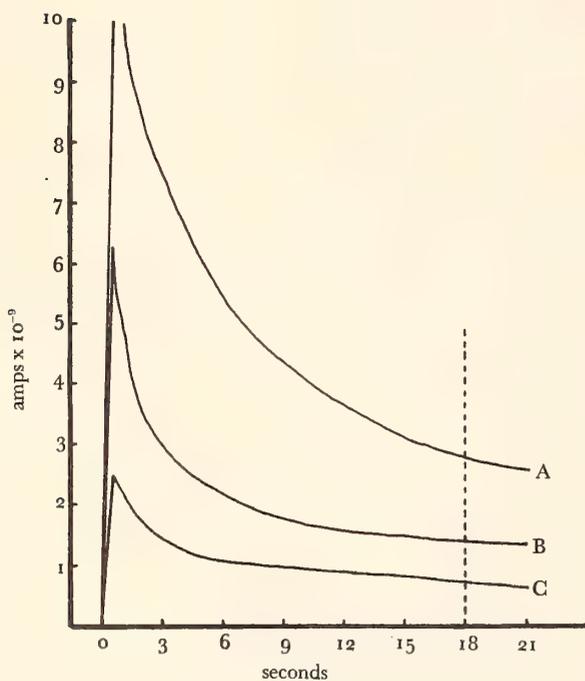


Figure 5

Recorder tracings at three different oxygen tensions.

A = 2 cc  $O_2$ /l; B = 1 cc  $O_2$ /l; C = 0.5 cc  $O_2$ /l.  
The dotted line at 18 seconds indicates the amperage value used for construction of the  $O_2$  calibration curve.

samples of different oxygen concentrations, the reading at 18 seconds was found to be the shortest time yielding such a linear relationship (Figures 5 and 6). In the final calibration step, an amps. *vs.*  $O_2$ /curve (Figure 6, line C) was determined for each cathode, using 3 to 5 sea water samples of known  $O_2$  content (by Winkler method). These cathodes were very stable, with little or no changes evident in the calibration curve from day to day.

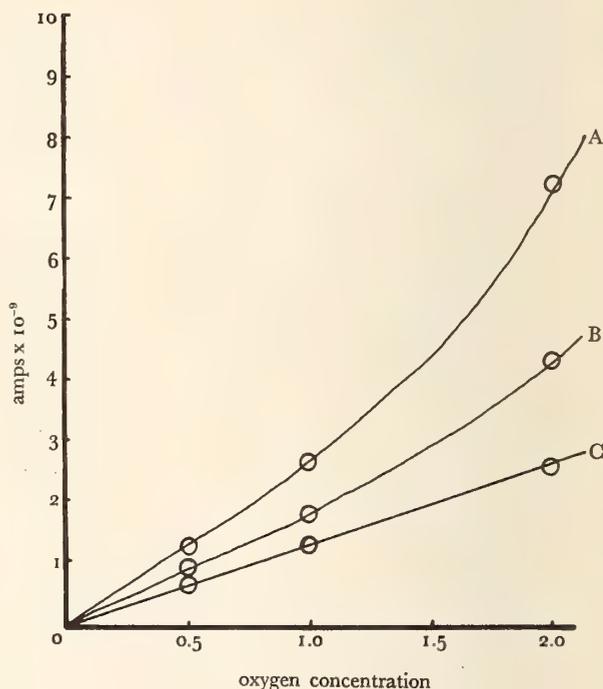


Figure 6

Output of the oxygen electrode at different  $O_2$  tensions and different times after closing of circuit.

A = 3 seconds; B = 9 seconds C = 18 seconds

All experiments and calibrations were performed at  $21^\circ C$ . To compensate for minor temperature fluctuations during an experiment (a one degree change resulted in as much as a 10% change in current flow), the  $O_2$  tension at 2 to 3 different sites on the animal were simultaneously measured with 2 to 3 different cathodes.

In each experiment the animal, previously kept in the desired environment for a minimum of 4 hours, was placed upside-down and the cathodes were inserted into the desired tissue or circulatory vessel, through holes previously made with a dissecting needle. The cathodes were left in place and supported in small ring stands, whereas

the anode was placed on the tissue only at the time of measurement.

In the first experiments, comparisons were made of blood oxygen in the visceral cavity, the anterior afferent vessel, and the pericardial sinus. The results (Table 1) show that the  $O_2$  tension is higher in the pericardial sinus in both aerobic and aquatic conditions. Comparing the visceral cavity with the anterior afferent vessel showed that the  $O_2$  tension was higher in the latter area. Because blood flows from the visceral cavity to the anterior afferent vessel through the vessels of the mantle fold, gaseous exchange must occur at the mantle-fold surface.

In the next group of experiments the ctenidial respiration was eliminated, and the respiratory efficiency of the mantle fold determined. Small lead clamps, cut from thin sheets of lead and bent in "V" shapes, were pinched around the ctenidial afferent and efferent vessels of animals relaxed in  $MgCl_2$ . The treated animals, kept overnight in either wet or dry environments, were tested during the following days and then sacrificed and examined to ascertain whether the clamps were still in place and functioning.

Of 20 animals kept under water, 19 were still alive one day after the clamping operation. Testing of 10 of these animals showed (Table 1) the  $O_2$  tension of the pericardial sinus to be somewhat higher than that of the visceral cavity, although the differences were less than in normal, unclamped animals.

The second day after the operation the remaining 9 limpets kept under water were dead. This experiment

was performed twice, and each time all the animals were dead by the second day.

All 20 animals with clamped ctenidia kept under dry conditions were still alive one day after the clamping operation. Testing of 10 of these animals showed the  $O_2$  tension of the pericardial sinus to be higher than that of the visceral cavity, this difference being greater than in submerged animals with clamped ctenidia, and about the same as in normal animals under dry conditions.

The second day after the clamping operation 2 of the 10 dry animals had died. Testing of the remaining 8 showed the  $O_2$  tension of the pericardial sinus to still be greater than that in the visceral cavity, although the absolute tension in both was slightly lower than the previous day.

## DISCUSSION

Four lines of evidence indicate that the mantle fold serves a respiratory role in the limpet *Acmaea*. These are (1) the presence of a capillary system close to the surface of the mantle fold, (2) a counter-current ciliary system passing over the mantle fold, (3) the dilation of the mantle fold with blood, and the concomitant decreased size of the ctenidia, when the animal is dry, and (4) the polarographic evidence that the  $O_2$  tension of the blood is higher after passage through the mantle fold, and before passage through the ctenidium.

These results demonstrate that better gaseous exchange occurs at the mantle surface in air than in water, and

Table 1

Mean Oxygen Tensions (cc/l)

Respiring surface	Conditions	<i>Acmaea</i> species	Trials	Visceral cavity oxygen tension		Pericardial sinus oxygen tension		Ant. afferent vessel oxygen tension	
ctenidium and mantle	dry	<i>A. scutum</i>	14	1.08 ± 0.39	12	1.67 ± 0.43	2	1.40 ± 0.28	
		<i>A. pelta</i>	4	0.55 ± 0.20	2	0.85 ± 0.07	2	1.60 ± 0.21	
		<i>A. limatula</i>	1	1.00	1	2.85			
ctenidium and mantle	wet	<i>A. pelta</i>	6	0.83 ± 0.44	6	1.67 ± 0.55	4	1.57 ± 0.54	
		<i>A. limatula</i>	2	1.35 ± 0.21	2	1.80 ± 0.14			
mantle only	dry	<i>A. pelta</i>	7	1.70 ± 0.36	7	2.24 ± 0.48			
		<i>A. limatula</i>	3	1.50 ± 0.28	3	2.20 ± 0.28			
mantle only	dry	<i>A. pelta</i>	3	1.37 ± 0.03	3	1.73 ± 0.02	3	1.80 ± 0.13	
		<i>A. scabra</i>	3	1.23 ± 0.13	3	1.60 ± 0.10			
		<i>A. digitalis</i>	2	1.60 ± 0.14	2	2.05 ± 0.21	1	2.2	
mantle only	wet	<i>A. pelta</i>	8	2.23 ± 0.93	8	2.50 ± 0.95			
		<i>A. limatula</i>	2	1.60 ± 0.70	2	1.80 ± 0.80			

that *Acmaea* has physiological and behavioral adaptations which allow it to better expose the mantle in air and the ctenidium in water.

That *Acmaea* uses both ctenidium and mantle fold as respiratory organs is evolutionarily interesting, pointing to similarities with the related limpets *Lottia gigantea* SOWERBY, 1843, which respire with ctenidium and pallial gills (ABBOTT, 1956), and *Patella*, which has no ctenidium and respire solely with ciliated flaps fringing the margin of the circumpallial vessel (YONGE, 1962).

### SUMMARY

The circulatory system of *Acmaea* was injected with colloidal carbon. Two areas — the ctenidium and the mantle — were found where a large amount of blood flows close to the animals' external surfaces. Blood flows through one or the other of these surfaces immediately before it returns to the heart. A ciliary counter-current was found associated with each of these surfaces.

When observed in the field, the mantle fold was found to expand and the ctenidium to contract when the animal was out of water. Conversely, the ctenidium elongates and the mantle fold flattens under water. Low intertidal species of *Acmaea* have larger ctenidia and smaller mantle respiratory capacities than higher intertidal species.

Oxygen polarography was used to measure the oxygen tensions of the blood in different parts of *Acmaea*. These

measurements indicate that both the mantle and ctenidium are respiratory surfaces, and that the mantle is more effective in aerial conditions and the ctenidium more effective in submerged conditions.

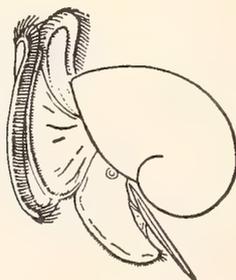
### ACKNOWLEDGMENTS

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# Manometric Measurements of Respiratory Activity in *Acmaea digitalis* and *Acmaea scabra*

BY

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(3 Text figures; 2 Tables)

BOTH *Acmaea digitalis* ESCHSCHOLTZ, 1833 and *Acmaea scabra* (GOULD, 1848) spend a considerable proportion of the day exposed to the desiccating elements of the environment. Several field studies have shown that, although the two species are always found in close proximity above about the +5 foot level of the intertidal, *A. scabra* is usually the only species found on exposed horizontal rock surfaces (HAVEN, 1964). This difference in exposure suggests possible physiological differences between the two that might be revealed by respiratory studies. To determine such differences, laboratory measurements of oxygen consumption were made under varying conditions of temperature and dehydration. The results showed a significant difference in metabolic rate between the two species, as well as differences in submerged and aerial respiration rates.

## METHODS

Respiration rate was measured with Warburg-Barcroft manometers in a refrigerated water bath. Carbon dioxide was absorbed in the vessel side arm using 0.3 ml of a 30% KOH solution, with a wick of starch-free Whatman no. 40 filter paper. The vessel constants for each run were determined for the conditions of the experiment, the variables being the experimental temperature, volume of flask content, and the volume of the flask plus manometer arm. The latter was determined by Umbreit's method of calibration with water (UMBREIT, 1945), and the appropriate vessel constants then found from Dixon's nomogram (DIXON, 1951). During each run the vessels were agitated at the rate of approximately 60 to 70 oscillations per minute. The experiments were done during April and May, 1966.

The organisms used were medium sized *Acmaea scabra* and *A. digitalis* collected about 1 hour before low tide from the +4 to +6 foot region of the intertidal zone near Hopkins Marine Station. They were then transferred to aquaria supplied with running seawater at 15°C where they were allowed to equilibrate for at least 24 hours before being tested.

Submerged and damp runs were made with animals temperature-equilibrated for 3 hours before the experiment in a finger bowl in the water bath. Animals used for determining submerged respiration rates were then placed in the Warburg vessels and covered with 6 ml of millipore filtered seawater. Animals used for determining damp respiration rates were shaken to remove excess filtered sea water and then placed in dry flasks. Animals used in aerial runs (no water included in Warburg vessel) were placed foot down on a stack of 6 paper towels for 3½ hours at room temperature (approximately 22°C) before being placed in the dry vessel. With this procedure they were still able to attach to the sides of the vessels.

For each experimental variable, animals were collected under the same conditions and subjected to the same treatment in order to minimize variables. Five separate vessels were used containing 3 limpets per vessel. Each run was repeated a minimum of 3 times using different animals so that each rate is an average of approximately 45 similar-sized animals taken from the same intertidal area.

All respiratory measurements reported were made in the dark, since preliminary runs measuring aerial respiration of *Acmaea scabra* showed that photosynthesis of algal growths on the animals' shells was producing significant amounts of oxygen. Measurements of respiration of empty limpet shells in the dark showed that the volume of oxygen consumed was about 0.05% of the total amount used by limpets during a 2 hour run. This

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negligible amount was therefore not taken into consideration in the final rate calculations.

In all experiments shell sizes of animals used were 14 to 17 mm in length. Dry weights were determined by removing the shell and drying the animal to constant weight. Rates are expressed in  $\mu\text{l O}_2$  per mg dry weight.

### RESULTS

Respiration rates of the two species as related to the state of their environment are shown in Figure 1 and Table 1. This depicts average oxygen consumption at 30 minute intervals during the 2 hour measuring period. It is seen that the damp and aerial respiration rate of *Acmaea*

*scabra* is considerably less than the submerged rate, the submerged rate being 2.25 times greater than the damp rate, and 4.36 times greater than the aerial rate. By contrast, it is apparent that the respiration rate of *A. digitalis* is not so markedly affected by these conditions, the submerged rate being only 1.12 times greater than the damp rate and 1.42 times greater than the aerial rate. It is also seen that although the rate of submerged *A. scabra* is slightly greater than the submerged *A. digitalis* rate, the damp and aerial rates of *A. digitalis* are greater than the damp and aerial rates of *A. scabra*.

Figures 2 and 3 and Table 2 depict respiration rates of submerged *Acmaea scabra* and *A. digitalis* as a function of temperature. In these experiments, the same group of animals was used for the different temperatures, and

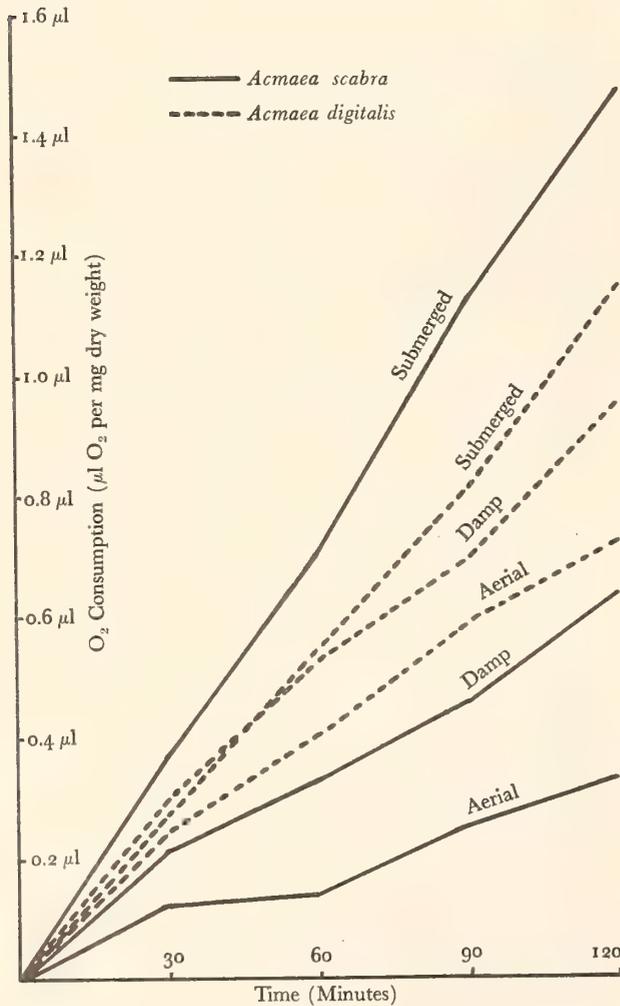


Figure 1  
Oxygen consumption of *Acmaea digitalis* and *Acmaea scabra* at different degrees of hydration

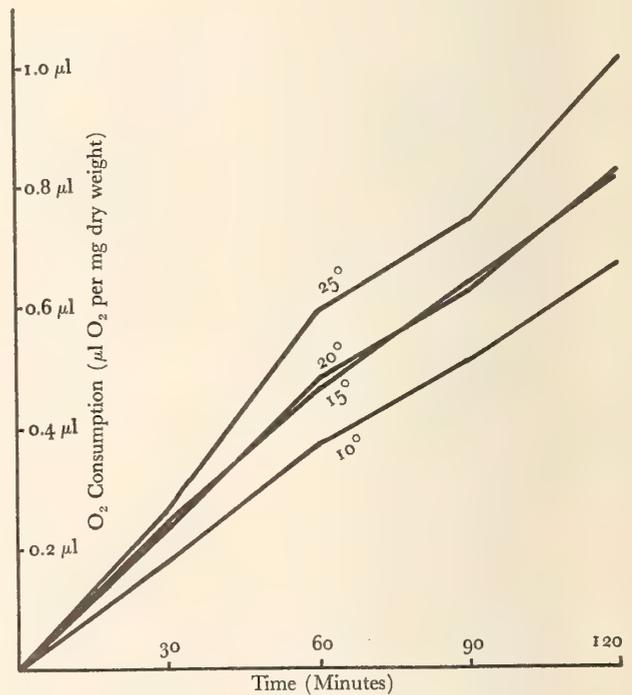


Figure 2  
Oxygen consumption of *Acmaea scabra* at different temperatures

they were equilibrated for 3 hours at the experimental temperature before being placed in the Warburg vessels. In *A. scabra* (Figure 2), it is seen that although respiration rate generally increases with temperature, it is not very pronounced, the initial rate increasing only about 60% in going from 10°C to 25°C. It is also seen that in *A. digitalis* (Figure 3), the respiratory rate is again relatively insensitive to temperature, and between 20°C and 25°C it actually drops to a rate less than was found at

Table 1

Oxygen consumption at different degrees of hydration

Time (Minutes) :		30	60	90	120
<i>Acmaea digitalis</i>	Aerial	0.241±0.019	0.404±0.024	0.587±0.042	0.728±0.071
<i>Acmaea digitalis</i>	Damp	0.297±0.056	0.538±0.097	0.701±0.155	0.955±0.205
<i>Acmaea digitalis</i>	Submerged	0.280±0.033	0.544±0.049	0.818±0.145	1.148±0.155
<i>Acmaea scabra</i>	Aerial	0.127±0.092	0.142±0.091	0.258±0.088	0.335±0.039
<i>Acmaea scabra</i>	Damp	0.216±0.034	0.334±0.048	0.466±0.089	0.644±0.121
<i>Acmaea scabra</i>	Submerged	0.380±0.081	0.712±0.136	1.132±0.226	1.476±0.314

Values are  $\mu\text{l O}_2$  consumed per mg dry weight and each is an average of approximately 45 animals

10°C. This same drop of rate between 20°C and 25°C was observed whether the first measurements were made at 10°, then at 15°C, etc., or whether the first measurements were made at 25°, then at 20°C, etc.

## DISCUSSION

### Respiration Under Varying Exposure to Air

The data indicate that in both species the maximum rate of respiration occurs under submerged conditions and the minimum rate under aerial conditions. In both species the rate during damp conditions falls between that of submerged and aerial.

*Acmaea scabra* showed a much lower rate of respiration than *A. digitalis* under damp and aerial conditions, a finding which suggests a possible mechanism for conservation of food reserves while out of water. This also fits in with WHITE's (1968) observations on glycogen content of high and low forms of *A. scabra*.

All animals of both species were chosen with a similar shell size, but the mean weight (without shell) of *Acmaea scabra* was later found to be 23 mg as compared to 59 mg for *A. digitalis*. This could account for the finding that

*A. scabra* had a higher submerged rate than *A. digitalis* in that it may be a reflection of a higher surface to volume ratio for the former, rather than a higher metabolic rate.

### Respiration Under Varying Conditions of Temperature

The increased respiratory rate at higher temperatures was not as great as would be expected on thermodynamic grounds. The reason for this is not known. The marked drop in submerged rate of *Acmaea digitalis* between 20° and 25°C is interesting, since *A. digitalis* has a lethal temperature during prolonged exposure of around 32°C (HARDIN, 1968). It is possible, therefore, that the decreased respiratory rate is related to physiological derangements leading to death. As no such decrease was observed in *A. scabra*, it would appear that it is better suited to withstand elevated temperatures than is *A. digitalis*. This finding also agrees with the field observations on distribution, and the lower tolerance to high temperatures of *A. scabra* as compared to *A. digitalis* (HARDIN, 1968). SOUTHWARD (1958), in a study on intertidal animals, found that during exposure to increasing temperature, the animal's activity was the first and

Table 2

Oxygen consumption at different temperatures

Time (Minutes) :		30	60	90	120
<i>Acmaea digitalis</i>	10°C	0.215±0.060	0.407±0.079	0.583±0.106	0.765±0.136
<i>Acmaea digitalis</i>	15°C	0.217±0.061	0.450±0.048	0.675±0.062	0.919±0.100
<i>Acmaea digitalis</i>	20°C	0.259±0.088	0.511±0.133	0.711±0.145	0.942±0.171
<i>Acmaea digitalis</i>	25°C	0.161±0.050	0.352±0.081	0.535±0.121	0.767±0.235
<i>Acmaea scabra</i>	10°C	0.187±0.003	0.371±0.001	0.510±0.009	0.672±0.012
<i>Acmaea scabra</i>	15°C	0.243±0.020	0.480±0.048	0.632±0.061	0.830±0.059
<i>Acmaea scabra</i>	20°C	0.258±0.025	0.475±0.046	0.639±0.052	0.829±0.063
<i>Acmaea scabra</i>	25°C	0.265±0.063	0.593±0.131	0.743±0.081	1.094±0.097

Values are  $\mu\text{l O}_2$  consumed per mg dry weight and each is an average of approximately 45 animals

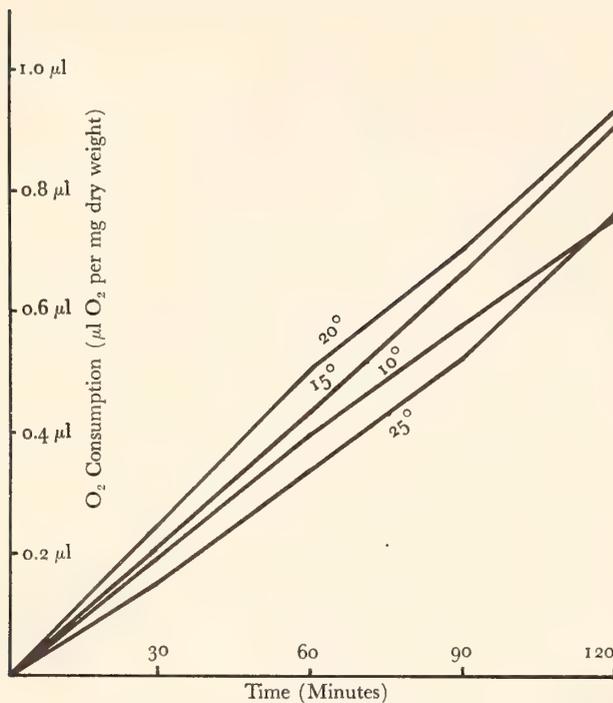


Figure 3

Oxygen consumption of *Acmaea digitalis* at different temperatures

most sensitive body function to be affected. This would be reflected in a decreased respiration rate as the critical mortality temperature was approached. Further measurements at closer increments from 15° through 30° should be made in order to determine the exact point of rate decrease and the maximum tolerable temperatures for both of these species.

In these limited laboratory observations it was impossible to take into consideration daily and tidal rhythms in respiratory rates that almost certainly were present (SANDEEN, STEPHENS & BROWN, 1958). Other uncontrolled variables that might have influenced the observed results are body weight and nutritional state of the animals, since the intervals between last feeding and respiratory measurements were unknown.

### SUMMARY

Respiration in two species of limpets, *Acmaea digitalis* ESCHSCHOLTZ, 1833 and *A. scabra* (GOULD, 1848), was studied under varying conditions by means of Warburg

manometers. In both species it was found that the maximum respiration rate occurs when the animal is submerged, and the least occurs when it is exposed to air. Under damp and aerial conditions *A. scabra* showed a much lower rate of respiration than did *A. digitalis*. Under conditions of increasing temperature from 10°C to 25°C, *A. scabra* increased its respiratory rate approximately 60%. *Acmaea digitalis*' rate increased 23% from 10°C to 20°C but at 25° its rate decreased to less than that at 10°C.

### ACKNOWLEDGMENTS

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# A Comparative Study of Lethal Temperatures in the Limpets *Acmaea scabra* and *Acmaea digitalis*

BY

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(4 Text figures; 1 Table)

## INTRODUCTION

THE IMPORTANCE OF TEMPERATURE as a limiting factor for the geographical distribution of intertidal animals is generally recognized (MOORE, 1958). Many studies have been conducted in an effort to correlate the distribution of some intertidal organisms with either air or sea temperature, among them those of HUTCHINS (1947), SOUTHWARD (1950), and SOUTHWARD & CRISP (1954). SEGAL (1956, 1961, 1962) has indicated that there is a difference between the highest and lowest intertidal members of *Acmaea limatula* CARPENTER, 1864 with respect to such body functions as heart rate and oxygen consumption. Intraspecific differences in these processes have also been detected in animals from different latitudes. SEGAL has further demonstrated that these differences are a possible effect of temperature and that acclimation of these processes to different tidal levels takes place.

Studies have also been conducted to determine the lethal temperatures of many intertidal animals (MAYER, 1918; GOWANLACH & HAYES, 1926; BROEKHUYSEN, 1940). However, these investigators have not dealt satisfactorily with intraspecific variation or niche difference. For instance, there may be a difference between lethal temperatures of members of a single species from the extreme boundaries of its vertical intertidal distribution at a single latitude. There may also be differences between different species which are found at the same intertidal levels.

This study was undertaken in an attempt to answer three main questions: 1) Is there a difference in lethal temperatures of animals of the same species and body size taken from different tidal levels? 2) Is there a dif-

ference between lethal temperatures of two species occupying the same intertidal levels? 3) How does an organism's lethal temperature relate to the temperature of its microhabitat?

The organisms used in this study, the limpets *Acmaea digitalis* ESCHSCHOLTZ 1833, and *A. scabra* (GOULD, 1846), are ideal for answering these questions, since they occupy the same vertical range in the intertidal zone (+2 to +10 feet) along the central California coast (TEST, 1945).

## MATERIALS AND METHODS

The members of each species were collected from areas visibly dominated by one or the other (at Point Pinos and Mussel Point on the Monterey Peninsula) in order that the results gained by laboratory experiments would be as representative as possible of the normal population.

For the purposes of testing intraspecific differences in lethal temperatures with respect to differences in intertidal location, animals were taken from the extreme upper (above +7 feet) and lower (below +4.5 feet) limits of the species in that area, and from a region midway between. Collected animals were placed in running sea water at approximately 15°C in the laboratory and used within 24 hours.

Two types of laboratory experiments were conducted to determine lethal temperatures. The first type was run with the animals submerged. High, mid, and low members of each species were placed in continuously aerated beakers of sea water in a water bath and allowed to equilibrate to constant temperature. The temperature was then raised at a rate of 1°C per 5 minutes, to allow for complete equilibration of the internal and external temperatures of the animals (SOUTHWARD, 1958). Fifteen high, mid, and low members of each species were

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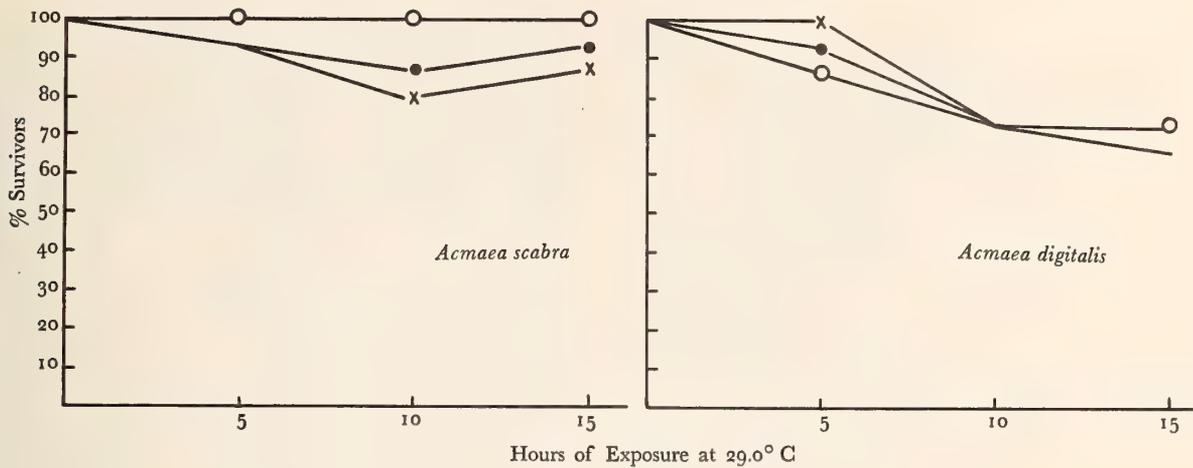


Figure 2

Prolonged Exposure to 29° C Air Temperature  
 ○ = high intertidal animals; ● = mid-intertidal animals;  
 x = low intertidal animals

To check any possible correlations between size and survival, shell dimensions of all animals used in the submerged temperature experiments were determined with vernier calipers readable to 0.1 mm. Twenty individuals from each group were randomly chosen (by random selection of pieces of paper containing data on each individual), and the respective size distributions are shown in Table 1. The data show a marked tendency for the low intertidal group to be smaller than the high intertidal group.

The mean temperatures for 10 randomly selected limpets are represented in each graph of Figure 4. *Acmaea scabra* consistently exhibited higher microhabitat temperatures than did *A. digitalis* at similar air temperatures. It can also be seen that in all cases the actual temperature of the limpet (mantle cavity temperature) is higher than the microhabitat temperatures. No significant temperature differences of any of the measured variables were found between the upper and lower areas (the temperatures were taken from +3 to +7 feet).

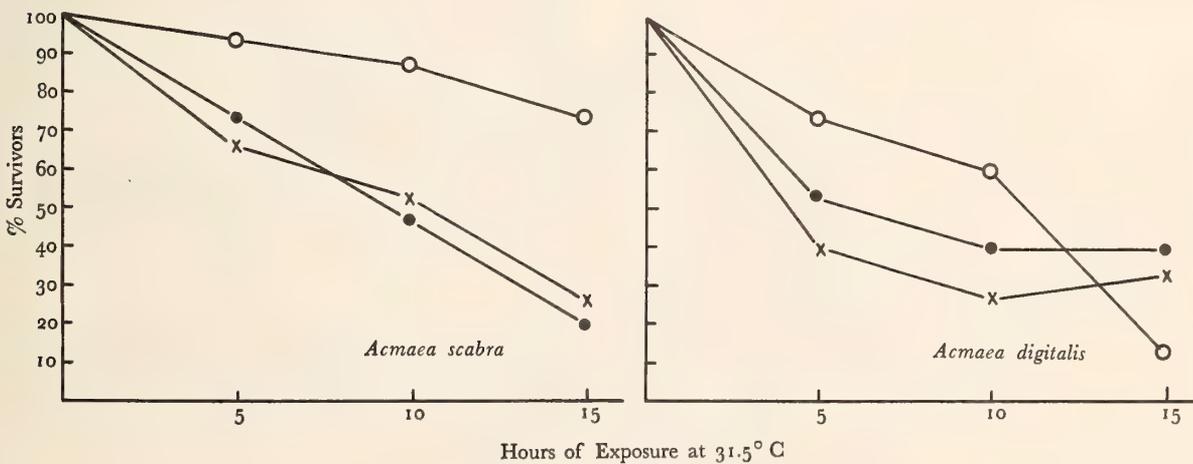


Figure 3

Prolonged Exposure to 31.5° C Air Temperature  
 ○ = high intertidal animals; ● = mid-intertidal animals;  
 x = low intertidal animals

Table 1

Mean Shell Dimensions for 20 Randomly Selected High, Mid, and Low Intertidal Members of Each Species.

All measurements are in centimeters, and the standard deviation is indicated in parentheses.

Microhabitat and Size of Limpets			
	Length	Width	Height
<i>Acmaea scabra</i>			
High	1.35 ( $\pm 0.26$ )	1.05 ( $\pm 0.21$ )	0.47 ( $\pm 0.12$ )
Mid	1.34 ( $\pm 0.17$ )	1.03 ( $\pm 0.15$ )	0.45 ( $\pm 0.10$ )
Low	1.27 ( $\pm 0.19$ )	0.98 ( $\pm 0.16$ )	0.36 ( $\pm 0.05$ )
<i>Acmaea digitalis</i>			
High	1.46 ( $\pm 0.19$ )	1.11 ( $\pm 0.15$ )	0.51 ( $\pm 0.10$ )
Mid	1.37 ( $\pm 0.19$ )	1.03 ( $\pm 0.18$ )	0.45 ( $\pm 0.10$ )
Low	1.20 ( $\pm 0.17$ )	0.95 ( $\pm 0.15$ )	0.40 ( $\pm 0.05$ )

## DISCUSSION

In all experiments *Acmaea scabra* survived high temperatures, in both air and water, better than did *A. digitalis*. These differences between the two species correlate with the higher mean microhabitat temperatures which were found. HAVEN (1964) has observed that *A. scabra* is seen in greatest abundance on surfaces which are more horizontal than the areas of highest *A. digitalis* concentration. This also correlates well with the observed lethal

temperatures, since *A. scabra* would therefore receive more and stronger sunlight than would *A. digitalis*.

The observed intraspecific differences in ability to survive high temperatures possibly result from temperature acclimation. Thus, even though the high and low members within each species experience much the same temperatures, the higher members would experience any high temperatures for longer periods of time than their lower counterparts. An alternative explanation, not involving temperature acclimation, is that the greater resistance of the higher forms is a consequence of their greater size or age or both. This is suggested by the results in Table 1, showing a continuum in size, with higher animals having larger shells than lower animals, and the work of FRANK (1965), which showed that *Acmaea digitalis* move higher in the intertidal zone with age. However, these results do not establish a causal relationship between size and ability to withstand high temperatures, and further research is planned.

The intraspecific differences in ability to survive high temperatures were much less clear in the prolonged temperature trials. This was especially evident with *Acmaea digitalis*, and can possibly be traced to the fact that, even though the *A. digitalis* population presents a size continuum (with the smallest animals lowest in the intertidal zone and the largest animals highest), this species is probably more mobile than *A. scabra*. This results in

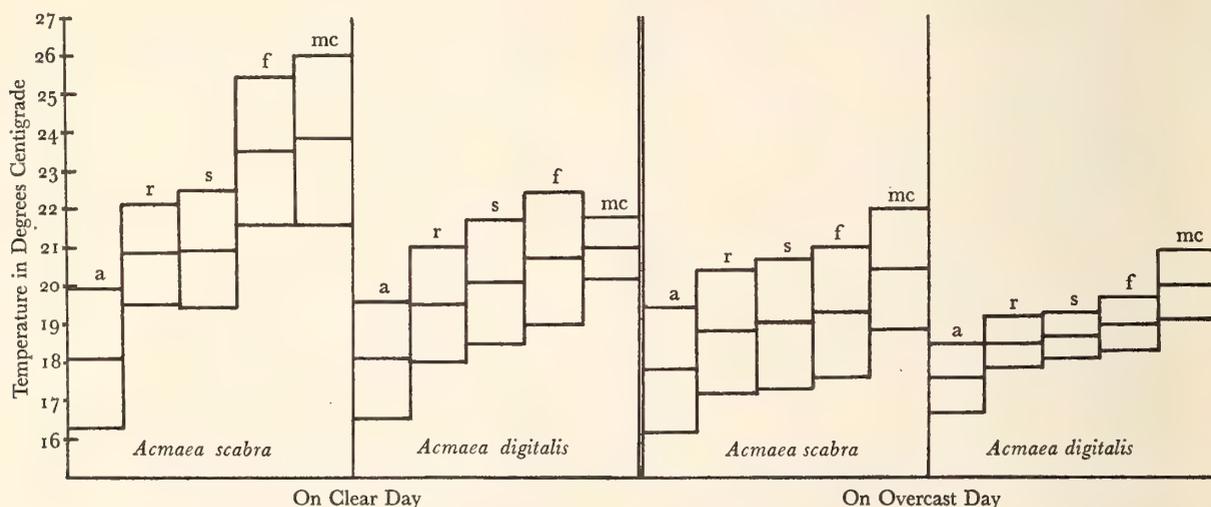


Figure 4

### Microhabitat and Body Temperatures

The midline of each bar indicates the mean of 10 measurements, and the portion of each bar above and below the midline indicates the standard deviation.

a = air; r = rock; s = shell; f = foot; mc = mantle cavity

part from the greater percentage of homing behavior found in *A. scabra* than in *A. digitalis* (HAVEN, 1964; JESSEE, 1968; MILLER, 1968). It can be expected, therefore, that the effects of acclimation would be more clearly defined in a population of animals which remain in rather fixed positions. This agrees with the greater intraspecific differences in survival at high temperatures seen in *A. scabra*.

### SUMMARY

Lethal temperatures of the limpets *Acmaea scabra* and *A. digitalis* were studied. *Acmaea scabra* was found to survive high temperatures better than *A. digitalis*. These results correlate with field studies which showed that *A. scabra* experiences higher microhabitat and body temperatures than does *A. digitalis* at similar temperatures. It was also found that the internal temperatures of limpets are consistently above the external surrounding temperatures.

Members of the species coming from the highest intertidal ranges of the species were found to survive high temperatures better than members from the lowest intertidal ranges of the species. These intraspecific differences may be a result of acclimation to the length of exposure to high environmental temperatures. There are indications that the size or age, or both of these variables, may have an effect on the ability to survive high temperatures.

### ACKNOWLEDGMENTS

This work was made possible by Grant GY806 from the Undergraduate Research Participation Program of the National Science Foundation. My sincerest thanks are given to the faculty and staff of Hopkins Marine Station of Stanford University for allowing me the opportunity to do this study, and especially to Dr. David Epel who advised and directed me in my research. I am also gratefully indebted to Dr. A. Todd Newberry of Cowell College at the University of California, Santa Cruz, without whose encouragement and advice this work would not have been possible.

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# Studies on the Jaw, Digestive System, and Coelomic Derivatives in Representatives of the Genus *Acmaea*

BY

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(13 Text figures; 1 Table)

PREVIOUS AND CONTEMPORARY WORKERS have noted some dietary differences between California species of *Acmaea* (e. g., TEST, 1945, 1946; CRAIG, 1968; EATON, 1968), and have noted some species differences in the radula and radula strap (e. g., TEST, 1945, 1946; FRITCHMAN, 1960, 1961), which also may be related to diet. No similar attempts have been made to compare the digestive tracts or jaws of California limpets, though these too might be expected to show some variation possibly related to food habits. In the present work, the gross morphology of the gut has been compared in the following species: *A. asmi* (MIDDENDORFF, 1849); *A. digitalis* ESCHSCHOLTZ, 1833; *A. limatula* CARPENTER, 1864; *A. pelta* ESCHSCHOLTZ, 1833; *A. scabra* (GOULD, 1846) *A. scutum* ESCHSCHOLTZ, 1833. The jaws have been studied in these six species and also in *A. insessa* (HINDS, 1842); *A. paradigmatis* FRITCHMAN, 1960; and *Lottia gigantea* SOWERBY, 1843. Additional observations have been made on the development of the digestive tract in very young *Acmaea*, and on the interconnection of the coelomic cavities in the 6 species.

## METHODS

All specimens examined were collected on Mussel Point, near the Hopkins Marine Station, Pacific Grove, California, and at Point Piños and Point Joe nearby on the Monterey Peninsula, during April and May, 1966. Dissections were made on animals killed in 95% alcohol. Since this type of preservation, making tissues more pliable, introduced distortion of certain structures, comparisons were made with fresh material also. Injection of Ward's latex suspension was used to determine placement of gland ducts and intestinal loops. Starvation of the animals for 3 to 5 days before injection proved helpful in clearing

the digestive tract, though injection of the entire tract was never successful.

## GENERAL DESCRIPTION OF THE DIGESTIVE SYSTEM

The gross anatomy of the digestive tract of the *Acmaea* species studied (Figures 1 and 2) is similar to that of *A.*

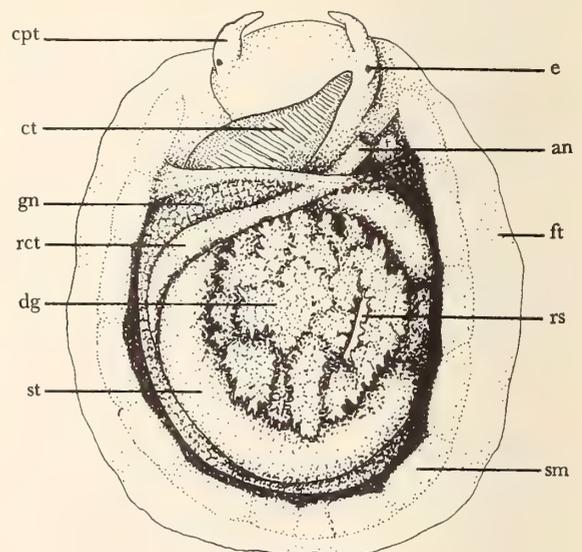


Figure 1

Generalized dorsal view of an *Acmaea*  
with shell and mantle removed:

an - anus	cpt - cephalic tentacles	ct - ctenidium
dg - digestive gland	e - eyespot	ft - foot
gn - gonad	rct - rectum	rs - radula sac
	sm - shell muscle	st - stomach

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*virginea* (MÜLLER) as described by FRETTER & GRAHAM (1962; pp. 149 - 239, 477 - 509), and *Lottia gigantea* (see FISHER, 1904). The mouth opens ventrally on the head. The lips completely encircle the elliptical opening with a wide wrinkled border. The mouth leads into the buccal or oral cavity which contains a series of pouches, grooves, the radula, and the jaw. The radula sac begins just behind the buccal mass and cavity. Passing straight

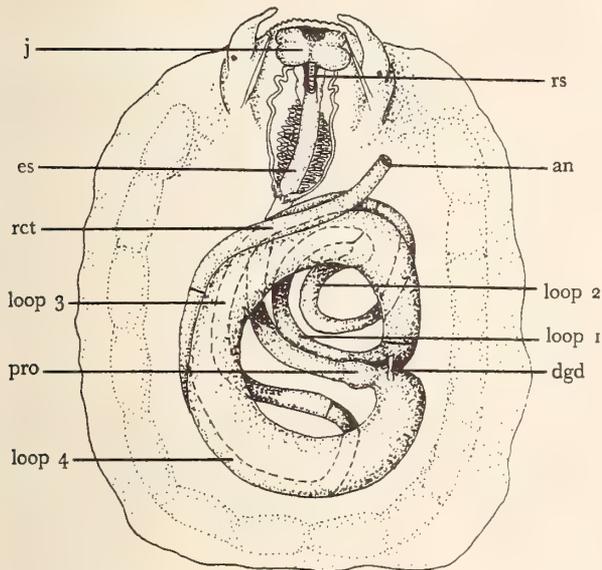


Figure 2

Generalized dorsal view of the digestive tract of an *Acmaea* with the dorsal body wall, kidneys, gonad, digestive gland, and pericardium removed:

an - anus	dgd - digestive gland duct	es - esophagus
j - jaw	pro - proventriculus	rct - rectum
	rs - radula sac	

back over the head muscles, it enters the anterior side of the visceral mass. It passes between the intestinal loops and stomach, curves to the right and forms a loop surrounded by the digestive gland, and extends anteriorly again, rising up over the top of the gland where often it can be seen when the shell and dorsal body wall are removed. Reentering the head cavity at the same point at which it left, the sac ends in a fleshy knob or caecum which secretes the radula (FRETTER & GRAHAM, *op. cit.*, p. 173; RUNHAM, 1963).

The jaw, a pliable chitinous structure, is positioned anterodorsal to the oral cavity (Figures 2 and 6). It protects the upper lip from the scraping movement of the

radula teeth, and prevents food from escaping the buccal cavity (FRETTER & GRAHAM, *op. cit.*, p. 168). In *Acmaea* and *Lottia gigantea* there is a single symmetrical jaw, with 4 wing-like extensions that are opaque, white, and fairly flexible. The smaller anterior wings overlap the posterior wings on the dorsal side. Muscles attached to the bases of the wings run to the body wall and buccal mass. Across the anterior mid-portion of the jaw runs a harder band, which becomes reddish-brown in older animals.

Behind the oral cavity lie the pharynx dilation and the esophagus. The latter extends backward to enter the visceral mass slightly to the left of the midline. Curving to the right it passes upward through the digestive gland and ends just under the dorsal surface of the gland in a mid-dorsal position. The greater and lesser folds of the esophagus follow the course described by FISHER (1906, pp. 10 - 11) in *Lottia*. The lesser fold begins in the mid-ventral region of the pharynx, twists counter-clockwise as viewed from the rear, and finally reaches a dorsal position just before it ends. The greater folds, which begin dorsally, are twisted so they come to lie ventrally at the posterior end of the esophagus. The twisting of the esophagus is the result of torsion in the veliger stage. Lateral pouches or sacculations are found between the greater and lesser folds. They become smaller and finally disappear as the esophagus curves to the right in the mid-visceral region (Figure 7).

The paired buccal glands lie along or under the mid-esophagus. Their ducts, which overlie the head muscles and empty into the oral cavity on dorso-lateral folds, are white and prominent.

The numerous posterior salivary or esophageal glands lie on either side of the pharynx dilation and esophagus. They are conspicuous, small, finger-like processes that extend out laterally and, in some species, ventrally around the esophagus. These glands open into tiny pockets, which in turn are divisions of the lateral sacculations of the esophageal wall. Like the esophageal folds, the glands and pharynx dilation appear twisted as a result of torsion.

The esophagus passes into the proventriculus or fore-chamber of the stomach, which opens into the stomach by way of a contractable aperture encircled by numerous small folds. The stomach forms a wide loop, encircling the digestive gland. In sexually mature individuals the gonad is sometimes visible laterally beyond the stomach margin.

The digestive gland occupies the central region of the visceral hump, slightly overlapping the inner margin of the stomach and almost completely covering the right lateral portions. A single digestive gland duct enters the

stomach near its junction with the esophagus. The volume and color of the gland vary somewhat with species and with the size of the animal.

The hind gut makes a series of 4 loops before it empties into the rectum (Figure 2). Loops 1 and 4 circle clockwise, while loops 2 and 3 circle counter-clockwise. The rectum begins where loop 4 crosses the anterior portion of the stomach. Constriction of the feces into linked pellets occurs in the latter portion of loop 4 and the rectum. The pellets are readily broken up, making them easily washed out of the nuchal cavity when the animal is splashed. Often if the animal has not been exposed to water for some time, fecal pellets may fill both sides of the mantle cavity.

In the comparative study of the gut in species of *Acmaea*, differences between species were observed in the length and placement of the radula sac in the body, the jaw, the salivary glands, and the gut loops.

LENGTH AND PLACEMENT OF THE RADULA SAC

The radula sac of all species except *Acmaea scutum* extends only to the mid-visceral region (Figures 3 and 4). The radula sac of *A. scutum* passes farther to the left and extends to the posterior visceral region before passing

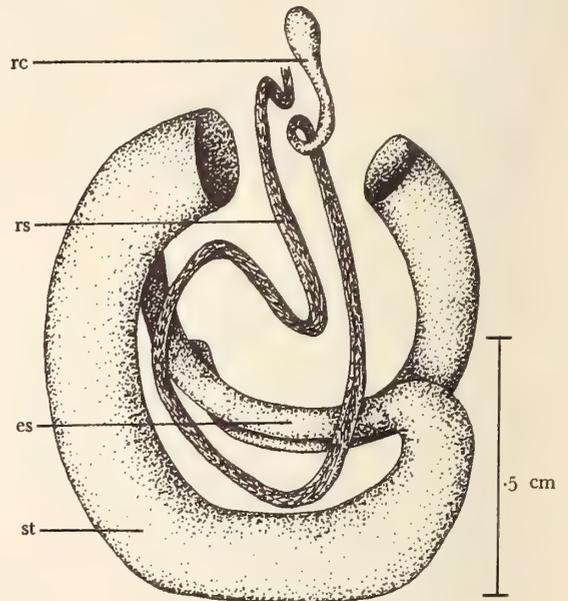


Figure 4

Dorsal view showing the placement of the radula sac in the visceral cavity of *Acmaea scutum* (shell length 25 mm; ratio of radula length to shell length 1.9).

es - esophagus      rc - radula caecum      rs - radula sac  
st - stomach

anteriorly again (Figure 4). The radula sac of *A. scutum* is almost twice the length of the shell, while in the other species it rarely exceeds 1.5 times the shell length (Table 1).

THE JAW

For species comparison, the jaw was removed, the muscle scraped away from the lower side, and the jaw then examined microscopically. When jaws were mounted in water on slides, the cover slips were slightly propped up to avoid breaking the jaws or bending them severely. Approximately 10 jaws, taken from animals ranging from small to large, were examined for each species (Figure 5). The jaw remains the same size, relative to the size of the animal, as growth occurs. With age, however, the

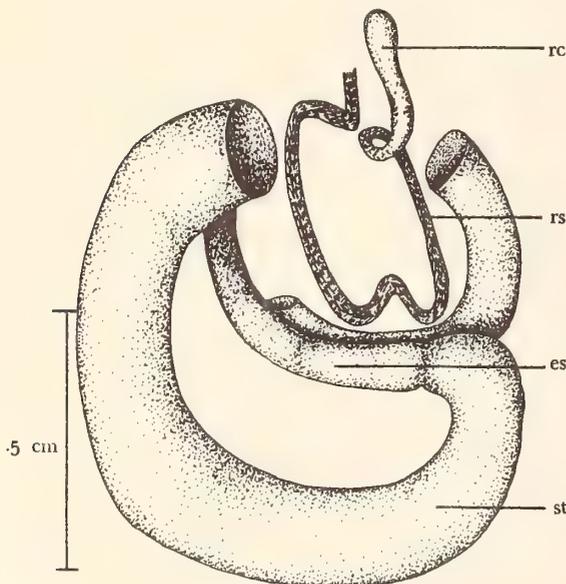


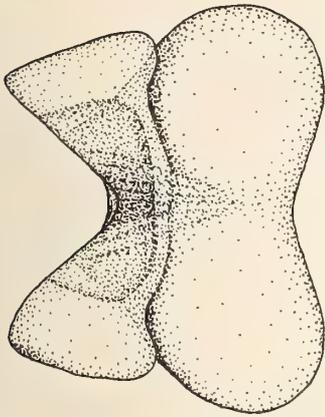
Figure 3

Dorsal view showing the placement of the radula sac in the visceral cavity of *Acmaea limatula* (shell length 25 mm; ratio of radula length to shell length 1.11). Placement of the sac here is similar to that in *A. pelta*, *A. scabra*, *A. digitalis*, and *A. asmi*.

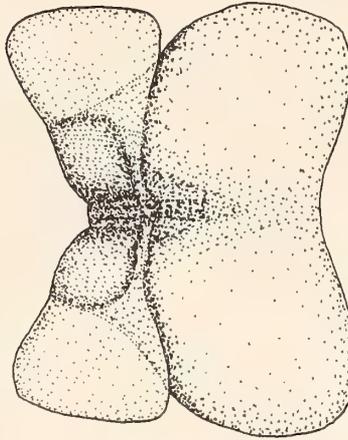
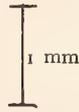
es - esophagus      rc - radula caecum      rs - radula sac  
st - stomach

Figure 5  
(on facing page →)

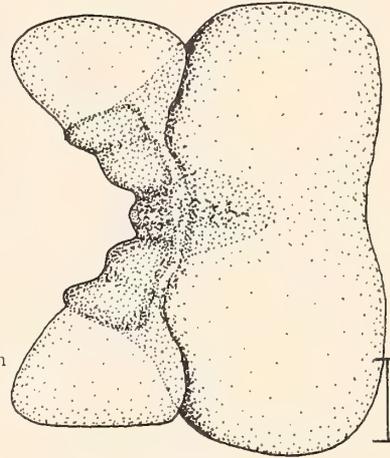
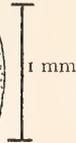
Dorsal view of the jaws of large adult limpets. The jaws are partially flattened out on slides and drawn with a camera lucida. Dark areas represent the most opaque areas.



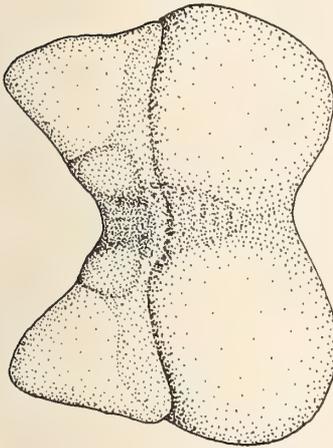
*Acmaea scutum*



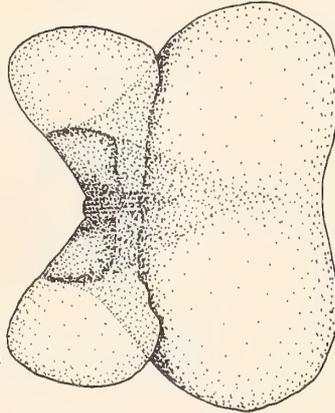
*Acmaea paradigitalis*



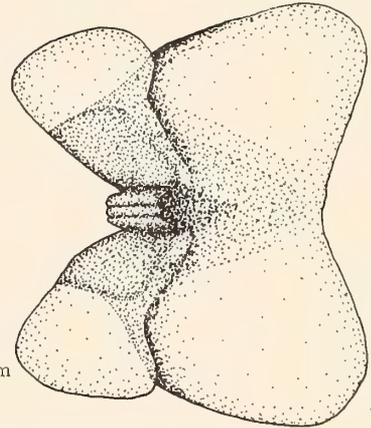
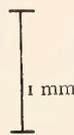
*Lottia gigantea*



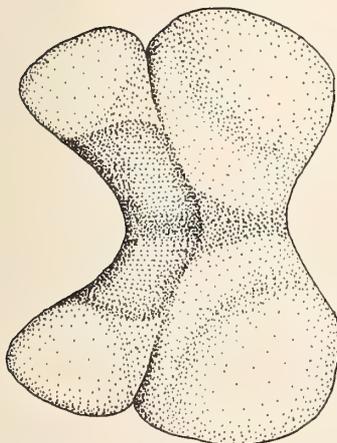
*Acmaea limatula*



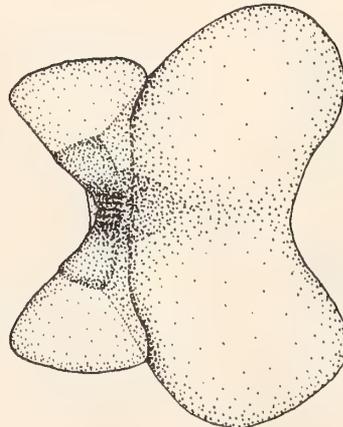
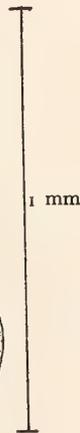
*Acmaea digitalis*



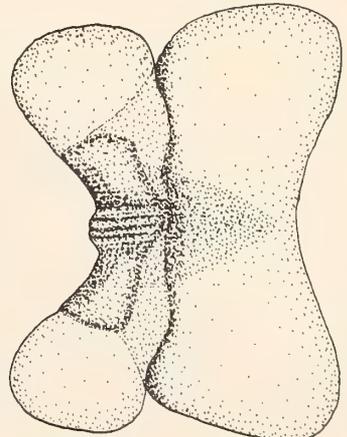
*Acmaea pelta*



*Acmaea asmi*



*Acmaea scabra*



*Acmaea insessa*

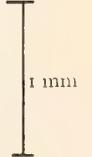


Table 1

Species	Number of specimens examined	Size range shell length cm	Ratio of $\frac{\text{radula length}}{\text{shell length}}$	
			mean	Range
<i>Acmaea limatula</i>	10	2.0 - 3.5	1.06	0.90 - 1.29
<i>Acmaea insessa</i>	10	1.5 - 2.0	1.07	0.97 - 1.33
<i>Acmaea digitalis</i>	10	1.5 - 2.5	1.13	0.90 - 1.25
<i>Acmaea paradigitalis</i>	10	1.0 - 2.0	1.19	1.00 - 1.40
<i>Acmaea scabra</i>	10	1.0 - 2.5	1.20	1.00 - 1.40
<i>Acmaea asmi</i>	10	0.5 - 2.5	1.23	1.06 - 1.37
<i>Acmaea pelta</i>	10	1.3 - 3.5	1.24	1.06 - 1.50
<i>Lottia gigantea</i>	10	3.0 - 5.6	1.29	1.07 - 1.58
<i>Acmaea scutum</i>	14	1.5 - 4.0	1.90	1.71 - 2.00

anterior band gets harder and darker. The median ridges of the band become more prominent, possibly as a result of wear caused by scraping of the radula.

In Figure 5 the jaws of large adult specimens of the species studied are arranged according to an increase in the irregularity of the anterior band. *Acmaea asmi*, *A. limatula*, and *A. scutum* show little or no marking on the band, and the anterior margin is smooth and unridged. *Acmaea scabra*, *A. digitalis*, and *A. paradigitalis* have small ridges in the medial region of the band, causing the margin to be slightly uneven. In *A. insessa*, *A. pelta*, and *Lottia gigantea* the anterior band bears a conspicuous median tooth with smaller ridges running along it. This characteristic is most apparent in *A. pelta*, which also has a darker anterior band. The undulating anterior border of the anterior band in the jaw of *Lottia* is rather distinctive. Species differences in shape of the lateral wings of the jaw are less apparent, though variations occur in wing shape and in the relative size of the anterior and posterior wings.

#### ANTERIOR AND POSTERIOR SALIVARY GLANDS

The anterior and posterior salivary glands show some variation in placement and size in the species examined. In *Acmaea digitalis*, *A. limatula*, and *A. scutum* the two buccal or anterior salivary glands extend along the esophagus, their posterior ends lying on the esophagus to the rear of the posterior salivary glands (Figure 6). The ducts of the buccal glands of *A. pelta*, *A. scabra*, and *A. asmi* are shorter and wind back and forth several times across the head muscles (Figure 7). In these species the distal extremities of the buccal glands terminate near the posterior margin of the buccal mass on either side.

In *Acmaea digitalis*, *A. scabra*, and *A. limatula* the very numerous posterior salivary glands extend straight

out from the pharynx dilation and anterior esophagus (Figure 6). In *A. pelta* and *A. scutum* the pharynx dilation is wider than in the above species and the twist of the esophagus more apparent (Figure 7). The glands here are slightly smaller, and curve ventrally around the foregut. In *A. asmi* the posterior salivary glands are very small, and light green in color, while in the other species they are white. A count of glands was attempted, but an accurate number was difficult to obtain. The left side of the esophagus has about 65 glands, while the right side has a slightly smaller number, the number varying somewhat with size of the animal and the species.

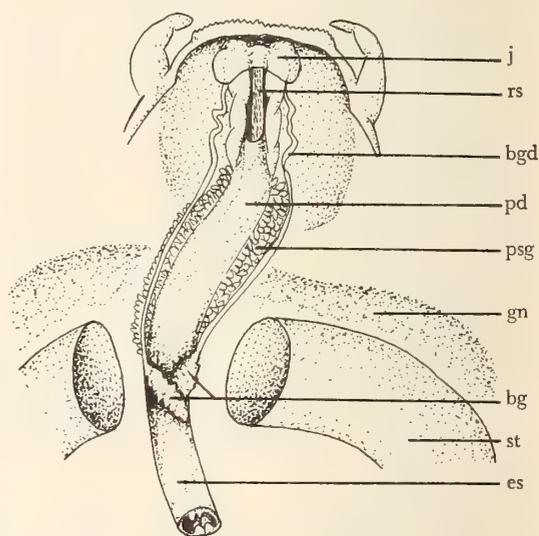


Figure 6

Dorsal view of the anterior end of *Acmaea digitalis*, showing the location of the anterior (buccal) and posterior salivary glands. Semi-diagrammatic; based on 15 adult individuals.

bg - buccal gland    bgd - buccal gland duct    es - esophagus  
gn - gonad    j - jaw    pd - pharynx dilation  
psg - posterior salivary glands    rs - radula sac    st - stomach

#### LOOPING OF THE GUT

The length and placement of the gut loops in 6 species of limpets is shown in Figure 8. Some variation in the exact placement of the loops occurs within the individual species depending on the size of the animal and the degree of maturity of the gonad. However, there is a characteristic placement of the loops in each species. In *Acmaea pelta*, *A. scutum*, and *A. scabra* loop 1 circles farther posteriorly. Loop 2 is considerably smaller in *A. digitalis*, *A. scabra*, and *A. asmi*. In *A. asmi* the anterior portions of loops 1 and 2 extend farther anteriorly relative to the stomach. In *A. digitalis*, and *A. scabra* loop 3 does not

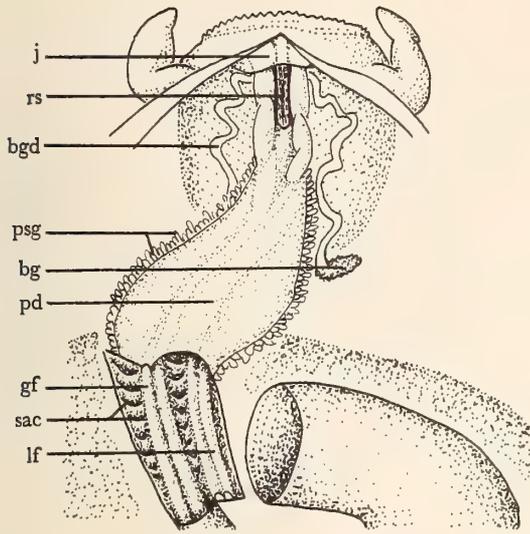


Figure 7

Dorsal view of the anterior end of *Acmaea pelta*, showing the location of the anterior (buccal) and posterior salivary glands.

Semi-diagrammatic; based on 15 adult individuals.

bg - buccal gland      bgd - buccal gland duct  
 gf - greater folds    j - jaw      lf - lesser folds  
 pd - pharynx dilation    psg - posterior salivary glands  
 rs - radula sac      sac - sacculations

lie as far to the left as in the other species. Dorsoventral thickness of the visceral mass is difficult to show in the drawings; in *A. limatula* and *A. scutum*, both rather flat species, the loops lie beside or just beneath one another. In the other species with taller shells the loops pass back and forth from ventral to dorsal regions as they twist through the digestive gland. The digestive tracts in *A. pelta*, *A. limatula*, and *A. scutum* are somewhat greater in diameter than those of the other species; this is a consistent difference, which appears related to diet but shows up whether the gut is full or empty. The stomach of *A.*

Figure 8  
(see page 94)

Diagrams of the stomach, intestine, and rectum of individuals of six species of *Acmaea*, based on dissections of 25 to 30 animals of each species. Exact placement of the loops is subject to slight variation within each species; the diagrams show the typical condition in large adults.

rct - rectum      st - stomach

*pelta* often contains sizable fragments of larger algae, and this species has been shown by CRAIG (1968) to feed mainly on larger red, brown, and green algae. The gut in *A. limatula*, which feeds mainly on flat encrusting algae (EATON, 1968) contains smaller fragments. The gut contents of *A. scutum* observed in the present study resembled those found in *A. limatula*. In contrast *A. digitalis*, *A. scabra*, and *A. asmi* are known to eat microscopic green and blue-green algae and diatoms (CASTENHOLZ, 1961; FRITCHMAN, 1961; HAVEN, 1965). The gut contents of these three species consists of very finely divided material.

Looping of the gut is less complex in the smallest limpets. In order to determine at what stages the small limpets develop the adult pattern of intestinal coils, small limpets, 0.5 to 5.0 mm in shell length, were examined. Animals 3 to 5 mm long could be identified to species. They were preserved and dissected in the usual way. Results showed that the digestive tract was present in essentially the adult condition. Still smaller individuals (1 to 2 mm in shell length) probably representing *Acmaea scabra* and *A. digitalis* were collected in a small splash pool in the high intertidal zone, which contained only large *A. scabra* and *A. digitalis*. Minute specimens (0.5 to 2.0 mm in shell length) of these species and probably *A. pelta* as well, were also collected in the crevices among *Mytilus californianus* CONRAD, 1837, *Tetraclita squamosa* DARWIN, 1854, and *Pollicipes polymerus* SOWERBY, 1833. These tiny limpets were killed and preserved in 70% alcohol, dehydrated in an alcohol series (the shells being removed in 95% alcohol), and cleared in cedar wood oil. The foot and mantle were removed by dissection, and the animals mounted on slides in cedar wood oil. The limpets had been feeding, and the digestive tract was dark and clearly distinguishable through the remaining more transparent tissues.

The results of these studies are shown in Figures 9 to 11. Limpets 0.5 to 1.5 mm in shell length have a relatively short stomach and intestine, and intestinal loops 1, 2, and 3 are not evident, though there are twists in the gut. Loop 4 and the rectum are in the normal position. Limpets 1.5 to 2.5 mm in length have loops 1, 2, and 3 but these are not fully developed (Figure 12). When the shell reaches 2.5 to 3.5 mm in length the digestive tract is almost the same as that in the larger animals (Figure 13). Placement of the inner loops 1, 2, and 3 may be slightly out of regular orientation, but this displacement is random. The radula sac is easily seen in its normal position.

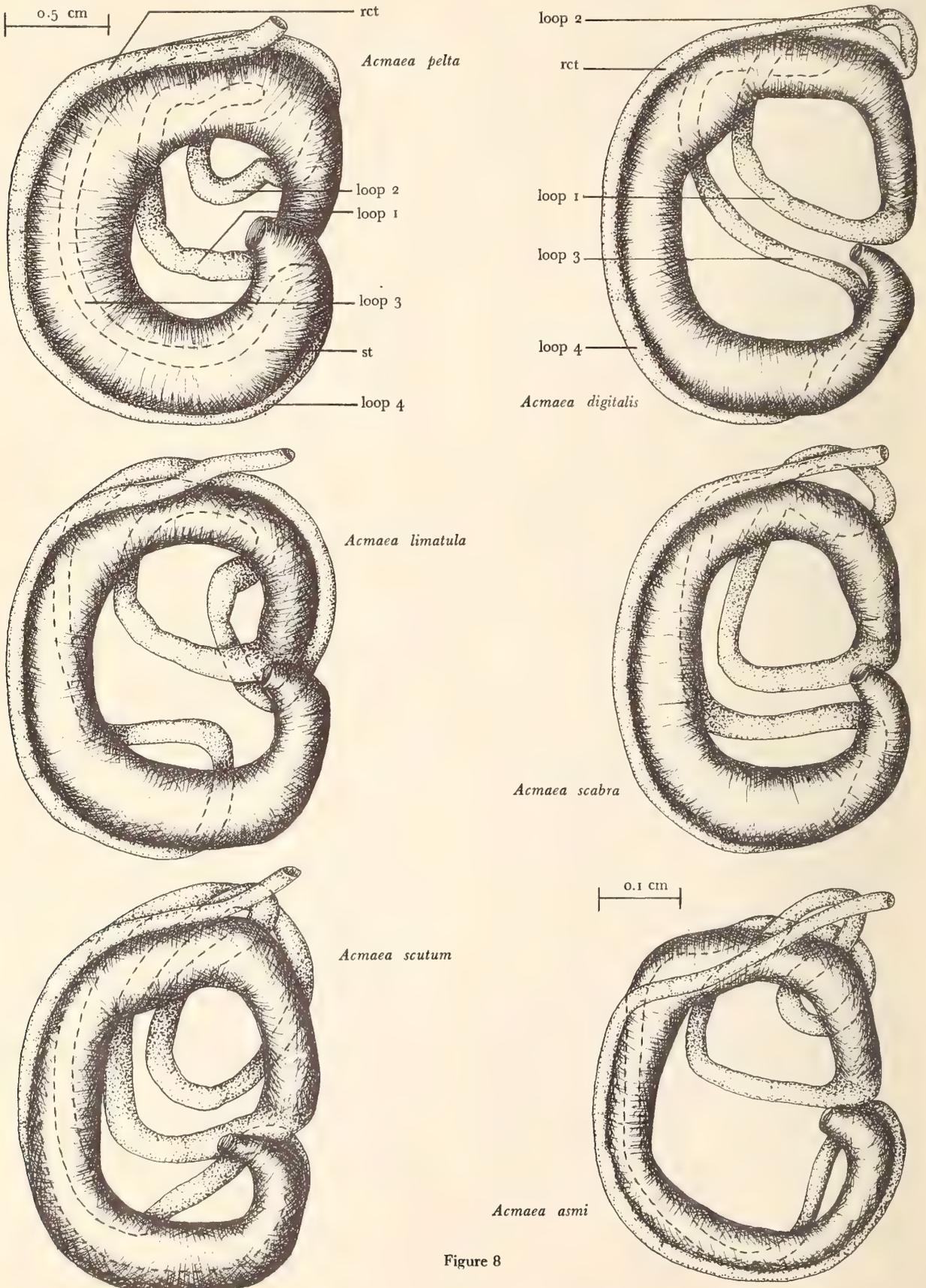


Figure 8

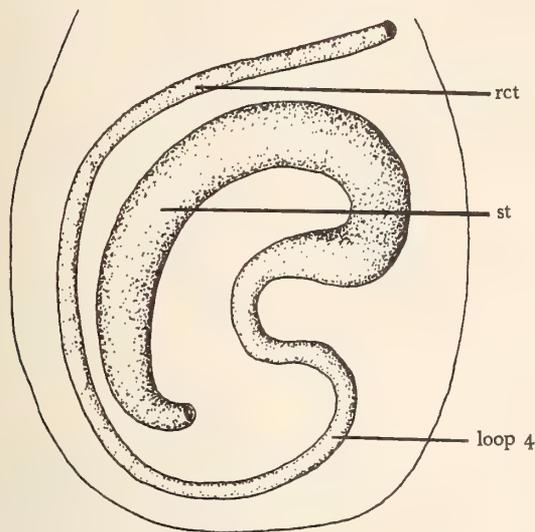


Figure 9

Generalized diagram of *Acmaea digitalis* and *A. scabra*, showing the development of the digestive tract in dorsal view.  
rct - rectum    st - stomach

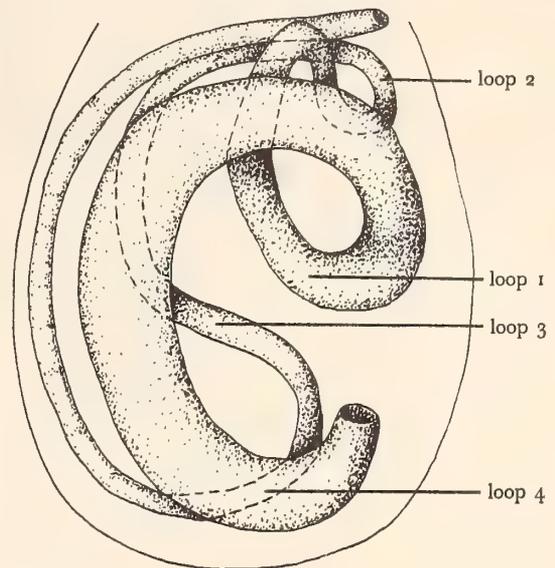


Figure 11

Generalized diagram of *Acmaea digitalis* and *A. scabra*, showing the development of the digestive tract in dorsal view.  
rct - rectum    st - stomach

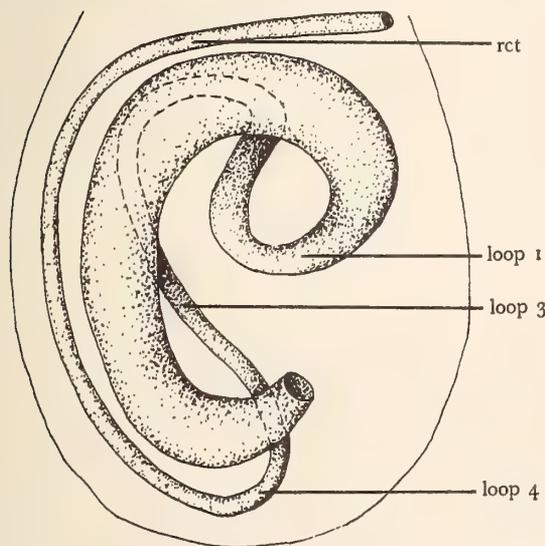


Figure 10

Generalized diagram of *Acmaea digitalis* and *A. scabra*, showing the development of the digestive tract in dorsal view.  
rct - rectum    st - stomach

### COELOMIC SYSTEM

The coelomic system consists of 4 interconnected cavities in the visceral mass: the right and left kidneys, the pericardium, and the gonad. The points of interconnection lie anteriorly on the right side of the visceral mass, just behind the muscular wall of the nuchal cavity (Figure 12).

The right kidney, easily identified by its dark green to brown color, encircles the whole visceral mass, with its distal end just posterior to the pericardium. Internally its surface is quite irregular. The large right renal-genital pore has thick muscular lips and is just to the right of the anal opening. The pore on *Acmaea scabra* is much larger than that in the other species, and even exceeds the size of the anus. The left kidney is much smaller and difficult to distinguish from the muscular wall separating the pallial and visceral cavities. It is situated just back of the nuchal cavity, between the midline and the anus, and overlies part of the rectum (Figure 12). *Acmaea digitalis* and *A. scabra* both have relatively large left kidneys. The left renal pore is a narrow slit opening on the anterior surface of the middle of the kidney (Figure 13). Only in *A. digitalis* are there very small bulbous lips on each side of the slit.

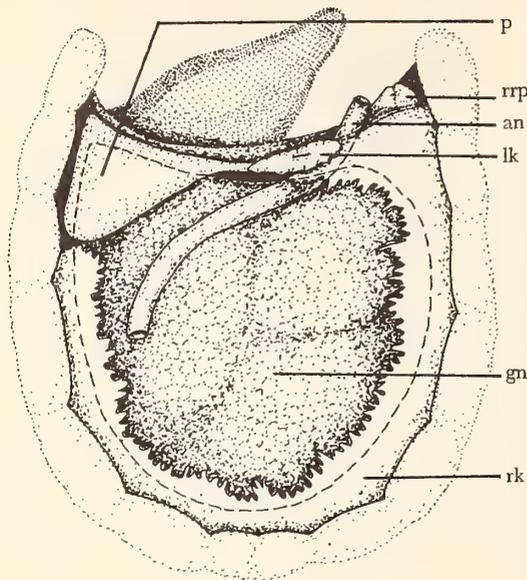


Figure 12

Generalized dorsal view of the coelomic system in *Acmaea*, with the dorsal body wall, digestive system, and posterior wall of the nuchal cavity removed.

an - anus      gn - gonad      lk - left kidney  
p - pericardium      rk - right kidney      rrp - right renal pore

The pericardium occupies the space above the gonad in the left dorsal anterior region of the visceral mass. From it the reno-pericardial canal (Figure 13) crosses the body from left to right and, as in *Lottia gigantea* (see FISHER, 1904), divides just under the left kidney, one canal going to the left kidney and one under the rectum to the right kidney.

The gonad is situated in the hollow above the foot beneath the digestive system. During the breeding season when it is greatly enlarged it extends up around the gut. The duct is merely an extension of the thin epithelium that surrounds the gonad (Figure 13). It arises on the antero-dorsal wall of the gonad in the midline and extends to the right to open into the right kidney. Gametes pass from the gonad to the right kidney, and leave the kidney through the right renal-genital pore.

### SUMMARY

1. Placement and extent of the radular sac, the form of the jaw, the arrangement of the salivary glands, the looping of the gut, and the interconnections of coelomic derivatives are described and illustrated for *Acmaea*: *A. asmi*, *A. digitalis*, *A. limatula*, *A. pelta*, *A. scabra*, *A. scutum*, *A. insessa*, *A. paradigitalis*. Some notes on *Lottia gigantea* are included.

2. The radula of *Acmaea scutum* is almost twice the length of its shell, while in the other species the radula ranges from 1 to 1.5 times the length of the shell.

3. Limpet jaws vary primarily in the shape and character of the hard anterior band. The jaws of *Acmaea pelta*, *A. insessa*, and *Lottia gigantea* bear an anterior median tooth.

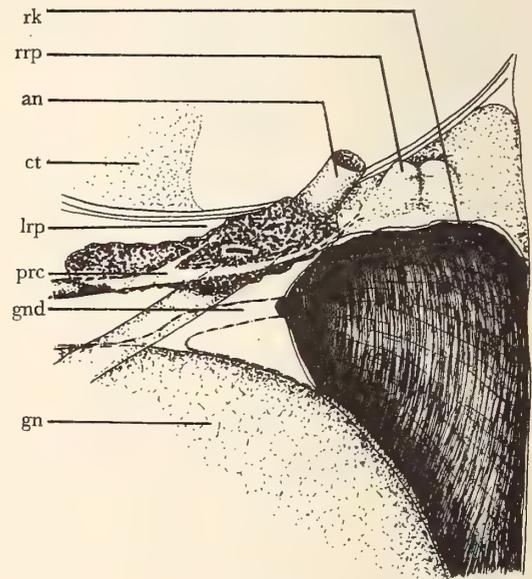


Figure 13

Enlarged view of the right upper corner of Figure 12, showing the interconnection of the coelomic cavities. Kidneys and anus open into the nuchal cavity.

an - anus      ct - ctenidium      gn - gonad  
gnd - gonad duct      lrp - left renal pore  
prc - pericardial renal canal      rk - right kidney  
rrp - right renal pore

4. The limpets studied can be separated into two groups on the basis of the salivary glands. *Acmaea digitalis*, *A. limatula* and *A. scutum* have buccal glands that extend down the esophagus posterior to the esophageal glands, while in the other species they terminate near the posterior margin of the buccal mass. In *A. pelta* and *A. scutum* the posterior salivary glands are slightly smaller, and curve ventrally around the foregut, while in the other species the glands extend straight out from the pharynx and esophagus.

5. The pattern of gut loops shows minor but consistent differences between *Acmaea* species. Development of the adult pattern of loops is essentially completed before animals attain a shell length of 4 mm. The gut tends to be thicker and heavier in species feeding on

larger erect or encrusting algae (*Acmaea pelta*, *A. limatula*, *A. scutum*) than in species feeding on films of microscopic algae (*A. digitalis*, *A. scabra*, *A. asmi*).

6. The coelomic cavities and ducts are described and diagrammed. Both right and left kidneys of *Acmaea digitalis* and *A. scabra* are relatively larger than those of other *Acmaea* species.

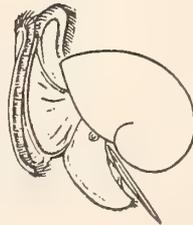
An excellent paper by RIGHI (1966), published after the present study was completed, describes the internal anatomy of the Brazilian species of *Acmaea* and provides illustrations permitting comparisons to be made between Brazilian and Californian species. RIGHI also finds that the shape of the jaw and the looping of the gut provide means of distinguishing *Acmaea* species.

### ACKNOWLEDGMENTS

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# A Comparison of Carbohydrate Digestion Capabilities in Four Species of *Acmaea*

BY

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(2 Tables)

AN EXAMINATION OF THE DISTRIBUTION of the genus *Acmaea* in the intertidal revealed that the various species have their highest population densities in different tidal zones. One of the characteristics of these zones is a difference in their algal flora. These various intertidal algae, which synthesize a variety of polysaccharide materials (see PEAT & TURVEY, 1966) constitute a potential source of food to herbivorous scrapers such as *Acmaea*. The hypothesis that assimilation of these materials would require a variety of carbohydrases led to the present comparative study of the carbohydrases present in the digestive tracts of four species of *Acmaea*.

## MATERIALS AND METHODS

The species chosen for this study were: *Acmaea scabra* (GOULD, 1846); *A. digitalis* ESCHSCHOLTZ, 1833; *A. limatula* CARPENTER, 1864; and *A. scutum* ESCHSCHOLTZ, 1833. Large individuals of these species were collected from areas of their maximum population density at Pescadero Point, Monterey County, California.

To remove the digestive tract, the animals were anaesthetized in magnesium chloride solution isotonic with seawater, and the entire digestive tract along with associated glands (digestive gland, buccal salivary gland, and esophageal salivary gland) excised and placed in 3% sodium chloride in an ice bath. An enzyme extract was prepared, containing 1 part tissue, 8 parts 3% sodium chloride, and 1 part of a saturated solution of ovomucoid (ovomucoid dissolved either in 0.1 M acetate buffer, pH 5.5, or 0.1 M Tris buffer, pH 7.2). This mixture was homogenized in a tissue grinder and then centrifuged for 30 seconds at half maximum speed in an International

Clinical Centrifuge. Ovomucoid, prepared by the method of FREDERICQ & DEUTSCH (1949), was used as an inhibitor of proteolytic enzymes since initial experiments indicated low carbohydrase activity, possibly resulting from degradation of the enzymes by proteolysis. Use of ovomucoid permitted detection of higher levels of enzyme activity, and was therefore used in all reported experiments.

One ml of enzyme extract was incubated with 1 ml of 0.5% polysaccharide solution in 3 ml buffer. Two buffers were used, either 0.1 M acetate at pH 5.5 or 0.1 M Tris at pH 7.2. The polysaccharides included in this study were starch (Baker and Adamson, Reagent grade), agar (Difco), carrageenin (Kappa-fraction) isolated from *Rhodoglossum* spp., and laminarin, fucoidin, and alginic acid isolated from *Pelvetia* spp. The mixtures were incubated for 1 hour at 20°C. Enzyme and substrate controls were similarly incubated. All tubes, including enzyme and substrate controls, were layered with toluene as a bactericide, after GALLI (1956).

At the end of the incubation period enzyme activity was stopped by sodium hydroxide-zinc sulfate precipitation, and aliquots were assayed for reducing sugar by a modification of NELSON's (1944) procedure. This modification was the substitution of sodium hydroxide for barium hydroxide to prevent precipitation of sulfated oligosaccharides formed during enzymatic hydrolysis of K-carrageenin and fucoidin. The optical density of the final solution was measured in a Klett-Summerson photoelectric colorimeter using a green filter. Two standard glucose solutions, 150 $\gamma$  and 50 $\gamma$ , were run with each set of assays, and all readings were evaluated by comparison with a glucose standard curve. Values represent an average of two determinations of total reducing sugar released in the incubation mixture, and correspond to the activity of 0.1 ml of tissue extract. The assay was found to be reproducible within 10%. The minimum value

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considered to be significant in the tables was taken to be 50  $\mu\text{g}$ .

## RESULTS

**Algal Associations:** To correlate diet with digestive activity, a rough survey was made of the collection area. Heights for maximum concentration of larger specimens were estimated as 5 feet for *Acmaea scabra*, 4 feet for *A. digitalis*, 3 feet for *A. limatula*, and 2.5 feet for *A. scutum*. Algae growing in the respective collection areas were: *A. scabra* – microscopic algae only; *A. digitalis* – microscopic algae, *Ralfsia* spp., and some *Peyssonnelia* spp.; *A. limatula* – microscopic algae, *Ralfsia* spp., *Gigartina* spp., *Endocladia* spp., and *Pelvetia* spp.; and *A. scutum* – microscopic algae, *Ralfsia* spp., *Gigartina* spp., and *Porphyra* spp. The microscopic algae in this area were varied green and red algae (HAVEN, 1966). This is not meant to be a definitive list of the dietary constituents of these animals, but rather an observation of the algae available for food in the limited areas from which the organisms were collected.

**Enzyme Activity:** In the first experiment, the animals were "starved" in an aquarium scraped free of algae, and kept in the dark. After 4-6 days of starving, extracts of the gut were made, and enzyme activity determined. The results are recorded in Table 1.

The results show the presence of a powerful amylase with higher activity at pH 5.5 in all species. An alginase was also demonstrated in all species. All species examined except *Acmaea limatula* exhibited a fucoidinase with highest activity at pH 5.5. Small amounts of agarase and laminarinase were found in *A. scutum*, *A. scabra*, and *A. digitalis*, a K-carrageeninase also being present in the two latter species. *Acmaea digitalis* and *A. scabra* show the greatest overall activity, with significant amounts of enzyme activity demonstrated for all substrates tested.

In a second experimental series, animals were collected fresh from the field and extracts prepared within 3 hours. Enzyme activities determined are shown in Table 2.

A powerful amylase was again evident, but with these animals small amounts of activity for all substrates were found in all species. *Acmaea digitalis* showed the greatest overall activity.

A comparison of the starved animals with the non-starved animals shows, in general, greater activity in non-starved animals. However, some species differences are evident. Although there are some deviations for particular substrates, there is a general grouping in the effect of starvation, starvation causing a much greater decrease in activity in *Acmaea limatula* and *A. scutum* than in *A. scabra* and *A. digitalis*. Amylase activity was particularly affected.

Table 1

Carbohydrase Activity of Starved Animals

Species	pH	Substrates					Starch
		K-carrageenin	Agar	Laminarin	Fucoidin	Algimic Acid	
Total Reducing Sugar Released in 1 Hour							
<i>Acmaea scabra</i>	5.5	132 $\mu\text{g}$	197 $\mu\text{g}$	197 $\mu\text{g}$	132 $\mu\text{g}$	66 $\mu\text{g}$	3520 $\mu\text{g}$
	7.2	0	0	44 $\mu\text{g}$	0	109 $\mu\text{g}$	1530 $\mu\text{g}$
<i>Acmaea digitalis</i>	5.5	168 $\mu\text{g}$	112 $\mu\text{g}$	298 $\mu\text{g}$	298 $\mu\text{g}$	224 $\mu\text{g}$	3420 $\mu\text{g}$
	7.2	0	0	0	0	56 $\mu\text{g}$	1440 $\mu\text{g}$
<i>Acmaea limatula</i>	5.5	0	0	0	21 $\mu\text{g}$	0	2760 $\mu\text{g}$
	7.2	0	0	0	0	239 $\mu\text{g}$	811 $\mu\text{g}$
<i>Acmaea scutum</i>	5.5	41 $\mu\text{g}$	103 $\mu\text{g}$	82 $\mu\text{g}$	82 $\mu\text{g}$	62 $\mu\text{g}$	1660 $\mu\text{g}$
	7.2	0	0	0	0	0	681 $\mu\text{g}$

Table 2

Carbohydrase Activity of Non-Starved Animals

Species	Substrates						
	pH	K-carrageenin	Agar	Laminarin	Fucoidin	Alginic Acid	Starch
<i>Acmaea</i>	5.5	51 $\mu$ g	51 $\mu$ g	77 $\mu$ g	128 $\mu$ g	0	3440 $\mu$ g
<i>scabra</i>	7.2	0	0	0	0	77 $\mu$ g	1980 $\mu$ g
<i>Acmaea</i>	5.5	250 $\mu$ g	100 $\mu$ g	275 $\mu$ g	275 $\mu$ g	225 $\mu$ g	3850 $\mu$ g
<i>digitalis</i>	7.2	100 $\mu$ g	25 $\mu$ g	0	150 $\mu$ g	350 $\mu$ g	1950 $\mu$ g
<i>Acmaea</i>	5.5	51 $\mu$ g	51 $\mu$ g	103 $\mu$ g	103 $\mu$ g	103 $\mu$ g	3880 $\mu$ g
<i>limatula</i>	7.2	0	0	0	0	206 $\mu$ g	1870 $\mu$ g
<i>Acmaea</i>	5.5	300 $\mu$ g	175 $\mu$ g	198 $\mu$ g	150 $\mu$ g	150 $\mu$ g	3320 $\mu$ g
<i>scutum</i>	7.2	50 $\mu$ g	50 $\mu$ g	0	125 $\mu$ g	100 $\mu$ g	1775 $\mu$ g

## DISCUSSION

Green and red algae, which contain starches, K-carrageenin, and agar (PEAT & TURVEY, 1965), are available to all species of *Acmaea* studied, and the high recorded amylase activity correlated well with this. Brown algae, containing alginic acid, fucoidin, and laminarin (PEAT & TURVEY, *op. cit.*), are available to all species except *A. scabra*. Although this correlates well with the low alginase activity found in *A. scabra*, it is not reflected in the laminarinase or fucoidinase activity. EATON (1968) has made a careful study of the diet of *A. limatula* and *A. pelta*. A similar study of the other species would be desirable.

The data on changes in carbohydrase activity due to starvation suggest that the degree of these changes is a function of height in the intertidal zone, higher species (*Acmaea scabra* and *A. digitalis*) showing less drop in activity than lower species (*A. limatula* and *A. scutum*). Two possible reasons for this difference are food retention time and feeding behavior. In fresh animals, the lower species eliminated food that was only partly digested at a much greater rate than the higher species. In the starved animals, the remains of well-digested food were still found in the gut of the two higher species, whereas nothing was found in the gut of the two lower species. This difference in food retention time correlates well with the changes in enzyme activity during starvation.

The feeding behavior of the higher species has also been found to be more sporadic than that of the lower

species. The lower species, being splashed or under water a greater amount of the time than the higher species, are thus able to move and feed for longer periods of time. The higher species must retain and digest food more efficiently, as was found, and are, therefore, not as susceptible to the effects of deprivation of food.

## SUMMARY

The carbohydrate digestion of four species of limpets, *Acmaea scabra*, *A. digitalis*, *A. limatula*, and *A. scutum* was studied. The presence of a K-carrageeninase, an agarase, a laminarinase, a fucoidinase, an alginase, and an amylase was demonstrated for all species. Some correlation of foods available with enzyme activity was found. Carbohydrase levels in starved animals were compared with levels in non-starved animals. A decrease in enzyme activity during starvation was found, and the amount of this decrease could be correlated with height in the intertidal, higher species (*A. scabra* and *A. digitalis*) showing less decrease than lower species (*A. limatula* and *A. scutum*). This correlation might be related to food retention time and feeding behavior.

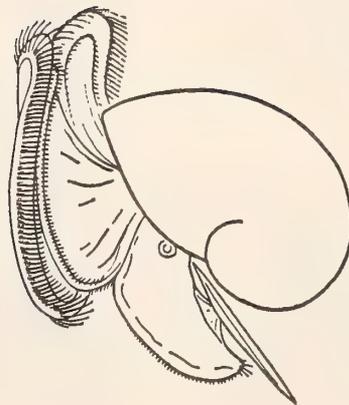
## ACKNOWLEDGMENTS

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work was made possible by Grant GY806 from the Undergraduate Research Participation Program of the National Science Foundation.

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# Metabolic Activity and Glycogen Stores of Two Distinct Populations of *Acmaea scabra*

BY

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(2 Tables)

## INTRODUCTION

OF THE SEVERAL SPECIES of *Acmaea* found in the intertidal area of Monterey County, *A. scabra* (GOULD, 1846) occupies the highest zone on the rocks (HEWATT, 1935). It ranges between the tide levels of +2 and +6 feet. HEWATT has observed, and my own observations have confirmed, that these limpets make excursions in search of food only when submerged or amidst heavy wave action. Lower populations move more often, and more regularly, than do populations located at higher tide levels. For example, individuals of the higher populations have been observed to remain immobile for periods up to three days.

In April and May, 1966, studies were carried out at the Hopkins Marine Station of Stanford University, Pacific Grove, California, to determine what physiological factors enable these populations to sustain themselves over prolonged periods between feeding. Two parameters were investigated: oxygen consumption and glycogen stores. These parameters were chosen in conjunction with the hypothesis that a decrease in metabolic activity and larger stores of glycogen may allow the animals to survive under conditions that preclude frequent feeding.

## METHODS

All specimens were collected off Mussel Point, Pacific Grove, California. Individuals were taken from two different parts of the intertidal zone: below +2 and above +6 feet. Within 20 minutes after collection, individual animals were placed in a separate water-filled jar and

their oxygen consumption measured for a 3 hour period by the Winkler method (SMITH & WELSH, 1949, p. 146). The water temperature remained constant at 14°C. In order to minimize oxygen evolution and consumption by algae on the shells, the shells were scraped and the animals kept in the dark. After the respiratory measurement was completed, each animal was removed from its shell, blotted and weighed. These same individuals were then used to determine the amount of glycogen present in the entire body. The whole animal was homogenized in 2 ml of 10% trichloroacetic acid. After centrifugation, the glycogen was precipitated from the trichloroacetic acid supernatant by the addition of an equal volume of 95% ethanol. The precipitate was dissolved in 4 ml of water and the carbohydrate content determined using the phenol-sulfuric acid method of DUBOIS (1956).

## RESULTS AND DISCUSSION

The results of measurements of oxygen consumption are represented in Table 1 and glycogen content is shown in Table 2.

The findings show a difference in metabolic activity and glycogen stores in animals from the two intertidal locations. The results show that the higher forms have higher glycogen stores and lower metabolic activity than the animals from the lower intertidal area. This combination could be the critical factor permitting maintenance of the animal between feedings. Measurements of glycogen in the higher group also show a somewhat greater range, as is reflected in the higher standard deviation. A greater degree of variation in glycogen content would be anticipated if life between feedings is dependent upon the use of glycogen stores. The higher mean glycogen content

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Table 1

Glycogen Stores from High and Low Populations  
(these figures correspond to the same animals as the first 21 figures  
in Table 2)

Glycogen as $\mu\text{g}$ glucose/0.1 gm body weight	
<i>Acmaea scabra</i> + 6ft	<i>Acmaea scabra</i> + 2ft
490	170
490	330
360	160
700	270
840	110
300	225
630	105
770	145
600	85
660	224
316	135
440	330
640	58
690	194
218	224
344	214
640	164
585	195
320	470
410	490
	214
$\Sigma = 10443$	$\Sigma = 4513$
$\bar{X} = 522.15$	$\bar{X} = 214.9$
S. D. = 177.25	S. D. = 111.9
S. E. = 8.8625	S. E. = 5.233
S. E. diff = 10.29	
$\bar{X}$ diff/S. E. diff = 29.8	
P = < 0.001	

in the limpets from higher portions of the intertidal zone also suggests an increased storage potential in these animals.

The glycogen and respiratory data also permit calculation of the minimum time these higher populations could survive between feedings, if glycogen were the sole respiratory substrate. Assuming 6 moles of oxygen consumed per mole of glucose utilized, this could permit submerged maintenance for 60 hours. The lower population of *Acmaea scabra*, with its higher respiratory activity and lower glycogen store, could maintain itself for 17 hours. Since BALDWIN (1966) has found that the aerial respiration rate in *A. scabra* is several times less than the submerged rate, the above values are certainly minimal for aerially breathing limpets.

Table 2

Metabolic Activity of High and Low Populations

Oxygen Consumption as $\mu\text{l O}_2/1$ gm body weight/3 hrs.	
<i>Acmaea scabra</i> + 6ft	<i>Acmaea scabra</i> + 2ft
440	115
132	230
222	194
190	292
165	242
77	230
129	200
160	156
120	148
194	238
167	157
176	378
220	315
144	124
143	202
150	200
176	330
315	240
318	296
164	250
180	450
315	278
234	206
336	175
172	222
332	550
254	317
130	322
150	440
139	570
108	435
92	480
	416
$\Sigma = 6244$	$\Sigma = 9398$
$\bar{X} = 195.12$	$\bar{X} = 284.78$
S. D. = 81.04	S. D. = 120.11
S. E. = 2.531	S. E. = 3.67
S. E. diff = 4.45	
$\bar{X}$ diff/S. E. diff = 20.01	
P = < 0.001	

Lower metabolic activity and higher glycogen content are not the only characteristics of high populations that suggest adaptations for survival in the higher portions of the intertidal. HEWATT (1940) has observed that the shells of *Acmaea scabra* from higher intertidal areas are higher and thicker, while the shells of this species living

in low, moist areas are thinner and flattened. In addition, the study of JESSEE (1968) indicates that *A. scabra* of the higher intertidal shows a greater tendency to home than *A. scabra* found in the low intertidal. Animals with a strong homing tendency present a greater irregularity of shell shape that is complementary to the home site. The resulting better fit of animal to substratum may decrease susceptibility to desiccation. A third adaptation of the higher forms, shown by the study of HARDIN (1968), demonstrates that higher populations of *A. scabra* can withstand greater temperatures than populations lower in the intertidal.

### SUMMARY

1. Metabolic activity and glycogen content of populations of *Acmaea scabra* from the higher and lower portions of the intertidal area were determined.
2. Animals from +6 feet showed lower metabolic activity and larger glycogen stores than animals from +2 feet.
3. The possible survival value of this difference is discussed.

### ACKNOWLEDGMENTS

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National Science Foundation. I am also happy to acknowledge the guidance of Dr. John Phillips.

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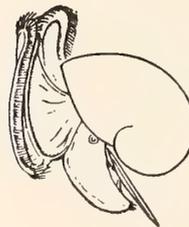
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# Laminarinase and Hexokinase Activity during Embryonic Development of *Acmaea scutum*

BY

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(2 Text figures; 1 Table)

THIS REPORT DESCRIBES some enzyme activities related to carbohydrate metabolism during embryonic development of the gastropod *Acmaea scutum* ESCHSCHOLTZ, 1833. Data are presented on activities of enzymes releasing free glucose from various polysaccharides, on hexokinase<sup>2</sup> (an enzyme common to both the pentose shunt and glycolysis), glucose-6-phosphate dehydrogenase (an enzyme of the pentose shunt), and isocitrate dehydrogenase (an enzyme of the citric acid cycle).

## MATERIAL AND METHODS

**Obtaining and Culturing Gametes:** Adult *Acmaea scutum* were collected from an area on Cypress Point in Pebble Beach, California. The animals were removed from their shell, and the dorsal body wall ruptured near the posterior end of the animal, with care taken not to puncture the nearby digestive gland. Parts of ovaries from 5 females were removed with forceps, agitated in alkaline sea water to remove the mature eggs, and then treated as described below to remove the chorion. Sperm were obtained by agitating the testes in 25 ml of normal filtered sea water, and filtering the suspension through a double layer of cheesecloth.

Removal of the chorion by the alkaline sea water treatment of WOLFSOHN (1907) was necessary to obtain fertilization and normal development. For *Acmaea scutum*, best results were obtained by 90 minutes incubation in alkaline sea water (2.5 ml of 0.1N NaOH in 100 ml of filtered sea water, final pH 9.2). The alkaline sea

water was then removed, the eggs re-suspended in filtered sea water, and 1 ml of sperm suspension added. After 2 hours the eggs were agitated, allowed to settle, and the supernatant water containing most of the sperm removed and filtered sea water added. After about 36 hours, the embryos were gently centrifuged, and re-suspended in freshly filtered sea water. Above operations were all performed at 15° to 17° C.

**Enzyme Preparation:** Assays of one large group of eggs were made before fertilization, 24 hours after fertilization at the trochophore stage, and 48 hours after fertilization at the veliger stage. In initial experiments, enzyme instability was noted, which suggested the possibility of degradative enzymes (such as proteases). Therefore, in some experiments 6 to 9 mg ovomucoid prepared by the method of FREDRICK & DEUTSCH (1949) were added to one half of the samples as a specific trypsin inhibitor.

The eggs, trochophores, or veligers were divided into two parts and centrifuged. One sample was suspended in 2.5 ml buffer (0.05M TEA, pH 7.6), the other in 2 ml buffer-0.5 ml of ovomucoid solution (also suspended in buffer), and both samples then disrupted in a Dounce homogenizer at 0° C. The volumes of embryos used ranged from 0.05 to 0.1 ml packed cells per 2.5 ml. All preparations were assayed for enzyme activity at 26° C.

**Fluorometric Assays:** Because of the small amounts of embryos obtainable, sensitive fluorometric techniques, based upon the absorption of TPNH at 366 m $\mu$  and its fluorescence at wavelengths greater than 420 m $\mu$  were utilized to measure enzyme activity. Using incubation mixtures described below, glucose-6-phosphate dehydrogenase (G6PDh) and isocitrate dehydrogenase activity were determined by direct measurement of TPNH formation; hexokinase (HK) activity was measured by coupling its product, glucose-6-phosphate, with excess G6PDh, to yield TPNH; and carbohydrases releasing free

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<sup>2</sup> The following abbreviations will be used: HK - hexokinase; G6PDh - Glucose-6-phosphate dehydrogenase; TEA - tri-ethanolamine buffer; TPN - triphosphopyridine nucleotide (NADP), and G-6-P - glucose-6-phosphate.

glucose measured by coupling glucose production to HK and G6PDh in the presence of excess ATP.

(1) Glucose-6-phosphate dehydrogenase: The 2 ml reaction mix contained, per ml, 43  $\mu$ moles TEA buffer (pH 7.6), 0.25  $\mu$ moles TPN, 5  $\mu$ moles  $MgCl_2$ , and 4  $\mu$ moles glucose-6-phosphate (G-6-P).

(2) Hexokinase: Same as (1), except 10  $\mu$ moles/ml glucose were substituted for G-6-P, plus 2  $\mu$ moles/ml ATP, and 0.5  $\mu$ gm/ml G6PDh.

(3) Carbohydrase: Same as (2), plus 2.5  $\mu$ gm/ml HK, and in place of glucose were substituted either maltose (1  $\mu$ mole/ml), lactose (1  $\mu$ mole/ml), laminarin or starch (0.05%, final concentration). Negligible glucose was present in the starch, laminarin, or cell extract. Appreciable amounts, however, were found in the maltose and lactose. To eliminate this contribution, the reaction mix (minus cell extract) was incubated with sufficient substrate to remove all free glucose, and the reaction then initiated with cell extract.

(4) Isocitrate dehydrogenase: Same as (1), except 2  $\mu$ moles/ml isocitric acid was substituted for the G-6-P.

**Other Assays:** Unfertilized and veliger stages were also assayed for carbohydrases by measuring reducing sugars released, using the method of SOMOGYI as modified by NELSON (1944). As a further check, amylase activity was also measured qualitatively by adding iodine to samples of extract and starch at 5-minute intervals, and using decolorization of the blue iodine-starch complex as an indicator of amylase activity.

The fluorometric technique was capable of detecting the production of as little as  $6 \times 10^{-11}$  moles glucose per minute. The reducing sugar test of SOMOGYI as modified by NELSON could detect about  $3 \times 10^{-8}$  moles of reducing sugar.

Protein was measured spectrophotometrically by both the method of WARBURG & CHRISTIAN (1941) and LOWRY *et al.* (1951). Protein content was assumed to reflect enzyme content, since the observed rates were closely related to enzyme concentration (a twofold increase in extract approximating a two-fold increase in rate). The activities are therefore expressed as moles TPNH formed per minute per milligram protein.

Commercial sources of enzymes and substrates: Hexokinase and glucose-6-phosphate dehydrogenase (Boehringer und Söhne), isocitrate, glucose, TPN, and ATP (Sigma Chemical Company), lactose (Nutritional Biochemical Corporation), and maltose (Difco Laboratories). The laminarin and starch were prepared by Dr. John Phillips of Hopkins Marine Station.

## RESULTS AND DISCUSSION

Enzyme activities determined fluorometrically are listed in Table 1. The lack of amylase activity in unfertilized eggs and in veligers was also supported qualitatively by the lack of decolorization of the iodine-starch complex, and quantitatively by the reducing sugar test. This latter test indicated that, if amylase were present, it produces less than  $30 \times 10^{-9}$  moles reducing sugar per hour per mg protein.

Enzyme extracts assayed for carbohydrase activity were prepared from embryos obtained from different females. For comparison of enzyme activity during development, eggs of 5 females were pooled, and enzyme activity was determined in the unfertilized egg, trochophore, and veliger stages. The rates of enzyme activity at these various stages are shown in Table 1 and Figure 1. Table 1 shows that by the veliger stage, regardless of previous activity, the embryos homogenized in ovomucoid show a greater activity than the extracts prepared without this

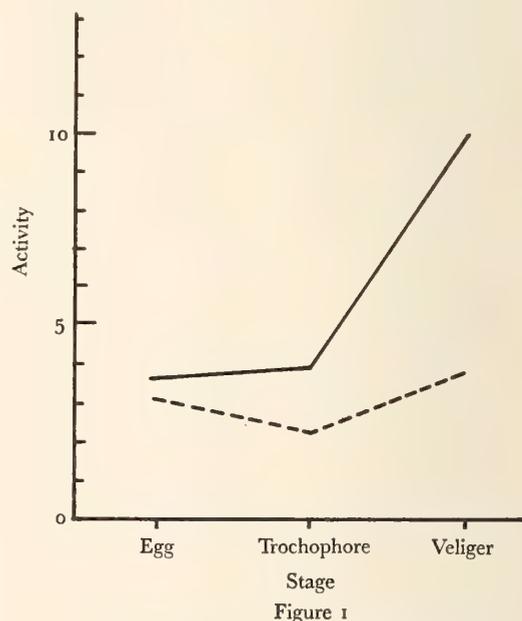


Figure 1  
Activity of Hexokinase During Development.  
Activity expressed as  $10^{-11}$  moles TPNH/minute/milligram protein.

trypsin-inhibiting compound. This could indicate the development of a trypsin-like protease. This conclusion is also supported by Figure 2, which shows that storage of HK at  $0^{\circ}C$ , without ovomucoid, results in considerable loss of activity, and that this loss does not occur with

Table 1  
Enzyme Activities at Various Embryonic Stages

Enzyme	Stage	egg extract time - 0	same extract after 2½ hours	trochophore extract time - 0	same extract after 2½ hours	veliger extract
Hexokinase						
with mucoïd		3.7	3.5	3.8	3.8	11
without mucoïd		3.5		2.2	0.8	3.7
Laminarinase						
with mucoïd		10+		5 to 30		
without mucoïd						
Maltase						
with mucoïd		0		0		0
without mucoïd		0		0		0
Galactosidase						
with mucoïd		0		0		0
without mucoïd		0		0		0
Amylase						
with mucoïd		0		0		0
without mucoïd		0		0		0
Isocitrate dehydrogenase						
with mucoïd		63		43	35	56
without mucoïd		33		31		16
G-6-P dehydrogenase						
with mucoïd		42		43	43	48
without mucoïd		52		36		28

Enzyme rates expressed as  $10^{-11}$  moles TPNH/minute/mg protein  
The table also shows enzyme activity after "aging" of extract at  
0°C for 2½ hours in the presence and absence of ovomucoid

ovomucoid present. Similar stabilization of activity was also observed with laminarinase activity. Isocitrate dehydrogenase activity in the unfertilized eggs (Table 1) shows anomalous behavior in ovomucoid which is unlike the activity of HK and ZF. The reasons for this are unclear.

Perhaps the most interesting discovery is that the eggs and early embryonic stages of *Acmaea scutum* possess an enzyme(s) capable of releasing glucose from laminarin. The rate of activity of this enzyme, however, was non-linear under the present assay conditions, and therefore no comparison of activity was attempted.

The role of the embryonic laminarinase in the egg and early trochophore stages is difficult to visualize, since

neither of these is a feeding stage. The veliger, however, does feed, and could certainly utilize a laminarinase-type enzyme for digestion of the large amount of algal laminarin found near the surf zone in small fragments of brown algae. If the veligers are degrading laminarin to free glucose in their natural environment the sharp increase in HK activity (Figure 1), also found at this stage, could result from free glucose made available by laminarinase. ZF activity, however, appears to remain relatively constant during development.

Another possibility, which is presently being investigated, is that the observed laminarinase activity actually reflects  $\beta$ -1,3 glucanase activity involved in the breakdown of cellular glyco-proteins.

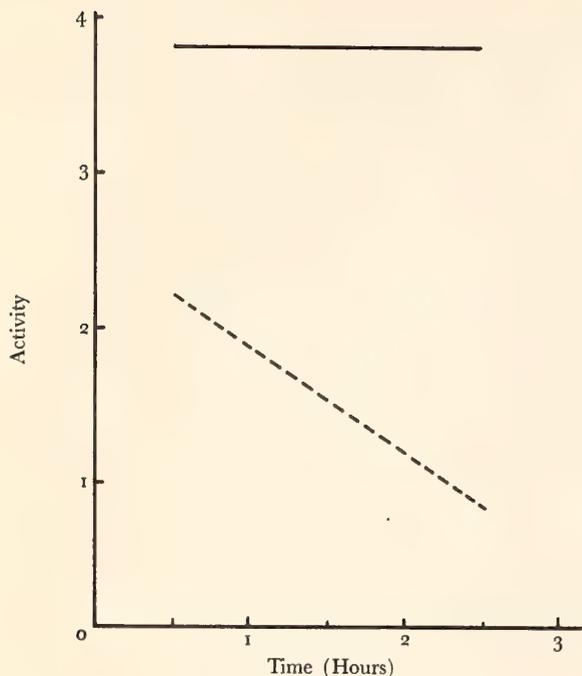


Figure 2

Inactivation of Trochophore-Stage Hexokinase During Storage at 0° C.

Activity expressed as  $10^{-11}$  moles TPNH/minute/milligram protein. The top line is with ovomucoid, the bottom without ovomucoid.

### SUMMARY

Activity of embryonic carbohydrases, hexokinase, glucose-6-phosphate dehydrogenase, and isocitrate dehydrogenase were analyzed fluorometrically in the unfertilized eggs, trochophores, and veligers of the limpet *Acmaea*

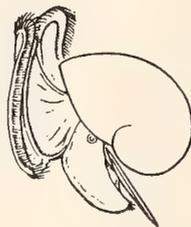
*scutum*. Glucose-6-phosphate dehydrogenase and isocitrate dehydrogenase activity was constant at all stages. Hexokinase activity increased almost threefold between the trochophore and veliger stage. No maltase, galactosidase, or amylase activity was detectable at any stage. A laminarinase type enzyme, capable of degrading algal laminarin to free glucose was found in all embryonic stages. The role of laminarinase in unfertilized eggs is puzzling since the substrate for this enzyme is not available until feeding begins at the veliger stage.

### ACKNOWLEDGMENTS

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# Nitrogen Excretory Products in the Limpet *Acmaea*

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(1 Text figure; 2 Tables)

## INTRODUCTION

EXAMINATION OF EXCRETORY PRODUCTS in the mollusks indicates that more terrestrial forms tend to be uricotelic and aquatic forms tend to be ammonotelic. This is especially evident in NEEDHAM's (1935) classic study of the littorinid snails where an ascending order of uric acid production was found to be directly correlated with increasing height in the intertidal zone. In this regard, members of the genus *Acmaea* also present an interesting continuum. On the Monterey Peninsula, populations of the five common species of this genus are found varying from +0.0 feet to +5.0 feet. To see if any correlations could be found between intertidal zonation and nitrogen excretory patterns, a comparative analysis of excretory products in this genus was made.

## METHODS AND MATERIALS

The five species of *Acmaea* used in this investigation were *A. digitalis* ESCHSCHOLTZ, 1833; *A. limatula* CARPENTER, 1864; *A. scutum* ESCHSCHOLTZ, 1833; *A. pelta* ESCHSCHOLTZ, 1833; and *A. scabra* (GOULD, 1846). All species were collected from the intertidal area at periods of low tide on Pescadero Point in Monterey County, California.

The same collection procedure was followed for all animals studied. Each animal was removed from the rock substratum, with care to avoid loss of fluid from the mantle cavity, and placed into a capped glass vial containing 5 ml of artificial sea water (HARVEY, 1954). The animals were kept in the vials at sea water temperature for 2 to 3 hours. The artificial sea water (ASW hereafter) was decanted into a graduated centrifuge tube, the limpet removed, and each vial rinsed with 2 ml of ASW. Each animal was then gently squeezed, anterior end

down over the centrifuge tube, to empty the mantle cavity of any remaining fluid. The pooled fluid was brought to 10 ml with ASW, centrifuged to remove any particulate matter, and the supernatant decanted and frozen until used for analyses.

Total nitrogen, ammonia nitrogen, and urea nitrogen were assayed by the microdiffusion method of TERNBERG (1964). The total nitrogen was determined on a 1 ml sample after wet ashing with 1 ml of sulfuric acid saturated with copper sulfate. Ashed samples were diluted with 5 ml of distilled water, and 4 ml of 10 *M* sodium hydroxide were substituted for the sodium carbonate solution used in TERNBERG's original method.

Urea was determined as ammonia after a 30 minute incubation with 0.1 ml of a 50 mg per ml solution of urease (obtained from Matheson, Colman, and Bell) in distilled water. Uric acid was determined colorimetrically by the method of SOBRINHO-SIMÕES (1965). Prior removal of proteins was found to be unnecessary.

Gaseous excretion of ammonia was determined by placing the intact animal into a dry diffusion apparatus for 24 hours. Gaseous ammonia was collected by absorption on alkaline filter paper, and analyzed for ammonia by TERNBERG's procedure.

The microdiffusion assay procedure was calibrated with ammonium sulfate solutions of known concentrations and found to be extremely reproducible (Figure 1) with triplicate and duplicate determinations on the same sample revealing small standard deviations (e.g., *Acmaea scutum* NH<sub>3</sub> - N, 7.0 ± 0.25 μg).

## RESULTS

Ammonia, urea, and uric acid were found to be excretion products in all species of *Acmaea* investigated. Some species showed wider individual variations in amounts of certain excretory products than other species. For example, the values for ammonia nitrogen in *A. digitalis*

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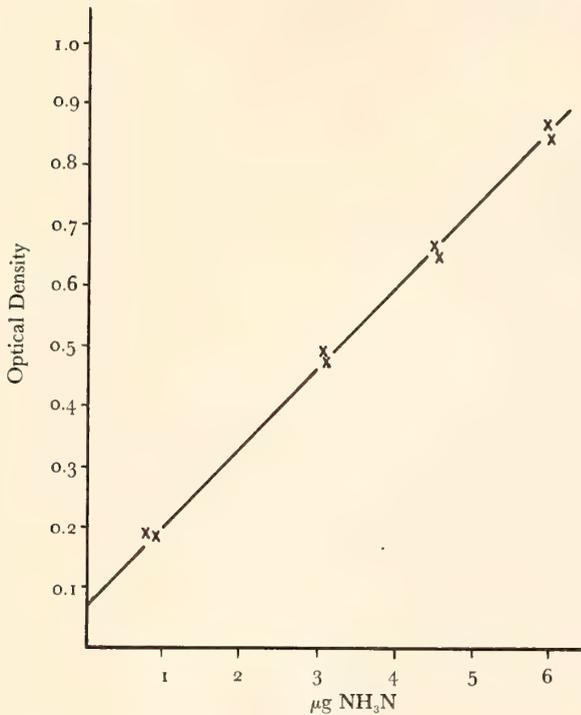


Figure 1

Calibration of diffusion apparatus with ammonium sulfate solutions using optical density as a measure of  $\mu\text{g}$  concentration

ranged from 0.4 to 25  $\mu\text{g}$  N, whereas for *A. scutum* the range was much less, varying from 6 to 10  $\mu\text{g}$  N (Table 1). In terms of individual variation the greatest variability in ammonia nitrogen was found in *A. digitalis*, followed by *A. scabra*, *A. pelta*, *A. limatula*, and *A. scutum*.

Variation in uric acid was greatest in *A. pelta*, followed by *A. scabra*, *A. limatula*, *A. scutum*, and *A. digitalis*.

The percentage distribution of the various nitrogenous compounds, based upon mean values, is shown in Table 2. The comparison between the five species indicates that relatively more ammonia is excreted by *Acmaea digitalis* and *A. scabra*, that less uric acid is excreted by *A. digitalis*, and that *A. scabra* and *A. scutum* excrete less urea. It can also be seen that greater than 80% of the total non-protein nitrogen can be accounted for by these methods.

Gaseous ammonia was found to be excreted in the five species of *Acmaea* examined with the dry diffusion technique described above. Those forms found higher in the intertidal zone, *A. scabra* and *A. digitalis*, produced more of the gaseous product than the lower intertidal forms, *A. scutum*, *A. limatula*, and *A. pelta*. The mean value after 24 hours was 2.9  $\mu\text{g}$  NH<sub>3</sub>-N for the higher species and 2.0  $\mu\text{g}$  NH<sub>3</sub>-N for the lower ones.

## DISCUSSION

The microdiffusion and colorimetric techniques, coupled with the collection procedure, have provided a means of analysis of individual specimens of the genus *Acmaea*. The extreme standard deviations obtained (e. g., in *A. digitalis*, NH<sub>3</sub>-N  $8.9 \pm 8.3\mu\text{g}$ ) indicate very large individual differences. The deviations did not result from the assay procedure, which, as previously indicated, was highly reproducible. These maximal and minimal components of variability, only seen in comparing individuals, would be masked by methods that measure pooled material. In particular, these results demonstrate the value of individual analysis. Although average values can be

Table 1

Relative  $\mu\text{g}$  Nitrogen Excretory Products  
with Standard Deviations for Eight Animals for Each Species.  
Rest nitrogen is the difference between the total nitrogen and the  
sum of the three products.

Nitrogenous Excretory Products in <i>Acmaea</i>					
	<i>Acmaea scabra</i>	<i>Acmaea digitalis</i>	<i>Acmaea limatula</i>	<i>Acmaea scutum</i>	<i>Acmaea pelta</i>
NH <sub>3</sub> N	10.6 $\pm$ 6.3	8.9 $\pm$ 8.3	8.5 $\pm$ 3.9	7.5 $\pm$ 1.8	9.25 $\pm$ 4.6
Urea N	3.75 $\pm$ 3.2	5.9 $\pm$ 4.2	7.3 $\pm$ 5.2	4.6 $\pm$ 1.9	11.1 $\pm$ 4.2
Uric Acid N	14.6 $\pm$ 4.9	7.9 $\pm$ 1.7	14.7 $\pm$ 4.3	13.3 $\pm$ 2.9	17.8 $\pm$ 6.7
Rest N	5.0 $\pm$ 3.5	5.0 $\pm$ 2.9	2.8 $\pm$ 2.7	5.7 $\pm$ 4.2	5.75 $\pm$ 4.7
Total N	33.0 $\pm$ 7.9	27.8 $\pm$ 7.1	33.3 $\pm$ 8.2	32.0 $\pm$ 7.3	43.9 $\pm$ 9.1

Table 2  
Percentage Excretory Products Calculated From Mean Values  
of Table 1

	Percentage Distribution of Nitrogen Products				
	<i>Acmaea scabra</i>	<i>Acmaea digitalis</i>	<i>Acmaea limatula</i>	<i>Acmaea scutum</i>	<i>Acmaea pelta</i>
NH <sub>3</sub> N	32	32	25.5	23.5	21
Urea N	11.4	21	22	14.5	25.5
Uric Acid N	44.5	28.5	44.5	42.5	40.5
Rest N	12	18.5	8	19.5	13
Total N	100	100	100	100	100

obtained, as in Table 2, the interspecific differences observed might therefore be misleading.

Several hypotheses can be advanced to explain the large individual variation in the relative amounts of excretory nitrogen. One is that enzyme content varies within the population, resulting in different distributions of excretory nitrogen products when comparing individuals. This is especially suggested by WILLIAM's (1966) work on biochemical variation within individuals of the same species. Another explanation is that individual variations in the diet would result in the observed distribution of nitrogen products. A third possibility is that the limpet changes its major products of nitrogen excretion as a result of exposure to air during the tidal cycle. This is suggested by NEEDHAM's (1935) proposal that more terrestrial animals tend to excrete uric acid, whereas more aquatic forms tend to excrete ammonia. This hypothesis is also suggested by the larger variations in the higher forms, which would have been exposed to longer, and more variable, periods of dryness. If correct, this could suggest a unique adaptation of the higher forms to secrete more uric acid when dry than when wet. Such a transition would be of adaptive value, since ammonia and urea at high concentrations are toxic.

Although urea was found to be excreted by all species examined, the metabolic mechanism of its formation is not clear. CAMPBELL (1966) found no arginase in the digestive gland of *Acmaea* spp., suggesting the absence of the ornithine cycle in this species. However, CAMPBELL only examined the digestive gland, leaving open the possibility that the enzyme might be present in other tissues. While his results possibly eliminate the ornithine cycle, other pathways based upon purine degradation could be alternative mechanisms for urea production. For example, in fishes, products of purine breakdown from uric acid give rise to urea via the enzyme allantoicase (LASKOWSKI, 1951). This pathway is also suggested by preliminary

experiments indicating formation of urea by minced digestive gland in an ASW-uric acid solution.

The finding that all three products are present, and the reported absence of arginase, suggests the possibility that the source of ammonia nitrogen is by protein catabolism, whereas the urea and uric acid products might result from purine catabolism.

The nature of the "rest" nitrogen is not clear. Qualitative tests with 0.25% ninhydrin solution show the presence of peptides and amino acids in the samples from the limpets. This may account for some of the 13-18% unidentified nitrogen.

## SUMMARY

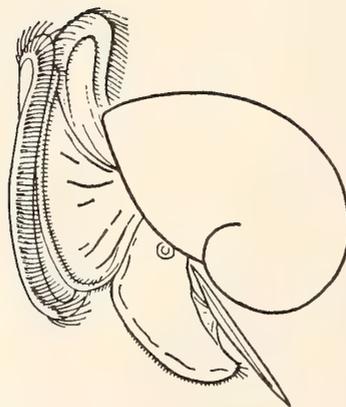
Nitrogen excretory products were examined in the genus *Acmaea* by a microdiffusion technique for urea and ammonia and a colorimetric technique for uric acid. Five species were examined, varying in vertical distribution. Similar concentrations of ammonia, urea, and uric acid were found in all five species. A large amount of intraspecific variation in the distribution of these compounds was noted, especially in the high intertidal forms *A. scabra* and *A. digitalis*. These variations might reflect individual differences in enzyme activity, individual differences in diet, or adaptive responses to the environment.

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