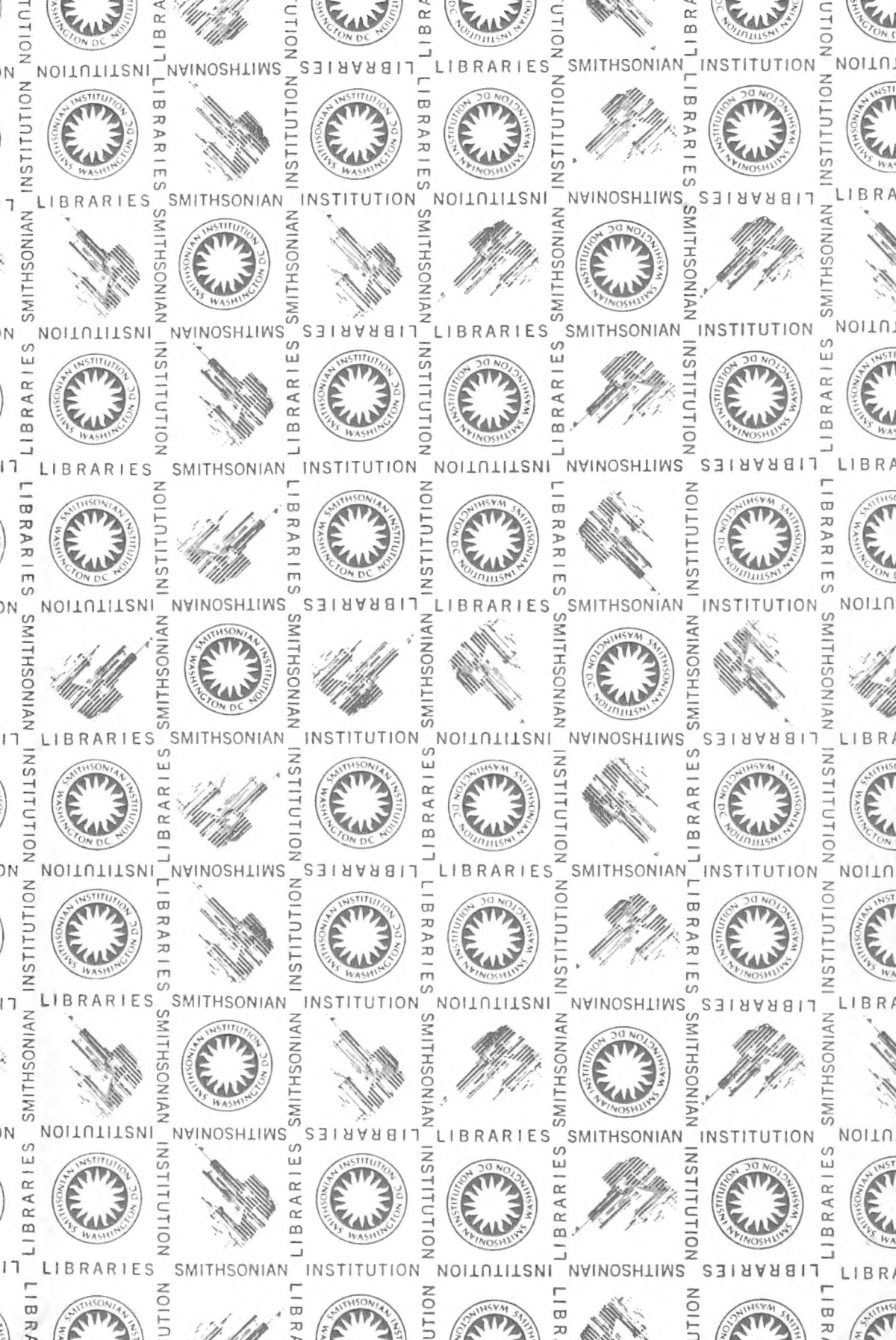
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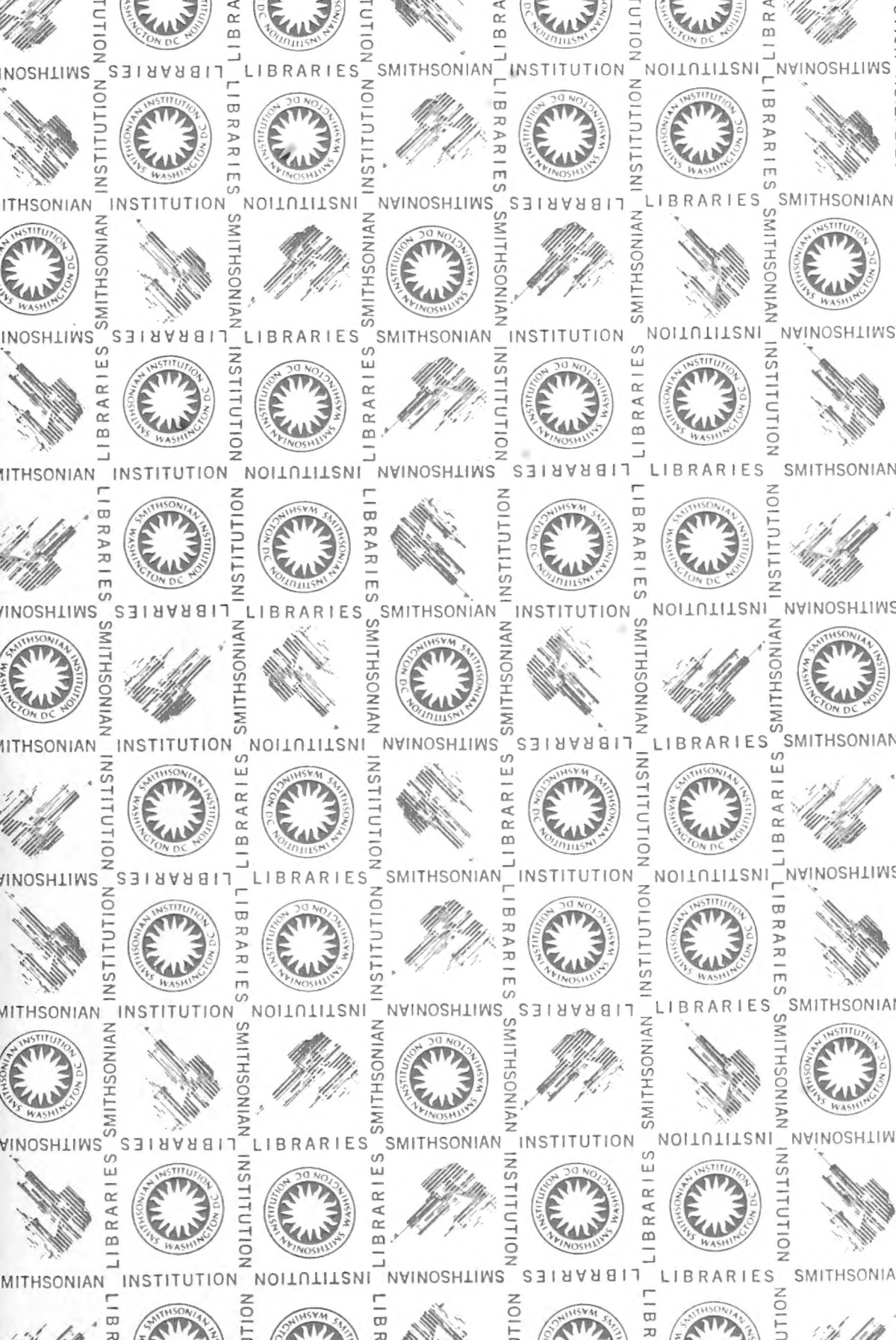
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AMERICAN MALACOLOGICAL UNION SYMPOSIUM PROCEEDINGS
INTERNATIONAL SYMPOSIUM ON LIFE HISTORY
SYSTEMATICS AND ZOOGEOGRAPHY OF CEPHALOPODS
IN HONOR OF S. STILLMAN BERRY
Organized and Edited by Roger T. Hanlon

1988

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INTRODUCTION TO THE INTERNATIONAL SYMPOSIUM ON LIFE HISTORY, SYSTEMATICS AND ZOOGEOGRAPHY OF CEPHALOPODS

IN HONOR OF S. STILLMAN BERRY

2–5 July 1987
Monterey, California

Roger T. Hanlon, Organizer and Editor

Marine Biomedical Institute and Department of Psychiatry and Behavioral Sciences, University of Texas Medical Branch, 200 University Boulevard, Galveston, Texas 77550-2772, U.S.A.

S. Stillman Berry may have liked this symposium. The broad title attracted a wide spectrum of researchers and students who reported on many facets of cephalopod biology that revolve around life history, systematics and zoogeography. Curiously, there have never been symposia on the latter two subjects despite their obvious importance and their long history of publication. Perhaps the greatest benefit of such a gathering is that there is vigorous exchange between those who work primarily on live animals and those who work on dead specimens. This is a necessary enterprise and, among cephalopod workers, it is conducted in a relaxed and fun manner and has led to fruitful collaborations.

Research on cephalopods is flourishing. This is evident from several recent books and symposia (e.g. Roper, 1982; Unipub, 1982; Boyle, 1983, 1987; Caddy, 1983; Roper, Lu & Hochberg, 1983; Clark, 1984; Roper, Sweeney & Nauen, 1984; Mangold & Boletzky, 1985). In the Monterey symposium there were 38 presentations on cephalopods, 22 of which are reported herein and 11 of which appeared as abstracts in Volume 4(2) (1986) of the *American Malacological Bulletin.* Attendees hailed from Asia, Europe and North and South America. Although there are only 50 or so full-time cephalopod specialists worldwide, a reasonably large and growing number of scientists (a few hundred) are studying the group. The reasons are varied, but include (1) the increased interest in cephalopods as human food due to a general decrease in finfish, crustacean and other molluscan fisheries worldwide and (2) the usefulness of cephalopods in experimental biology. This resurgence in cephalopod research has led to the establishment in 1983 of the Cephalopod International Advisory Council (CIAC) whose principal aims are "to stimulate, speed up and influence the direction of cephalopod research, to provide help and advice on aspects of cephalopod biology including those relevant to the management of fisheries and to spread information on past and current research." This symposium was co-sponsored by CIAC and the American Malacological Union (AMU) and was held during the 57th Annual Meeting of AMU.

A critical review process was undertaken for each paper in this volume. On average, three anonymous referees (both within and outside of cephalopod research) were consulted for each submission. The resulting revisions improved the quality of the publications and I thank the reviewers for their diligence and precise, constructive criticisms.

I also thank the American Malacological Union, particularly Jim Nybakken (President), for co-sponsoring this symposium, and George Davis and Robert Robertson for arranging publication in *Malacologia.* The rapporteurs performed a commendable job and I thank Joanne Hollyfield and Laura Koppe for clerical assistance before and after the meeting. Finally, I appreciate the efforts of Bill Gilly, Judy Thompson, Lynn Mather and a host of others for the salmon and squid cookout at Hopkins Marine Laboratory and a lasting memory that night of an ad hoc group dissecting a frozen 5-ft. *Moroteuthis robusta* after the cookout. Dr. Berry would have approved!

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MALACOLOGIA, 1988, 29(1): 3–5

IN THE SPIRIT OF S. STILLMAN BERRY

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Why is it appropriate to honor S. Stillman Berry (Fig. 1) with an international symposium on cephalopods? The answers are as diverse as the man. We all know of S. Stillman Berry because he was a productive teuthologist for over half a century and many of his publications are considered classics in the field. In his first publication on cephalopods in 1909, he described 1 new genus and 7 new species from the Hawaiian Islands. In total, Berry described 70 new species and 13 new genera and subgenera of cephalopods (Sweeney, Roper & Hochberg, 1988). His last cephalopod publication, which appeared in 1963, discussed the *"Doratopsis"* larval stage in the life history of the mesopelagic squid, *Chiroteuthis*, from California waters. Berry's last paper, although not published, was presented at the joint AMU/WSM meeting held in San Diego in 1975. The topic was the systematics, distribution and life history of the pelagic octopus, *Ocythoe*. These firsts and lasts in Berry's cephalopod work addressed many of the topics that will be covered in this symposium. However, Berry's cephalopod research certainly was not limited to systematics, zoogeography and life history any more than the papers to be presented here are limited to these topics. For example, S. S. Berry worked extensively on bioluminescence in cephalopods in the teens and early 1920s.

Berry's work on cephalopods covered most zoogeographic zones and oceans of the world and included major monographic works on cephalopods from the eastern Pacific, Hawaii, Japan, Australia and Antarctica. A number of the species being discussed during this symposium either were described as new species by Berry or knowledge about them was expanded greatly by his research.

Not only is it appropriate to honor S. S. Berry with a symposium on cephalopods, it is most fitting it be held in Monterey. Four of the first five papers Berry published were about the molluscan fauna of Monterey Bay. These were published in the *Nautilus* in 1907, when Berry was 20 years old. Furthermore, for decades Monterey Bay has been the center of the fishery on the squid *Loligo opalescens*, which Berry described as a new species in 1911. In addition, S. S. Berry studied for his Bachelor and Ph.D. degrees just a few miles from here at Stanford University.

For some diversity in his educational experience, Berry took his Master's degree at Harvard University. While back east Stillman was fortunate to meet at Yale the pioneer in North American cephalopods, A. E. Verrill.

As a further indication of the man's diversity, it is interesting that only about one-fourth of Berry's 209 zoological publications dealt with cephalopods. An association test given to a cross-section of malacologists would elicit responses such as chitons, West American land snails, fossil mollusks and Eastern Pacific marine gastropods and bivalves. Other zoologists might recall sparrows, magpies and beavers, for he published on these groups as well. Berry's other careers as a bibliophile, horticulturist, genealogist and rancher are noted in a number of other publications (e.g. Brookshire, 1984; Coan, 1984; Hochberg, 1985; Roper, 1984; Sweeney & Roper, 1984). Berry's publication record of nearly 50 papers and monographs on cephalopods is indeed impressive. Equally impressive, and perhaps tragic, were the many substantial yet unfinished manuscripts found in his house in Redlands following his death. On the envelope of each was a notation of what was needed for completion: 1 or 2 illustrations or a reference to an ancient, obscure paper. The man was thorough and precise. For example, Berry had completed, save for a few illustrations, a monograph on Philippine cephalopods in the late 1920's, 40 years before that fauna eventually was monographed by Gil Voss in 1963. Number 3 in his series on "Light Production in Cephalopods" was nearly ready to submit in the 20's as well. Unpublished monographs on Australian sepiids and octopods hold the answers to a number of systematic and zoogeographic questions that remain unanswered in print even today. An additional large number of partial manuscripts and notes were found

FIG. 1. S. Stillman Berry with a specimen of the squid *Moroteuthis robustus* (Verrill, 1876) on the front walkway of his home in Redlands, CA, 5 January 1964. (Photographer unknown; photograph courtesy of S. S. Berry Estate)

in various stages of completion. Perhaps there is a lesson for all of us in these unfinished manuscripts, and even today we can learn from Berry's legacy.

So we honor S. S. Berry for his published works and his unpublished works. We honor him because he is a direct link with all the teuthologists of the first half of the 20th Century, e.g. Hoyle, Joubin, Chun, Naef, Verrill, Grimpe, Robson, Taki, Adam and others, all of whom he corresponded with more or less regularly. We honor him because he shared his encyclopedic knowledge with generations of malacologists. He encouraged and inspired them and us to pursue research in the fascinating phylum Mollusca.

Finally, S. S. Berry viewed diversity as a biological phenomenon that enriches the living world. Diversity certainly enriched and typified his own life and work. He would be very proud and pleased that this symposium in his honor is so enriched by the diversity of topics about his favorite group of animals, the cephalopods.

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MALACOLOGIA, 1988, 29(1): 7–19

A CATALOG OF THE TYPE SPECIMENS OF RECENT CEPHALOPODA DESCRIBED BY S. STILLMAN BERRY

Michael J. Sweeney[1], Clyde F. E. Roper[1] & F. G. Hochberg[2]

ABSTRACT

The primary type specimens of Recent cephalopods described by S. Stillman Berry (1887–1984) have been traced and the museums in which they are deposited verified. Specimen data, collection data and museum catalog numbers are given for all specimens. Specimens known to be no longer extant and those types that could not be located are noted. A bibliography of Berry's cephalopod publications is included.

Key words: cephalopods, type specimens, museum(s), collections.

INTRODUCTION

S. Stillman Berry's contributions to cephalopod systematics rank as the most important of any of the past American teuthologists. Berry studied cephalopods and other mollusks for over 75 years. While publishing 47 papers on cephalopods, he introduced 105 new names, 70 of which were new species. This is a significant contribution to cephalopod systematics for someone never employed for research at an academic institution! Brookshire (1984), Coan (1984) and Roper (1984) provided biographies of S. Stillman Berry, and Sweeney & Roper (1984) listed all zoological taxa described by Berry.

The cephalopod types described by Berry were deposited in several museums as well as his own home, a virtual museum itself. The extent and physical condition of many of the type specimens kept in his home were unknown prior to his death, because during his lifetime he accumulated a huge collection of specimens that eventually occupied nearly every available space in his 17-room house, from cellar to attic and even in his garden shed. While a few types were kept in a small, alligator leather satchel next to the front door ("in case of fire"), most were scattered among the general collection. Curatorial attention to the collections became inconsistent and infrequent as the collections grew and with Berry's generally fragile health. With the bequest of the Berry cephalopod collection to the National Museum of Natural History, a thorough search for type material produced a number of specimens for which Berry had designated no museum depository.

This paper summarizes the status of Berry's Recent cephalopod type material from his collection as well as from all known museum repositories. The literature was reviewed to determine the number and location of type specimens designated by Berry. The museums that he published as recipients for his types were contacted to verify deposition.

While examining the literature, several problems arose concerning the type status and designation of specimens from Berry's collection. In several cases, paratypes were not listed in the original publication but were in subsequent papers. In other cases, no paratypes were listed in print but specimens were found labeled as such in Berry's handwriting. We were fortunate to have Berry's card catalog that listed specimen data, collecting data, type status and the date of examination for all specimens. Inconsistencies in type status from published vs. specimen information were resolved on an individual basis. Questions concerning interpretations of the International Code of Zoological Nomenclature (ICZN, 1985) were taken to Dr. F. M. Bayer, National Museum of Natural History, who is a U.S. member of the International Commission on Zoological Nomenclature.

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TABLE 1. Location of extant S.S. Berry cephalopod type material. Abbreviations: H = holotype; P = paratype; S = syntype.

Species, genus	Catalog no.	Type	Not illustrated
A. National Museum of Natural History (NMNH).			
alecto, Octopus	815707	H	*
alecto, Octopus	815708	P	
alecto, Octopus	816381	P	
astrolineata, Abralia	816428	H	
astrosticta, Abralia	214313	H	
australis, Rossia	815719	P	
austrinum, Sepiadarium	816456	P	
californiana, Opisthoteuthis	816426	P	
chiroctes, Loliolopsis	815722	H	
chiroctes, Loliolopsis	815723	P	
compacta, Teleoteuthis	214381	H	
corona, Teuthowenia	338695	H	
diegensis, Rossia pacifica	214376	S	
ecthambus, Sandalops	338697	H	
etheridgei, Loligo	816590	P	
fisheri, Helicocranchia	214316	H	
fitchi, Octopus	815713	H	*
fitchi, Octopus	815715	P	
fitchi, Octopus	816591	P	
fitchi, Octopus	815714	P	
galaxias, Enoploteuthis	816352	P	
galaxias, Enoploteuthis	816355	P	
gilbertianus, Polypus	214320	H	
globula, Cranchia	214315	H	
harrissoni, Moschites	815724	P	
hastula, Chiroteuthoides	338693	H	*
hawaiiensis, Ommastrephes	214382	H	
hawaiiensis, Stephanoteuthis	214311	H	
heathi, Eledonella	214318	H	
hedleyi, Sepia	815720	P	
hoylei, Polypus	214310	H	*
hubbsorum, Octopus	816360	H	
hubbsorum, Octopus	816480	P	
hubbsorum, Octopus	816400	P	
kermadecensis, Polypus	816461	H	
lemur, Pyrgopsis	338698	H	
lugubris, Laetmoteuthis	214385	H	
macrope, Cirroteuthis	214317	H	
micropyrsus, Octopus	815704	H	*
micropyrsus, Octopus	815706	P	
micropyrsus, Octopus	815705	P	
nipponianum, Sepiadarium	816462	H	
nipponianum, Sepiadarium	816359	P	
oliveri, Polypus	816455	H	
opalescens, Loligo	214388	P	
opalescens, Loligo	816384	P	
pardus, Megalocranchia	816465	H	
pathopsis, Sandalops	338696	H	
pathopsis, Sandalops	729182	P	
penicillifer, Octopus	815717	H	*
persephone, Opisthoteuthis	816361	P	
phyllura, Galiteuthis	214325	H	
pluto, Opisthoteuthis	815718	P	
pricei, Polypus	214680	P	
regalis, Nematolampas	815721	H	
regalis, Nematolampas	816592	P	
rubescens, Octopus	815709	H	*

TABLE 1 (Continued).

Species, genus	Catalog no.	Type	Not illustrated
A. National Museum of Natural History (NMNH) (Continued).			
rubescens, Octopus	815710	P	
rubescens, Octopus	815711	P	
rubescens, Octopus	815712	P	
scintillans, Abraliopsis	816498	P	
scolopes, Euprymna	214380	H	
scolopes, Euprymna	727393	P	
scorpio, Polypus	338699	H	
spinicauda, Enoptroteuthis	338694	H	
veligero, Octopus	815716	H	*
veligero, Octopus	816432	P	
B. California Academy of Sciences (CASIZ).			
apollyon, Polypus	021808	P	
californiana, Opisthoteuthis	021666	H	
californicus, Polypus	017971	P	*
diegensis, Rossia pacifica	021807	S	
formosana, Sepia	021668	H	
gilbertianus, Polypus	021806	P	
globula, Cranchia	017978	P	
heteropsis, Calliteuthis	029130	P	
magister, Gonatus	017965	H	
nipponensis, Stoloteuthis	017976	H	
oliveri, Polypus	021805	P	
opalescens, Loligo	017970	H	
pacifica, Rossia	018796	P	
panamemsis, Lolliguncula	017968	H	
panamemsis, Lolliguncula	017969	P	
pricei, Polypus	017966	H	
pricei, Polypus	017967	P	
scintillans, Abraliopsis	021667	H	
scolopes, Euprymna	017975	P	
C. Australian Museum Sydney (AMS).			
adelieana, Moschites	C40889	H	
albida, Moschites	C40888	H	
aurorae, Moschites	C40891	H	
australis, Rossia	C148246	H	
dannevigi, Sepia	C148249	H	
etheridgei, Loligo	C148250	H	
galaxias, Enoploteuthis	C148251	H	
harrissoni, Moschites	C40892	H	
harrissoni, Moschites	C40893	P	
hedleyi, Sepia	C148252	H	
mawsoni, Stauroteuthis	C40886	H	
persephone, Opisthoteuthis	C148253	H	
D. South Australian Museum (SAM).			
austrinum, Sepiadarium	D17493	H	*
austrinum, Sepiadarium	D17494	P	
nipponianum, Sepiadarium	D17496	P	
notoides, Idiosepius	D17495	H	
E. San Diego Natural History Museum (SDNHM).			
chiroctes, Loliolopsis	49460	P	

The key portion of the Code required to resolve the paratype question was Art. 74(b)(i) pertaining to the type series. In a situation where Berry published a new species and listed several specimens, one of which he designated type (holotype), the fact that he gave the remaining specimens no type status excludes them from the type series and therefore they cannot be listed as paratypes, even though they may have been examined along with the original material at the time of description. In most species descriptions both holotype and paratype (often listed as type and cotype) were designated clearly. An exception to this is where Berry listed a paratype in a subsequent, but not in the original, publication. The fact that a specimen bears a label in Berry's handwriting identifying it as a paratype *and* that his card catalog shows that the specimen was used in the species description (examination date, illustration notes, etc.) meets the requirements for the Code's definition of type series (Art. 72(a)(i)&(v)). A specimen (not designated in print at any time as a type specimen) found labeled as a paratype *and* shown to be used in the original description (supported by his card catalog) is justified in the Code in the same manner. This interpretation of the new code has changed the status of several Berry specimens published previously as types in Smith (1974), Roper & Sweeney (1978) and Zeidler (1983). These changes are noted under the pertinent species.

The format for the presentation of data for type-specimens is similar to that used in Roper & Sweeney (1978) as follows: Species, genus, author, year of publication. Publication citation for that species. Category(ies) of type(s), number and sex of specimens, dorsal mantle length in mm, reference to original illustration(s) of type(s), S. Stillman Berry catalog number (e.g., SSB 438), depository catalog number, collector (ship or person) and station number, locality (latitude and longitude or descriptive location, occasionally with compass bearing and distance in nautical miles ('')), sampling gear, depth collected (in original unit and converted to m), date collected. Comments.

Berry's published mantle lengths for octopods were measured as "length of body to base of dorsal arms." Therefore, his measurements are not equivalent to the currently defined octopod mantle length as given in Roper & Voss (1983). Where mantle length or sex of specimens were not indicated in print by Berry, we have determined sex and measured the specimen as defined by Roper & Voss (1983). Specimens that are too immature to distinguish sex are listed as juveniles (juv.). These recently determined, additional elements of data are designated by an asterisk (*).

Several phrases are used repeatedly throughout this catalog and require definition:

A. "Specimen is no longer extant." Where type specimens originally deposited at the NMNH are known to have been lost through desiccation or have not been located. Unfortunately, some of Berry's "Albatross" types were among material lost during the long period (early 20th century until 1962) when the molluscan collections preserved in alcohol were housed apart from the shelled (dry) molluscs. Also tragically, approximately 30% of the cephalopod type specimens retained at home by Berry were found to have dried out. An attempt has been made to reconstitute these specimens using a commercial wetting/penetrating agent, and in general some softening of the specimens has been achieved.

B. "Specimen was unique." Only one specimen was available at the time of the species description.

C. "No paratypes designated." No paratypes were designated from material on hand at the time of the species description in the original publication. In several instances, e.g. *Polypus californicus* and *Loligo opalescens*, Berry did not list a paratype in the original publication but did so in a subsequent paper. Specimen and collecting information given in the subsequent publication *plus* data from his specimen card catalog verifies that he had the specimen in question at hand and used it in the description of the species. Prior to approximately 1915 Berry used the designation "cotype" to indicate what we now term a paratype.

D. "Location of specimens unknown." In several cases, the museum that Berry selected for deposition of a type specimen is known, but the museum representative we contacted has been unable to locate and verify the specimen. In these instances we have used this phrase followed by the name of the author who cited the collection involved.

E. "Deposition of specimen unknown." Several type specimens were published with no museum deposition, nor were they located in any of the museums that contain Berry cephalopod type material.

Table 1 lists all of Berry's extant cepha-

lopod types that we were able to locate and the museums in which they are deposited. Those species that have not been illustrated are noted. A number of the octopod species described by Berry in his journal, *Leaflets in Malacology,* were not illustrated. These are being illustrated in a review of the octopods of the eastern North Pacific by F. G. Hochberg.

The California Academy of Sciences recently changed to a 6-digit catalog numbering system for all its holdings (Dunn, 1981). Berry's types deposited at CAS were listed by Smith (1974) under the original 3-digit catalog numbers; these are indicated here in brackets for ease of tracing specimens.

Abbreviations used for institutions that are depositories of Berry's cephalopod types are as follows:

AMS—Department of Malacology, Australian Museum, 6-8 College Street, Sydney, N.S.W., Australia 2000. CASIZ—Department of Invertebrate Zoology, California Academy of Sciences, Golden Gate Park, San Francisco, CA 94118, U.S.A. SAM—Department of Marine Invertebrates, South Australian Museum, Adelaide, S.A., Australia 5000. SDNHM—Department of Marine Invertebrates, San Diego Natural History Museum, Balboa Park, San Diego, CA 92112, U.S.A. USNM—Division of Mollusks, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560, U.S.A.

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CEPHALOPOD TYPE SPECIMENS OF S. S. BERRY

adelieana, Moschites Berry, 1917. *Sci. Rep. Australasian Ant. Exped.,* C4(2): 17–20. HOLOTYPE: 1♀, 38 mm, text figs. 10–13, pl. 11 fig. 5, pl. 12 figs. 6 and 7, SSB 438, AMS C40889, "Aurora" sta. 2, Antarctic Ocean, Adelie Land, off Mertz Glacier, 66°55′S 145°21′E, trawl, 288–300 fm (526–548 m), 28 December 1913. No paratypes designated.

albida, Moschites Berry, 1917. *Sci. Rep. Australasian Ant. Exped.,* C4(2): 15–17. HOLOTYPE: 1♀, 45 mm, text figs. 6–9, pl. 10 figs. 2 and 3, pl. 11 fig. 4, SSB 436, AMS C40888, "Aurora" sta. 5, Antarctic Ocean, off Wilkes' Land, 64°34′S 127°17′E, trawl, 1700 fm (3107 m), 14 January 1914. Specimen was unique.

alecto, Octopus Berry, 1953. *Leaf. Malac.*, 1(10): 56–57. HOLOTYPE: 1♂, 14 mm, SSB 896, USNM 815707, B. W. Walker *et al.* sta. W52-12, Pacific Ocean, Mexico, Sonora, S of Estero Soldado, 27 January 1952. PARATYPE: 1♀, 22* mm, SSB 922, USNM 815708, from same lot as holotype. PARATYPE: 1♂, 9* mm, SSB 924, USNM 816381, B. W. Walker *et al.* sta. W52-14, from same locality as holotype, 28 January 1952. Specimen has dried and is in very poor condition. This species has not been figured.

apollyon, Polypus Berry, 1912. *Bull. Bur. Fish.*, 30(1910): 284. HOLOTYPE: 1♂, 81 mm, pl. 35 fig. 3, pl. 36 fig. 1, pl. 39 fig. 4, SSB 142, USNM 214319, "Albatross", Pacific Ocean, Alaska, Kadiak Island (misspelling of Kodiak), Uyak Bay, seine, 14 August 1903. Specimen is no longer extant. PARATYPE: 1♀, 76 mm, SSB 145, CASIZ 021808 [461], from same lot as holotype. Specimen was designated as a "cotype" by Berry both in his card catalog and on the specimen label at the time of the species description.

astrolineata, Abralia Berry, 1914. *Trans. N.Z. Inst.*, 46: 145–148. HOLOTYPE: 1♀, 34 mm, pl. 10, SSB 408, USNM 816428, R. S. Bell, Pacific Ocean, Kermadec Islands, Sunday Island, on beach. Specimen has dried and is in poor condition. Specimen was unique.

astrosticta, Abralia Berry, 1909. *Proc. U.S. Natl. Mus.*, 37(1713): 412–414. HOLOTYPE: 1♀, 34 mm, figs. 4–7, SSB 171, USNM 214313, "Albatross" sta. 4122, Pacific Ocean, Hawaii, off Oahu Island, 8 ft Blake trawl, 192–352 fm (350–643 m), 26 July 1902. Unique specimen has dried and is in very poor condition.

aurorae, Moschites Berry, 1917. *Sci. Rep. Australasian Ant. Exped.*, C4(2): 20–24. HOLOTYPE: 1♂, 36 mm, text figs. 14–20, pl. 12 fig. 9, pl. 13 figs. 1 and 2, SSB 437, AMS C40891, "Aurora" sta. 8, Antarctic Ocean, 66°08′S 94°17′E, dredge, 120 fm (219 m), 27 January 1914. No paratypes designated.

australis, Rossia (Austrorossia) Berry, 1918. *Biol. Res. Fish. Exp. "Endeavour"*, 4(5): 253–258. HOLOTYPE: 1♂, 32 mm, text fig. 43, pl. 69 figs. 3 and 4, pl. 70 figs. 2 and 5, SSB 538, AMS C148246, "Endeavour" E3636, Indian Ocean, Western Australia, Great Australian Bight, south of Eucla, —°—′S 130°50′E, 250–300 fm (457–548 m), 6 May 1913 (date from AMS Register Book). PARATYPE: 1♀, 50 mm, SSB 537, USNM 815719, "Endeavour" E3635, from same lot as holotype. PARATYPE: 1♀, 50 mm, text figs. 44–46, pl. 70 fig. 1, SSB 539, "Endeavour" E3637, from same lot as holotype. Location of specimen unknown; not listed by Rudman (1983) in AMS collections.

austrinum, Sepiadarium Berry, 1921. *Rec. S. Aust. Mus.*, 1(4): 354–355. HOLOTYPE: 1♂, 12 mm, SSB 716, SAM D17493, A. Zietz, South Australia, St. Vincent's Gulf, dredge, September 1885. PARATYPE: 1♂, 133 mm, SSB 717, USNM 816456, from same lot as holotype. PARATYPE: 1♀, 17 mm, SSB 718, SAM D17494, from same lot as holotype. SAM type material not located by Zeidler (1983); recently found in Berry collection and returned to SAM. Specimen was designated as a paratype by Berry both in his card catalog and on the specimen label at the time of the species description. This species has not been figured.

californiana, Opisthoteuthis Berry, 1949. *Leaf. Malac.*, 1(6): 23–26. HOLOTYPE: 1♀, 295 mm (total length), SSB 858, CASIZ 021666 [548], "M/V Andrew Jackson" (Capt. N. Franklin), Pacific Ocean, California, Humboldt County, NW by E of Eureka Bar, trawl, 188 fm (343 m), 25 April 1948. Illustrated in Berry, 1952, figs. 1–4. PARATYPE: 1♀, 340 mm (total length), SSB 859, USNM 816426, from same lot as holotype. Photograph in Berry, 1952, fig. 5.

californicus, Polypus Berry, 1911. *Proc. U.S. Natl. Mus.*, 40(1838): 590. HOLOTYPE: 1♂, 89 mm, SSB 131, USNM 214321a, "Albatross" sta. 4325, Pacific Ocean, California, San Diego County, Point La Jolla, off Soledad Hill, SE, 4.4 mi, 9 ft Tanner trawl, 275–292 fm (502–533 m), 8 March 1904. Specimen is no longer extant. PARATYPE: 1♂, SSB 131, USNM 214321b, from same lot as holotype. Sepcimen is no longer extant. PARATYPE: 1♂, 77* mm, SSB 131, CASIZ 017971 [460], from same lot as holotype. Paratypes designated by Berry, 1912c: 287. Original label on CASIZ lot and Berry's card catalog list this specimen as a "cotype." This species has not been figured.

chiroctes, Loliolopsis Berry, 1929. *Trans. S.D. Soc. Nat. Hist.*, 5(18): 267–278. HOLOTYPE: 1♂, 50 mm, pl. 32 fig. 1, SSB 851, USNM 815722, T. Craig, Pacific Ocean, Mexico, Baja California Sur, Puerto Escondido, entrance to inner bay, dipnet and night light, surface, 4 March 1928. PARATYPES: 3♂, 2♀, 45–61 mm, text figs. 1–9, pl. 32 fig. 2, pl. 33 figs. 3, 5 and 6, SSB 852, USNM 815723, from same lot as holotype. PARATYPES: 1♂, 1♀, 46 and 55 mm, pl. 33 figs. 1, 2, and 4, SSB 853, SDNHM 49460, from same lot as holotype.

chirotrema, Sepia Berry, 1918. *Biol. Res. Fish. Exp. "Endeavour"*, 4(5): 268–276. HOLOTYPE: 1♂, 183 mm, pl. 74 figs. 6–9, pls. 75 and 76, SSB 522, "Endeavour" E2459, Indian Ocean, South Australia, S of Kangaroo Island, January/February 1912. Specimen is no longer extant in AMS collections according to Rudman (1983). PARATYPE: 1♀, 150 mm, text figs. 60 and 61, SSB 523, "Endeavour" E2460, from same lot as holotype. Specimen is no longer extant in AMS collections according to Rudman (1983). Additional specimens (E2454, E3621, E3622) listed in Rudman (1983) as paratypes are actually "additional material."

compacta, Teleoteuthis Berry, 1913. *Proc. U.S. Natl. Mus.*, 45(1996): 565. HOLOTYPE: 1♀?, 21 mm, SSB 238, USNM 214381, "Albatross" sta. 3989, Pacific Ocean, Hawaii, off Kauai Island,

Hanamaula warehouse, S 33°, W 9.5′, 8 ft Tanner trawl, 385–500 fm (703–914 m), 11 June 1902. Specimen is unique, has dried and is in very poor condition, with only a portion remaining. Illustrated in Berry, 1914c, text fig. 32, pl. 52 figs. 4 and 5. No paratypes designated.

corona, Teuthowenia (Ascoteuthis) Berry, 1920. *Proc. U.S. Natl. Mus.*, 58(2335): 296. HOLOTYPE: 1 juv.*, 27 mm, pl. 16 fig. 7, SSB 618, USNM 338695, "Bache" sta. 10173, Atlantic Ocean, W of Bermuda, 32°27′N 68°22′W, plankton net, 0–100 m, 4 February 1914. Specimen has dried and is in poor condition. No paratypes designated.

dannevigi, Sepia Berry, 1918. *Biol. Res. Fish. Exp. "Endeavour"*, 4(5): 264–268. HOLOTYPE: 1♀, 82 mm, text figs. 51, 52, 54 and 55, pl. 73, pl. 74 figs. 1 and 2, SSB 493, AMS C148249, "Endeavour" E2466, Indian Ocean, South Australia, S of Kangaroo Island, area S of Investigator Strait, January/February 1912. PARATYPE: 1♀, 74 mm, SSB 492, AMS C148248, "Endeavour" E2465, same locality as holotype, date unknown. Location of specimen is unknown; not listed by Rudman (1983) in AMS collections.

diegensis, Rossia pacifica Berry, 1912. *Bull. Bur. Fish.*, 30(1910): 293. SYNTYPES: 1♂, 6♀, 12–31* mm, pl. 42 figs. 2–5, SSB 19, USNM 214376, "Albatross" sta. 4356, Pacific Ocean, California, San Diego County, Point Loma lighthouse, N 82°30′ E, 5.9 mi, 8 ft Tanner trawl, 120–131 fm (219–239 m), 15 March 1904. SYNTYPES: 1♂, 1♀, 24 and 29* mm, pl. 42, SSB 19, CASIZ 021807 [457 & 458], from same lot as other syntypes. In Berry's card catalog 2 specimens from the type series (SSB 19) are identified as "cotypes" (1♂, 1♀, 23 and 32.5 mm). These would normally be designated as paratypes. However, in light of errors of record and the failure to designate a holotype we are applying a syntype designation to the entire lot.

ecthambus, Sandalops Berry, 1920. *Proc. U.S. Natl. Mus.*, 58(2335): 297–298. HOLOTYPE: 1 juv.*, 22 mm, pl. 16 fig. 2, SSB 627, USNM 338697, "Bache" sta. 10208, Atlantic Ocean, off Florida, 27°46′N 78°46′W, plankton net, 0–100 m, 21 March 1914. Specimen was unique.

etheridgei, Loligo Berry, 1918. *Biol. Res. Fish. Exp. "Endeavour"*, 4(5): 243–249. HOLOTYPE: 1♂, 132 mm, text figs. 29, 30, 33, 34 and 38, pls. 67 and 68, pl. 69 figs. 1 and 2, SSB 489, AMS C148250, "Endeavour" E6068, "Australian Seas (?S.E.)," date unknown. PARATYPE: 1♂, 143 mm, text-figs. 28, 31, 32, 35–37, SSB 490, USNM 816590, from same lot as holotype.

famelica, Chiroteuthis Berry, 1909. *Proc. U.S. Natl. Mus.*, 37(1713): 414–415. HOLOTYPE: 1 sex undetermined, 39 mm, fig. 8, SSB 260, USNM 214314, "Albatross" sta. 3989, Pacific Ocean, Hawaii, off Kauai Island, Hanamaula warehouse, S33°, W 9.5′, 8 ft Tanner trawl, 385–500 fm (703–914 m), 11 June 1902. Unique specimen is no longer extant.

fisheri, Helicocranchia Berry, 1909. *Proc. U.S. Natl. Mus.*, 37(1713): 417. HOLOTYPE: 1 sex undetermined, 47 mm, SSB 106, USNM 214316, "Albatross" sta. 3883, Pacific Ocean, Hawaii, off Maui Island, Mokuhooniki Islet, S 80°30′, W 7.8′, 9 ft Tanner trawl, 277–284 fm (506–519 m), 16 April 1902. Illustrated in Berry, 1914c, pl. 53 figs. 5 and 6, pl. 55 fig. 2. Specimen was unique.

fitchi, Octopus Berry, 1953. *Leaf Malac.*, 1(10): 54–55. HOLOTYPE: 1♂, 22 mm, SSB 887, USNM 815713, J. E. Fitch, Pacific Ocean, Mexico, Gulf of California, Baja California Norte, Punta San Felipe, under cobbles, intertidal, 12 December 1951. Specimen has dried and is in poor condition. PARATYPES: 5♂, 2♀, 21–30* mm, SSB 888, USNM 815715, from same lot as holotype. PARATYPES: 6♂, 15♀, ML measurements not possible, SSB 889, USNM 816591, from same lot as holotype. Listed as uncataloged in Hertz (1984). Specimens are macerated, dried, and in very poor condition. PARATYPES: 1♂, 1♀, 9 and 14* mm, SSB 895, USNM 815714, S. S. Berry & J. E. Fitch, from same locality as holotype, 2 April 1953. Specimens have dried and are in poor condition. Female with eggs on substrate. PARATYPE: 1♂, SSB 999, from same lot as holotype. Deposition of specimen is unknown; not found in Berry collection. This species has not been figured.

formosana, Sepia Berry, 1912. *Proc. Acad. Nat. Sci. Phila.*, 64: 420–422. HOLOTYPE: 1♂*, 72 mm, pl. 9, SSB 361, CASIZ 021668 [465], H. Sauter, Pacific Ocean, Formosa (Taiwan), Takao fishmarket, 12 January 1907. Specimen was unique.

galaxias, Enoploteuthis Berry, 1918. *Biol. Res. Fish. Exp. "Endeavour"*, 4(5): 211–221. HOLOTYPE: 1♀, 72 mm, text fig. 2, pl. 59, pl. 60 figs. 1 and 3, SSB 543, AMS C148251, "Endeavour" E5723, Australia, Tasman Sea, Victoria, Gabo Island to Everard Grounds, 200–250 fm (365–457 m), October 1914. PARATYPE: 1♂, 73 mm, pl. 60 figs. 2, 4 and 5, SSB 544, USNM 816352, from same lot as holotype. PARATYPE: 1♀, 78 mm, text-figs. 1–4, pl. 60 figs. 6 and 7, SSB 462, USNM 816355, from same lot as holotype. Specimen dissected completely by Berry.

gilbertianus, Polypus Berry, 1912. *Bull. Bur. Fish.*, 30(1910): 284–286. HOLOTYPE: 1♂, 65 mm, pl. 35 figs. 4 and 5, pl. 36 fig. 2, pl. 37, SSB 139, USNM 214320, "Albatross" sta. 4228, Pacific Ocean, Alaska, vicinity of Naha Bay, Behm Canal, Indian Point, N 18°E, 0.9 mi, 8 ft Tanner trawl, 41–134 fm (74–244 m), 7 July 1903. PARATYPE: 1♂, 79 mm, SSB 140, CASIZ 021806 [510], "Albatross" sta. 4253, Pacific Ocean, Alaska, Alexander Archipelago, Stephens Passage, Thistle Ledge, N 53°E, 1.7 mi, 8 ft Tanner trawl, 131–188 fm (239–343 m), 14 July 1903.

globula, Cranchia (Liocranchia) Berry, 1909. *Proc. U.S. Natl. Mus.*, 37(1713): 415–416. HOLOTYPE: 1 juv.*, 20 mm, fig. 9, SSB 262, USNM 214315, "Albatross" sta. 3878, Pacific Ocean, Hawaii, S of Lanai Island, Molokini Islet, N 81°, E 51.2′, plankton net, surface, 14 April 1902. Specimen has dried and is in poor condition. PARATYPE: 1 juv.*, 11 mm, SSB 282, CASIZ 017978 [520], from same lot as holotype. Cited as only paratype by Berry, 1914c: 347. A specimen (USNM 214608) was listed incorrectly as a paratype in Roper & Sweeney (1978).

harrissonia, Moschites Berry, 1917. *Sci. Rep. Australasian Exped.*, C4(2): 24–27. HOLOTYPE: 1♀, 70 mm, text figs. 21, 22 and 24, pl. 14 fig. 15, SSB 440 AMS C40892, "Aurora" sta. 10, Antarctic Ocean, off Shackleton Ice Shelf, 65°06′S 96°13′E, trawl, 325 fm (594 m), 29 January 1914 (published date), December 1913 (label date). PARATYPE: 1♀, 54 mm, pl. 13 figs. 13 and 14, SSB 442, USNM 815724, "Aurora" sta. 11, Antarctic Ocean, off Shackleton Ice Shelf, 66°44′S 97°28′E, trawl, 358 fm (654 m), 31 January 1914. PARATYPE: 1♀, 65* mm, text-figs. 23 and 25, SSB 441, AMS C40893, C. T. Harrison, Antarctic Ocean, off Shackleton Glacier, "Western Base" Queen Mary Land, trapped, 270 fm (491 m), January 1913. Specimen was designated as a paratype by Berry both in his card catalog and on the specimen label at the time of the species description.

hastula, Chiroteuthoides Berry, 1920. *Proc. U.S. Natl. Mus.*, 58(2335): 293–294. HOLOTYPE: 1 juv.*, 10 mm, SSB 637, USNM 338693, "Bache" sta. 10187, Atlantic Ocean, Sargasso Sea, 28°59′N 69°22′W, plankton net, 0–200 m, 23 February 1914. Specimen was unique. This species has not been figured.

hawaiiensis, Ommastrephes Berry, 1912. *Proc. Acad. Nat. Sci. Phila.*, 64: 434. HOLOTYPE: 1♀*, 138 mm, SSB 243, USNM 214382, "Albatross" sta. 4117, Pacific Ocean, Hawaii, off Oahu Island, Kahuku Point, S 69°30′, E 9.0′, 10 ft Blake trawl, 253–282 fm (462–515 m), 25 July 1902. Holotype designated and illustrated in Berry, 1914c: 340, text fig. 38, pl. 54 fig. 2. No paratypes designated.

hawaiiensis, Stephanoteuthis Berry, 1909. *Proc. U.S. Natl. Mus.*, 37(1713): 409–410. HOLOTYPE: 1♀, 22 mm, fig. 2, SSB 30, USNM 214311, "Albatross" sta. 3989, Pacific Ocean, Hawaii, off Kauai Island, Hanamaula warehouse, S 33°, W 9.5′, 8 ft Tanner trawl, 385–500 fm (703–914 m), 11 June 1902. Holotype has been found in Berry collection; listed in Roper & Sweeney (1978) as no longer extant. Unique specimen is dried, in pieces, and in very poor condition.

heathi, Eledonella Berry, 1911. *Proc. U.S. Natl. Mus.*, 40(1838): 589–590. HOLOTYPE: 1♀, 50 mm, SSB 118, USNM 214318, "Albatross" sta. 4396, Pacific Ocean, California, off San Diego, 33°01′35″N 121°32′W, 8 ft Tanner trawl, 2228 fm (4072 m), 31 March 1904. Illustrated in Berry, 1912c, pl. 32 fig. 4, pl. 33 figs. 3 and 4. Unique specimen has dried and is in poor condition.

hedleyi, Sepia Berry, 1918. *Biol. Res. Fish. Exp. "Endeavour"*, 4(5): 258–264. HOLOTYPE: 1♂, 81 mm, text figs. 48 and 49, pl. 71, pl. 72 figs. 1, 3 and 6, SSB 499, AMS C148252, "Endeavour" E2464, Indian Ocean, South Australia, S of Kangaroo Island, area S of Investigator Strait, January/February 1912. PARATYPE: 1♂, 83 mm, SSB 497, USNM 815720, "Endeavour" E2461, from same locality as holotype, date unknown. PARATYPE: 1♂, 79 mm, SSB 502, "Endeavour" E2463, from same locality as holotype, date unknown. PARATYPE: 1♂, 74 mm, SSB 503, "Endeavour" E2462, from same locality as holotype, date unknown. Location of SSB 502 and 503 is unknown; not listed by Rudman (1983) in AMS collections.

heteropsis, Calliteuthis (Meleagroteuthis) Berry, 1913. *Proc. Acad. Nat. Sci. Phila.*, 65: 75–76. HOLOTYPE: 1♀, 59 mm, SSB 108, USNM 214620, "Albatross" sta. 4416, Pacific Ocean, California, off Santa Barbara Island, SW rock, N 49° W, 4.7 mi, 9 ft Tanner trawl, 323–448 fm (590–818 m), 12 April 1904. Illustrated in Berry, 1912c, pl. 50 figs. 1–3, pl. 51 figs. 2 and 3. Specimen is no longer extant. PARATYPE: 1♀, 48 mm, SSB 110, CASIZ 029130, "Albatross" sta. 4538, Pacific Ocean, California, Monterey Bay, Point Pinos lighthouse, S 85° E, 6.5 mi, 8 ft Albatross-Blake trawl, 795–871 fm (1453–1592 m), 31 May 1904. This specimen is designated as the "cotype" on the original label and in Berry's card catalog. Illustrated in Berry, 1912c, pl. 51 fig. 1.

hoylei, Polypus Berry, 1909. *Proc. U.S. Natl. Mus.*, 37(1713): 407–408. HOLOTYPE: 1♂, 65 mm, text fig. 1, SSB 166, USNM 214310, "Albatross" sta. unknown, Pacific Ocean, vicinity of Hawaiian Islands, no date. Illustrated in Berry, 1914c, pl. 47 fig. 1, pl. 48 figs. 2 and 3, pl. 55 fig. 2. No paratypes designated. Paratypes listed incorrectly in Roper & Sweeney (1978).

hubbsorum, Octopus Berry, 1953. *Leaf. Malac.*, 1(10): 53–54. HOLOTYPE: 1♂, 48 mm, SSB 943, USNM 816360, A. O. Flechsig & K. S. Norris sta. W50-50, Pacific Ocean, Mexico, Gulf of California, Sonora, Bahia San Carlos, S side of Punta San Guillermo, 1 February 1950. PARATYPE: 1♀, 18* mm, SSB 926, USNM 816480, A. Allanson *et al.* sta. H51-387, Pacific Ocean, Mexico, Gulf of California, Sonora, Puerto San Carlos, midway between Punta Paredones and Punta de las Cueras, 17 October 1951. Specimen has dried and is in very poor condition. PARATYPE: 1♀, 55* mm, SSB 941, USNM 816400, A. O. Flechsig sta. W50-35, Pacific Ocean, Mexico, Gulf of California, Sonora, Bahia San Carlos, W side of Punta de las Cueras, 27–29 January 1950. This species has not been figured.

iris, Stoloteuthis Berry, 1909. *Proc. U.S. Natl. Mus.*,

37(1713): 410–412. HOLOTYPE: 1 sex undetermined, 7 mm, fig. 3, SSB 31, USNM 214312, "Albatross" sta. 3832, Pacific Ocean, Hawaii, off Molokai Island, Lae-o Ka Laau light, N 69°30′, W 14.5′, 8 ft Tanner trawl, 142–153 fm (259–279 m), 2 April 1902. Unique specimen is no longer extant.

kermadecensis, Polypus (Pinnoctopus?) Berry, 1914. *Trans. N.Z. Inst.*, 46: 138–139. HOLOTYPE: 1♀, 50 mm, pls. 7 and 8, SSB 399, USNM 816461, collector undetermined (Iredale, Oliver or Bell), Pacific Ocean, Kermadec Islands, Sunday Island, stranded on beach, no date. Specimen was unique.

leioderma, Polypus Berry, 1911. *Proc. U.S. Natl. Mus.*, 40(1838): 590–591. HOLOTYPE: 1♀, 45 mm, SSB 137, USNM 214322, "Albatross" sta. 4293, Pacific Ocean, Alaska, Shelikof Strait, Cape Uyak, S 10°W, 5.8 mi, 8 ft Tanner trawl, 106–112 fm (193–204 m), 15 August 1903. Illustrated in Berry, 1912c, pl. 35 fig. 1, pl. 40 fig. 4. Specimen is no longer extant. No paratypes designated.

lemur, Pyrgopsis Berry, 1920. *Proc. U.S. Natl. Mus.*, 58(2335): 298–299. HOLOTYPE: 1 juv.*, 33* mm, pl. 16 fig. 5, SSB 582, USNM 338698, "Bache" sta. 10161, Atlantic Ocean, off North Carolina, 35°27′N 73°14′W, plankton net, surface, 28 January 1914. No paratypes designated.

lugubris, Laetmoteuthis Berry, 1913. *Proc. U.S. Natl. Mus.*, 45(1996): 563. HOLOTYPE: 1 sex undetermined, ML undetermined, SSB 211, USNM 214385, "Albatross" sta. 3904, Pacific Ocean, Hawaii, off Molokai Island, Mokapu Islet, S 76°, W 13′, dipnet, surface, 30 April 1902. Illustrated in Berry, 1914c, text-figs. 1 and 2. Partially decomposed when collected. No paratypes designated. Paratype listed incorrectly in Roper & Sweeney (1978).

macrope, Cirroteuthis Berry, 1911. *Proc. U.S. Natl. Mus.*, 40(1838): 589. HOLOTYPE: 1 juv.*, 99 mm (total length), SSB 120, USNM 214317, "Albatross" sta. 4393, Pacific Ocean, California, off San Diego, 32°54′20″N 121°11′15″W, 8 ft Tanner trawl, 2113–2259 fm (3862–4129 m), 30 March 1904. Illustrated in Berry, 1912c, text-fig. 1, pl. 32 figs. 1–3. No paratypes designated.

magister, Gonatus Berry, 1913. *Proc. Acad. Nat. Sci. Phila.*, 65: 76–77. HOLOTYPE: 1♀*, 153 mm, SSB 88, CASIZ 017965 [463], shrimp fishermen, Pacific Ocean, Washington, Puget Sound, 1909. Illustrated in Berry, 1912c, pl. 52 figs. 1 and 2, pl. 53 fig. 1, pl. 54 figs. 1–3, pl. 55 figs. 1, 3, 4 and 7. No paratypes designated.

mawsoni, Stauroteuthis (?) Berry, 1917. *Sci. Rep. Australasian Ant. Exped.*, C4(2): 8–11. HOLOTYPE: 1 juv.*, 15 mm, text figs. 1–4, pl. 10 fig. 1, SSB 447, AMS C40886, "Aurora" sta. 2, Antarctic Ocean, Adelie Land, off Mertz Glacier, 66°55′S 145°21′E, trawl, 288–300 fm (526–548 m), 28 December 1913. Specimen was unique.

megaleia, Lampadioteuthis Berry, 1916. *Proc. Acad. Nat. Sci. Phila.*, 68: 52–58. HOLOTYPE: 1 sex undetermined, 30 mm, SSB 416, R. S. Bell, Pacific Ocean, Kermadec Islands, Sunday Island, stranded on beach, 1910. Deposition of specimen unknown; unique specimen not found in Berry collection.

microlampas, Pterygioteuthis Berry, 1913. *Proc. U.S. Natl. Mus.*, 45(1996): 566. HOLOTYPE: 1♀, 18 mm, SSB 277, USNM 214386, "Albatross" sta. 4105, Pacific Ocean, Hawaii, Kaiwi Channel, Molokai Island, Lae-o Ka Laau light, S 45°30′, E 10.6′, 8 ft Tanner trawl, 314–335 fm (573–612 m), 24 July 1902. Illustrated in Berry, 1914c, text fig. 36, pl. 52 figs. 1 and 3. Specimen is no longer extant. No paratypes designated.

micropyrsus, Octopus Berry, 1953. *Leaf. Malac.*, 1(10): 52–53. HOLOTYPE: 1♂, 10 mm, SSB 995, USNM 815704, C. Limbaugh, Pacific Ocean, California, San Diego County, La Jolla Cove, by hand from kelp holdfast, 6 fm (10 m), 27 October 1950. PARATYPE: 1♂, 14* mm, SSB 900, USNM 815706, B. M. McConnaughey sta. 1-60, Pacific Ocean, California, San Diego County, La Jolla, Devil's Slide, intertidal, 3 March 1947. PARATYPE: 1♂, 10* mm, SSB 996, USNM 815705, same lot as holotype. This species has not been figured.

miranda, Calliteuthis Berry, 1918. *Biol. Res. Fish. Exp. "Endeavour"*, 4(5): 221–228. HOLOTYPE: 1♀, 140 mm, text-figs. 5–9, pls. 61 and 62, SSB 545, "Endeavour" E5605, Australia, Tasman Sea, Victoria, SSE of Gabo Island, 270 fm (493 m), 15 September 1914. Specimen is no longer extant in AMS according to Rudman (1983). No paratypes designated. Neotype designated by Voss (1969: p. 801).

nipponensis, Stoloteuthis Berry, 1911. *Zool. Anz.*, 37(2): 39–41. HOLOTYPE: 1♂, 17 mm, 1 fig., SSB 32, CASIZ 017976 [459], "Albatross" (J. O. Snyder?), Pacific Ocean, Japan, Honshu Island, Suruga Bay, 1900. Specimen was unique.

nipponianum, Sepiadarium Berry, 1932. *Philipp. J. Sci.*, 47(1): 42–46. HOLOTYPE: 1♂, 11 mm, pl. 1 figs. 2 and 3, SSB 724, USNM 816462, M. Sasaki, Pacific Ocean, Japan, Honshu Island, "Prov. Sagami", 10 April 1918. PARATYPES: 1♂, 1♀, 16.2 and 16 mm, pl. 1 figs. 4 and 5, SSB 725, USNM 816359, from same lot as holotype. PARATYPE: 1♀, 15 mm, SSB 725, SAM D17496, from same lot as holotype. Not located in SAM by Zeidler (1983); recently was found in Berry collection and returned to SAM. Seven specimens (SSB 725) are listed as paratypes by Berry in his card catalog and the lot was labeled as such at the time of the species description. However, only three of these specimens were designated by Berry, 1931: 44 as paratypes.

notoides, Idiosepius Berry, 1921. *Rec. S. Aust. Mus.*, 1(4): 361–362 HOLOTYPE: 1♂, 16 mm, fig. 67, SSB 719, SAM D17495, A. Zietz, South Australia, Goolwa, no date. Not located in SAM by Zeidler (1983); recently was found in Berry collection and returned to SAM. PARATYPE:

1♀, 21 mm, SSB 720, USNM 816458, from same lot as holotype.

oliveri, Polypus Berry, 1914. *Trans. N.Z. Inst.*, 46: 136–137. HOLOTYPE: 1♀, 45 mm, SSB 405, USNM 816455, W. R. B. Oliver, Pacific Ocean, Kermadec Islands, Sunday Island, intertidal, 1908. Illustrated in Berry, 1916b, pl. 6, fig. 2. PARATYPE: 1♀, 40 mm, SSB 405, CASIZ 021805 [464], from same lot as holotype.

opalescens, Loligo Berry, 1911. *Proc. U.S. Natl. Mus.*, 40(1838): 591–592. HOLOTYPE: 1♂, 132 mm, SSB 101, CASIZ 017970 [547], shrimp fishermen, Pacific Ocean, Washington, Puget Sound, 1908. PARATYPE: 1♀, 146 mm, SSB 101, USNM 214388, from same lot as holotype. PARATYPE: 1♂, 126 mm, SSB 101, USNM 816384, from same lot as holotype. Paratypes designated and species illustrated in Berry, 1912c.

pacifica, Rossia Berry, 1911. *Proc. U.S. Natl. Mus.*, 40(1838): 591. HOLOTYPE: 1 sex undetermined, SSB 21, USNM 214323, "Albatross" sta. 4233, Pacific Ocean, Alaska, Alexander Archipelago, Yes Bay, Behm Canal, Cannery Point, N 55°W, 1 mi, 8 ft Tanner trawl, 39–45 fm (71–82 m) 8 July 1903. Specimen is no longer extant. PARATYPES: 8 sex undetermined, SSB 21, USNM 214323, from same lot as holotype. Specimens are no longer extant. PARATYPES: 1♂*, 1♀*, both 30* mm, SSB 21, CASIZ 018796 [495 and 496], from same lot as other types. Original label reads "cotypes." Species illustrated by Berry, 1912c, pl. 41 figs. 1–4, pl. 43 figs. 2–4, with unspecified specimens from SSB 21.

panamensis, Lolliguncula (?) Berry, 1911. *Proc. Acad. Nat. Sci. Phila.*, 63: 100–105. HOLOTYPE: 1♀, 102* mm, text figs. 1, 2 and 7 (not 5 as listed), pl. 6, SSB 58, CASIZ 017968 [538], Hopkins Expedition, R. E. Snodgrass & E. Heller, Pacific Ocean, Panama, 1898–1899? PARATYPE: 1♀, 103* mm, SSB 58, CASIZ 017969 [537], from same lot as holotype. PARATYPE: 1♀, ML undetermined, text figs. 1, 2 and 7 (not 5 as listed), pl. 6, SSB 58, USNM 214324, from same lot as holotype. Specimen is no longer extant.

pardus, Megalocranchia Berry, 1916. *Proc. Acad. Nat. Sci. Phila.*, 68: 61–64. HOLOTYPE: 1 juv.*, 50 mm, text figs. 19–22, pl. 9 fig. 2, SSB 415, USNM 816465, R. S. Bell, Pacific Ocean, Kermadec Islands, Sunday Island, stranded on beach, 1910. Specimen was unique.

patagiatus, Scaeurgus Berry, 1913. *Proc. U.S. Natl. Mus.*, 45(1996): 564. HOLOTYPE: 1♂, 46 mm, SSB 204, USNM 214379, "Albatross' sta. 4079, Pacific Ocean, Hawaii, off Maui Island, Puniawa Point, S 31°30′, E 6′, 10 ft Blake Trawl, 143–178 fm (261–325 m), 21 July 1902. Illustrated in Berry, 1914c, pl. 47 figs. 2 and 3, pl. 48 fig. 1. Specimen is no longer extant. No paratypes designated.

pathopsis, Sandalops Berry, 1920. *Proc. U.S. Natl. Mus.*, 58(2335): 297. HOLOTYPE: 1 juv.*, 8 mm, pl. 16 fig. 1, SSB 624, USNM 338696, "Bache" sta. 10166, Atlantic Ocean, Sargasso Sea, 32°33′N 72°14′W, plankton net, 0–1100 m, 30 January 1914. PARATYPE: 1 sex undetermined, ML undetermined, SSB 625, USNM 729182, from same lot as holotype. One of six specimens (mentioned in remarks on p. 297) that was designated in Berry's card catalog and on the specimen label as a paratype at the time of the species description. The specimen recently has been found in the Berry collection.

penicillifer, Octopus Berry, 1954. *Leaf. Malac.*, 1(11): 66. HOLOTYPE: 1♂, 15 mm, SSB 1014, USNM 815717, "Stranger" (M. W. Williams), Pacific Ocean, Mexico, Gulf of California, Baja California Sur, off Punta Arena, trawl, 17 fm (31 m), 23 April 1937. Specimen has dried and is in poor condition. No paratypes designated. This species has not been figured.

persephone, Opisthoteuthis Berry, 1918. *Biol. Res. Fish. Exp. "Endeavour"*, 4(5): 290–294. HOLOTYPE: 1 sex undetermined, 330 mm (tip of right dorsal arm to tip of left ventral arm), pl. 81 fig. 7, pl. 82 fig. 9, pls. 85 and 86, SSB 480, AMS C148253, "Endeavour" E5718, Australia, Bass Strait, Victoria, 42 mi SE of Genoa Peak, 260 fm (475 m), 24 October 1914. PARATYPE: 1 sex undetermined, 225 mm (tip of right dorsal arm to tip of left ventral arm), text-fig. 66, pl. 87 fig. 2, pl. 88 fig. 2, SSB 481, "Endeavour" E5719, from same lot as holotype. Location of specimen unknown; not listed by Rudman (1983) in AMS collections. PARATYPE: 1 juv.*, 150* mm (tip of right dorsal arm to tip of left ventral arm), SSB 482, USNM 816361, "Endeavour" E5720, from same lot as holotype.

phyllura, Galiteuthis Berry, 1911. *Proc. U.S. Natl. Mus.*, 40(1838): 592. HOLOTYPE: 1 sex undetermined, 230 mm, SSB 113, USNM 214325, "Albatross" sta. 4529, Pacific Ocean, California, Monterey Bay, Point Pinos lighthouse, S 61°E, 10.9 mi, 9 ft Tanner Trawl, 780–799 fm (1425–1460 m), 27 May 1904. Illustrated in Berry, 1912c, text figs. 17 and 18, pl. 46 figs. 1–3, pl. 56 figs. 1 and 2. Unique specimen has dried and is in poor condition.

pluto, Opisthoteuthis (Teuthidiscus) Berry, 1918. *Biol. Res. Fish. Exp. "Endeavour"*, 4(5): 284–290. HOLOTYPE: 1 sex undetermined, 540 mm (tip of right dorsal arm to tip of left ventral arm), pl. 82 fig. 5, pls. 83 and 84, SSB 464, "Endeavour" E3628, Indian Ocean, Great Australian Bight,, —°—′S 129°28′E, 350–450 fm (632–822 m), 14 May 1913. Specimen is no longer extant in AMS collections according to Rudman (1983). PARATYPE: 1 sex undetermined, 265 mm (tip of right dorsal arm to tip of left ventral arm), SSB 465, USNM 815718, "Endeavour" E3629, Indian Ocean, Great Australian Bight, S of Eucla, —°—′S 126°10′E, 200 fm (365 m), 13 May 1913.

pricei, Polypus Berry, 1913. *Proc. Acad. Nat. Sci.*

Phila., 65: 73–75. HOLOTYPE: 1♀, 18 mm, fig. 2, SSB 189, CASIZ 017966 [454], C. H. Gilbert, Pacific Ocean, California, Monterey Bay, off Point Pinos, salmon stomach, 23 June 1911. Specimen has dried and is in poor condition. PARATYPES: 2♀, both 20* mm, fig. 2, SSB 189, CASIZ 017967 [455 & 456], from same lot as holotype. PARATYPE: 1♀, 7* mm, fig. 2, SSB 189, USNM 214680, from same lot as holotype. Specimen has dried and is in very poor condition.

regalis, Nematolampas Berry, 1913. *Biol. Bull.,* 25(3): 208–211. HOLOTYPE: 1♂*, 32 mm, SSB 409, USNM 815721, R. S. Bell, Pacific Ocean, Kermadec Islands, Sunday Island, stranded on beach, 1910. Illustrated in Berry, 1914b, text figs. 2 and 3, pl. 9. PARATYPE: 1♂*, 26 mm, SSB 410, USNM 816592, from same lot as holotype. Paratype designated and illustrated in Berry, 1914b, text fig. 1. Specimen dissected completely by Berry.

rubescens, Octopus Berry, 1953. *Leaf. Malac.,* 1(10): 51–52. HOLOTYPE: 1♂, 32 mm, SSB 969, USNM 815709, "Orca" (C. L. Hubbs *et al.*) sta. H49-167, Pacific Ocean, Mexico, Baja California Norte, off S end of South Coronado Island, trawl, 15–17 fm (27–31 m), 27 November 1949. PARATYPE: 1♀, 14* mm, SSB 968, USNM 815710, "E. W. Scripps" (C. L. Hubbs *et al.*) sta. H48-222, from same locality as holotype, trawl, 15 fm (27 m), 11 August 1948. Specimen has dried and is in poor condition. PARATYPES: 3♂, 5♀, 5 juv., 11–29* mm, SSB 970, USNM 815711, from same lot as holotype. PARATYPES: 1♂, 1♀, ML undetermined, SSB 972, "Orca" (C. L. Hubbs *et al.*), from same locality as holotype, trawl, 12 fm (21 m), 18 October 1949. Deposition of specimens is unknown, not found in Berry collection. PARATYPES: 2♀, 12 and 23* mm, SSB 976, USNM 815712, "Orca" (C. L. Hubbs *et al.*) sta. H49-36, from same locality as holotype, trawl, 7–13 fm, 23 April 1949. This species has not been figured.

scintillans, Abraliopsis Berry, 1911. *Nautilus,* 25(8): 93–94. HOLOTYPE: 1♀, 59 mm, SSB 147, CASIZ 021667 [453], Alan Owston?, "Japan, probably off Misaki" "Label lost." Illustrated in Berry, 1912d, pl. 7 figs. 3 and 4, pl. 9 figs. 2 and 3. PARATYPES: 2♀, 52 and 55 mm, SSB 147, USNM 816498, from same lot as holotype. Paratypes designated by Berry, 1912d: 430 (specimens 2 and 3). Specimens have dried and are in very poor condition. One specimen (52 mm) dissected completely by Berry. Illustrated in Berry, 1912d, text figs. 2 and 3, pl. 7 figs. 1 and 2, pl. 9 figs. 1, 4–6.

scolopes, Euprymna Berry, 1913. *Proc. U.S. Natl. Mus.,* 45(1996): 564–565. HOLOTYPE: 1♂, 25 mm, SSB 320, USNM 214380, "Albatross" sta. 3905, Pacific Ocean, Hawaii, Molokai Island, off Kalanpapa leper settlement 0.75 mi, dipnet and night light, surface, 30 April 1902. Illustrated in Berry, 1914c, text figs. 23 and 24, pl. 49 fig. 5. PARATYPES: 6♂, 3♀, 7–13* mm, SSB 323, USNM 727393, from same lot as holotype. PARATYPES: 2♂, 1♀, 18–25* mm, SSB 323, CASIZ 017975 [497, 498 and 499], from same lot as holotype. Paratypes designated by Berry, 1914c: 314.

scorpio, Polypus Berry, 1920. *Proc. U.S. Natl. Mus.,* 58(2335): 299–300. HOLOTYPE: 1 juv.*, 4 mm, pl. 16 fig. 4, SSB 682, USNM 338699, "Bache" sta. 10204, Atlantic Ocean, off Florida, 25°33′N 80°03′W, plankton net, 0–75 m, 20 March 1914. No paratypes designated.

spinicauda, Enoptroteuthis Berry, 1920. *Proc. U.S. Natl. Mus.,* 58(2335): 295. HOLOTYPE: 1 juv.*, 17 mm, pl. 16 fig. 6, SSB 638, USNM 338694, "Bache" sta. 10188, Atlantic Ocean, Sargasso Sea, 28°51′N 70°08′W, plankton net, 0–75 m, 24 February 1914. Unique specimen has dried and is in poor condition.

trigonura, Abralia Berry, 1913. *Proc. U.S. Natl. Mus.,* 45(1996): 565–566. HOLOTYPE: 1♀, 34 mm, SSB 275, USNM 214387, "Albatross" sta. 4087, Pacific Ocean, Hawaii, Pailolo Channel (Between Molokai and Maui Islands), Mokuhooniki Islet, S 85°45′, W 21.2′, 10 ft Blake trawl, 306–308 fm (559–563 m), 21 July 1902. Illustrated in Berry, 1914c, text fig. 33. Unique specimen is no longer extant.

veligero, Octopus Berry, 1953. *Leaf. Malac.,* 1(10): 57. HOLOTYPE: 1♂, 37 mm, SSB 1000, USNM 815716, B. Fukuzaki, Pacific Ocean, Mexico, Baja California Sur, off San Juanico, 50 fm (91 m), August 1952. PARATYPES: 2♀, 24 and 25* mm, SSB 947, USNM 816432, from same lot as holotype. Specimens have dried and are in poor condition. This species has not been figured.

CEPHALOPOD PUBLICATIONS OF S. STILLMAN BERRY

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BERRY, S. S., 1911a, A new sepiolid from Japan. *Zoologischer Anzeiger,* 37(2): 39–41, 1 text fig.

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BERRY, S. S., 1911c, Preliminary notices of some new Pacific cephalopods. *Proceedings of the United States National Museum,* 40(1838): 589–592.

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BERRY, S. S., 1911e, Note on a new *Abraliopsis* from Japan. *Nautilus,* 25(8): 93–94.

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MALACOLOGIA, 1988, 29(1): 21–22

OCTOPUS LIFE HISTORIES

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Life histories are not simple to work out. Laboratory and field data must be corroborated and this has been accomplished only rarely due to logistical difficulties and problems finding or following cephalopods in their natural habitats. Shallow-water, benthic octopods that produce large eggs and large adult-like hatchlings (no planktonic stage) are probably the most straightforward to study, but by no means easy. Species with small planktonic hatchlings are more difficult to understand; no one knows how long they stay in the plankton or which factors influence settlement. Five of the six papers reported here (Forsythe & Hanlon the exception) deal with species that produce small, numerous planktonic hatchlings.

The case of *Octopus bimaculatus - O. bimaculoides* is intriguing. They are sympatric, sibling species with different life history tactics, yet their life cycles are unknown and their general appearance is so similar that it is very difficult to identify the species. R. Ambrose has done a masterful job of collecting and analyzing field data on *O. bimaculatus*. He postulates two models of life cycles ("alternating generations" vs. "alternating years") based upon his six years worth of data on adult population densities, reproduction and recruitment and growth. This paper will stimulate much thought and experimental design in future ecological studies of octopuses. The study by J. Forsythe & R. Hanlon on *O. bimaculoides* is markedly different in approach. Octopuses were cultured through the life cycle in the laboratory, permitting observations on the early post-hatching period. Many aspects of behavior, body patterning and reproduction were described along with some field observations. It is clear that we now need complementary data on these sibling species: long-term field data on *O. bimaculoides* and extensive laboratory data on *O. bimaculatus*. As described in oral presentations, M. Lang is conducting a field study of *O. bimaculoides* near San Diego, and F. G. Hochberg delineated characters of live animals and their distributions that can help us understand interrelationships of other 2-spotted octopus species worldwide.

Another common E. Pacific species, *O. dofleini*, has been studied extensively in the field by B. Hartwick and coworkers. Here they report differential abundance, growth and distribution during winter and attempt to correlate these observations with den availability, predators and prey. Life history estimates of this large, commercially important octopus are hampered by (1) its early planktonic phase, (2) large adult size, (3) low temperatures and (4) differential abundance and behavior of males vs. females. In an oral presentation for S. Snyder, J. Eddy described the first successful rearing of *O. dofleini* from eggs to adult size, providing information on the enigmatic planktonic phase. D. Whitaker and L. DeLancy described catch rates and seasonality in a pot fishery for *O. vulgaris* off the coast of the Carolinas.

J. Mather found that the daytime activity of juvenile *O. vulgaris* in Bermuda over a 5-week period in summer was characterized by inactivity (70% time in shelters) interspersed with hunting and feeding episodes not unlike other high-level predators in areas of abundant foods. Although this study did not include important night-time observations, her data will be useful in the future in evaluating important questions on activity patterns, foraging efficiency and bioenergetics.

P. Boyle, K. Mangold & M. Ngoile provide the first study in which it is possible to compare temporal aspects of reproduction with morphometric characteristics of one species to determine clinal as well as life cycle variations. They concluded that the longer life cycle in the northern range of *Eledone cirrhosa* is not due exclusively to lower temperatures. Theirs is a fine example of combining systematics and life history studies. The paper by S. Kuehl also concentrated on seasonality of repoduction and geographical distribution. She found for five Antarctic species that fecundity was low and egg size large compared to other octopodids in temperate and

warmer seas. Such a contribution is important, especially considering the dearth of information of any type available on Antarctic cephalopods.

In summary, these six papers represent solid contributions that will add substantially to information collated in Volume I of the book *Cephalopod Life Cycles* (Boyle, 1983). These highly adapted, dynamic creatures are both a challenge and a joy to study. Collectively, the papers and oral presentations emphasize the importance of bringing forth new methods and perspectives to understand the complex life histories of octopods.

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POPULATION DYNAMICS OF *OCTOPUS BIMACULATUS:* INFLUENCE OF LIFE HISTORY PATTERNS, SYNCHRONOUS REPRODUCTION AND RECRUITMENT

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ABSTRACT

A six-year field study of a subtidal octopus population in Southern California has provided information on reproductive biology, recruitment, size composition and population fluctuations of *Octopus bimaculatus*. There was distinct seasonality in mating frequency, although mating was observed throughout the year. Most matings occurred in May and June, when water temperatures began rising. Most females laid eggs from April through August. Development time of the eggs was shortest during this period because water temperatures were relatively high. Octopuses that laid eggs earlier in the year were much less likely to be reproductively successful; 70 to 100% of all females that laid eggs before April died before the eggs hatched. Females stayed with their eggs throughout development and died shortly after the eggs hatched. All broods hatched between June and September. After hatching, the young octopuses spend one to several months in the plankton before assuming a benthic existence. Kelp holdfasts, a primary habitat of benthic juveniles, were sampled for two years to estimate the abundance and size distribution of juveniles. Settlement occurred throughout the year. Juveniles were most abundant in early summer; a second peak in abundance occurred in winter. Juveniles inhabited kelp holdfasts from settlement until they grew to a mantle length of approximately 50 mm, after which they moved into habitats used by the adult population. The density of adults fluctuated greatly, ranging from 0.5/100 m^2 to 3.0/100 m^2. The abundance cycles followed two patterns: during years of low recruitment, the semelparous life history of *O. bimaculatus* and synchronous brooding resulted in a precipitous decline in abundance during Fall. In contrast, there was no decline in abundance during years of high recruitment, in spite of the death of post-reproductive adults.

Key words: life history; octopuses; population biology; recruitment; reproductive biology; semelparity.

INTRODUCTION

Octopuses are abundant in temperate and tropical oceans throughout the world. They are effective predators that can influence the abundances of many other species, particularly molluscs and crustaceans (Fotheringham, 1974; Schmitt, 1982; Ambrose & Nelson, 1983; Fawcett, 1984; Ambrose, 1986). Octopuses also constitute a valuable fishery resource in many parts of the world, particularly Japan (Mottet, 1975), the Mediterranean (Mangold-Wirz, 1963) and Africa (Hatanaka, 1979). Despite their importance, however, relatively little is known about octopus population biology. A recent compilation of information on the life histories of cephalopods (Boyle, 1983b) summarizes our knowledge for many species. Some of the most detailed studies have relied on trawling as a means of sampling octopuses (Mangold-Wirz, 1963; Mangold & Boletzky, 1973; Guerra, 1975, 1981; Hatanaka, 1979; Boyle & Knobloch, 1982a, 1982b, 1983; Mangold, 1983). Recently, *in situ* studies of octopus populations have been undertaken using SCUBA. Short-term population studies have focused on *O. vulgaris* in South Africa (Smale & Buchan, 1981; Buchan & Smale, 1981) and *O. briareus* in a marine lake in the Bahamas (Aronson, 1986, *in press*). Hartwick *et al.* (1984a) studied the dynamics of the giant octopus, *O. dofleini,* in British Columbia over a six-year period; in that population, there were no breeding females at the site studied and the density fluctuated considerably due to immigration and emigration of adults.

Several aspects of octopus biology are likely to affect the dynamics of octopus populations. Nearly all octopuses grow rapidly, are relatively short-lived and with few exceptions reproduce only once (Rodaniche, 1984).

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Females protect ("brood") their eggs during development. In many species the juveniles have a planktonic dispersal stage that lasts from a few weeks to several months. Octopus species differ in the amount of movement undertaken during their lives. Some species are highly mobile (Hartwick *et al.*, 1984b; Aronson, *in press*) or undertake massive seasonal migrations (Mangold-Wirz, 1963; Kanamaru & Yamashita, 1967). In contrast, other species appear to move relatively little as adults (Van Heukelem, 1966; Ambrose, 1982a).

In this paper, I report observations made over a six-year period on a subtidal population of the two-spotted octopus, *Octopus bimaculatus* Verrill, 1883. *O. bimaculatus* is a typical octopus in many ways. It grows to a moderate size in shallow, rocky-bottom habitats. Because it lives in temperate waters, with distinct cold- and warm-water seasons, seasonality in its population processes is expected. My observations were made with three objectives in mind: (1) to determine the pattern of reproductive activities, including mating, egg-laying, brooding and hatching of eggs, (2) to evaluate the pattern of recruitment and growth of juveniles and (3) to document the variability of adult densities. These objectives correspond to three of the major stages in the life cycle of *O. bimaculatus;* the fourth stage, which is spent in the plankton, has remained virtually intractable.

Natural history of Octopus bimaculatus

Octopus bimaculatus is common in intertidal and subtidal habitats throughout Southern California, U.S.A., and Baja California, Mexico (Hochberg & Fields, 1980). *Octopus bimaculatus* occupies holes and crevices in a wide range of hard-substrate habitats. Most octopuses inhabit the same shelter for an extended period of time (from one to five months; Ambrose, 1982a); even octopuses that change shelters usually remain in the same area. Female octopuses lay strands of eggs in protected rock shelters and care for the eggs until they hatch. The eggs are relatively small, approximately 4 mm long, or about 5% of the adult mantle length (ML). At Santa Catalina Island, with an average octopus weight of 260 g (71 mm ML), the average clutch size is approximately 20,000 eggs (Ambrose, 1981). Eggs develop in one to two months, with development time dependent on water temperature (Ambrose, 1981). After hatching, the young octopuses spend from one to several months in the plankton (F. G. Hochberg, personal communication) before settling to the bottom. Settlement from the plankton is thought to be triggered when the octopuses contact the kelp canopy and move down the stipes to set up residence in the kelp holdfasts (F. G. Hochberg, personal communication).

Juvenile *O. bimaculatus* live in small shelters and kelp holdfasts, and probably feed on small crustaceans. Adult *O. bimaculatus* consume a wide variety of motile benthic invertebrates; at Santa Catalina Island, snails make up 75% of octopus diets, with chitons, bivalves, crabs and hermit crabs comprising most of the remaining portion (Ambrose, 1984). Although *O. bimaculatus* discards mollusc shells and crab exoskeletons outside its shelters, the discarded prey items rarely accumulate to form a conspicuous midden as observed in other species of octopuses (Ambrose, 1983). *Octopus bimaculatus* is a very effective predator that can dramatically reduce the abundances of its prey (Ambrose, 1986).

METHODS

Octopuses were studied on Santa Catalina Island (33°27′N, 118°29′W) near the Catalina Marine Science Center, about 30 km S of Los Angeles, California, U.S.A. A permanent study site was established on the E end of Bird Rock, an islet 0.5 km off the N side of Santa Catalina Island (Fig. 1). Since 1976, giant kelp (*Macrocystis pyrifera*) has been nearly absent from East Bird Rock and an introduced brown alga, *Sargassum muticum,* has been very abundant seasonally (Ambrose & Nelson, 1982). The study site depth varied from approximately 4 m (at the lower edge of an *Eisenia arborea* bed) to a maximum of 10 m; the substrate consisted primarily of bedrock with a network of cracks, interspersed with occasional patches of sand (for more detail see Ambrose, 1982b). East Bird Rock was chosen because it supported a large octopus population.

The water temperature at Santa Catalina Island normally varies from about 13°C to 20°C (Fig. 2). The lowest temperatures occur between November and early spring. In late spring, the temperature increases, reaching a maximum in August or September.

SCUBA diving was used to census octopuses. Each hole and crevice occupied by an

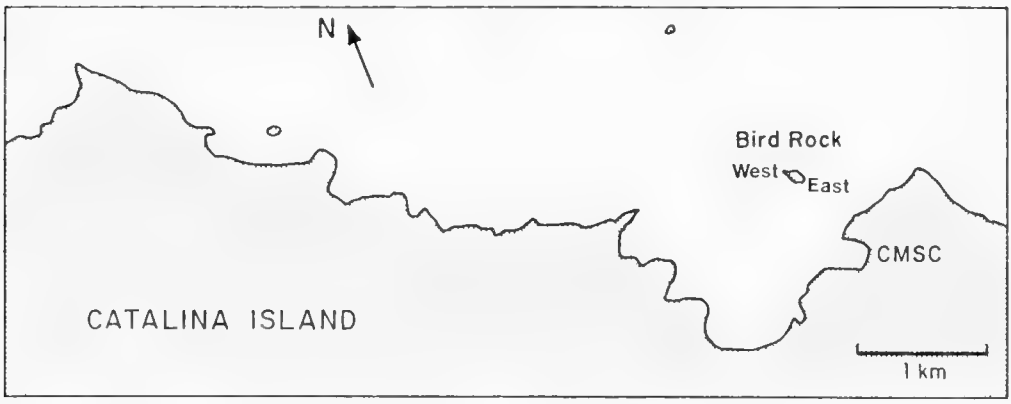

FIG. 1. Location of the study sites on the northwest shore of Santa Catalina Island, California. The primary study site at East Bird Rock, and a second site at West Bird Rock, are indicated, as is the Catalina Marine Science Center (CMSC).

octopus at East Bird Rock was marked permanently with a lettered tag. This procedure was repeated until octopuses in unmarked shelters were found rarely, indicating that most suitable shelters had been marked. During censuses of the octopuses at Bird Rock, all known and potential locations of octopus shelters were examined carefully along an established census route, and the location of each octopus recorded. Additional reconnaissance confirmed that virtually all octopuses in the study were included in each census. The census area measured approximately 1500 m^2. All censuses were taken during the day, when *O. bimaculatus* is relatively inactive (unpublished data). The study site was censused on 67 occasions between June 1976 and January 1982.

The reproductive status of female octopuses was monitored during regular censuses from 1978 to 1981. The date of egg laying, the developmental stage of the eggs, date of hatching, physical appearance of the female, and date of death or disappearance were recorded for each brooding female. The dates of all observed matings were also recorded.

In 1979 to 1981, the size of each octopus in the Bird Rock census was estimated. Actual measurements, which would have required the removal of each octopus from its den, were avoided because of the potentially adverse effects on subsequent behavior. Octopuses were assigned to one of six size categories; although subjective, the size estimates were based on experience with hundreds of octopuses, and I believe it reflects the overall pattern of sizes accurately. Buchan & Smale (1981) also estimated the size of octopuses in the field; their relationship between estimated and actual mass had an $r^2 = 0.83$ ($n = 38$).

Young *O. bimaculatus* usually occurred in a different type of habitat than adults. Holdfasts of the giant kelp (*Macrocystis*) provided the most abundant and predictable source of juveniles, although they were sometimes found in other refuges, such as very small holes or empty gastropod or bivalve shells. Octopuses occurred in kelp holdfasts from the time of settlement until they were about 50 mm ML; octopuses of these sizes (hereafter considered "juveniles") were rarely encountered in the Bird Rock census. The density of juvenile octopuses was estimated by sampling kelp holdfasts. Because giant kelp disappeared from the main study site on the E end of Bird Rock in 1976 (Ambrose & Nelson, 1982), juveniles were sampled on the W end of Bird Rock, which was similar to the main study site except for the presence of a kelp forest (see Ambrose, 1982b). Juvenile density was estimated by sampling 9 to 17 (Mean = 10.7) haphazardly-chosen kelp holdfasts distributed throughout the shallow portion of the kelp bed. A small amount of bleach was introduced into each holdfast, inducing any octopuses in the holdfast to exit. Up to three octopuses occupied a single holdfast. Each octopus was captured as it exited the holdfast and its dorsal mantle length (ML; the distance from a point midway between the eyes to the

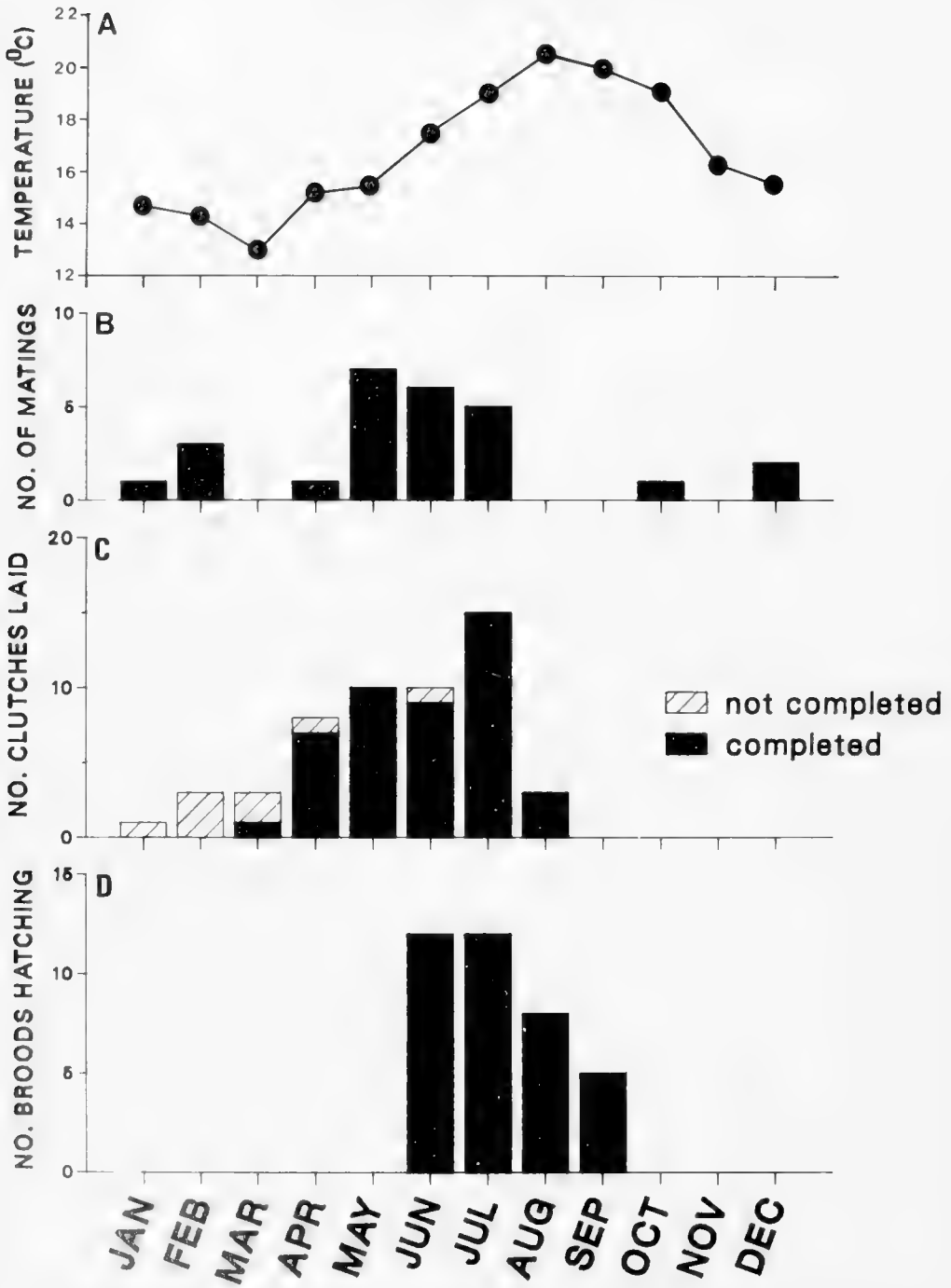

FIG. 2. Seasonality of reproductive activities. (*A*) Average water temperatures at Catalina Island. (*B*) Monthly occurrences of mating. All matings observed between 1976 and 1982, including those away from the Bird Rock study site, are included. (*C*) Monthly occurrences of egg laying. Only broods from the East Bird Rock study site are included. (*D*) Monthly occurrences of hatching. Only broods from the East Bird Rock study site are included.

posterior tip of the mantle) measured, after which it was released into a nearby holdfast. Juvenile octopuses were censused 15 times between July 1979 and May 1981.

Voucher specimens of all stages of *O. bimaculatus* examined during this study are archived at the Santa Barbara Museum of Natural History, California.

RESULTS

Reproductive biology

I observed *O. bimaculatus* mating in the field on 26 occasions between 1976 and 1982. Mating occurred throughout the year (Fig. 2b). However, there was a distinct seasonal peak in mating activity in May, June and July. Because female octopuses can store sperm for months (Dew, 1959; Wolterding, 1971; Wodinsky, 1972, 1977; Van Heukelem, 1976; Smale & Buchan, 1981 for other species; personal observations for *O. bimaculatus*), the timing of egg-laying is not related necessarily to the timing of mating.

There was a distinct seasonality to egg-laying (Fig. 2c), even though females laid eggs throughout much of the year. No egg-laying was recorded from September to December. Most eggs were laid in April through July, when the water temperature increased (Fig. 2a). All eggs at the Bird Rock study site hatched between June and September (Fig. 2d).

Not all periods of laying eggs were equally successful. A very high percentage of broods laid in the early part of the year, when water temperatures were still low, were not successful (Fig. 3). In most unsuccessful broods, both mother and the eggs disappeared. In the few cases in which the mother was discovered missing but the eggs remained, subsequent observations revealed that the eggs were eaten quickly. The cause of a brooding female's disappearance generally could not be determined. On several occasions, however, moray eels (*Gymnothorax mordax*) appeared immediately before an octopus disappeared, and the morays are assumed to have eaten the females. Moray eels did not inevitably kill brooding octopuses, though, since a number of octopuses successfully brooded eggs over a period of several years in one large crevice that occasionally contained moray eels.

The seasonality of reproduction is indicated clearly in Fig. 4, which presents the abundance of brooding octopuses at Bird Rock from 1978 to 1981. Each year, the first brooding octopuses were found in March or April. The total number of brooding octopuses increased until summer. By late summer, the number of brooding octopuses declined precipitously as the last-laid broods hatched. Approximately half of the adult population was brooding each year; assuming a 50 : 50 sex ratio, this suggests that nearly all adults at the study site were sexually mature during summer.

All females apparently died after reproducing. Some females built substantial barricades around their shelters during brooding, and the bodies of several of these octopuses were found within the barricaded shelters a few weeks after their broods had hatched. In cases where no body was found, the female disappeared shortly after her eggs had hatched. Most females were missing on the second or third census following hatching of the eggs; the average time after hatching for the disappearance of the females was 17.8 days (range 6 to 33, SD = 6.7) for 21 brooding females censused in 1978 through 1979, when censuses were most frequent. Furthermore, the physical condition of females inevitably deteriorated during the course of brooding, as the skin became greyer (particularly around the eyes), lost its tone, and occasionally developed lesions; these conditions have preceded death in the laboratory in a number of *Octopus* species (*O. briareus:* Wolterding, 1971; *O. cyanea:* Van Heukelem, 1973; *O. vulgaris:* O'Dor & Wells, 1978; *O. bimaculatus:* personal observations). Large males also became grey, and it is presumed that they died at about the same age as the females, as occurs in other species (Van Heukelem, 1973; Opresko & Thomas, 1975; Joll, 1977; Wodinsky, 1977; Hanlon, 1983).

Juveniles

Juveniles were sampled for two years. Abundances varied widely throughout the year (Fig. 5). High densities occurred consistently in early summer of 1979, 1980 and 1981, although the timing differed slightly among the years. In 1979 and 1980, a second peak of high density equal to the summer peak occurred in winter.

Because juveniles could be sampled very shortly after they settled, these data can be

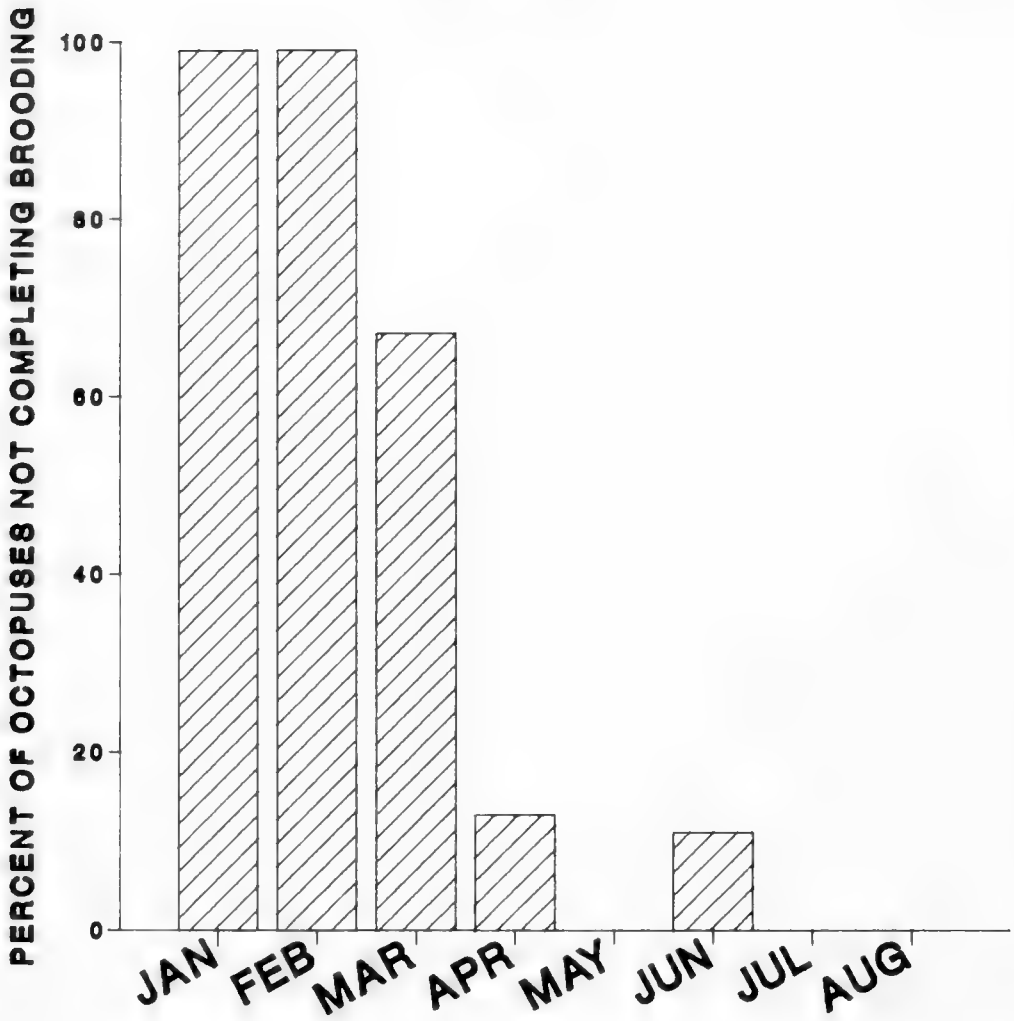

FIG. 3. Mortality of brooding octopuses. In all cases, the eggs disappeared at the same time as, or shortly after, the female.

used to indicate the time of settlement from the plankton. One or two octopuses <10 mm ML were sampled in all but three sampling periods (Fig. 6). The presence of these newly settled octopuses in nearly all months indicates that juveniles settled throughout the year.

The juvenile size data can provide a crude estimate of the growth of young octopuses, particularly for 1979 and 1980, when more frequent samples were taken. Assuming that many octopuses settled around July 1979, as indicated in Fig. 5, one can follow a regular progression in the maximum octopus size sampled through May 1980 (Fig. 6). If this progression does indeed reflect the growth of a cohort, then it took approximately 8 to 10 months for the octopuses to grow from 5 mm ML (the size shortly after settlement) to 50 mm ML. Octopuses greater than 50 mm ML left the holdfasts, and were no longer considered juveniles.

Adults

Between June 1976 and July 1982, the octopus population at East Bird Rock varied

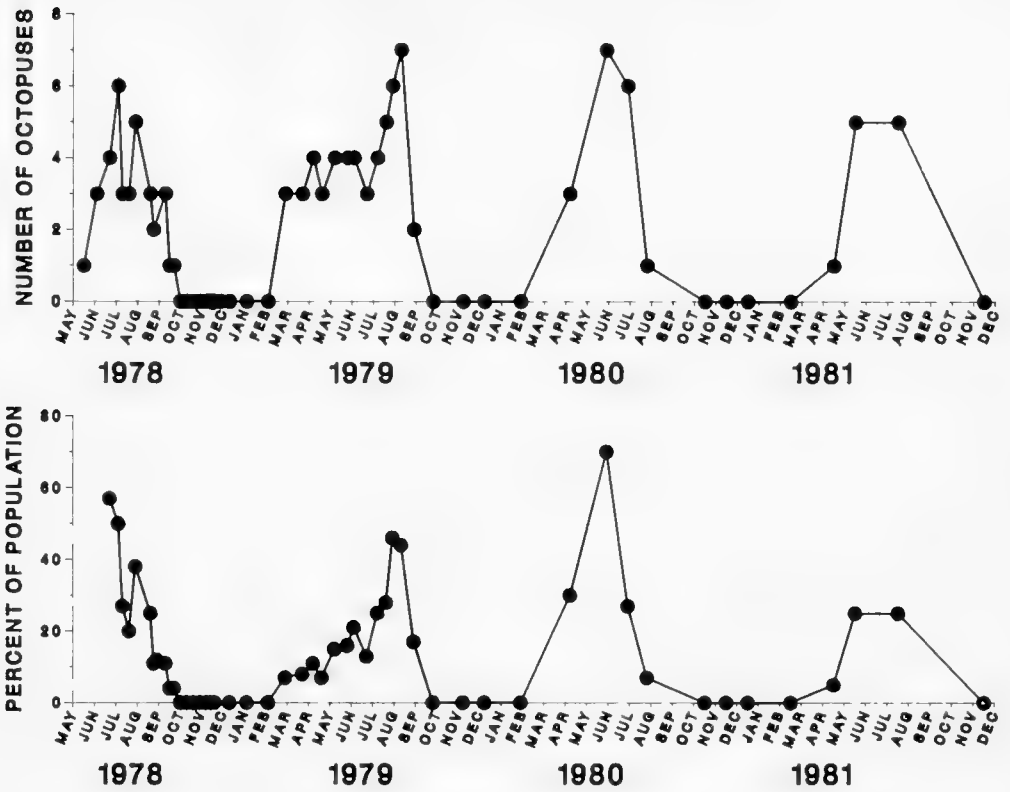

FIG. 4. Abundance of brooding octopuses at East Bird Rock.

from 7 to 45 octopuses (Fig. 7), with a Mean (± SD) abundance of 23.2 (± 11.5). Expressed as density, the highest density was 3.0 octopuses/100 m^2 in 1978, with a mean of 1.55/100 m^2. This variation in abundance occurred in distinct cycles. Abundance generally decreased markedly at the end of summer; this was noticeable in 1976, 1977, 1979 and 1981.

The decline in abundance at the end of summer coincided with the death of post-reproductive adults. Fig. 4, showing the abundance of brooding octopuses at Bird Rock, indicates that females generally stopped brooding by August or September in all years from 1978 to 1981; these females would have died by October. In four of the six years (1976, 1977, 1979 and 1981), October was a period of low abundance.

However, the general pattern of population abundance did not occur in two years, 1978 and 1980. During 1978 and 1979, the peak abundance of octopuses was 45, compared to 20–30 during normal years. The large population size in 1978 and 1979 was due to a large influx of small octopuses in 1978 (which occurred before adult size frequencies were recorded). Although post-reproductive adults died at the end of summer in 1978, this did not result in a population decline because the loss of old adults was compensated by the large number of young octopuses entering the adult population. Octopuses from the heavy recruitment in 1978 survived to reproduce in 1979; after this reproductive season, the population abundance declined due to post-reproductive mortality. In 1980, most clutches hatched by June, so females would have died by July or August. However, a large recruitment of small octopuses in July through November (Fig. 8) resulted in a sustained population size rather than a decline.

Because of variability in growth rates and the time of recruitment, variability in size distributions of adult *O. bimaculatus* is expected (Fig. 8). There is no consistent pattern

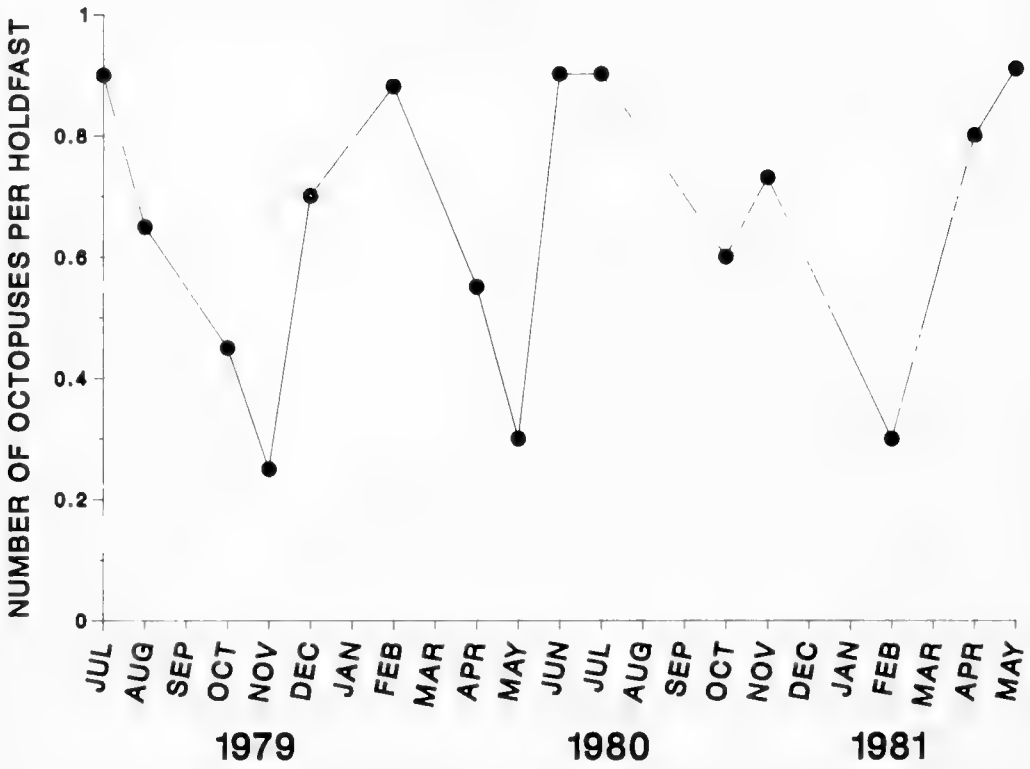

FIG. 5. Abundance of juvenile octopuses at West Bird Rock. Mean densities are based on samples of kelp holdfasts.

of sizes among years. For example, the size distributions in the same month, April, were very different in 1979, 1980 and 1981. In April 1979, most octopuses were medium-sized, with some small ones. In April 1980, most octopuses were large. In April 1981, again most octopuses were medium-sized, but there were no small octopuses.

The size distributions may provide some indication of cohorts. For example, a pulse of small octopuses can be distinguished in April 1979. The progression of this cohort is obscured by the presence of other size classes (e.g. large octopuses in April–September 1979 and small octopuses in February to April 1980), perhaps from other age classes. Nonetheless, by November 1979 only medium-sized octopuses were found; by April 1980, this pulse appeared to have moved to the larger size classes. If these data reflect the growth of a single cohort, the adult lifespan of the cohort was approximately one year.

DISCUSSION

The abundance of a semelparous species in a seasonal environment fluctuates in response to many factors. In *Octopus bimaculatus*, predictable changes in abundances result from life history patterns, the seasonality of reproduction and recruitment. The influences of these factors, as well as some of the factors that limit population size, are discussed below.

Reproductive biology

Reproductive activities of octopuses at Santa Catalina Island were very seasonal. Most eggs at the Bird Rock study site were laid between April and July, and hatched between June and September. The narrower period of hatching, compared to the time over which eggs were laid, results from the inverse relationship between development time and water temperature (Ambrose, 1981). Broods

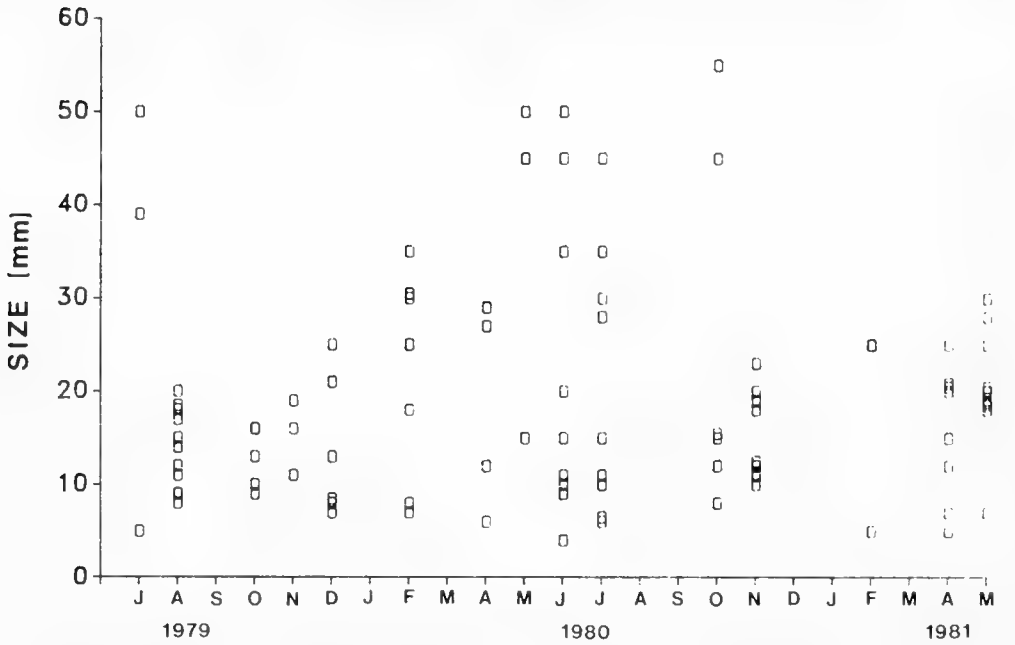

FIG. 6. Size distribution of juvenile octopuses at West Bird Rock. Each octopus measured is represented by one point, indicating its size and month of capture.

that were laid early in the year, when water temperatures were low, took 60 to 100 days to develop and hatch (*ibid.*). In contrast, broods laid later in the year, when water temperatures were higher, hatched after only 30 to 40 days. Thus, all young octopuses at Bird Rock hatched during summer, regardless of when the eggs were laid.

Among brooding octopuses, mortality was related strongly to the time of egg-laying. Nearly all clutches laid in late spring and summer were successful. In contrast, the earliest clutches, which would have hatched earlier than June, were never successful.

The relationship between development time and water temperature has important consequences for the reproductive success of *O. bimaculatus*. Much of the mortality of brooding octopuses was probably caused by predation; moray eels were associated with the disappearance of several brooding females (see also MacGinitie & MacGinitie, 1968). Because brooding females constantly guard their eggs, they are very susceptible to predation. If the probability of being eaten is constant over time, then the longer an octopus broods her eggs, the greater her chance of being eaten. Because of the inverse relationship between development time and water temperature, females laying eggs early in the year, when water temperature is low, have a much higher probability of being eaten before the brood is successful. The high mortality rate of octopuses that lay eggs early in the year appears to be a strong selective force for the timing of *O. bimaculatus* reproduction, particularly because each female has only one chance to reproduce.

It is generally assumed that most octopuses are semelparous (but see Rodaniche, 1984, for a species that is iteroparous), based on observations of the physical conditions of brooding females ("greying") and laboratory studies (Wolterding, 1971; Mangold & Boletzky, 1973; Van Heukelem, 1973; Wodinsky, 1977; O'Dor & Wells, 1978). Observations in the laboratory of physical deterioration and death of *O. bimaculatus* after brooding parallels the information on other species. Moreover, post-reproductive mortality is also indicated by the field observations. All females disappeared shortly after their eggs hatched; the consistency of the pattern and timing of the disappearances

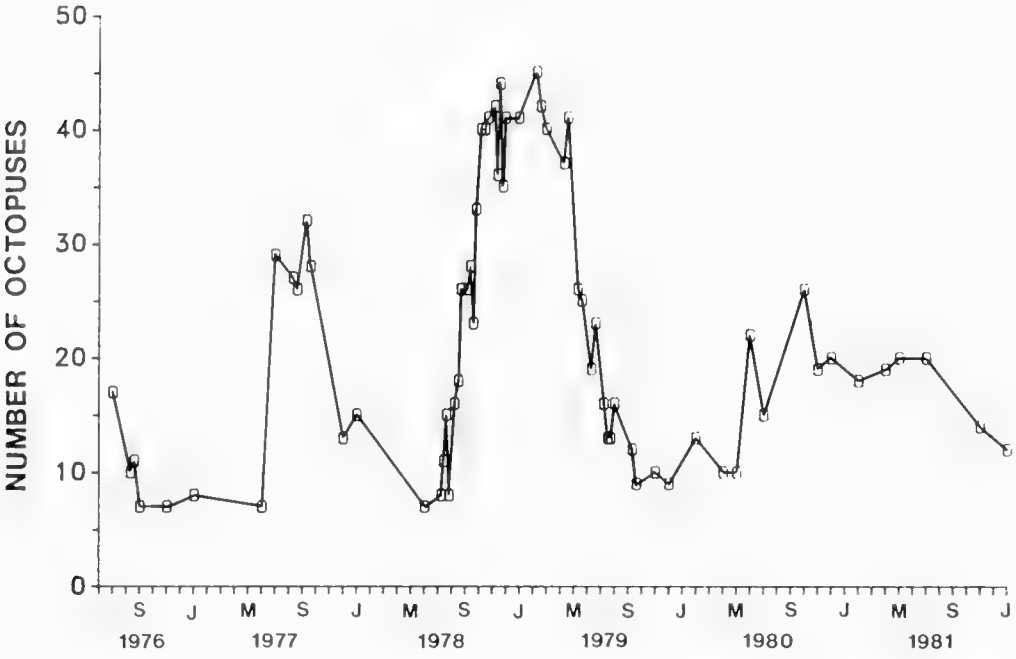

FIG. 7. Abundance of adult octopuses at East Bird Rock. Abundances were determined by censuses of all suitable shelters.

indicates that they were the result of post-reproductive mortality, rather than the females simply moving. A number of dead post-reproductive females were also discovered in their shelters. Thus, the data presented here demonstrate that *O. bimaculatus* is semelparous in nature.

Population dynamics

Increases in the numbers of adults at Bird Rock could have resulted from either the growth of juveniles that recruited to Bird Rock or the immigration of adults into the area. Although immigration and emigration were not explicitly measured for *O. bimaculatus*, adults were fairly sedentary at Catalina (Ambrose, 1982a), particularly in comparison to species such as *O. dofleini* (Hartwick *et al.*, 1984b). Nonetheless, some movement of adults did occur, and immigration was particularly noticeable when distinctively marked or unusually large individuals entered the study site. In addition, some of the increase in numbers of adults at Bird Rock can be tied to the growth of juveniles. For example, in July 1980, as juveniles grew too large for the kelp holdfasts at West Bird Rock, small octopuses began occurring in the censuses at East Bird Rock; these small octopuses were apparently juveniles that settled and grew unnoticed at East Bird Rock, although juveniles probably moved from West Bird Rock as well. Thus, both immigration and recruitment contributed to the increases in number of adults at Bird Rock.

Although octopuses undoubtedly emigrated from Bird Rock, this movement apparently had little influence on the patterns of adult abundances. Rather, the conspicuous declines in the number of adult octopuses resulted from synchrony of reproduction and the death of post-reproductive adults. As discussed earlier, females die shortly after reproducing; males presumably die at about the same time as females (see Van Heukelem, 1973; Wodinsky, 1977). Because egg-laying occurs in a restricted time period, and nearly all broods hatch in summer, most mature adults die at the same time, leading to precipitous declines in abundance recorded in Fall 1976, 1977, 1979 and 1981.

Although mortality following the summer reproductive period explains the observed

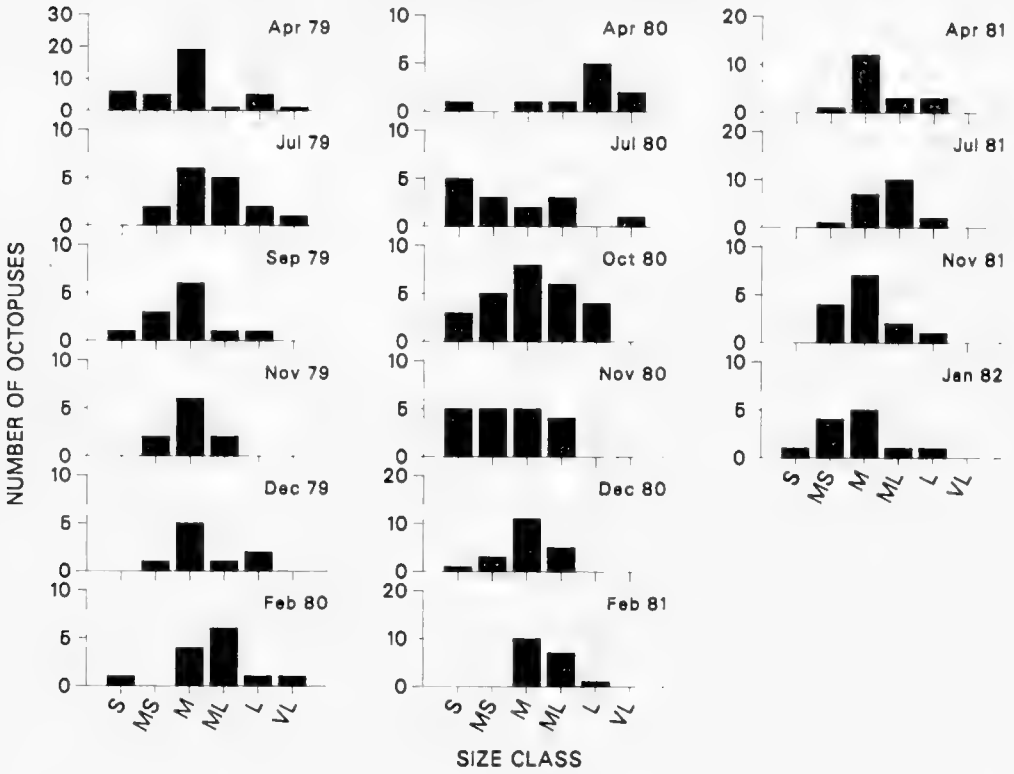

Fig. 8. Size distribution of adult octopuses at East Bird Rock. The six size categories are small (S), medium-small (MS), medium (M), medium-large (ML), large (L) and very large (VL).

declines in abundance that occurred during Fall in many years, in 1978 and 1980 there was no decline. Reproduction occurred as normal during these years, and adults died after reproducing. However, in these years their loss was compensated by young octopuses entering the adult population. Year 1978 was distinguished by a very large recruitment of small octopuses in early summer. A similar, but smaller, recruitment episode occurred in July 1980, as indicated by the smaller size classes in Fig. 8.

Thus, the pattern of abundances during a year depends on the success of recruitment. During years of relatively low recruitment, the abundance of *O. bimaculatus* declines in Fall, whereas during years of relatively high recruitment abundance does not decline, and may even increase depending on the timing and strength of the recruitment event (as in 1978).

Changes in abundance similar to that presented here for *O. bimaculatus* have been reported for *O. dofleini* (Hartwick *et al.*, 1984a) and *O. briareus* (Aronson, 1986). As in *O. bimaculatus*, the abundance of *O. briareus* declined immediately following the brooding period, and increased as juveniles (which do not have a planktonic stage) grew into the adult population. However, the cycles in *O. briareus* were not as distinct as for *O. bimaculatus*, perhaps because some females brooded at an "anomalous" time (Aronson, 1986). The absence of a planktonic stage results in a closer link between brooding and recruitment, and this link could also influence the dynamics of the populations. In *O. dofleini*, the mechanisms leading to changes in adult abundances in the studies by Hartwick and co-workers were fundamentally different from those in *O. bimaculatus* and *O. briareus*. In these studies, *O. dofleini* abundances were not closely tied to the seasonality of reproductive activities, since the *O. dofleini* studied by Hartwick *et al.* (1984a, 1984b) did not breed in the study area.

Instead, the abundance patterns were driven by the large amount of immigration and emigration to the study site (Hartwick *et al.,* 1984b).

Life history

The growth of juvenile *O. bimaculatus* was not determined in the lab, but a rough estimate can be attempted from the field data on size frequencies. These data suggest that octopuses that settled (at 5 mm ML) in July–September grew to 50 mm ML by May–June of the following year, a period of 8–10 months. This growth rate is slower than that reported for many octopus species (Itami *et al.,* 1963; Wells & Wells, 1970; Mangold & Boletzky, 1973; Van Heukelem, 1973, 1976; Smale & Buchan, 1981; Hanlon, 1983), but it also occurs at a much lower temperature than those species (13 to 18°C, compared to 20 to 27°C), and low temperatures reduce growth rates (Mangold & Boletzky, 1973; Van Heukelem, 1976; Hartwick *et al.,* 1981). Yamashita (1975) estimated that *O. dofleini,* which also occurs in cooler water, grew between 120 and 130 g in one year; this is roughly equivalent to the growth rate estimated for small *O. bimaculatus. Octopus bimaculoides,* the sibling species of *O. bimaculatus,* grew to a length of 50 mm in about 6.5 months when raised at 18°C (Forsythe & Hanlon, unpublished data); this temperature is several degrees higher than Catalina (which averaged about 16°C between September and June), suggesting that the growth rate of *O. bimaculatus* may be comparable to that of *O. bimaculoides,* and that 8 months is a reasonable estimate of the time needed to reach 50 mm ML.

The information on the growth of juvenile *O. bimaculatus,* in conjunction with estimates for the length of the adult stage, can be used to estimate the lifespan of *O. bimaculatus.* One estimate of the length of the adult stage, based on the growth of a cohort from April 1979 to April 1980 (see Fig. 8), suggests that the adult stage lasts 12 months. A second estimate, based on the presence of octopuses from the high recruitment year from August 1978 to July 1979 (Fig. 7), indicates that the adult stage lasts 11 months. Combined with the estimate of 8 to 10 months for growth from settlement to adult, a preliminary estimate of the overall lifespan of *O. bimaculatus* is 19 to 22 months, plus several months in the plankton.

The actual lifespan of *O. bimaculatus* is likely to vary, depending on the time (and hence temperature) of hatching and settlement, and the time available between settlement and the next reproductive season. The influence of these variables have been described in two alternative models of the life cycle of *O. bimaculatus* (Fig. 9). Both of these models incorporated the information known about the timing of reproductive activities of *O. bimaculatus,* and the availability of planktonic young (M. Ninos, unpublished data).

The "alternating generation" model of cephalopod life cycles (Fig. 9A) has been proposed in various forms for various cephalopod species, including *Illex illecebrosus* (Mesnil, 1977), *Sepia officinalis* (Mesnil, 1977; Boletzky, 1983), *Loligo pealei* (Summers, 1983), *Eledone cirrhosa* (Boyle, 1983b) and *Eledone moschata* (Mangold, 1983). In this model, octopuses that hatched in June would spend one month in the plankton and spawn 13 months after settling, at the end of the reproductive season of the following year (in August). These eggs would hatch in September, with the octopuses spending two months in the plankton, skipping the first reproductive season, and spawning 17 months after settling, in April of the second season. The eggs would then hatch in June, and the cycle would be repeated. In any cohort, the octopuses that hatch first initially grow more rapidly due to better conditions (higher water temperatures), and spend less time in the plankton. The octopuses that hatch last would not reach a sufficient size for spawning in the first breeding season, and hence would lay eggs at the beginning of the breeding season the following year.

Although the alternating generation model is appealing, it requires rapid growth by *O. bimaculatus* so that spawning can occur 13 months after settling. This lifespan is considerably less than the 19 to 22 months lifespan estimated from the field data. Because the estimate from the field data is so crude, the shorter lifespan is possible. In fact, *O. bimaculoides* raised at 18°C (slightly higher than Catalina conditions) do spawn in as little as 13 months (Forsythe & Hanlon, unpublished data). However, confirmation of a sufficiently high growth rate for *O. bimaculatus* under natural conditions at Catalina Island is necessary before the alternating generation model can be considered likely.

The "alternating years" model (Fig. 9B) differs from the alternating generation model

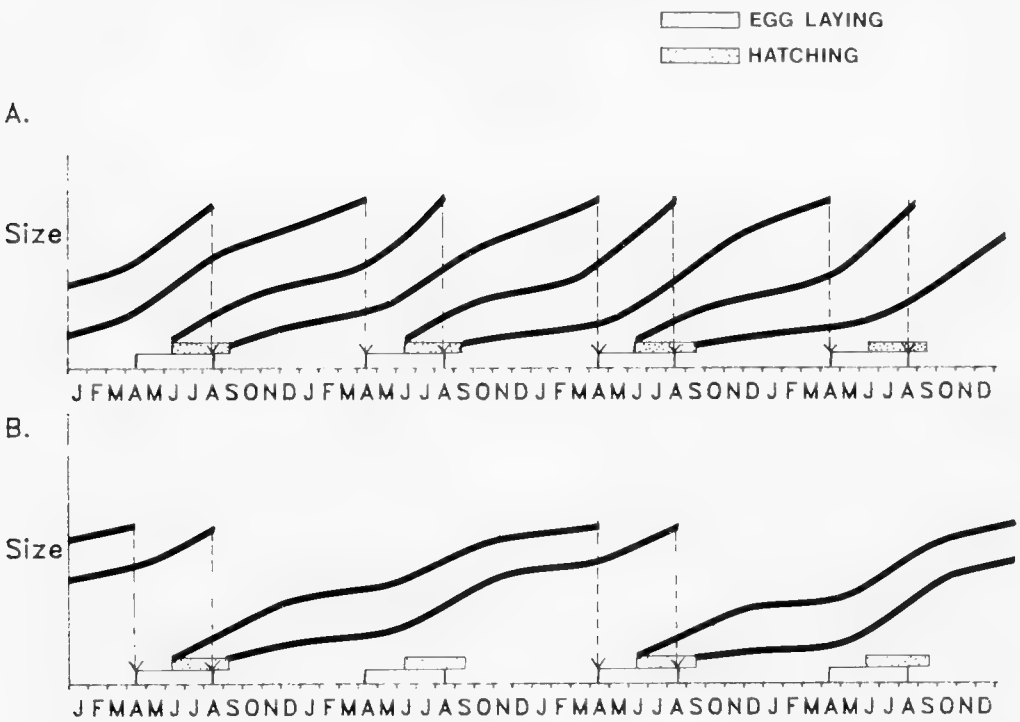

Fig. 9. Life history models for *Octopus bimaculatus*. (A) "Alternating generation" model. In each cohort, the octopuses that hatch in June spend one month in the plankton and spawn 13 months after settling, with their eggs hatching the following September, whereas the octopuses that hatch in September spend 2 months in the plankton, skip the first reproductive season and spawn 17 months after settling, with their eggs hatching in June. (B) "Alternating years" model. In each cohort, all octopuses skip the first reproductive season and spawn the following year, with the octopuses that hatched first spending one month in the plankton and spawning first, and those hatching last spending two months in the plankton and spawning last. For clarity, the growth of one year-class is omitted from B, although egg-laying and hatching times are presented. Vertical arrows represent egg-laying; although not indicated, adults survive past egg-laying, but die shortly after the eggs hatch. See text for further explanation.

in several critical facets. First, no octopuses spawn in the year following hatching. Octopuses that hatch in June spawn 21 months after settlement, while octopuses that hatch in September also spawn 21 months after settlement. Second, "lineages" retain their relative spawning times *i.e.* early-spawning octopuses always spawn in the beginning of the breeding season. This latter aspect could be relaxed to allow flexibility in spawning time, so there is no relationship between the relative hatching time and relative spawning time. The alternating years model is consistent with the estimated lifespan of *O. bimaculatus*. This model, rather than the "alternating generations" model, must be closer to reality for longer-lived octopuses such as *O. dofleini,* which must skip several breeding seasons before reproducing at an age of 4 or 5 years (B. Hartwick, personal communication).

Both models predict that settlement would occur only from late summer to fall; in reality, settlement occurs throughout the year. Year-round settlement probably results from planktonic young arriving at Bird Rock from other areas, where spawning may not be synchronized with Catalina. Depending on when these recruits settled, and which life cycle model is more realistic, the recruits may spawn during the first reproductive season after settlement, or they may have to wait until the following season. Similarly, if planktonic *O. bimaculatus* drift into other areas, they would presumably spawn according to the local breeding season.

Factors limiting the size of O. bimaculatus *populations*

Some octopus populations may be limited by shelters (Mather, 1982; Aronson, 1986). Shelters are most likely to be limiting in soft-bottom habitats or when the individuals are so large that adequate refuges are rare, as may be the case for *O. dofleini* (Hartwick *et al.*, 1988, this volume). Aronson's experimental study (1986) demonstrated clearly that the number of dens limited the density of *O. briareus* in a soft-bottom marine lake without predators. The addition of thousands of pots to an area off the coast of Japan increased the catch of *O. dofleini,* suggesting that dens limited the size of this population as well (Tauchi & Matsumoto, 1954). However, in rocky habitats shelters probably do not limit *O. bimaculatus* populations (Ambrose, 1982a), nor the populations of a number of other octopuses (Aronson, 1986; Hartwick *et al.*, 1978, 1984b).

Based on the growth of wild individuals compared to laboratory-reared individuals, food does not appear to limit populations of *Sepia officinalis* (Boletzky, 1983), *Eledone cirrhosa* (Boyle & Knobloch, 1982b), *O. vulgaris* (Mangold & Boletzky, 1973), *O. dofleini* (Hartwick *et al.*, 1981) or *O. cyanea* (Van Heukelem, 1973, 1976); this may be the case for *O. bimaculatus* as well. Food was abundant at East Bird Rock in 1978, even though the octopus density was 1.4 times higher than in any other year and five times higher than the density in 1982 (Ambrose, 1986). Even if food limited the number of octopuses in 1978, food was probably not limiting in other years, when octopus density was lower and food density was higher (Ambrose, 1986). Of course, because the *O. bimaculatus* population fluctuates, both temporally and spatially, the density of octopuses may occasionally be quite high. Under these circumstances, octopuses can reduce the abundances of their prey (Ambrose, 1986). When *O. bimaculatus* are dense, therefore, food could be limiting locally.

However, *O. bimaculatus* can be very mobile. At least some of the time, the high density of octopuses will be only very local; in these circumstances, to alleviate the food shortage, octopuses need only move away. During the six-year study at Santa Catalina Island, octopuses never appeared to be dense enough to be severely limited by food. However, food could become limiting if *O. bimaculatus* experiences population explosions such as those reported by Forrest & Waterston (1934), Rees & Lumby (1954), and Arntz (1984).

The impact of predation on benthic octopus populations has been studied rarely. Hartwick *et al.* (1988, this volume) have suggested that the giant Pacific octopus, *O. dofleini,* may suffer substantial predation in some habitats. Aronson (*in press*) studied a population of *O. briareus* in a salt lake (Sweetings Pond) in the Bahamas. The salt lake presented a unique environment because it was virtually free of predators. Aronson noted that the density of *O. briareus* was far higher in Sweetings Pond than in the surrounding ocean, which did contain a number of predators on octopuses. It seems reasonable to conclude that the absence of predators in Sweetings Pond contributed to the high octopus densities there.

In southern California, the situation with regard to octopus predators is somewhat problematic. Many species eat octopuses; in southern California, at least 10 common fish species found in kelp beds are reported to include cephalopods in their diets (Quast, 1971). However, octopuses comprised a substantial portion of the diet in only a few of these fish, primarily the cabezon (*Scorpaenichthys marmoratus*) and the sculpin (*Scorpaena guttata*), with 70% and 20% of the stomachs examined, respectively, containing octopuses or squids (Quast, 1971). At Catalina, I rarely observed fish feeding on octopuses, although they occasionally followed active octopuses, and on very rare occasions bit octopus arms. Sculpins and cabezons frequently had octopuses in their stomachs (unpublished data), but these were usually very small octopuses rather than adults. One predator that could affect adult octopuses is the moray eel (*Gymnothorax mordax*). Moray eels are presumed to have preyed upon several brooding females at Catalina Island. However, brooding females are particularly vulnerable, and it seems unlikely that moray predation on other adults is significant.

In the past, fish may have been more important predators on adult octopuses. Most of the large fish in southern California have been removed by fishing. In Baja California and the distant offshore islands of California, where fishing pressure is relatively low, the size distribution of potential octopus predators such as sheephead (*Semicossyphus pulcher*) and kelp bass (*Paralabrax clath-*

ratus) is dramatically different from Catalina; the larger fish are much more effective predators on sea urchins (Nelson & Vance, 1979), and presumably on octopuses as well. Thus, fish may have been *historically* important predators on adult octopuses, but they are unlikely to be important now.

Marine mammals also eat octopuses (Kenyon, 1965, 1975), and where abundant could limit octopus population sizes. Anecdotally, I have noted at other areas in southern and Baja California that octopuses are frequently rare near high densities of seals and sea lions, such as occur around rookeries; however, these areas are limited. At Bird Rock, seals and sea lions were seldom abundant, were never observed feeding in the area, and probably had no effect on octopus abundance. Even locations where marine mammals occurred regularly (although not in high densities) on Catalina supported relatively high densities of octopuses.

I conclude that few factors seem to be limiting the number of adult octopuses at Santa Catalina Island. Rather, the population-regulating processes appear to be occurring in the larval and juvenile stages. The importance of early life stages is supported by the fact that the heavy recruitment in 1978 resulted in unusually high octopus densities at Bird Rock. Heavy recruitment has been reported for other octopus species (Rees & Lumby, 1954; Boyle & Knobloch, 1982b; Arntz, 1984).

The abundance of juveniles could be limited by the number of octopuses that arrive to settle, and by post-settlement processes. There are many known hazards for young benthic octopuses, and mortality is probably high. Cannibalism occurs in *O. bimaculatus* (Ambrose, 1984) as well as other species (Aronson, 1986, *in press*), although its importance is not known. The risk of predation by other species, especially fish, is likely to be great; at Catalina, cabezon and sculpins consume small octopuses regularly (unpublished data), and many other fish species probably consume juvenile octopuses whenever possible. Although there are no data on the factors determining the number of settling octopuses, the direction and speed of ocean currents and the abundance of food and predators in the plankton are likely to be important. As in many marine species, varying oceanographic conditions probably result in a very wide range of settlement densities, so that density-independent factors may have the greatest influence on the number of octopuses at Catalina.

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BEHAVIOR, BODY PATTERNING AND REPRODUCTIVE BIOLOGY OF *OCTOPUS BIMACULOIDES* FROM CALIFORNIA

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ABSTRACT

Octopus bimaculoides is a common predator in subtidal and intertidal communities along the southern California coast, yet there is little information on the biology of this species. Laboratory culture experiments combined with field observations provided information on behavior, body patterning and reproductive biology. The benthic hatchlings are adult-like in appearance and behavioral capabilities. They are active in the first month and swim commonly. This species is tolerant of crowding in the lab, although cannibalism is seen in the first 40 to 60 days and rarely afterwards. Thirty-six components of body patterning were identified, being combined variably to produce eight categories of body patterns.

In a lab population cultured at 18° C, the hectocotylus on males (mean weight and size 54 g and 55 mm ML) was seen at five months, but mating was not seen until 10 months. During mating, which lasted typically one hour, males and females sat adjacent to each other with the third right arm of the male being the only point of contact. Mating did not stimulate immediate egg-laying. Egg-laying began at 13 to 14 months of age with an average brood size of 380 eggs (maximum was 774 eggs). Eggs were typically between 10 and 12 mm long. Embryonic development required 82 days at 17.8° C, but only 46 days at 23.4° C. Generally, development time decreased by 10% with each rise in temperature of 1° C.

Field observations were made on subtidal and intertidal spawning populations and indicated a broader spawning season for this species than thought previously. The spawning population observed at San Quintin, Baja California, Mexico, extends the S known range of this species by about 25%. Some populations of this species spawn at a smaller size and produce significantly smaller eggs.

Key words: *Octopus;* culture; behavior; body patterning; reproduction.

INTRODUCTION

The California coast (including the Baja peninsula) has a rich cephalopod fauna, with octopods represented by at least ten species (Hochberg, 1976). Two of these, *Octopus bimaculoides* (Pickford & McConnaughey, 1949) and *Octopus bimaculatus* (Verrill, 1883) form a sibling-species complex with a sympatric distribution from San Simeon and the Channel Islands S to at least Ensenada on the northern Baja peninsula (Hochberg & Fields, 1980). These species are nearly indistinguishable from a morphological standpoint, and for over 60 years they were thought to be a single species (*O. bimaculatus*). The existence of two species was determined finally by (1) egg size and mode of life of the hatchlings (*O. bimaculoides* produces eggs 10 to 17 mm long and benthic hatchlings; *O. bimaculatus* produces eggs 2 to 4 mm long and planktonic hatchlings), and (2) the characteristic mesozoan parasites found in the kidneys of adults (Pickford & McConnaughey, 1949).

Since the original description of *Octopus bimaculoides* (Pickford & McConnaughey, 1949), few data have been added to our knowledge of the biology and life history of this active carnivore. McConnaughey (1941, 1951, 1960) studied the mesozoan kidney parasites and Peterson (1959) described the anatomy and histology of the reproductive system. Pilson & Taylor (1961) reported that *O. bimaculoides* could inject venom through holes it drills in the shells of certain molluscan prey species. MacGinitie & MacGinitie (1968) provided anecdotal natural history observations. Packard & Hochberg (1977) described aspects of skin patterning, while Hochberg & Fields (1980) reviewed information available on *O. bimaculoides.* Conversely, *O. bimaculatus* has been studied recently by Ambrose, who described the egg stages and

hatchlings (Ambrose, 1981) and conducted a thorough study of its ecology at a site off Catalina Island (Ambrose, 1982a, b; 1983, 1984, 1988, this volume). In some of these studies, authors have pointed out that species identification may be uncertain due to the difficulty in differentiating field-caught *O. bimaculoides* from *O. bimaculatus* (Peterson, 1959; Pilson & Taylor, 1961). Clearly there is need for information on the biology of *O. bimaculoides* derived from animals of known identity.

The majority of the data reported here are based upon observations of large lab populations of *Octopus bimaculoides* cultured from eggs to sexual maturity and egg-laying. Field observations were made on spawning populations of *O. bimaculoides* during collecting trips to California and Mexico. This study arose as part of our ongoing research to evaluate various large-egged octopus species as candidates for large-scale laboratory culture (Hanlon & Forsythe, 1985).

MATERIALS AND METHODS

Octopus bimaculoides was studied at this lab from 1982 to 1986. Live material was obtained and studied from throughout the geographic range. We collected eggs or gravid females from populations near Santa Barbara, Los Angeles and Carlsbad, California and from San Quintin, Baja California, Mexico. Field observations on these spawning populations were made during two collecting trips in 1984 and 1985. Live animals from all four locations were returned via air freight to Galveston, Texas for subsequent lab culture experiments. A single major full-life-cycle culture experiment was conducted from late June 1982 to mid-August 1983, with 47 *O. bimaculoides* reared successfully from hatching to full sexual maturity. The majority of observations on reproductive biology were made on this population. Three other lab populations were reared to various points in the life cycle, but not to maturity. During partial and full-life-cycle culture experiments, chronological observations on certain aspects of behavior and body patterning were possible. Notes from visual observation periods plus photo-documentation were used in analyzing behavior and body patterning.

Most aspects of behavior are associated with specific body patterns. Each body pattern (the appearance of the animal at any given moment) is made up of chromatic (color) components, textural components, postural components and locomotor components (see Packard & Sanders, 1971). The body patterns and their components are physiological entities; that is, they are under control of the nervous system and therefore can change rapidly. Although many of the components of patterns, and some patterns themselves, are common among related octopus species, others are species-characteristic and useful for identification (see Hanlon, 1988, this volume). Many of the patterns are predictive of specific types of behavior. Details of the hierarchical organization of body patterning are found in Packard & Sanders (1971) and Packard & Hochberg (1977). In this paper we restrict our observations to the component and body pattern level of patterning. For standardization, Components and BODY PATTERN names are capitalized as shown. We favor the term "DEIMATIC" rather than "DYMANTIC" (Young, 1950) for the threat pattern because it has gained general acceptance with ethologists (e.g. McFarland, 1982).

Culture work was carried out in closed, recirculating seawater systems, consisting typically of a circular 2,000 liter capacity water-conditioning tank above which were supported shallow culture trays. These systems were described by Hanlon & Forsythe (1985). In the conditioning tanks, the sea water was subjected continuously to physical, biological and chemical filtration (cf. Spotte, 1979). Generally, each system had one 400 liter capacity (240 × 60 × 28 cm) and one 200 liter capacity (120 × 60 × 28 cm) culture tray. Water depth was kept shallow, ranging from 4 to 5 cm for hatchlings and juveniles to a maximum of 18 to 20 cm for adults. Hatchlings were provided with small ceramic cylinders (1 cm long × 1 cm diameter) for dens. As the animals grew they were provided with progressively larger dens made from short lengths of PVC plastic pipe. Dens were always provided in excess (typically 1/3 to 1/4 remaining unoccupied) and fairly evenly distributed throughout the culture trays. Artificial sea water (Instant Ocean® brand) was used exclusively, with sea salts dissolved in deionized water. The culture systems received overhead fluorescent lighting and natural, indirect sunlight from large adjacent windows. The light cycle was approximately the same as that outdoors since the fluorescent lights were only on from 0800 to 1700 hours.

Octopuses were grown on a diet of live food, primarily crustacean shrimps supplemented with crabs, fish and gastropod molluscs (see Forsythe & Hanlon, 1980; Hanlon & Forsythe, 1985).

LABORATORY OBSERVATIONS

Behavior and body patterning

General behavioral observations. At hatching, *Octopus bimaculoides* weighs approximately 70 mg (live wet weight) and has a mantle length of 6.0 mm. The hatchlings assume a benthic life style immediately and are adult-like in appearance and behavioral capabilities; they can immediately swim, crawl, eject ink, capture prey and change color. The hatchlings are alert and agile, quickly perceiving and reacting to activity around them. Upon hatching, the octopuses seek cover quickly, but during the first month the animals are quite active and mobile even during the day. Night observations typically revealed nearly all octopuses out of their dens, whereas during the day it was unusual to see more than 10% of the population out at any one time. During the day young octopuses remain at the opening of their dens in an observant posture. The octopuses swim a great deal in the first month. Two types of swimming are seen. The first is that used as an escape response when startled. From the sitting position, the octopus first ejects ink in one direction and quickly swims off in the opposite direction with the mantle foremost and the arms trailing straight back. The second and more common type of swimming is slower and more controlled. It is a general searching locomotion used in conjunction with crawling on the bottom. The mantle is held nearly horizontal to the bottom with the arms held together and directed downward. The octopus then lifts off the bottom in a hovering fashion and proceeds either forward with the arms and head preceding the mantle, or occasionally backwards (mantle first) or laterally. These excursions usually cover 10 to 15 cm, with intermittent bottom searching, although excursions up to 30 or 50 cm are sometimes seen. This searching behavior does not always seem to be associated with hunting prey because potential food organisms are sometimes ignored or captured and released. It is a restless behavior that may have been evoked by the relatively high crowding encountered in lab culture.

Swimming decreases after the first two months. Swimming becomes reserved for short bursts or hops to capture prey (one or two arm-spans distant) or for situations requiring a quick escape. Benthic crawling or walking become the principal means of locomotion.

Octopuses were highly tolerant of crowding under the high-density culture conditions. In one experiment, a population remained stable at a stocking density of 44 octopuses per 0.23 m^2 (equivalent to nearly 200 octopuses per m^2) from approximately two weeks post-hatching until three months. Thereafter the animals were given more space (0.72 m^2). The octopuses grew from a wet weight of 0.07 g to nearly 3 g over this period. At one year post-hatching, this same group of octopuses (now adults) numbered 30 animals, had a mean wet weight of 505 g and was distributed over 2.2 m^2 (13.6 octopuses per m^2). The animals were non-territorial and essentially nonaggressive towards conspecifics, although exceptions did occur. Aggressive behavior between conspecifics, though observed rarely, usually consisted of animals tugging and pulling at one another. This behavior often left sucker marks on the skin of combatants. Competition for hiding places sometimes resulted in one octopus being evicted by a newcomer. It was not uncommon to see two or more octopuses sharing large dens. Large animals were usually dominant over smaller conspecifics.

Cannibalism was seen primarily during the first month and rarely thereafter; in the largest experiment it represented 8% of all mortalities throughout the study, with 70% of the cannibalism occurring during the first 30 days of culture. This same trend has been observed in numerous broods. Hatchling cannibalism occurred despite high food abundance (mysid shrimps) and almost always in the early morning hours just before daylight. Other aggression-related mortalities represented fewer than 4% of all mortalities. These "murders" were often violent judging from the victim's condition and, with one exception, always involved sexually mature animals.

Although octopuses are renowned for their propensity to crawl out of aquaria, *Octopus bimaculoides* only exhibited this behavior when young. From approximately three months onward, no lids or deterrents to escape were needed. In fact, no animals es-

caped on two occasions when the water supply was cut off accidentally (resulting in poor water quality). In contrast, other species we have cultured (e.g. *Octopus maya* and *Octopus briareus*) would have escaped under these conditions. Only one adult *O. bimaculoides* ever crawled out of a culture system.

Feeding behavior. Hatchlings typically began feeding within 24 hr or sooner. They were well-equipped to capture live prey (having approximately 30 suckers per arm at hatching) and could subdue food organisms their own size. Most feeding occurred at night, but intermittent feeding was seen throughout the day, especially during the first month. When prey organisms were abundant (as was normally the case during this study) the octopuses tended to be opportunistic predators by waiting for prey to come near or blunder into their arms. Young hatchlings often accumulated a webful of prey, particularly when mysid shrimps (*Mysidopsis* spp.) were available. When food was less abundant or a particularly desirable food organism was sighted, well-executed attacks covering several cm were made. Frontal or lateral attacks were usually made with two or more arms thrown out over the prey organism when it was within reach. By two weeks of age, hatchlings were also seen parachuting over the top of small groups of prey organisms, catching several at a time. As the octopuses grew larger in the culture systems, the relatively crowded conditions, combined with the abundance of food, made it unnecessary for the octopuses to move far from their dens to feed.

Body patterning. A total of 36 recognizable and repeatable components of body patterning were identified and are listed in Table 1. These components were combined variably to produce eight body patterns (Table 1). Although most of the component names are self-explanatory, some of the more common ones deserve comment. Figs. 1 to 12, and those in Packard & Sanders (1971) and Packard & Hochberg (1977), illustrate many of these components.

Several white spots are found over the dorsal mantle, but there is a large conspicuous pair on the mid-dorsal mantle just anterior to two prominent mantle papillae. This pair is referred to as Mantle White Spots (e.g. Figs. 1, 2, 7, 9). They are generally round but their edges are not delineated sharply. Frontal White Spots (e.g. Figs. 1, 2, 6, 7) consist of an upper central grouping of spots and two lower crescents (Fig. 2) or oval spots. The edges of these spots are not delineated sharply. Arm White Spots are arranged linearly along the arms with the first (proximal) pair usually most conspicuous (Figs. 1, 2, 6, 12). White Longitudinal Center Stripe extends dorsally from the posterior mantle tip over the head and along the adjacent edges of the first pair of arms (e.g. Fig. 9). It is sometimes interrupted on the dorsal head by Dark Hood (Fig. 9). The Transverse Mantle Bar begins at the base of the two major mantle papillae and extends toward the ventral mantle surface (Figs. 4, 7, 8, 9). Yellow-Orange Spots (Fig. 11) are found on the dorsal mantle and arms and usually mark the location of major body papillae.

The Ocelli show brilliant blue iridescence with variable backgrounds. The iridescent portion of each ocellus is usually a series of rounded chainlinks (Fig. 11). Eye Bar is an extension of the Pupil slit and varies from spanning just the orbit to extending onto the arm base anteriorly and onto the mantle posteriorly (Figs. 2, 8). Integumental Trellis is the network of fine dark lines delineating the patch and groove system of the skin (Figs. 11, 12).

There are four Long Mantle Papillae forming a diamond in the center of the mantle (Fig. 7; see also diagram in fig. 25 in Packard & Hochberg, 1977). The postural component Two Raised Arms (first arm pair) is characteristic of hatchlings as they move about.

Eight basic body patterns are listed in Table 1 under two broad categories (chronic and acute) depending upon their duration. Chronic patterns are used generally for concealment and last long periods, while acute patterns are used in inter- and intraspecific encounters and last only seconds or minutes. UNIFORM LIGHT PHASE, UNIFORM DARK PHASE and CHRONIC GENERAL MOTTLE are typical of many octopuses. The acute pattern weak DISRUPTIVE is first seen in hatchlings (Fig. 1A) and is characterized by the component White Longitudinal Center Stripe (same as dark Longitudinal Stripes described in *O. vulgaris* by Packard & Sanders, 1971) bordered by uniform darkening on either side. A variation of DISRUPTIVE is seen where one side of the body is very dark brown and the opposite side is very pale light brown separated by the White Longitudinal Center Stripe. The darker side is usually toward the source of stimulus. The most commonly seen strong DISRUPTIVE pattern

is used for concealment in the field and consists of White Longitudinal Center Stripe, Dark Hood, Transverse Mantle Bar, extended Eye Bar, Prominent Mantle and Eye Papillae, Frontal and Mantle White Spots and Coiled Arms (Figs. 7, 8). The DEIMATIC pattern is illustrated by Packard & Hochberg (1977: fig. 26B). It is different from the classic DEIMATIC in *Octopus vulgaris* in that the overall body color is not pale, but mottled, and the Ocelli are present along with Dark Edged Suckers and Interbrachial Web Spread. It is similar to ACUTE MOTTLE. An early form of the DEIMATIC can be shown by hatchlings and consists of a weak Mottle with Ocelli being expressed strongly and the Interbrachial Web Spread with Head Flattened. The small animals orient strongly towards the stimulus. In the field, the fully formed FLAMBOYANT pattern and posture (to include swimming) has been seen only once in a small animal chased into the water column (Fig. 10). The FLAMBOYANT pattern may be seen with either uniform or disruptive coloration. PASSING CLOUD was seen mainly in young animals and consisted of a wave of expanded chromatophores beginning at the base of the arms and moving outward to the arm tips.

The ontogeny of patterning was followed in laboratory reared animals. In the first week post-hatching, the hatchlings can show the body patterns UNIFORM LIGHT PHASE, UNIFORM DARK PHASE and weak DISRUPTIVE. The latter is first seen as just unilateral or bilateral darkening of the adjacent edges of the first and second pairs of arms while all other portions of the arms remain light. By day 7 or 8, weak DISRUPTIVE is shown from the mantle tip across the head and onto the arms. Weak DISRUPTIVE can also be modified by unilateral darkening of only one side of the body, often towards a conspecific or disturbance. Hatchlings usually walk about in the Two Raised Arms posture. While in UNIFORM LIGHT PHASE, Extrategumental Chromatophores are usually evident over the viceral mass and between the eyes on the head.

At three weeks, the octopuses begin to show a complete DEIMATIC pattern consisting of a weak ACUTE MOTTLE with prominent Ocelli, extended Eye Bar and Interbrachial Web Spread. Dark Edged Suckers is absent. While in DEIMATIC they rock their head and mantle gently from side to side and direct strong blasts of water from the funnel at the source of stimulus. Prominent Eye Papillae are seen at this age. In the first month the octopuses tend to be in UNIFORM DARK PHASE (Fig. 3) when out and moving, and change to UNIFORM LIGHT PHASE when stationary or in their dens. DISRUPTIVE becomes a more distinct and complete pattern and extended Eye Bar often is shown while in UNIFORM LIGHT PHASE (Fig. 2). At five weeks, the pattern PASSING CLOUD was seen when animals moving about in UNIFORM LIGHT PHASE would interact with other octopuses by darkening the distal two-thirds of the arms and web. The pattern is distinctive but lasts only one to two seconds and is seemingly intended to startle or surprise conspecifics. Furthermore, this behavior was only shown by aggressive animals rather than those in a defensive posture.

CHRONIC GENERAL MOTTLE (Fig. 12), with its typical greenish hue, begins to be seen at three to four months of age and becomes stronger with age. Animals greater than 50 mm ML predominantly show CHRONIC GENERAL MOTTLE, UNIFORM LIGHT PHASE or ACUTE MOTTLE. The other patterns in Table 1 are rarely, if ever, seen in older animals. Field observations have confirmed all body patterns listed in Table 1 except PASSING CLOUD.

Reproductive biology

Sexual maturation. The earliest sign of sexual maturation is the formation of the

FIG. 1. A 3-week-old juvenile *Octopus bimaculoides* (8.0 mm ML) in a transition phase from CHRONIC GENERAL MOTTLE (note arms) to UNIFORM DARK PHASE (note mantle). Note that the white spots are evident at this young age.

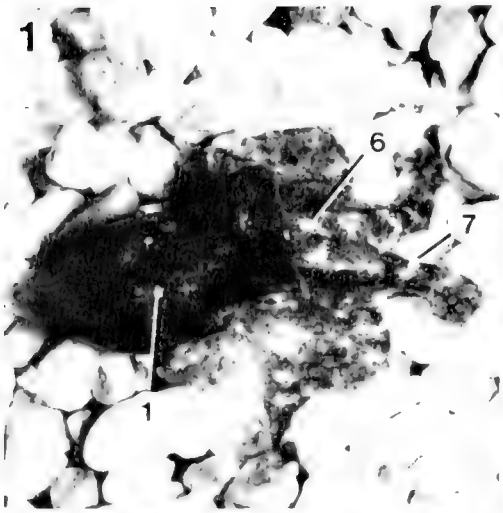
FIG. 2. A 4-week-old *Octopus bimaculoides* (9.0 mm ML) in UNIFORM LIGHT PHASE with extended Eye Bars. Note Frontal White Spots.

FIG. 3. Two 5-week-old *Octopus bimaculoides* (10.0 mm ML) moving about the culture tray in maximum UNIFORM DARK PHASE.

FIG. 4. A 10-week-old *Octopus bimaculoides* (13 mm ML) in moderately strong DISRUPTIVE.

FIG. 5. A 3-month-old *Octopus bimaculoides* (18 mm ML) in a light version of UNIFORM DARK PHASE.

FIG. 6. The same octopus in Fig. 5 in an exaggerated Standing posture resulting in a disruptive body outline Note Ocellus and Frontal White Spots.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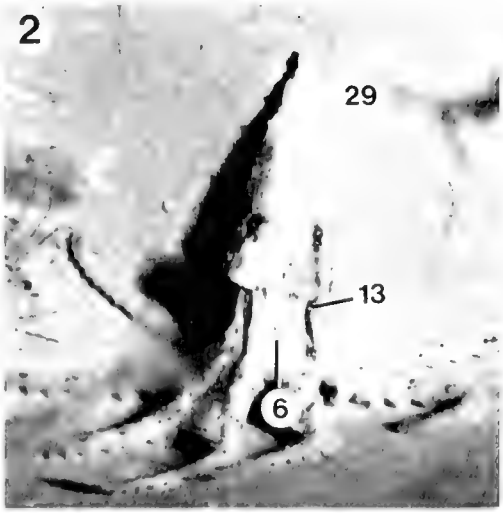
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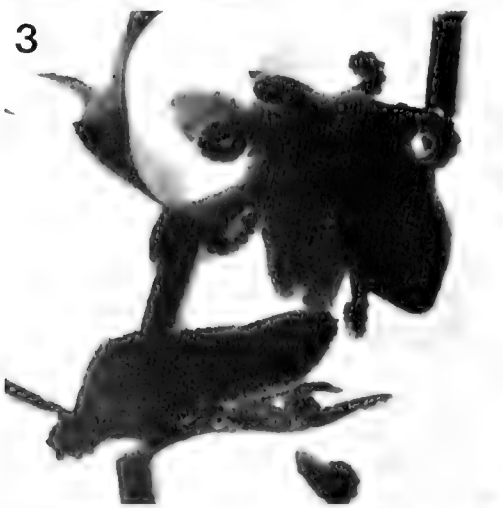
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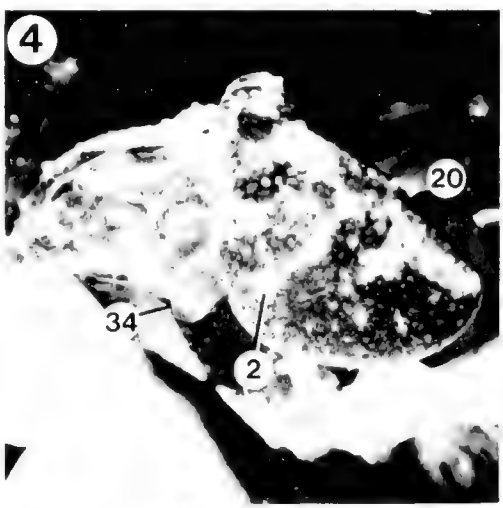
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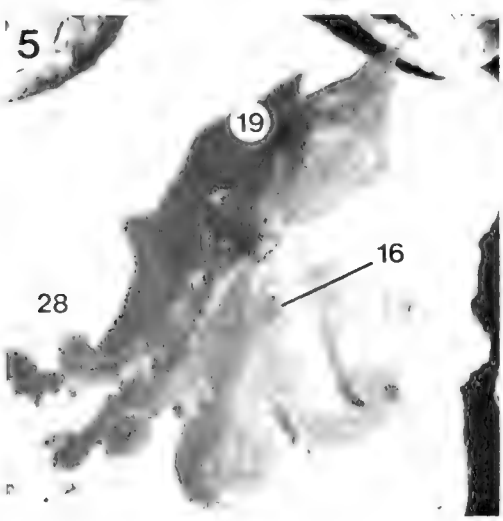
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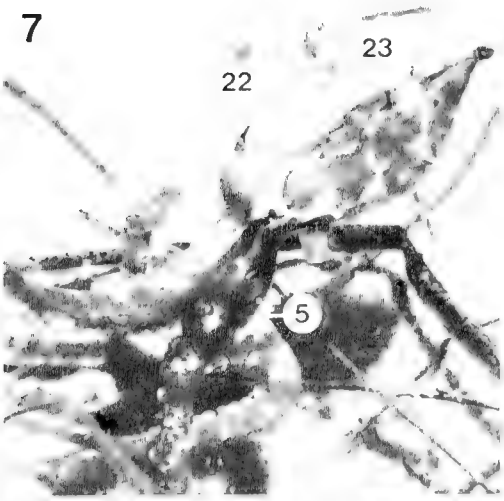
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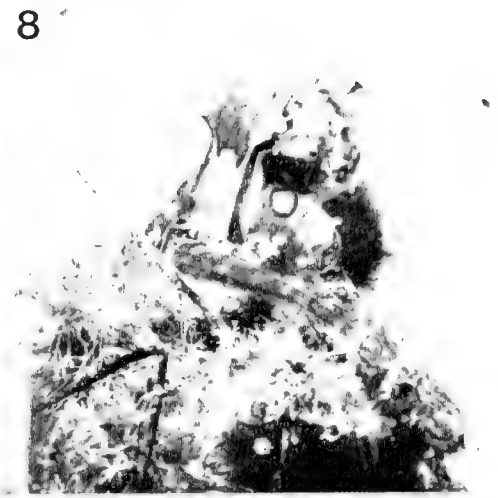
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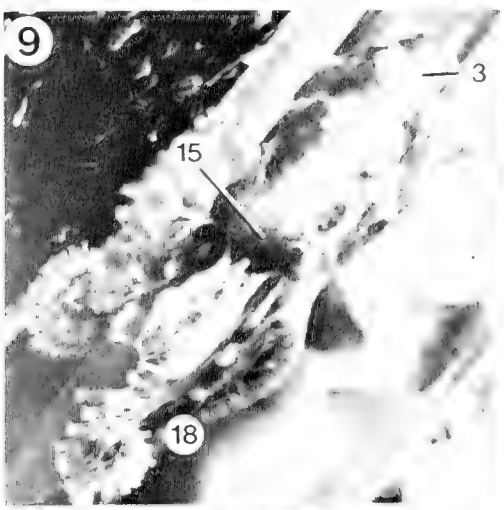
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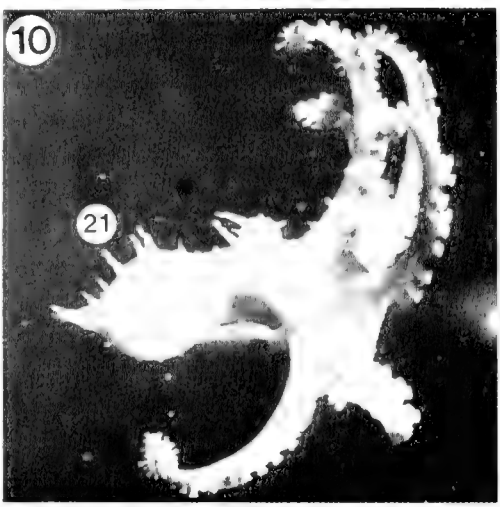
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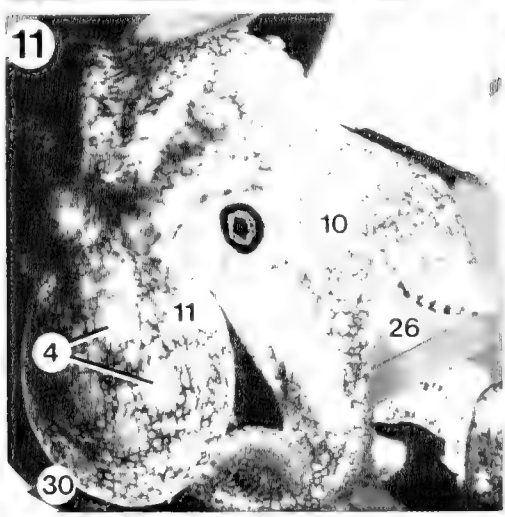
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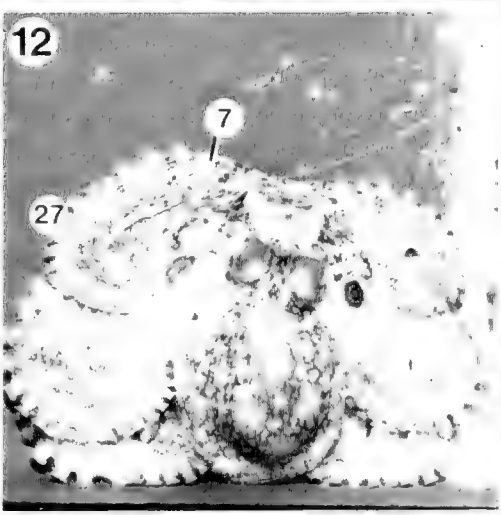
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TABLE 1. Body patterns and their components in *Octopus bimaculoides*.

CHROMATIC COMPONENTS	
Light	Dark
(1) Mantle White Spots*	(8) Branchial Hearts
(2) Transverse Mantle Bar	(9) Extrategumental Chromatophores
(3) White Longitudinal Center Stripe	(10) Mottle
(4) Yellow-Orange Spots	(11) Integumental Trellis*
(5) White Patches	(12) Pupil*
(6) Frontal White Spots*	(13) Eye Bar*
(7) Arm White Spots*	(14) Eye Ring
	(15) Dark Hood*
	(16) Ocelli*
	(17) Dark Edged Suckers*
	(18) Arm Bars*

TEXTURAL COMPONENTS	POSTURAL COMPONENTS	LOCOMOTOR COMPONENTS
(19) Smooth Skin	(24) Standing	(35) Water Jetting
(20) Coarse Skin	(25) Protective Posture	(36) Inking
(21) Papillate Skin	(26) Interbrachial Web Spread*	
(22) Prominent Eye Papillae	(27) Coiled Arms	
(23) Long Mantle Papillae*	(28) Twisted Arm Tips	
	(29) Ogive Mantle	
	(30) Rounded Mantle	
	(31) Two Raised Arms	
	(32) Head Raised	
	(33) Head Flattened	
	(34) Funnel Directed at Stimulus	

BODY PATTERNS	
Chronic	Acute
1. UNIFORM LIGHT PHASE	3. UNIFORM DARK PHASE*
2. CHRONIC GENERAL MOTTLE	4. ACUTE MOTTLE*
	5. DISRUPTIVE
	6. DEIMATIC*
	7. FLAMBOYANT
	8. PASSING CLOUD

*Described or mentioned by Packard & Hochberg (1977)

FIG. 7. A small *Octopus bimaculoides* (approx. 35 mm ML) in nature showing strong DISRUPTIVE while moving admist bottom detritus. This pattern shares some of the textural and postural components of the FLAMBOYANT pattern (Fig. 10).

FIG. 8. Octopus in Fig. 7 in a stationary posture showing strong DISRUPTIVE while clinging to clump of algae. Note concealment and strong expression of body papillae. Ocellus and extended Eye Bar.

FIG. 9. A 4-month old laboratory-reared octopus showing strong DISRUPTIVE. This octopus is about the same size as the animal shown in Figs. 7 and 8.

FIG. 10. A small *Octopus bimaculoides* (approx. 30 mm ML) in nature showing the FLAMBOYANT. This animal was pursued into the water column by the photographer. Note maximum expression of body papillae. This animal is missing its first right arm.

FIG. 11. An 8-month-old *Octopus bimaculoides* (approx. 65 mm ML) showing the ACUTE MOTTLE. Note the chainlink structure of the inner ring on the ocellus.

FIG. 12. An 8-month-old *Octopus bimaculoides* in CHRONIC GENERAL MOTTLE.

TABLE 2. Numbers of eggs in laboratory broods with some observations of festoon make-up.

Brood no.	Total no. eggs	No. festoons in brood	No. eggs per festoon			Female's origin
			n	$\bar{x}$	range	
1.	774	42	42	17.7	5–29	Los Angeles
2.	500	—	9	24.2	14–40	Los Angeles
3.	260	28	28	9.1	2–21	lab-reared
4.	490	24	24	16.9	3–32	lab-reared
5.	448	—	—	—	—	Los Angeles
6.	269	—	—	—	—	Baja, Mexico
7.	137	—	—	—	—	Baja, Mexico
8.	385	—	—	—	—	Carlsbad
9.	159	—	—	—	—	Carlsbad
$\bar{x}$	380	31.3		16.97		

hectocotylus on the third right arm of males. In our major culture experiment (at 18° C), the first males with an identifiable hectocotylus were found on day 156. All males could be identified by approximately day 200 (mean size 54 g and 55 mm ML). Final development of the penis, Needham's sac and testis seemed to coincide with the development of the hectocotylus, but spermatophores were not yet present. Once the hectocotylus appeared, the testis began to increase gradually over the following months. It is not known at what age spermatophores first appear in the penis. Another external feature of males associated with sexual maturation was the presence of one or two conspicuously enlarged suckers at the level of the web margin on the second and third pairs of arms. This feature became evident on some males at about the time the hectocotylus was fully formed, but it usually did not appear clearly until later in the life cycle.

Among females, the only visible external sign of sexual maturation was the rapid enlargement of the ovary in the distal end of the mantle. This enlargement did not become evident until the month prior to egg-laying; during this period the ovary increased from less than a tenth of the internal mantle space to almost 1/3 to 1/2 of the mantle space. The females became mature at a mean weight of about 500 g and size of 110 mm ML.

Mating and egg-laying. Matings were observed among group-cultured octopuses on day 317 in the main culture experiment. These first matings followed first hectocotylus formation by approximately 5.4 months. Observations of pre-copulatory behavior were made on day 313 during pairings of two male and two female octopuses that were being grown in individual chambers. These animals had been isolated since day 128. In both instances, highly aggressive behavior was observed almost immediately, with the female octopuses seeming to be the aggressors. The animals came together immediately upon visual contact and proceeded to grapple in a mirror-image fashion, arm to arm and mouth to mouth. After five minutes the two animals settled down adjacent to each other. In this configuration, separated by as much as 25 cm, the male would insert his third right arm into the mantle cavity of the female and mating would proceed. This somewhat aggressive initiation of mating was never observed among the group-reared octopuses, where mating occurred promiscuously and was always passive in nature. In fact, octopuses were sometimes observed mating without visual contact of any kind, with a male extending his third right arm out of his den into an adjacent den containing a female. Matings lasted from ten minutes to almost three hours, but one hour was typical. During mating the animals remained very calm without accelerated mantle ventilations or violent mantle contractions. Nearly all matings were during the day, although a few night observations were made. It does not appear that mating stimulated egg-laying, since egg-laying followed the initiation of mating behavior by two to four months. Only two broods of eggs were laid in this culture population before it was lost (due to a

TABLE 3. Measurements of egg length and egg width from 17 different broods.

Brood	N	Length (mm)		Width (mm)		Width/length ratio (%)	Stage of development	Female's origin
		$\bar{x}$	range	$\bar{x}$	range			
1	6	11.0	10.6–11.4	3.2	3.0–3.4	29	fresh laid	Los Angeles
2	50	11.9	10.3–13.2	3.8	3.3–4.4	32	infertile	lab-reared
3	10	14.5	14.1–14.8	5.2	4.6–5.6	36	completed	Los Angeles
4	20	12.7	10.7–14.0	4.0	3.2–4.4	31	mid-way	Carlsbad*
5	20	11.3	10.8–11.8	3.9	3.7–4.0	35	mid-way	Carlsbad*
6	20	12.3	10.6–13.9	3.9	3.2–4.3	32	fresh laid	Carlsbad*
7	20	10.1	9.3–11.4	3.4	3.1–4.1	34	fresh laid	Carlsbad*
8	20	12.4	10.3–13.8	3.5	2.9–4.1	28	fresh laid	Carlsbad
9	20	11.7	9.1–13.2	3.6	3.2–4.0	31	fresh laid	Carlsbad
10	20	11.4	10.7–12.2	3.4	3.2–3.6	30	mid-way	Carlsbad
11	20	11.8	10.9–12.8	3.5	3.1–3.9	30	mid-way	Carlsbad
12	20	10.3	9.1–11.2	3.3	2.9–3.6	32	fresh laid	Baja, Mexico*
13	20	9.8	9.0–10.4	3.4	3.1–3.6	35	mid-way	Baja, Mexico*
14	20	10.7	9.7–11.7	3.2	2.8–3.7	30	fresh laid	Baja, Mexico*
15	20	9.3	8.4–10.2	3.4	2.9–3.6	37	advanced	Baja, Mexico*
16	20	8.9	8.2– 9.6	2.8	2.3–3.0	32	mid-way	Baja, Mexico
17	20	10.4	9.5–11.7	3.3	2.9–4.1	32	fresh laid	Baja, Mexico

*Indicate eggs collected in the field. All other broods were laid in the laboratory.

TABLE 4. Egg development and hatching observations on laboratory broods of eggs maintained at different temperatures.

Mean temp. S.D. (°C)	Temp. range (°C)	First eyespots visible (d)	Second reversal (d)	Development time (d)	Hatching duration (d)	Percent hatching success	No. eggs	Female's origin
17.8 ± 0.51	16.0–19.0	—	70	82	8	~50%	~400	Los Angeles
19.0 ± 0.66	18.0–22.5	30	—	72	18	61%	269	Baja, Mexico
19.4 ± 1.12	18.0–24.0	—	53	68	16	70%	137	Baja, Mexico
19.4 ± 1.13	18.0–24.0	25	48	68	18	89%	385	Carlsbad
19.4 ± 1.35	18.0–24.0	—	52	65	22	75%	159	Carlsbad
22.0 ± 1.77	18.0–25.0	28	—	56	12	73%	448	Los Angeles
22.5 ± 0.83	21.5–26.0	31	—	51	4	—	—	lab-reared
23.4 ± 0.67	22.0–25.0	18	—	46	—	—	—	lab-reared

long-term power failure). These broods were laid on days 383 and 409, but perished before completing development. The 12 females in this group that died during the power failure were preserved and their ovaries examined for degree of maturation at a later date. Four females had ripe ovaries full of large eggs (10–12 mm). The diameter of the ovary equaled 40–55% of the dorsal mantle length in these animals. One of the females apparently died the day she would have begun egg-laying because there were eggs at the opening of each oviduct; the other three females did not yet have eggs in their oviducts. The eight remaining females were still immature. Two of these had eggs 3 to 8 mm long, while six females had immature ovaries with eggs only 3 mm or shorter. In these latter six females, ovary diameter ranged from 11 to 16% of mantle length.

Females typically ceased feeding four to five days prior to egg-laying. The females brooded their eggs throughout the developmental period by continuously running their

arms and suckers over the egg surfaces. Fecal strings produced by brooding females often contained the remains of egg cases, indicating that eggs had been eaten. It is unlikely that they represented infertile eggs since females brooded infertile broods the same as normal broods. Egg counts from nine laboratory broods ranged from 137 to 774, with a mean of 380 (Table 2). Eggs were laid in clusters or festoons, with the stalk of each egg woven and cemented into a central strand that was then attached to the substrate. The festoons were attached usually to an overhead surface, allowing them to hang in the water column. The number of festoons per brood ranged from 24 to 42, while the number of eggs per festoon ranged from 2 to 40 with a mean near 17 (Table 2). Egg size appeared to be quite variable, from 9 to 14 mm in length and 2.3 to 5.6 mm in width (Table 3). Part of this variation was due to eggs swelling in size over the course of embryonic development. However, a statistically significant difference was seen in field-laid eggs from Carlsbad versus those from Baja California (see *Reproductive biology* section under Field Observations).

Egg development and hatching. *Octopus bimaculoides* appears to go through a normal sequence of embryonic development in the egg (cf. Boletzky, 1974), although no detailed embryological observations were made. After an initial reversal in the egg shortly after fertilization, the embryos develop at the proximal end of the egg (nearest the stalk) and proceeded through most of the developmental period in this orientation. Three-fourths of the way through embryonic development, the nearly fully developed embryos reverse their position again, resulting in the mantle being pressed against the distal end of the egg. Embryonic development was clearly temperature-dependent (Table 4). Complete embryonic development required 82 days at a mean temperature of 17.8° C, but as few as 46 days at 23.4° C. Generally, development time decreases by about 10% for each increase in temperature of 1° C. A linear regression on log transformed data from Table 4 revealed that the decrease in development time with increasing temperature was described by the equation:

$$\text{Development Time (days)} = (2.3 \times 10^4)\ \text{Temp.}\ (^\circ\text{C})^{-1.96};\ r^2 = .9771.$$

Within the known range of this species it is unlikely that development time under 80 days occurs. Temperatures of 12 to 15° C are probably more typical and (extrapolating from the equation) would result in development times of 176 to 114 days, respectively. It is noteworthy that this species shows normal egg development, hatching and hatchling growth at temperatures up to 25° C, thus indicating the potential of a wider distribution to the south on the Baja California peninsula. Females added eggs continually to their broods for at least a week. This probably occurs for two to three weeks in some broods as evidenced by the duration of hatching seen in Table 4. Most hatching occurred at night.

There were often hatching-related mortalities. Some octopuses hatched prematurely, with the external yolk sac still present, and these usually died within 48 hrs. Premature hatching was usually the result of inadvertent physical stimulation on the part of the investigators while handling late-stage eggs or performing routine maintenance activities in the culture trays. Mortality due to hatchlings getting trapped half-in and half-out of the egg case was sometimes seen in eggs not being cared for by a female. Observations on broods attended by females showed only insignificant mortalities due to premature and incomplete hatching. A female obtained from a supplier in California in December laid a brood of eggs that hatched in early March. Of 244 hatchlings collected, only one was premature and no incomplete hatching was observed. However, there were 85 infertile eggs. Such infertile eggs are the main reason for rather low percent hatching success figures in Table 4.

FIELD OBSERVATIONS

Large populations of *Octopus bimaculoides* were observed at two locations: Santa Barbara Bay, Santa Barbara, CA (May, 1984) and San Quintin Bay (Bahia Falsa), San Quintin, Mexico (January, 1985). The observation of the *O. bimaculoides* population at San Quintin, Mexico, extends the southern known range of this species, which was previously Ensenada, Mexico (Hochberg & Fields, 1980), and increases the overall range by approximately 25 percent. The California population inhabited a subtidal area, while the Mexican population was living intertidally. The subtidal populations in California were observed using SCUBA on dives totalling 17.6 hours. Observations on the intertidal population were made during low tides over a 4-day period.

Habitat

Octopus bimaculoides was found inhabiting a range of habitats consistent with those reported in the literature (Pickford & McConnaughey, 1949; MacGinitie & MacGinitie, 1968; Hochberg & Fields, 1980). At the Santa Barbara site, the population was confined almost entirely beneath and within the perimeter of a large commercial pier in depths from 6 to 9 m. The surrounding seabed was a featureless, firm, sand plain where no octopuses were found. Beneath the pier, the bottom was littered with debris from human activity over many years. The octopus population was using an essentially man-made artificial habitat consisting primarily of plumbing pipe (approx. 8 cm diameter), cans, bottles, sheet metal and thousands of old discarded abalone shells. Octopuses were even found inside an old bicycle frame and the foot pocket of a swim fin. The plumbing pipe was the preferred site for egg-laying. The pier area supported a diverse biota including numerous species of potential octopus prey organisms, primarily crabs and bivalve molluscs. Octopus size ranged from 5 to 10 g juveniles to mature adults weighing 300 to 400 g. A small number of *Octopus rubescens* co-occurred with *O. bimaculoides* at this site, but the latter species was by far the dominant species. Water temperature was 12° C.

In San Quintin Bay, *Octopus bimaculoides* was found inhabiting an intertidal area having an underlying rock substratum with alternating areas of mud flats and oyster-encrusted rock reefs. Octopuses were generally found in (apparently) constructed burrows on the fringes of the rock reefs. The burrows typically had an 8 to 10 cm opening and extended down into the loose rock and mud substrate. Some octopuses were also found under large rocks. No octopuses were found in open mud-flat areas devoid of rock, pebbles or hard substrate. At this site, it seemed that nearly the entire population could be found above mean low tide. The tidal range observed was approximately 2.0 to 2.5 m daily. In one area, a group of females brooding eggs were as much as 30 m above the water line at low tide, although their dens still contained water. Potential food organisms included mysidacean shrimps, grapsid mud crabs and mussels on the oyster beds. The brooding females collected at this site were noticeably smaller than females collected at the California sites, but unfortunately no measurements were taken in the field. The water temperature was 14° C.

Reproductive biology

Mating was observed during the day on three occasions in the field, indicating that this is not strictly nocturnal behavior. Numerous females were found brooding eggs at both sites. At Santa Barbara, eggs were collected from 16 different broods. Four broods near hatching were not disturbed. Six broods were subsampled at San Quintin with three very late-stage broods left undisturbed. Broods representative of all developmental stages were seen (from freshly laid to near hatching) and were always near 100% fertile. During mid-day at San Quintin we came upon one undisturbed den during low tide and witnessed fresh hatchlings being blown out of the den, into the puddle surrounding the den opening, by the ventilation blasts of the female.

The following can be deduced based upon the range of egg stages observed in the field and assuming development times at the recorded ambient temperatures. During the spring of 1984 at Santa Barbara (which represents the northern range limit of this species), egg-laying took place from at least December through May, with hatching in May through September. In the southern range (San Quintin), spawning occurred from October 1984 through January 1985, with hatching from January through May 1985. These estimates by no means represent time limits of spawning, but they do indicate a broader spawning season for this species than reported previously (Hochberg & Fields, 1980). Caution is advisable in viewing these estimates because the 1983/84 "El Niño" conditions may have affected the normal spawning season.

Samples of 20 eggs from four broods collected at Carlsbad and four broods collected at San Quintin were measured to compare egg size, since adults at the Mexican location were noticeably smaller. Mean lengths of the four California broods were 12.7, 12.3, 11.3 and 10.1 mm, compared to means of 10.7, 10.3, 9.8 and 9.3 mm for the Mexican broods (see Table 3). Combining all eggs at each site gave a mean length of 11.6 vs 10.0 mm, respectively. A t-test on these means revealed the difference to be of high statistical significance ($p < 0.001$). A comparison in egg-length of broods laid in the laboratory by females from

TABLE 5. A numerical comparison of body patterning components among four species of *Octopus*. Numbers in parentheses represent the number of components the three last species have in common with *O. vulgaris*.

	Chromatic components only		Total no. components: chromatic, textural, postural, locomotor
	Light	Dark	
Octopus vulgaris[1]	7	11	41
Octopus bimaculoides[2]	7 (4)	10 (7)	36 (24)
Octopus briareus[3]	8 (2)	10 (5)	35 (22)
Octopus burryi[4]	6 (3)	6 (5)	26 (24)

[1]Packard & Sanders, 1971
[2]This report.
[3]Hanlon & Wolterding, unpublished.
[4]Hanlon & Hixon, 1980.

the two sites showed the same relationship. Carlsbad females laid significantly ($p < 0.001$) longer eggs (mean 11.8 mm) than Baja females (9.65 mm). Obviously, egg size is variable in this species and is determined probably by the size of the female that lays the brood.

DISCUSSION

Behavior and body patterning

The qualitative observations reported here are preliminary but illustrative of the types of behavior exhibited by octopuses grown in captivity. Of particular value are the observations possible on octopuses during the first three months of life. In nature, octopuses in this part of their life cycle are extremely difficult to find, much less observe for any period. More quantitative ethological studies on octopods are needed.

The two general behavioral traits of most interest were the high activity level of hatchlings and juveniles during the first month of life and their high tolerance of crowding. The rather high rate of movement, both benthic walking and swimming, may have been an artifact of confinement but could also be a mechanism to assure brood dispersal in an octopus species with benthic hatchlings. The tolerance of crowding observed in laboratory populations of *Octopus bimaculoides* is mirrored in the concentrated assemblages of this species we observed in nature. In general, the populations of *O. bimaculoides* we observed in nature have been restricted to relatively small areas of suitable habitat, resulting in high octopus densities and the need for behavior tolerant of crowding.

Packard & Hochberg (1977) described or mentioned approximately 12 components of body patterning and at least three body patterns for *Octopus bimaculoides*. We identified 24 additional components and five body patterns. The live animals they examined (*op. cit.*) were all larger than 35 g while the majority of new body patterning components we described were seen on animals below this size. Compared to other octopus species studied thus far, *O. bimaculoides* seems to have a body patterning reportoire that is fairly complex. Judging by the number of chromatic components (Table 5), *O. bimaculoides* ranks closely with *O. vulgaris* and *O. briareus* and is slightly more diversified than *O. burryi*. There are qualitative differences too, especially with *O. briareus*, which has quite a different appearance than the other three species (see also Hanlon, 1988, this volume). It is noteworthy how many components these four species have in common (Table 5). Although the same components in different species may be somewhat different in their details, it is clear that different species use many of the same visual tricks to fool their predators and prey and to communicate among themselves.

There has been a problem in differentiating live *O. bimaculoides* from *O. bimaculatus* (Pickford & McConnaughey, 1949; Hochberg & Fields, 1980). We have had limited opportunities to observe and photograph juvenile and adult *O. bimaculatus* in the field and in the laboratory. Based upon our observations and discussions with other investigators, the characters of potential value in separating these two species are the frontal white spots, mantle and arm white spots, and white spots over the eyes. There appears to be a difference in the fine structure of the irides-

cent blue rings of the ocelli, but more *O. bimaculatus* must be observed to confirm this. Also, the color of the ink may prove to be a very pragmatic identifying character. In two side-by-side comparisons of field-caught animals, the difference in ink color was most distinctive and obvious (*O. bimaculoides*-black; *O. bimaculatus*-reddish brown), but more comparisons with animals throughout the ranges of these two species are needed. We plan to document these differences in a future publication.

Reproductive biology

Similar information is available for four other species of *Octopus* having large eggs and benthic hatchlings (see reviews in Boyle, 1983; Hanlon & Forsythe, 1985). *Octopus joubini* (Hanlon, 1983a) and *Octopus digueti* (DeRusha *et al.*, 1988), are very small species (max. 55 and 85 g, respectively) with eggs (length 6–8 mm) that are attached individually to the substrate. Like *Octopus bimaculoides, Octopus briareus* (Hanlon, 1983b) and *Octopus maya* (Van Heukelem, 1983) grow to a larger adult size (1–2 kg) and have larger eggs (length 10–17 mm) laid in festoons, with 20 to 40 eggs sharing a common attachment point.

With respect to sexual maturation and spawning, *Octopus bimaculoides* followed the same general scheme observed in the other large-egged species. Males mature first and initiate mating behavior while females are still immature. Females do not become sexually mature and spawn until nearly the end of the life cycle. The first observed matings and spawning in *O. bimaculoides* occurred two to four months later in the life cycle than reported for *Octopus briareus* and *Octopus maya*, which have approximately one-year life cycles.

On a relative basis, all five species mentioned above have a similar rate of fecundity, producing about one to two eggs per gram body weight of the female. *Octopus maya* and *Octopus briareus* take nearly 60 days to develop at 25° C, while *Octopus bimaculoides, Octopus joubini* and *Octopus digueti* require only 40 to 45 days at this temperature. Our work at this laboratory with all five species has shown a clear inverse correlation between development time and water temperature. Ambrose (1981) found the same relationship among four species of small-egged octopuses.

Field observations

Octopus bimaculoides can be found in concentrated assemblages that are accessible for study by SCUBA diving or intertidal observation. Ambrose's (1982a, b) work with *Octopus bimaculatus* demonstrated the feasibility of detailed field studies along the southern California coast, and similar in-depth study is needed on *O. bimaculoides*. A notable aspect of the field populations of *O. bimaculoides* was the ability to use a relatively small and discrete area of suitable habitat maximally. At all such sites, it was by far the dominant cephalopod species if not the only one. The ability of several females of this species to release thousands of fully functional benthic hatchlings into a very small area each spawning season is certainly a competitive advantage over planktonically dispersed octopus species that might be able to drop only a few benthic juveniles into such a small area. The ecological relationship between *O. bimaculoides* and *O. bimaculatus* remains unclear. Although the geographic ranges of these two species overlap, the early literature suggested that the species occupied adjacent ecological zones and did not compete directly (Pickford & McConnaughey, 1949). In some areas, this is certainly the case (Ambrose, 1982a). However, the range of habitats they exploit does overlap (Hochberg & Fields, 1980) and, in fact, the species are known to co-occur (Lang, 1986). Feeding studies indicate that these two species feed on a very similar range of food organisms (Ambrose, 1984; Hochberg & Fields, 1980) making competition for food likely in areas of co-occurrence. Thus far, nothing is known of the nature and degree of ecological interaction between these two species.

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INSHORE-OFFSHORE COMPARISON OF *OCTOPUS DOFLEINI* WITH SPECIAL REFERENCE TO ABUNDANCE, GROWTH AND PHYSICAL CONDITION DURING WINTER

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ABSTRACT

Trapping and capture by *SCUBA* combined with a tag-release program provided information on the abundance, growth and physical condition of *Octopus dofleini* over a range of depths during winter. Catch data indicated that octopuses were abundant in deep offshore waters. In comparison to octopuses caught inshore, offshore individuals showed a lower frequency of positive growth, a higher growth rate among those growing and a higher mutilation rate. The size distribution of the catch offshore was shifted toward larger octopuses. These characteristics along with sex ratio differences and a trap size-octopus size relationship are discussed in relation to the availability of information on the movements and relative abundance of octopuses, their food sources, their predators and shelter availability inshore vs. offshore.

Key Words: *Octopus; depth; growth;* physical condition; abundance.

INTRODUCTION

There is ample evidence that cephalopods form a significant food resource for many marine vertebrates (Boyle, 1983). Like other octopus species, *Octopus dofleini* (Wülker) is both predator and prey with its predators including sea otters and seals (Kenyon, 1965), sea lions and mink (Wayne Campbell, Provincial Museum, Victoria, Canada, personal communication) and various fishes such as dogfish shark *Squalus* sp. (Chatwin & Forrester, 1953; Jones & Geen, 1977; Saunders *et al.*, 1984 and McFarlane *et al.*, 1984) and lingcod *Ophiodon elongatus* (personal observations and reports from commercial divers). Hartwick *et al.* (1978) reported scarring and missing arms in *O. dofleini* as possible indicators of high predation rates.

The importance of natural cavities or dens for protection of octopuses from predation has been confirmed in a number of studies (Hartwick *et al.*, 1978; Ambrose, 1982; Aronson, 1986). Octopuses leave their dens to forage and are at risk to predation during this time. Thus the availability and quality of shelters and the nature of the food resources in the area are both of considerable importance. The availability of natural dens may limit octopus populations in some areas. Mottet (1975) referred to the increased yield of octopuses to Japanese fisheries with the addition of large numbers of artificial lairs to the fishing areas. However, Hartwick *et al.* (1978, 1984) and Ambrose (1982) found no evidence of den limitation in shallow water populations of *O. dofleini* and *O. bimaculatus,* respectively. Increases in density of *O. briareus* occurred when artificial dens were added to a study area in a saltwater lake (Aronson, 1986), suggesting den limitation. Aronson also added artificial lairs to a coastal area of Eleuthera Island, Bahamas, but no octopuses occupied them. Large predatory fishes may limit populations of *O. briareus* in coastal waters (Aronson, 1986).

The size of available dens may also be important. Hartwick *et al.* (1978) reported a positive relationship between den volume and octopus size. Aronson (1986) found that cavity length and diameter were each important in determining suitability of dens.

Octopuses leave their dens to forage on abundant, easily accessible food items such as crustaceans and bivalves. Studies on various octopus species indicate that individual octopuses use the same den repeatedly, often for a month or more (Van Heukelem, 1976;

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Altman, 1967; Kayes, 1974; Ambrose, 1982; Hartwick *et al.*, 1984). In some populations there is considerable movement of individuals. Ambrose (1982) found that marked *O. bimaculatus* moved only short distances with no evidence of large-scale movements or migrations. *O. vulgaris* may be involved in seasonal migrations. Mangold (1983) provided evidence that *O. vulgaris* in the Mediterranean moves into deeper waters in the early winter and returns to coastal areas in the spring. Similar movements were also reported for *Eledone moschata* (Mangold, 1983) and *E. cirrhosa* (Boyle, 1983) in the Mediterranean. Hartwick *et al.* (1984) reported on frequent movements of *O. dofleini* but could not demonstrate evidence of mass seasonal migrations. However, seasonal onshore-offshore migrations are indicated in Japanese studies of *O. dofleini* (Mottet, 1975) and trap fishing shifts in response to these.

The time of exposure to predators of foraging octopuses may be reduced by returning to dense food patches and by bringing a number of prey items back to the den on any one trip. This tendency was observed in *O. dofleini* and used in part as an explanation for high individual growth rates in the field (Robinson & Hartwick, 1986). Growth rates in Robinson & Hartwick's study were similar at two different shallow-water sites but showed seasonal variation. In particular, the winter period, January through May, was a period of reduced growth in shallow-water *O. dofleini.*

A trap-fishing study in offshore waters combined with a trapping and *SCUBA* study in nearby inshore waters provided a unique opportunity to compare the occurrence, growth and physical condition of octopuses in shallow and deep waters during winter. The present study reports on these characteristics and discusses indirect evidence for offshore movements and the relative importance of den limitation and predators.

MATERIALS AND METHODS

This study used *SCUBA* and trapping to investigate shallow water (inshore) octopuses and trapping to study offshore octopuses from January through May 1982. The study site was Clayoquot Sound, British Columbia and adjacent offshore waters. Traplines consisting of groundlines with various types of traps attached individually by short lines and with float lines at either end were set at 7 inshore sites and 8 offshore sites (Fig. 1). Inshore sites were selected in relation to two long-term dive study areas; trap lines were set parallel to the shore at depths varying from 6.9 to 19.2 m, each line with 60 traps. Offshore traplines (each with 40 traps) were set at depths of 37 m, 55 m, 73 m, 92 m and 110 m at points located along 2 selected Loran C lines.

Each trapline included roughly equal numbers of eight different unbaited open trap types. The trap types and their volumes were large box (0.071 m^3) and small (0.025 m^3) box trap consisting of cedar slats nailed to box frames; whole tire traps (0.069 m^3) consisting of tires closed on either side with plywood and with an opening cut into the tread, and partial tire traps (0.004 m^3) made from part of a tire sewn together except for an entrance hole; tube traps made from plastic drain tiles either singly (0.014 m^3) or with two tiles lashed together, each tube being partitioned so that four chambers existed (chamber volume 0.0007 m^3 for large double tubes, 0.0015 m^3 for medium double tubes and 0.0005 m^3 for small double tubes).

Traplines were checked on a regular basis throughout the study period, every 4.8 days on average inshore and 8.1 days on average in the case of offshore traplines.

The two dive study sites (Fig. 1) were checked by *SCUBA* at approximately two week intervals. Characteristics of these sites are reported elsewhere (Hartwick *et al.*, 1984; Robinson & Hartwick, 1986).

Octopuses captured by trap or by *SCUBA* were examined for scars and missing arms (stumps), sexed and tagged with a numbered plastic disc (Petersen disc) pinned at the base of the third left arm. This method of tagging has been very successful with *O. dofleini*, the tags being retained for long periods (6 months) with no apparent effect. After draining water from the mantle cavity, the octopus was weighed using a brass spring scale. The octopus was then released (returned to a den in the case of *SCUBA* and to a trap in the case of traplines).

The development and analysis of growth curves of octopuses were based on methods of Kaufmann (1981) and are described in more detail elsewhere (Robinson & Hartwick, 1986). Growth rates are reported as an instantaneous specific growth rate (SGR) where

$$SGR = (\ln Wt_2 - \ln Wt_1)/\Delta t$$

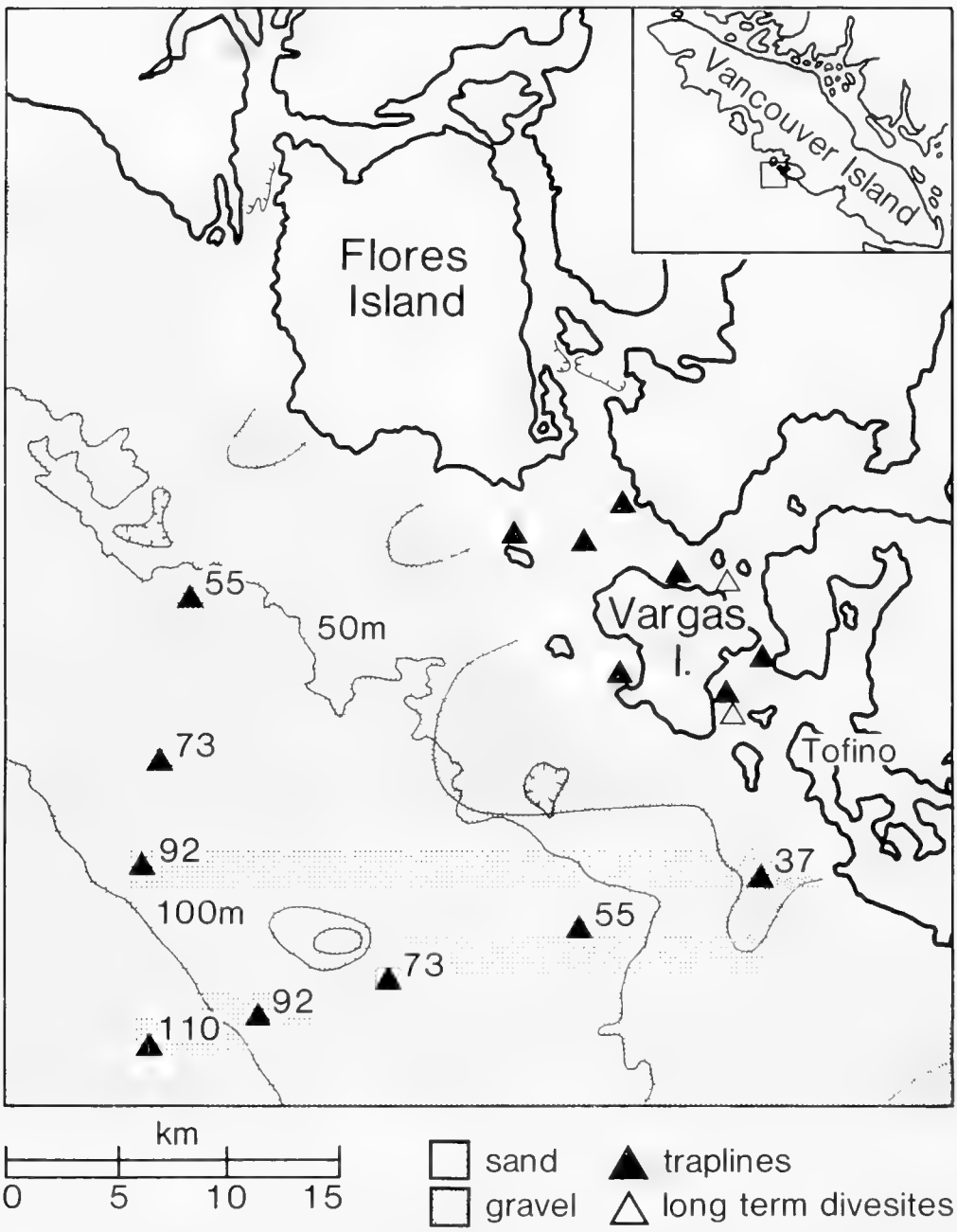

FIG. 1. The study area in Clayoquot Sound and adjacent waters showing locations of 2 dive sites, 7 inshore trap sites and 8 offshore trap sites. Bottom sediment types are from B.D. Bornhold, Geological Survey of Canada. Map O.F. 827.

TABLE 1. Catch rates of *Octopus dofleini* in traps set on longlines at various depths in the period January to May 1982. Total days fished were the same inshore and offshore but "soak time" (time between hauls) averaged 4.8 days inshore and 8.1 days offshore.

Location	Traps pulled	Total octopuses caught	New capture
Inshore (less than 37 m)	11832	60 (37 males)	36 (20 males)
Offshore (between 37 m and 110 m)	2073	131 (80 males)	81 (42 males)

Wt_2 and Wt_1 being weights of octopus at time 2 and time 1.

To investigate potential predators of octopuses in the offshore areas, jigging and longline fishing with baited hooks were carried out near traplines. Stomachs of all fish caught were examined for evidence of octopuses.

RESULTS

Comparison of inshore and offshore trap catch during the period January through May

Octopuses were caught singly in unbaited traps at both inshore and offshore sites. Catch per unit effort comparisons (Table 1, Fig. 2) indicate that traps were more efficient at catching octopuses in offshore waters than at shallow-water sites ($P < 0.001$, G test, Sokal & Rohlf, 1981: 707). When all trap types are combined for all depths at the offshore sites, the percentage of traps with octopuses was 6.3 compared with 0.5% inshore (recaptures included). Catch rates in offshore traps varied with trap type, depth and month (Hartwick *et al.*, unpublished data) with one type, the large cedar box traps averaging 14.3% occupancy (including recaptures) and reaching as high as 20.3% in January, many being caught at a depth of 73 m. Throughout the study period inshore catch remained variable but generally low.

Inshore trap catches showed a gradual decline from the fall period preceding this study through February followed by a gradual increase in the spring. *SCUBA* surveys at the dive sites during the same period showed a regular decline from a high of 40 octopuses in January to a low of 13 in May (Robinson, 1983).

Size frequency distributions for octopuses caught by traps are shown in Figs. 3 and 4. In comparison with offshore catch, the size distribution of the inshore catch is shifted towards smaller sizes. The range of sizes is comparable in both. Octopuses less than 10 kg in weight showed up in varying numbers in most trap types but the largest individuals were restricted to the largest traps (large box and whole tire traps). Table 2 summarizes the variation in size of octopus caught with various trap types. There is a significant positive relationship between the volume of the trap and the average size of octopuses caught for both inshore ($r = +0.92$, $n = 6$, $P < 0.05$) and offshore ($r = +0.99$, $n = 5$, $P < 0.05$). Moreover, octopuses caught offshore in large box traps, tire traps or small box traps were on average larger than those caught by the same trap type inshore ($P < 0.001$, Students t-test).

Growth of octopuses based upon tag-recaptures

At both inshore and offshore sites during winter some octopuses show positive growth while others show no growth or appeared to lose weight. Based upon recaptures of *SCUBA*-caught octopuses in shallow waters, the percentage showing positive growth during the period January through May varied from 60.0% in February to 91.7% in April with an average of 77.6% ($n = 76$) (Fig. 5). In contrast, the percentage of octopuses showing positive growth in offshore waters over the same period varied from 45 to 50% with an average of 47.8% ($n = 46$).

Because of the many factors that could be affecting individuals showing negative growth (illness, injury or parasitism), we restricted our attention to apparently healthy animals showing positive growth. If only octopuses showing positive growth are considered (see also Robinson & Hartwick, 1986), growth rates are higher for octopuses in deep water than at inshore sites (Table 3).

There was no significant relationship be-

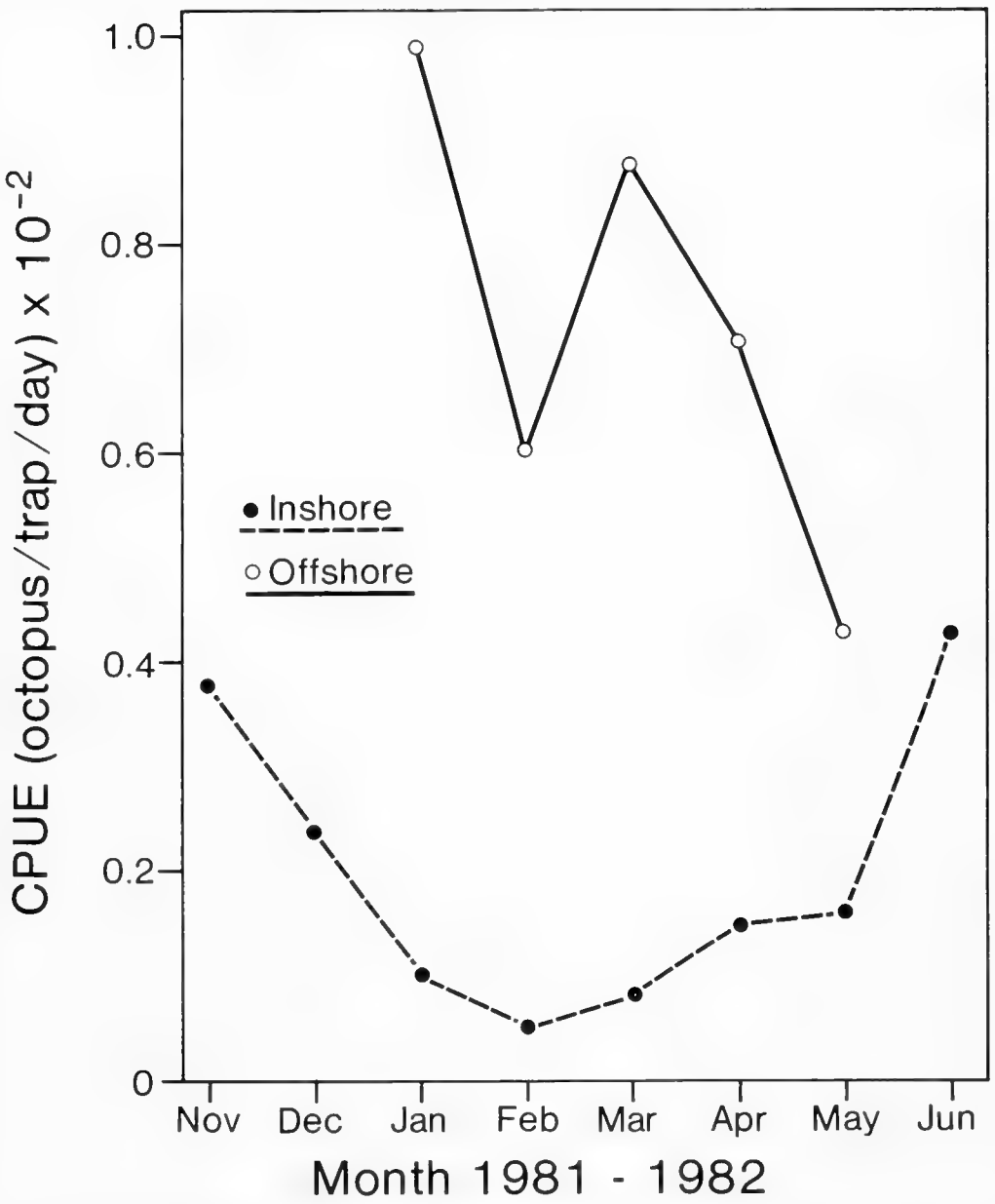

FIG. 2. Catch per unit effort (*CPUE*) for all trap types combined of *O. dofleini* in Clayoquot Sound and adjacent offshore waters.

tween specific growth rate and size of octopus in either inshore or offshore animals. Thus an exponential growth curve was fit with weight at time zero set arbitrarily at the weight of the smallest animal recaptured (1.6 kg). This arbitrary age of zero was necessary to position the curve on the time axis since age at some size information was not available. The equations describing the relationship were:

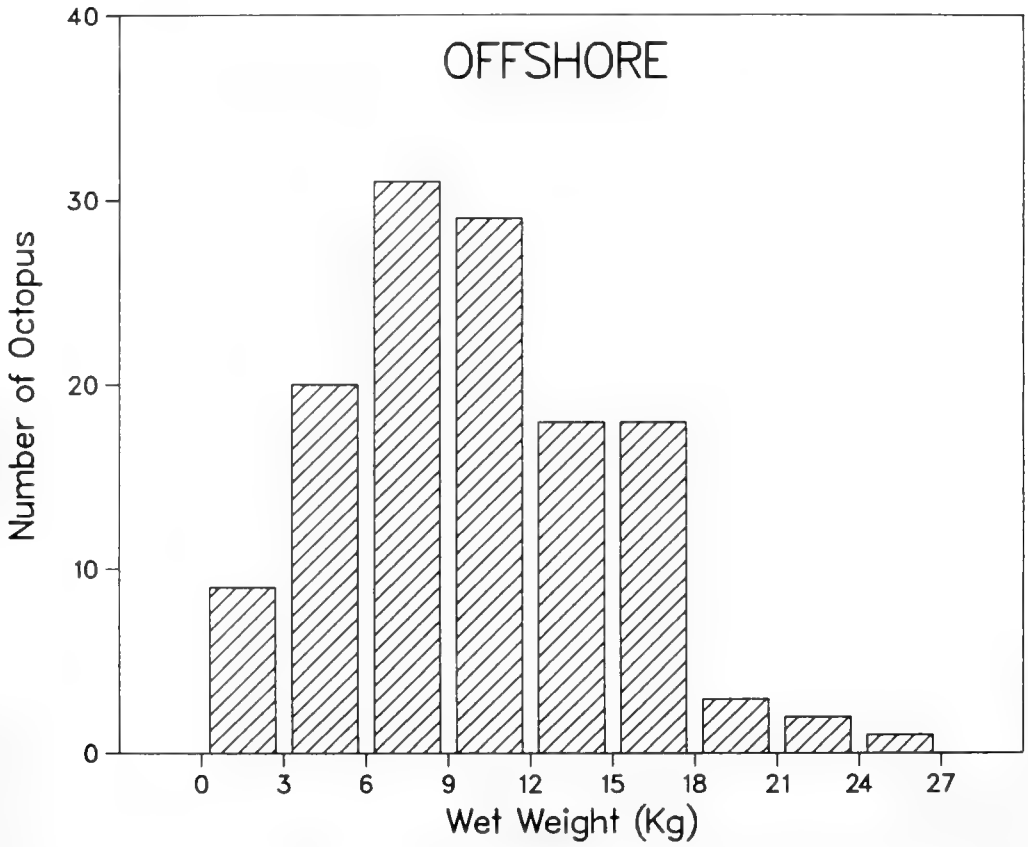

FIG. 3. Size frequency of octopuses caught by traps in offshore waters during the period January–May.

Inshore: WT = exp [0.00620 (t + 75.81)]
Offshore: WT = exp [0.00878 (t + 53.53)]

These are plotted in Fig. 6.

Injury rates and predation intensity

Octopuses caught in traps and by *SCUBA* showed evidence of attack by predators. Mutilations recorded included mantle scars, arm scars and missing arms (stumps). The extent of these injuries in octopuses caught at the different sites is shown in Fig. 7. Mutilation rates were significantly higher offshore than inshore ($P < 0.001$, G test Sokal & Rohlf, 1981: 707). If all types of mutilation are combined, 26.3% of the new captures of octopuses by *SCUBA* in shallow waters were affected, while 56.9% of the new octopuses trapped in deep waters in the same period were mutilated. There were no significant relationships between mutilation rate and size of octopus, sex or month in either inshore or offshore sites. The mean number of stumps recorded was 0.23 (SD = 0.60) for new octopuses caught by *SCUBA* (n = 57, $\bar{W}$ = 8.02 kg), while new octopuses caught in deep water by traps averaged 1.05 stumps (± 1.54) based upon 79 animals caught in large traps ($\bar{W}$ = 11.4 kg).

The presence of potential predators near offshore trap lines was indicated by the catch of fishes by jigging and hook-longline. Both lingcod *Ophiodon elongatus* and dogfish sharks *Squalus acanthias* were caught and both species showed evidence of feeding on octopuses (Table 4).

Sex ratio of octopuses

The sex ratio of new octopuses caught by *SCUBA* in shallow waters during winter was

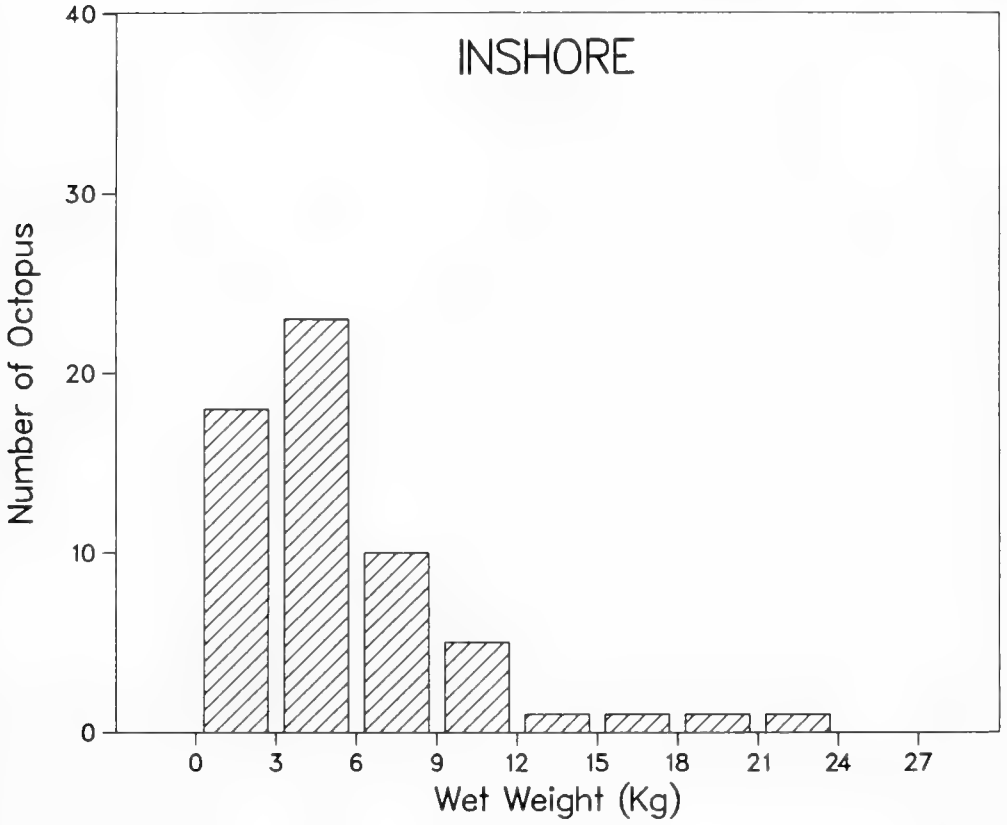

FIG. 4. Size frequency of octopuses caught by traps in inshore waters of Clayoquot Sound in the period January–May. Size distribution of *SCUBA*-caught octopuses for the same period is similar.

biased toward females (40 out of 57; 0.43 : 1, male : female with female normalized to 1), while inshore traps in the same area caught more males than females (20 of 36; 1.25 : 1). The sex ratio of new octopuses caught in deep water was 1.08 : 1 (41 of 79 octopuses caught in large traps were male).

When recaptures were considered, in both inshore and offshore traps, males were more likely to be recaptured (16 of 20 recaptures inshore were males, 4 : 1, while 36 of 46 offshore recaptures were males, 3.6 : 1). Recaptures in natural dens by *SCUBA* were more likely to be female (15 of 76 recaptures were males, 0.24 : 1).

An analysis of the mean number of stumps (missing arms) of new octopuses caught by *SCUBA* inshore and by trap in offshore waters did not reveal any difference between the sexes. Males caught inshore had an average of 0.24 stumps (± 0.66) while females averaged 0.23 (± 0.58). Offshore males averaged 0.81 (± 0.93), while females had 1.32 stumps (± 1.99).

Habitat, the availability of refuges

In shallow waters, observations by *SCUBA* indicated that natural dens were plentiful, especially for smaller-sized octopuses. These natural dens were occupied predominantly by females as indicated in the previous section. Unbaited, open traps operating in shallow waters as alternate refuges caught more males than females, but catches overall were generally low. Offshore traplines were located in areas of sand bottom (Fig. 1). Natural dens may have been in limited supply in deep waters.

TABLE 2. The relationship between size of octopus and trap volume for different trap types in inshore and offshore waters.

Trap type	Volume (m^3)	Individual octopus wet weight (kg)					
		Inshore			Offshore		
		n	$\bar{x}$	sd	n	$\bar{x}$	sd
Large box	0.071	31	8.00	6.54	70	11.85	4.81
Tire	0.069	36	6.43	3.32	27	10.97	4.29
Small box	0.025	29	4.09	2.17	30	5.97	3.08
Single tube	0.014	8	3.34	1.73	2	3.23	0.35
Double tube (large)	0.007 (×4)	13	3.30	2.03	2	2.00	0.35
1/3 tire	0.004	3	0.27	0.24	0	—	—
Double tube (medium)*	0.0015 (×4)	1	0.45	—	0	—	—
Double tube (small)	0.0005 (×4)	1	0.04	—	0	—	—

*Tubes were divided in the middle to give four chambers of equal volume

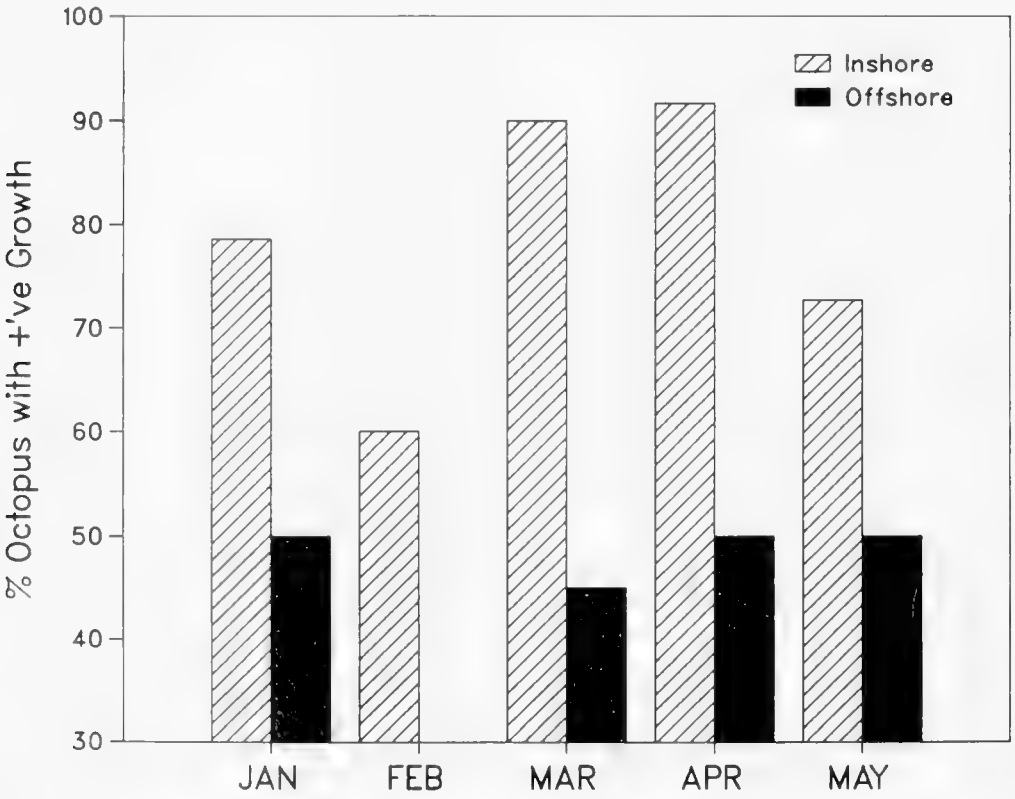

FIG. 5. The percentage of octopuses recaptured showing positive growth. No recaptures occurred offshore in February.

DISCUSSION

Previous studies have indicated considerable mobility in *O. dofleini* on the W coast of Canada (Hartwick *et al.*, 1984; Mather *et al.*, 1985). Although no evidence of a concerted inshore/offshore migration was reported in the study by Hartwick *et al.* (1984), the disap-

TABLE 3. Comparison of specific growth rates of recaptured inshore and offshore *O. dofleini*.

Location	Number of octopuses	Mean weight range (kg) ± SD	Mean weight range (kg)	Mean specific growth rate (×100) ± SD
Inshore	59	8.88 ± 3.52	1.63 – 18.88	0.620 ± 0.298
Offshore	22	7.82 ± 3.51	1.75 – 16.50	0.878 ± 0.567

pearance of tagged animals after a residence time of at least one month suggested large-scale movements interspersed with periods of residence in a relatively small area. The results of the trap catch data in the present study indicate that during the period January through May, many octopuses occur in deep water and may be caught using open unbaited traps. The lower catch rate with traps in inshore waters during the same period may indicate a lower abundance of octopuses or a greater availability of natural shelters. *SCUBA* surveys in rocky areas near traplines indicated that octopuses may at times be abundant and that this was not reflected in the inshore trap catch (Hartwick *et al.*, unpublished data). Natural shelters when available may be preferred by octopuses and such shelters do not seem to be limiting in our shallow-water study sites. However, the high offshore catch and the patterns of change in inshore trap catch and *SCUBA* censuses do suggest depth-related shifts in the population. Unfortunately, no octopuses tagged in shallow water were ever recaptured in deep-water sites, so the evidence of any such migration remains indirect. Interestingly, the catch-per-hour of octopuses by a commercial diver in an area near the shallow-water study sites showed a similar pattern to the inshore trap catch, declining from November to March and increasing again in the spring, thus supporting the idea for a movement out of shallow waters. Complex vertical movement patterns with possible age and sex differences were described for *O. vulgaris* (Mangold, 1983), although Hatanaka (1979) suggested that *O. vulgaris* simply moves from shallow water to deep water at about one year of age and spreads out over the area with no further migration.

Higher catch rates offshore may reflect a scarcity of suitable natural dens. Geological surveys off the W coast of Vancouver Island indicate that, in the general study area, sand covers flat sedimentary rock with little in the way of refuges, although there may be some rocks of glacial origin on top (Bornhold & Yorath, Pacific Geoscience Centre, Sydney, British Columbia, personal communication, 1986). In contrast, farther N from the study area an extensive and rugged bedrock belt extends 10–15 km offshore to depths of 100 m. Yorath *et al.* (1978) reported seeing many octopuses during submersible dives in the northern area (on 54 of 60 dives in a 2-1/2 year period, personal communication, 1986). In comparison with the study areas offshore from Clayoquot Sound, the N coast of Vancouver Island appears to have many potential shelters even in deep water.

The period January through May has been shown to be one of slow growth for inshore octopuses (Robinson & Hartwick, 1986). In that study and in the present one, some octopuses showed no growth during the period. The percentage of octopuses showing positive growth during the winter was greater inshore than offshore, but their growth rate was lower in comparison. The lack of information on the relative availability of food inshore and in offshore waters makes it difficult to explain such differences or to suggest any feeding or growth advantage for a movement offshore.

The mutilation data are of interest. The incidence of mutilation presumably reflects the frequency of non-fatal attacks by predators. How this is related to actual mortality is not clear. Schoener (1979) showed that under some conditions, injury frequencies may be related inversely to the efficiency of the predation process. Mutilation rates are much higher in offshore waters during the winter than inshore and this difference is independent of any size difference, but it is not clear whether this indicates a lower or higher predation intensity. Predators like the dogfish shark, *Squalus* sp., may be an important predator of octopuses in the study area. Most studies indicate that dogfish are opportunistic predators (Chatwin & Forester, 1953; Holden, 1966; Saunders *et al.*, 1984). Saunders *et al.* (1984) found octopus remains in 7.2% of

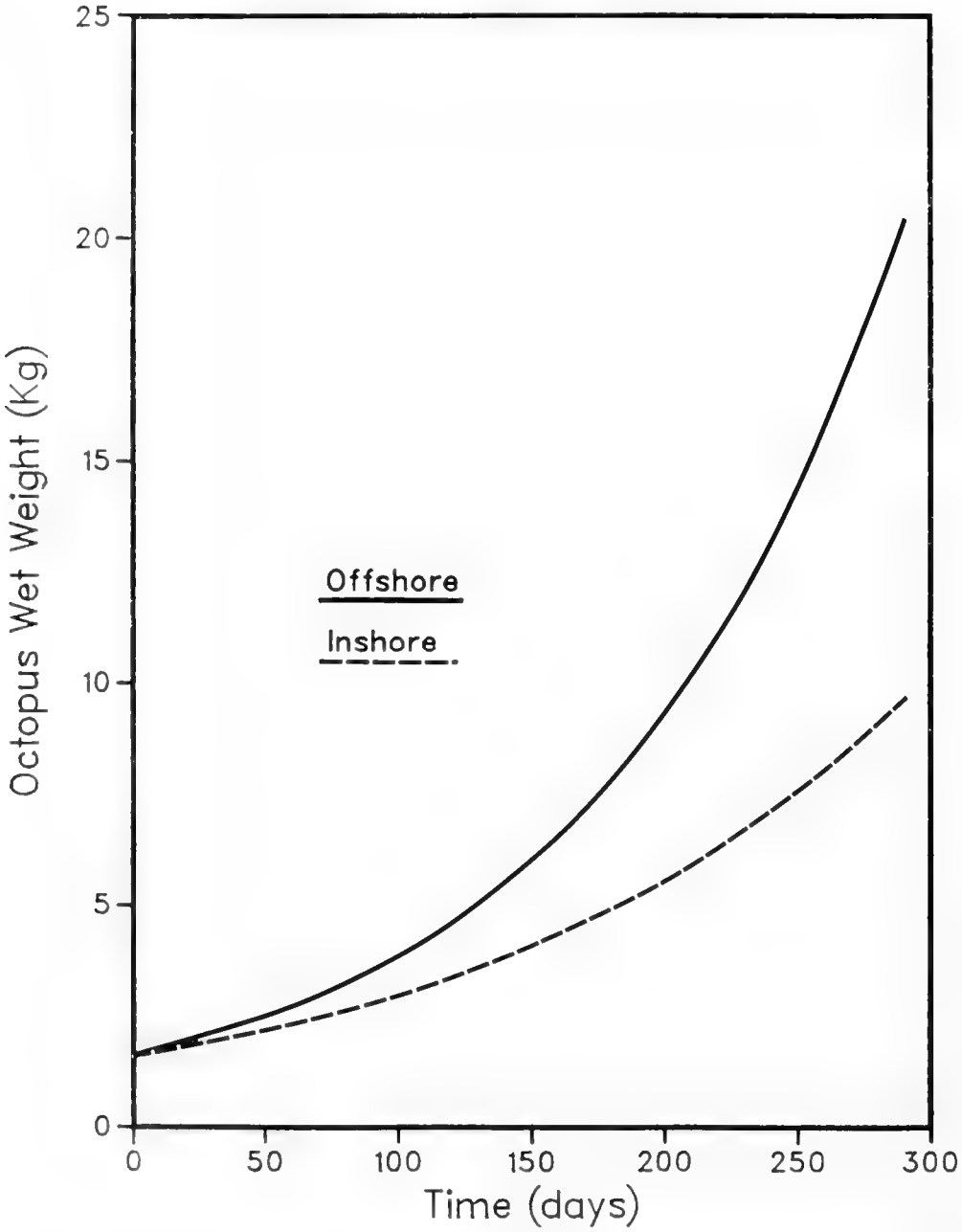

FIG. 6. Growth curves for *O. dofleini* in shallow water and deep water during winter. Time O is set arbitrarily at 1.6 kg, the weight of the smallest octopus recaptured.

adult dogfish sampled by trawl. Our findings for dogfish in offshore waters of Clayoquot Sound are comparable (6.9%). However, no information is available on the relative abundance of such predators inshore versus offshore. The relationship between mutilation

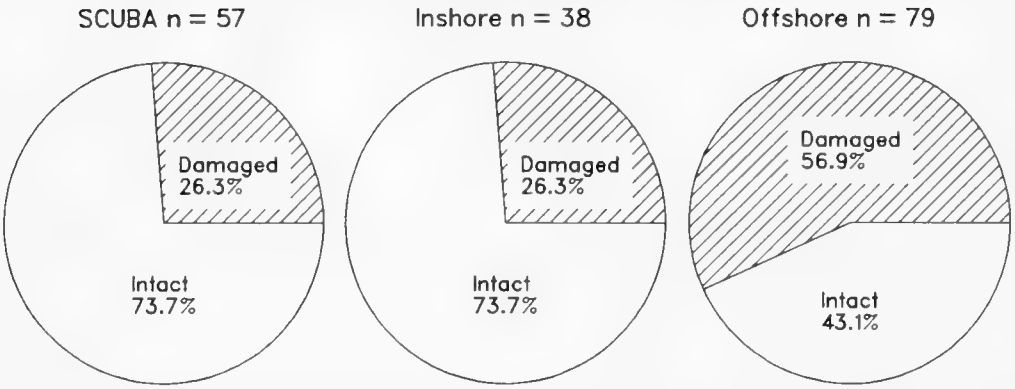

FIG. 7. The incidence of mutilation (scarring and loss of arms) in octopuses caught in shallow water (*SCUBA* and inshore trapping) and offshore (trap only).

rate and possible den limitation in deep waters is not clear at the present time.

The relationship between trap size and the average size of octopus was expected. A similar positive relationship had been found between natural shelter volume and octopus size (Hartwick *et al.*, 1978). Mottet (1975) suggested that dens are limiting to the abundant small size-classes of *O. dofleini;* however, our shallow-water observations do not support this. If large shelters were rarer in deep water, more large octopuses might take refuge in traps and this would explain the abundance of large octopuses in the offshore catch. Although large octopuses are generally restricted to larger traps, small octopuses show up in all trap sizes. Since a small octopus would have little defense against most predators, any available refuge is probably occupied quickly although, if not bothered by predators, they can dig their own dens (Hartwick *et al.*, 1978). Ambrose (1982) noted that *O. bimaculatus* was also able to dig out its own shelter.

Female *O. dofleini* were more likely to be caught in natural shelters in shallow water. This bias has been reported previously (Hartwick *et al.*, 1984; Robinson & Hartwick, 1986). Trapping in nearby shallow waters caught more males than females. The explanation for this is not clear. Females may prefer natural dens and may be better at defending them. Males use traps more and are more likely to be recaptured in them. The latter point applies even in offshore waters. Since the sex ratio of new captures in deep water was close to 1:1 yet males tended to be recaptured more often, the pattern may reflect differential mobility of the sexes similar to that reported by Mangold (1983) for *O. vulgaris.* Female *O. dofleini* in offshore waters had slightly more mutilations than males, while there was no difference inshore during the period, perhaps indicating differences in exposure to predators.

TABLE 4. Stomach analyses of fish caught in offshore jigging and hook-longline.

Fish species	Number caught	Number with octopus remains
Ophiodon elongatus	143	8
Squalus acanthias	72	5
Other spp.; mainly *Sebastes* spp.	67	0

Although the present study indicates that differences exist in the catchability, growth and physical condition of octopuses in offshore waters compared with nearshore shallow waters during the winter period of reduced inshore growth, more information on the movements and the relative abundance of octopuses, their food sources and their predators is required before something more substantial can be said about the occurrence and advantages of a winter movement offshore. Further studies should be carried out on the differential mobility and behavior of the sexes.

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DAYTIME ACTIVITY OF JUVENILE *OCTOPUS VULGARIS* IN BERMUDA

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ABSTRACT

To understand their life history through activity types and patterns, four juvenile *Octopus vulgaris* were observed over a five-week span in a nearshore environment in Bermuda. Observers watched the animals by snorkeling during daylight hours (0630–1830). Octopuses were generally inactive; on average they were within a sheltered "home" 70% of the time and away from it 30%. Locations were recorded every 10 min (2940 observations), and between observations octopuses had moved only 18% of the time. There was a significant difference in activity with time of day, with a peak of 30% movement at 0600–0800 and a minimum of 10% at 1600–1800. This high level of daytime activity is similar to that found for well-fed *O. vulgaris* in the lab but not previously for this species in the ocean, and the afternoon decline in activity matches that found for *O. dofleini* monitored in the wild by sonic tracking. Octopuses were most active on a high ebbing tide. The octopuses spent 53% of their time "asleep," 17% resting, 11% hunting, 13% eating, 3% on home maintenance and 2% on other activities. Smaller cycles of "sleep" and waking, averaging 6 hr (3.5 "asleep," 2.5 awake), were superimposed on this cycle. Their activity within these small cycles moved from "sleep" to rest, to hunting and feeding (once or a few times), to resting or other activity, and to "sleep" again. This pattern of bursts of activity superimposed on a longer cycle is also a common one in predatory mammals. Minimal daytime modulation of activity due to these cycles and the octopuses' speed in catching prey suggest that the octopuses were foraging in an area of high prey abundance.

Keywords: *Octopus vulgaris;* activity modulation; mini-cycles.

INTRODUCTION

The constraints acting upon the activity cycle of an animal are an important part of the adaptive function of its behaviour. Individuals of any species will not be active all the time, but will need some period of rest (Meddis, 1975). Two aspects of this activity will be examined here, the amount and the timing of periods of activity. In organizing when to be out moving around and when to rest, each animal must be responsive to the demands of survival, which are the necessity to find enough food to grow but also to survive predatory pressure; in evolutionary terms, they must "stay in the game" (Slobodkin, 1964). How do octopuses, in particular *Octopus vulgaris,* arrange their activity to survive and also to thrive? The evidence from the observations presented in this paper suggests that this pressure to organize their activity is not strong and thus their response is variable.

Environmental constraints are often the dominant ones that shape activity cycles. Animals are suited for a particular type of circadian activity cycle due to their utilization of predominantly high (diurnal) or low (nocturnal) light levels for vision (Daan, 1981). Octopuses' prey-catching method of speculative foraging (Yarnall, 1969; Hanlon, in press), using chemical and tactile cues but not always visual ones, does not select them for either diurnal or nocturnal activity. Yet, to survive the pressure of visual predators such as fish, many marine species who do not rely upon vision themselves adopt a nocturnal activity pattern. The soft-bodied octopuses presumably are vulnerable to predation by these species, but the complex and reactive adaptive colouration of *Octopus vulgaris* (see Packard & Saunders, 1969), which is most useful in the daytime, suggests that they resist fish predation pressure and are also diurnally active. In contrast, the smaller (15 g) *Octopus joubini,* which is probably more vulnerable to predation due to its size, has a strictly nocturnal activity cycle (Mather, 1984), and also has limited camouflage colouration to assume during the daytime (Mather, 1972). Tidal rhythms are another environmental variation that can control activity cycles, although they seem most important for intertidal species (Neumann, 1981)

and did not influence the subtidal *O. dofleini* (Mather et al., 1985).

Predatory species may respond to different non-environmental influences compared to herbivores or omnivores, for whom food is usually available. Many do not have obvious circadian cycles (Curio, 1976) but respond to ecological variables important to prey-catching. Some have cycles dependent on those of their prey, while others may have smaller activity and rest cycles within the day, such as the 8-hr cycle of the mole (Godfrey, 1955) and the 2-hr cycle of the vole (Daan & Slopsema, 1978). Rhythms of daily activity may also result from "timesharing," since domestic cats forage in common areas but avoid contact by time rather than space allocation (Leyhausen, 1965), and Houck (1982) suggests this for the three shallow-water octopus species of Hawaii. These biological constraints on activity may be more important for octopuses than the environmental ones.

Constraints on activity must be examined for *O. vulgaris* because its activity cycles appear variable. Altman (1967) and Kayes (1974) visited potential octopus homes in shallow-water (down to 6 m) areas off Malta, and concluded that the species had a nocturnal peak of activity. However, Wells *et al.* (1983a) monitored *O. vulgaris* activity in the lab at Banyuls and found a variable activity cycle. Starved octopuses had a nocturnal peak in the cycle, with a rise from a baseline of 20% activity to 60% around midnight. Conversely, animals fed *ad libitum* lost the nocturnal peak and showed equal activity across different times of the day. Could the octopuses in Malta have been living in a poor environment, and showing a nocturnal activity peak due to a low food supply? Could the Wells *et al.* (1983a) situation, with an excess of prey supplied in the lab, be atypical and suppress the normal cycle? Information from another natural situation, preferably one where octopuses had abundant prey, could help answer these questions.

Prey availability could change not just the pattern but also the amount of activity of octopuses. "Sleep" or rest probably serve to inactivate animals and keep them from risk of predation when they do not need to be finding food (Meddis, 1975). Thus an animal ideally should initiate foraging with the onset of hunger, stop when satiated and rest until hungry again. In fact, an efficient forager or one near an abundant food supply will be "lazy" and spend much of its time resting (Herbers, 1981). Lions, who spend up to 75% of their time resting or sleeping, are a well-known example of efficient "lazy" predators (Schaller, 1972). A direct manipulation of food availability confirmed Herber's (1981) hypothesis that more food would result in more sleep for herring gulls (Shaffery *et al.*, 1985). Gulls given food on their territory increased their time there from 83% to 93%, and the extra time was spent either in sleeping or in sitting alertly. If octopuses also have an abundant food supply, will they take a few short hunting trips and rest for a large proportion of the day?

To answer questions about activity amount, activity type and activity cycles, octopuses must be observed continuously for long periods, to assess not only where they are but what they are doing. Altman's (1967) and Kayes' (1974) studies used spot checks of octopus homes, and these authors thus only knew when the octopuses were at home, not what they were doing when away. The animals could have rested in other areas. Wells *et al.* (1983b), Houck (1982) in the lab, and Mather *et al.* (1985) on *O. dofleini* in the ocean, used indirect measures of activity and knew when the octopuses were active but not what they were doing. Direct observation, like that Yarnall (1969) used on *O. cyanea,* is needed to answer these questions about activity. Such observation has been conducted on juvenile *O. vulgaris,* and the results, which describe their activity types, percentages and diurnal cycles, are the subject of the present paper.

MATERIALS AND METHODS

Four juvenile *Octopus vulgaris* were observed over a five-week period in July and August 1985. They were part of a population of approximately 15 in the area, which was monitored by home occupancy during the study. Some octopuses apparently came and went and were replaced quickly, while others appeared to stay in one area throughout the study. Initially in the study six octopuses were captured, weighed and replaced in their "homes," but when this procedure was carried out they rapidly left the observation area and could not be located or studied. Thus the four individuals that are the subjects of this study were not captured but only observed. They were identified only by home except for # 2, who lost part of an arm in week 2 of the

study and was identifiable for 4 weeks thereafter. Octopus # 1 was followed for 12 days, # 2 for 32 days, # 3 for 5 days and # 4 for 8 days. Their weight was estimated at between 100 and 200 g and they were presumably juvenile, by the lack of differentiation of the hectocotylyzed arm, which indicates maturity in males (these measures were taken from the initially weighed and measured animals).

The area of observation was the region of Coney Island Bridge, a 10 m-long bridge between the main island of Bermuda and small Coney Island. Water depth ranged from intertidal to 2 m; because of the nearly constant tidal flow under the bridge (max. 90 cm/s), the tidal level variation (semidiurnal pattern) in Bermuda of 1 m was delayed (maximum flow at high or low tide), dampened and sometimes altered by wind pressure. The bottom in the study area was rock, coral rubble and some sand, with algae and soft corals growing on the rocks. Water temperature ranged from 25° to 28°C. Many locations were available as rocky "homes" for octopuses. Fish were common in the area, particularly the sergeant major (*Abudefduf saxatilis*), slippery dick (*Halichoeres bivittatus*), dusky damselfish (*Eupomacentrus dorsopunicans*), Bermuda angelfish (*Holacanthus bermudensis*) and squirrelfish (*Holocentrus rufus*). The octopuses foraged mainly in subtidal areas and ate mainly the bivalve *Lima pellucida,* a filter-feeder that sheltered under rocks and in crevices, and a variety of small snails and crabs.

Observations were carried out in shifts by teams of volunteer observers provided by Earthwatch, usually within 0630–2130 (dawn-dusk). An observer floated, snorkeling near the water surface, as far as possible from the octopus while still close enough to see what it was doing. The octopuses habituated quickly to the presence of observers, perhaps because scavenging fish were constantly pestering them. Observers generally remembered the type and timing of activities and recorded them after their 45-minute shift, but during periods of intense activity such as foraging they gave a oral description to a team member on the bridge abutment. Spot checks of home occupancy for all the population were made at night twice (2200).

Data on location and activity of octopuses were noted every 10 min, for a total of 2940 observations. These were entered into a computer program, along with time, tidal level (high or low), tidal flow (ebb or flood) and whether the animal was at Home and whether or not it had Moved in the last 10 min. Moved, Away from Home and Activity Type were considered dependent measures, and were sorted by tidal level, flow and time of day (using BMDP Multiway Frequency Tables). Chi-square analyses were carried out to find significant differences in the first two measures related to Tidal Level, Tidal Flow and Time of Day. Times of Day were grouped into two-hour periods, so that there were enough observations in each period. For evaluation of two influences at once, times were grouped into four-hour blocks to reduce the number of categories. Detailed evaluation of cycles of "sleep" and activity were then carried out. (1) Duration of cycles of rest and activity were used to construct a profile of these mini-cycles. These were collected as "sleep" periods (asleep to waking) and awake periods, all during one observation period. Analyses of variance were conducted to see if their duration differed among individuals. (2) A description of the sequence of changes of activity was extracted. Frequency counts were made to isolate significant transitions (between behaviours) to see whether one behaviour could be predictive of another to follow (see Castellan, 1979). (3) For animals 1 and 4, who used the same home range for 2 weeks, active periods were compared to see if they used "time sharing" of the same space.

RESULTS

Activity types and amounts

Behaviour was divided into six categories:

(a) "**Sleep**"—octopuses usually withdrew into their rocky "home," but occasionally to another protected location, curled one or two dorsal arms in front of them and often pulled small rocks in front of the entrance, assumed a uniform gray-green skin colour with purple on the ventral surfaces of the arms, narrowed the pupils of the eyes and became unreactive to environmental changes (see Houck, 1982, for similar observations on *Octopus ornatus*).

(b) **Rest**—octopuses moved forward but stayed within their "home" or another protected place, assumed an arms-down position with head elevated, skin colour mottled brown, gray-green and mustard and were reactive to stimuli from the environment.

(c) **Hunt**—octopuses left the protected area

TABLE 1. Percentage of time that four juvenile *Octopus vulgaris* were performing different activities (noted at 10-min intervals).

Animal number	(1)	(2)	(3)	(4)	Mean
N of observations	552	1453	360	575	—
"Asleep"	46	58	39	67	53
Resting	22	16	20	11	17
Eating	14	11	18	9	13
Hunting	9	11	15	9	11
Home maintenance	3	1	7	1	3
Other	5	1	1	0	1.5
Unknown	2	2	0	3	1.5

and moved across the rocky bottom, normally by using arms to grasp the substrate but sometimes by jet propulsion in the water. During most of this time the octopuses probed into crevices with one or several arms or surrounded small rocks with the arm web as described by Yarnall (1969). They showed changing but usually mottled camouflage colouration.

(d) **Feed**—octopuses moved to a protected location after hunting (not always the home), adopted a resting attitude and colouration and extracted and consumed prey. Duration of this activity was counted until mollusc shell or crustacean exoskeleton remains were discarded.

(e) **Home Maintenance**—octopuses modifying a "home" blew sand out of a crevice or from under a rock, collected rocks to put in front of a home and adjusted their position, or pulled algae off the rock surface.

(f) **Other**—most instances of this category were grooming of the arms and skin surfaces by octopuses, or category unknown.

Octopuses were usually inactive although the amount of different types of activity varied among individuals (Table 1). Sequencing of activity was not random. The prediction of all following behaviours (where 1.0 is perfect and 0.0 is no prediction) given an antecedent behaviour was 0.40 for all the octopuses and 0.39 for octopus # 2 only. Both values are significantly different from zero ($p < .05$), and the fact that they are the same for all observations and for those from one animal suggests that it is a general result. There were important transitions from "sleep" to rest (82%), rest to "sleep" (56%) and hunt (24%), hunt to rest (70%), and feed (27%), feed to rest (37%) or "sleep" (38%).

Activity patterns

(a) **Daily Patterns**—All three factors appeared to influence activity. When activity, as described by Moving or by being Away from Home, was examined across seven two-hour periods of the day, a daytime modulation appeared (Fig. 1). The octopuses moved most between 0600–0800 and significantly less as the day passed, to 10% between 1600–1800 (Chi-square = 69.01, 7 df, $p < .001$). They were also Away from Home most at this time (40%) and Away approximately 20% from 1000–1800 (significantly different also, chi-square = 78.67, 7 df, $p < .001$). They were also more significantly likely to be Moving when the tide was high (21%) than when it was low (12%) (Chi-square = 46.47, 1 df, $p < .001$), and when it was falling (23%) than when it was rising (9%) (Chi-square = 92.21, 1 df, $p < .001$). They were not much more likely to be Away from Home at high tide (29%) than at low (25%) ($p > .025$) (this is marginally significant considering the large n), but much more likely to be Away from Home on an ebbing tide (35%) than a flooding one (17%) (Chi-square = 119.43, df 1, $p < .001$).

When the influences of Time (in four-hour blocks) and Tide (by level and flow) were considered together, a complex pattern emerged. For dependent measure Moving, the pattern was mostly predictable from all three influences: octopuses were generally inactive on a low rising tide and most active on a high ebbing one, especially early and late in the day (Table 2). The interactions were more complex for the measure At Home. Ebbing tide was the phase when they were Away from Home most (38% high and 31% low) but Time of Day was a strong

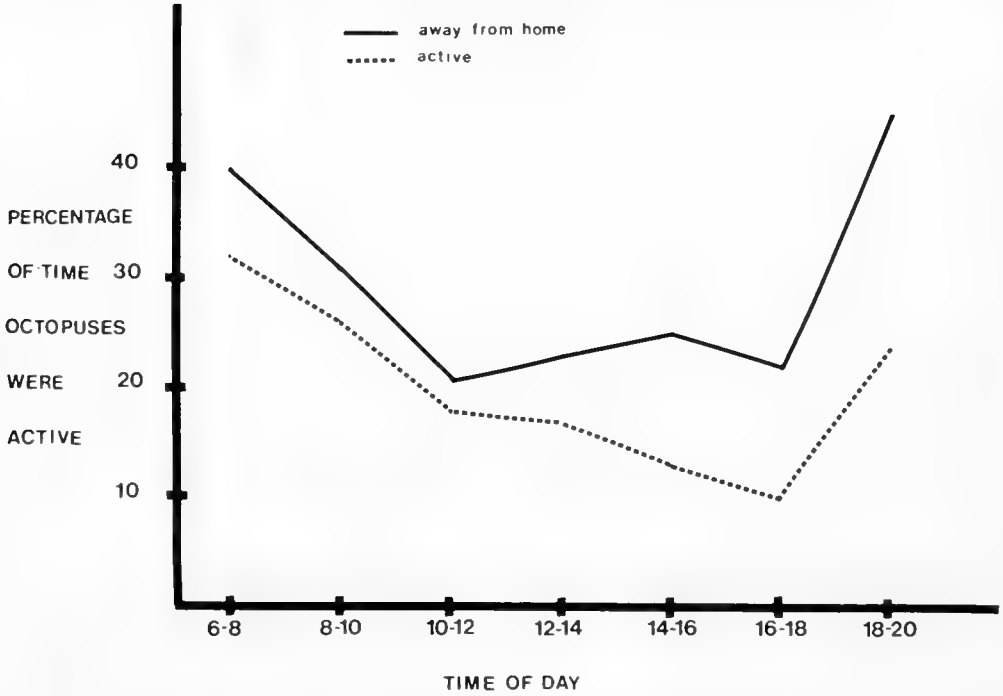

FIG. 1. Percentage of daytime activities, by time Away from home and observations during which the animal Moved, in two-hour blocks for four juvenile *Octopus vulgaris*.

TABLE 2. Percentage of time moving of four juvenile *Octopus vulgaris* during different tidal levels (low, high), tidal flows (flood, ebb) and times of day (0630–1000, 1010–1400, 1410–1800, 1810–2000)

	Time of day				
	0630–1000	1010–1400	1410–1800	1810–2000	Total
Low flood	10	6	6	10	7
Low ebb	31	19	9	(100)*	17
High flood	12	1	12	51	12
High ebb	32	23	29	(71)*	28

*Too few observations for consideration

modulator of this pattern of activity, particularly on low ebbing and high flooding tides (Table 3).

During the two spot-checks of night-time activity, four (or approximately 1/4) of the octopus homes that had been occupied in the daytime were empty. These checks and the lack of a sudden rise of activity around sunset and a return to home at sunrise, suggest a circadian modulation of activity and not an on-off shift.

(b) **Mini-Cycles**—within the day, every octopus we observed had shorter cycles of "sleep" and wakefulness (see Fig. 2 for examples). The 29 completely-observed periods of arousal lasted a mean of 140 min (median was also 140), with a standard deviation of 85 min and a range of 30 to 350 min. "Sleep" periods (n = 38) averaged 220 min (mean and median), with a standard deviation of 130 min and a range of 40–550 min. Individual octopuses varied in average Awake time (170, 155, 140 and 80 min) and "sleep" time (130, 215, 160 and 320 min). The dura-

TABLE 3. Percentage of observations of four juvenile *Octopus vulgaris* away from home during different tidal levels (low, high), tidal flows (flood, ebb) and times of day (0630–1000, 1010–1400, 1410–1800, 1810–2000)

	Time of day				
	0630–1000	1010–1400	1410–1800	1810–2000	Total
Low flood	19	12	12	48	19
Low ebb	43	20	38	100	31
High flood	31	7	9	27	15
High ebb	37	35	48	33	38

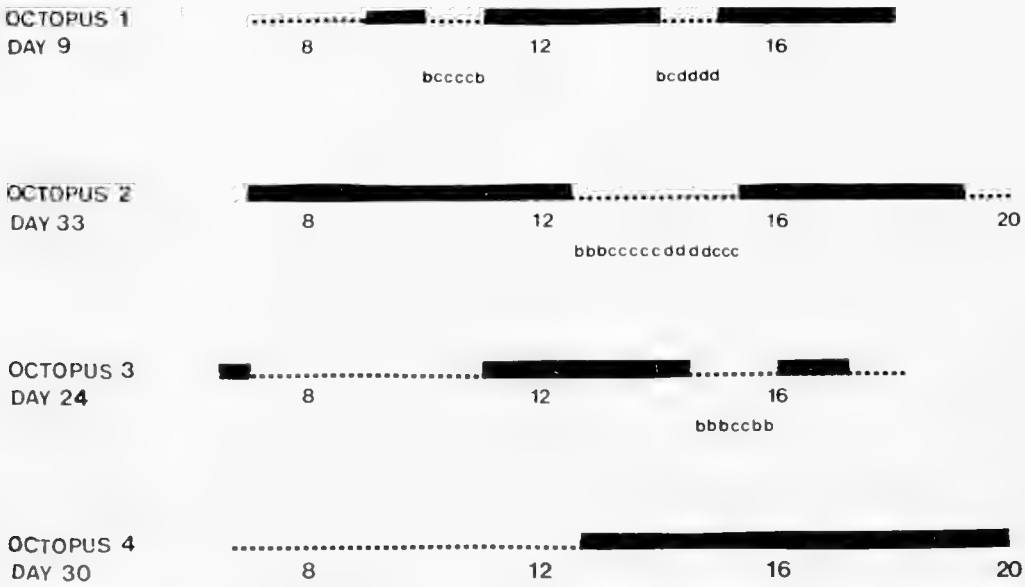

FIG. 2. Four examples of the daytime "sleep"-wake cycles of juvenile *Octopus vulgaris*. Periods of "sleep" are indicated by solid lines and of wakefulness by dashed ones. The sequence of behaviours in several wakeful periods, recorded every 10 min using the categories described in Results, are reproduced below each period's representation.

tions Awake were not significantly different among individuals ($p > .05$), but "Sleep" times were, $F(3,34) = 4.37$, $p = .01$. Since these are repeated observations not independent sampling, the analysis is not a strictly correct one, but the difference is suggestive.

(c) **Other Effects**—since octopuses 2 and 4 had homes less than 1 m apart, hunted in the same area and were observed concurrently, their times Away from Home were examined to test the possibility that they foraged in a common area at different times. Foraging periods (during which octopuses were out or at home with a 10-minute eating pause) took 18% of the time of octopus 4 and 19% of octopus 2. If their coincidence of activity was random, the expected time overlap would be 3.4%. Instead it was 31%, so they fed not time-exclusively but at common times, likely influenced by the environmental factors mentioned above.

DISCUSSION

While these are only daytime observations, the octopuses observed in this study were neither highly active nor inactive. They were "asleep" or resting for 70% of the observation time, a proportion similar to that of daytime

activity of successful mammalian predators (See Herbers, 1981). The small proportion of the time (11%) that they actually spent foraging for food suggests that they were "lazy" and were efficient predators at these particular times and location. This is in contrast to the octopuses observed by Altman (1967) whose activity over the daytime, while quite variable, probably averaged 50% (although these animals were sexually mature and could have been active for reasons other than food gathering). But the averages are more like the approximately 35% activity found by Wells *et al.* (1983a) for well-fed octopuses of variable ages in the lab. Like seagulls (Shafferey, *et al.*, 1985), *O. vulgaris* may be responding to an abundant food supply with reduced activity.

Do the cycles of activity show any major environmental influence? As one might expect of predators (Curio, 1976), the juvenile *O. vulgaris* were influenced by environmental constraints but only to modulate their activity. They were more active at dawn and dusk (when there are low light levels) than in the afternoon. This could fit either a crepuscular (Yarnall, 1969) or nocturnal (Altman, 1967) activity pattern. This modulation of activity over the day was also found by Altman (1967, see his fig. 3), and by Mather *et al.* (1985) for *O. dofleini,* and is an accumulating modulation (note the activity minimum in late afternoon, not midday). This is a direct contrast to the true nocturnal on-off pattern found in the much smaller and perhaps more vulnerable *O. joubini* (Mather, 1984), and apparently also in Kayes' (1974) *O. vulgaris.*

Tidal changes apparently also modulated the activity of these octopuses, since they were most active at ebbing high tides. This modulation is hard to match to direct environmental conditions, since the area near the bridge was affected strongly by tidal flow and wind, and thus did not exactly match ocean tidal conditions. Nevertheless it is interesting that tide did influence this group of animals that lived subtidally, and not the *O. dofleini* (Mather *et al.*, 1985) that were well below tidal level and thus need not have been affected by its variation. No reason for this modulation is apparent, but the octopuses might have been influenced secondarily by factors themselves dependent on tide.

Smaller "sleep"-wake cycles were an unexpected finding for these animals. They have only been recorded previously for moles (Godfrey, 1955) and shrews (Daan & Slopsema, 1978), small homeotherm mammals who may need frequent meals to balance their high energy expenditure in the terrestrial environment. Octopuses can live several days without food (e.g. Wells *et al.*, 1983a), and starved ones have an obvious activity peak near midnight. Perhaps the mini-cycles are the result of an abundant food supply, since when octopuses have food easily available they eat and then "sleep" approximately every 6 hours. The variation of these mini-cycles could account partly for the lack of a circadian activity cycle in Wells *et al.*'s (1983a) results, but mini-cycles would also have been obscured by their summaries of activity data. The 3.5 hour average "sleep" duration matches Wells *et al.*'s (1983b) report of peak oxygen consumption 1–3 hr after a crab meal, and Boucher-Rodoni & Mangold's (1977) report of peak absorption of nutrients 1½–3 hr after food was eaten. If prey were easy to capture, the juvenile octopuses, while appearing "lazy," were possibly an efficient survival machine (Hebers, 1981) engaged in foraging and feeding, then "sleeping" during digestion and awaking ready to feed again. Much more complete data on their circadian activity (including *in situ* activity at night) and an energy budget must be collected before this is proven.

Commonalities across the genus *Octopus* in such a basic aspect of natural history as an activity cycle are absent. *Octopus joubini* has a strictly nocturnal cycle (Mather, 1985), *O. cyanea* has a crepuscular (Yarnall, 1969) or diurnal (Houck, 1982) one, *O. briareus* has mainly a crepuscular or nocturnal cycle (Aronson, in press; Hanlon, in press) and *O. dofleini* has a somewhat nocturnal one, also unaffected by tidal influences (Mather, *et al.*, 1985). It now appears that *O. vulgaris* has a variable and somewhat nocturnal and tidal activity modulation, depending on the ecological parameters influencing the particular population. These differences emphasize the variability of behaviour both across the genus and within one species, and underline the necessity for direct observation and careful evaluation to understand octopod behaviour.

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BIOLOGICAL VARIATION IN *ELEDONE CIRRHOSA* (CEPHALOPODA: OCTOPODA): SIMULTANEOUS COMPARISON OF NORTH SEA AND MEDITERRANEAN POPULATIONS

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ABSTRACT

Reproductive maturity and morphometric characteristics were compared for two samples of the octopus *Eledone cirrhosa* from geographically widely separated sites, Aberdeen, Scotland (320) and Banyuls, France (285). At the two locations the collecting period was synchronized (13 months) and laboratory procedures standardized. In both samples the sex ratio was strongly biased to females, which were about twice the body size of the males. Both sexes were significantly larger in the northern population. Analysis of a limited selection of morphometric characters (mantle, arms, brain and body weight) showed significant differences, but these were insufficient to confirm any divergence in body shape between the two populations. The annual distribution of ovary maturity stages suggested that females of the Banyuls population tend towards a 1-year life cycle compared with nearer to 2 years at Aberdeen. Male maturity did not fluctuate annually. Estimated fecundity was close to 9000 eggs/female at Aberdeen and about 5500 eggs/female at Banyuls, or 10.5 eggs/g wet weight (Aberdeen) and 13.7 eggs/g wet weight (Banyuls). These differences and those for male spermatophore production were not significantly different.

Key words: life cycle; reproduction; geographical variation, *Eledone*.

INTRODUCTION

Eledone cirrhosa (Lamarck, 1798) is a common benthic octopod with a wide geographical distribution in the Mediterranean Sea, North Eastern Atlantic Ocean and the North Sea. Distributional records for the species are collected in Boyle (1983), Gamulin-Brida (1972), Grieg (1933), Mangold-Wirz (1963, 1973), Massy (1928), Rees (1956) and Stephen (1944).

In the western Mediterranean this species is fished commercially and analyses of market samples (Mangold-Wirz, 1963; Moriyasu, 1981, 1983) have provided a substantial body of data on abundance, seasonality, growth, reproduction and ecology, which have been supplemented by laboratory studies on maturation and embryonic development (Mangold & Boucher-Rodoni, 1973; Mangold *et al.*, 1971). Almost at the opposite end of the range, from the North Sea off Aberdeen, a series of studies on the growth, reproduction and ecology of *E. cirrhosa* has provided a parallel base of biological information (Boyle & Knobloch, 1982a, b, 1983, 1984a, b; Boyle & Thorpe, 1984; Boyle, 1986; Boyle *et al.*, 1986). General comparisons between these two series of studies are possible and have revealed interesting similarities and differences between the two populations (Boyle, 1983). Precise and detailed comparison and analysis is not possible for the usual reason that previous projects were not designed for the purpose and have used different methods.

Some years ago the close liaison existing between our two institutions (University of Aberdeen, Scotland and Laboratoire Arago, France) encouraged us to make a new joint study of *E. cirrhosa* in which samples of the population were taken over the same time period at each centre and processed by identical methods. This approach allows a more thorough and confident treatment of the results and in this paper we present selected aspects of the comparison for body size, morphometrics and reproductive maturity.

Although separated by only about 1° of longitude, Aberdeen (57°10′N) is some 1650 km almost due N of Banyuls (42°32′N). This distance and the general pattern of water circulation ensure that the populations of *E. cirrhosa* that we have sampled are reproduc-

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TABLE 1. Basic statistics of size by weight (g) of 605 *Eledone cirrhosa* sampled simultaneously from the North Sea off Aberdeen and the Mediterranean off Banyuls. The respective sampling periods were 4.11.78 to 10.12.79 (Aberdeen) and 3.11.78 to 12.12.79 (Banyuls).

		Aberdeen	Banyuls	Total
Females	sample n	278	249	527
	mean wt ± SE	702.8 ± 17.12	330.7 ± 9.33	
	range	29.8 – 1425.1	42.0 – 897.0	
Males	sample n	42	36	78
	mean wt ± SE	320.0 ± 25.81	199.9 ± 13.97	
	range	40.7 – 591.0	54.0 – 397.0	
Total		320	285	605

tively isolated from each other, yet form parts of a continuous geographical distribution of this species.

MATERIALS AND METHODS

Samples of *Eledone cirrhosa* were obtained by bottom trawling. In the North Sea these were supplied by inshore commercial fishing boats operating within a range of 100 km from the port of Aberdeen (a selection of the series previously reported on by Boyle & Knobloch, 1982a, 1983, 1984a, b), normally no more than 50 km from shore. The depth of most of the trawls was 60–100 m (total range 20–140 m). In the Mediterranean, samples of *E. cirrhosa* were obtained from the research trawler of Laboratoire Arago (M.V. "Lacaze-Duthiers") operating normally 5–15 km offshore and in 60–90 m of water (total range 20–105 m). In both cases the octopuses were returned to the respective seawater aquarium systems and either maintained alive, processed immediately or frozen.

The sampling period covered just over 13 months (4.11.78 to 10.12.79, Aberdeen; 3.11.78 to 12.12.79, Banyuls) and was coincident at each centre to within 2 days. The complete sample acquired at Aberdeen during this period comprised 983 females (mean body weight 764 g, range 28–1679 g) and 105 males (mean body weight 334 g, range 40–627 g). Of these, a total of 320 animals (Table 1), representing all size categories, were processed for reproductive components. This subsample therefore covered the reproductive aspects comprehensively but may not represent adequately the size frequencies present in the original population. At Banyuls, the total sample of 285 was processed (Table 1).

Each animal returned to the lab was assigned a number and its sex, date of capture and details of site of capture were recorded. It was weighed to the nearest 1 g after draining water and mucus from the surface and mantle cavity. At Aberdeen the animals kept for dissection were anaesthetized (2–3% methanol), killed and individually frozen. After thawing, each animal was washed briefly to remove mucus and re-weighed to the nearest 100 mg. This (second) weighing was taken as the body weight of the animal for all subsequent purposes. The Banyuls sample was almost entirely processed while the animals were still fresh, without freezing. Although, in general, the weight after thawing will be slightly less than the fresh weight (Boyle & Knobloch, 1983) no correction factors have been applied to these data.

The relative size of selected somatic, cardiac, gonadial and cerebral body components was determined by weighing after dissection from the body. The dead weight of each octopus (fresh or after thawing) was recorded and this (second) weight was the figure to which all component weights were related. The body components referred to in this paper are as follows: mantle (musculature cut cleanly from the back of the head); arms (cut as far towards their bases as possible); brain (supra- and sub-oesophageal parts separately dissected but weighed together); ovary (less oviducal glands and oviducts) and genital bag (testis, spermatophoric sac and associated glands). A sample of eggs from each ovary was weighed, the eggs were counted and assigned to incremental length categories of 1 mm. The mean egg length and the number of eggs contained in the ovary was

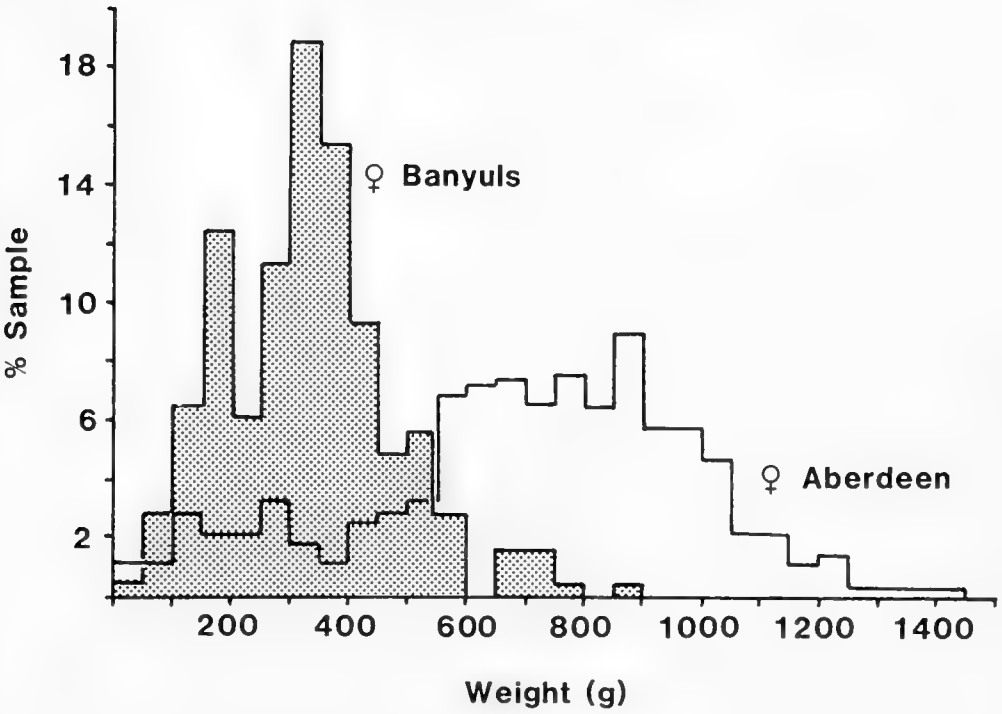

FIG. 1. Size distribution by total body weight of female *Eledone cirrhosa* sampled at Aberdeen (n = 278, white) and Banyuls (n 249, stippled). Individuals, weighed to the nearest 100 mg, are assigned to 50 g weight intervals and the numbers in each category expressed as a percentage of the sample.

estimated for each female. From each male a representative sample of spermatophores was counted and weighed, their length range recorded and mean length calculated.

The data collected in both centres was filed on computer (Honeywell) and standard statistics performed using the SPSS X package with additional manipulation by Fortran subroutines. The association of pairs of variables is given as estimated by the Pearson product moment correlation coefficient (r).

RESULTS

Body size

The basic statistics for body size by weight, separated for males and females, are shown in Table 1. Within sample comparison shows that females were, on average, substantially larger than males at both sites. Comparing stations it is clear that Aberdeen animals, males and females, were larger on average than the corresponding sex at Banyuls. In both samples the sex ratio was biased strongly towards females (about 7 : 1).

The distribution of body size within the two samples (Figs. 1, 2) shows that the size range of males and females was more restricted for the Banyuls animals. Compared with the Aberdeen sample there was a relatively clear modal size at Banyuls for males (150–200 g) but with possibly two modes for females (150–200 g and 300–350 g).

For the Aberdeen population, the data given in Table 1 and Figs. 1 and 2 are that for the sample of animals analysed for reproductive components. Although it is therefore comparable to the Banyuls sample it represents only a subsample of the total number of Aberdeen animals collected over this period.

Relative component size

The data in our samples will eventually permit a rather full analysis of the relative size of body components in the two populations.

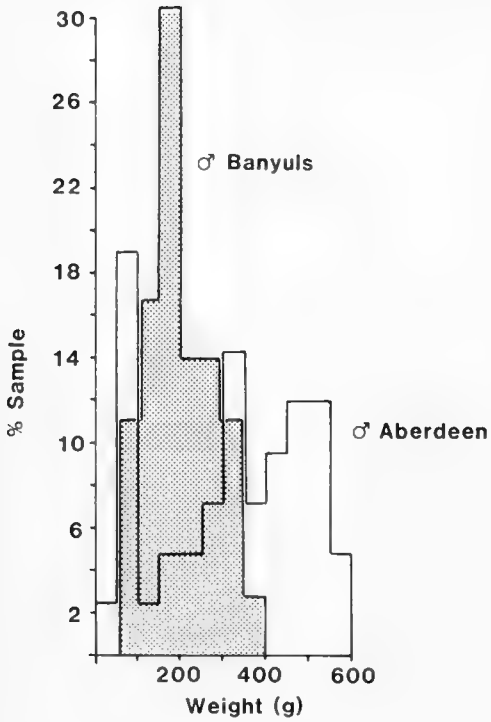

FIG. 2. Size distribution by total body weight of male *E. cirrhosa* sampled at Aberdeen (n = 42) and Banyuls (n = 36). Individuals, weighed to the nearest 100 mg, are assigned to 50 g weight intervals and the numbers in each category expressed as a percentage of the sample.

Here only mantle, arms and brain weights are related to total body weight. Table 2 gives the correlation coefficients and values for the linear regression equations of each of these components on total body weight. Figures are also given for mantle and arms on body weight less the correlated component. The linear regressions are plotted in Fig. 3. Alternative regression models were not tested, but scatter diagrams suggested that linear regression was a good fit.

In the case of brain-body weight, log transformation of body weight produced slightly altered correlations (r = 0.737, Aberdeen females; 0.792, Aberdeen males; 0.618, Banyuls females; 0.667, Banyuls males).

Analysis of covariance between the slopes and elevations of these bivariate regressions showed that for females, the Aberdeen and Banyuls samples were significantly different ($P < 0.01$) in every case except for the slope of mantle-total body weight. For males, only the slopes of arms-total body weight were significantly different ($P < 0.01$) as were the elevations of mantle-total body weight, mantle-body weight-mantle and brain-total body weight.

Reproductive maturity

The most commonly used index of reproductive maturity for cephalopods is the gonado-somatic index in which the weight of gonad is expressed as a percentage of total body weight. Eggs are present in females at all stages and the process of reproductive maturation is largely one of egg growth, yolk accumulation and consequent relative enlargement of the gonad. For females, the values for ovary index are plotted by time of capture in Figs. 4 & 5. Since the sampling period extended for more than one annual cycle, the results for November–December 1978 are superimposed on those for 1979. Monthly means of ovary index were also calculated for both Aberdeen and Banyuls samples.

For Aberdeen, the results show a rather wide range of maturity states throughout the year (Fig. 4). There is a progressive trend towards increasing values of ovary index, with a dislocated batch of low values appearing in November–December. Mean sample values for ovary index reach a maximum in the months of June to September (range 3.4–5.7%). Banyuls females also show a wide spread of maturity states at any particular time but there is a clearer annual trend. There is a relatively rapid increase in maturity during the first half of the year reaching a maximum in the months of April to June (mean ovary index range 5.8–8.1%). From July onwards there are very few maturing animals and only in December is there again an upwards trend.

A maturity index for males was also calculated from the relative size of the genital bag (genital bag weight/total body weight × 100). The total sample size was small (Aberdeen n = 41, Banyuls n = 36) and not very well distributed throughout the sampling period (two months at both sites with no sample). It is perhaps worth noting, however, that there was no observable annual trend in the maturity of Aberdeen males. The mean genital bag index of Banyuls males, however, showed a substantial drop between May and June (6.8% to 1.3%) and by October (the last

TABLE 2. Correlation coefficients (r) of selected body components (in grams) with total body weight (g) and body weight less the correlated component. Values for the intercept (a) and slope (b) of the linear regression are given. N is the number of cases and SE the standard error of the estimate of (b). Values for (a), (b) and SE are given to two significant figures without rounding. Males and females are treated separately and the Aberdeen females selected so that nearly coincident size ranges are compared (40 – 900 g).

		Aberdeen					Banyuls				
	Variables	r	a	b	SE±	N	r	a	b	SE±	N
♀	Mantle/body	0.984	24×10^{-1}	21×10^{-2}	83×10^{1}	206	0.974	65×10^{-1}	22×10^{2}	75×10^{1}	249
	Mantle/(body-mantle)	0.975	43×10^{1}	26×10^{-2}	10	206	0.957	98×10^{-1}	28×10^{-2}	96×10^{1}	249
	Arms/body	0.989	35×10^{1}	49×10^{2}	16	206	0.979	69×10^{1}	43×10^{2}	13	249
	Arms/(body-arms)	0.958	19	93×10^{2}	32	206	0.936	20	71×10^{2}	23	249
	Brain/body	0.730	42×10^{3}	18×10^{-5}	37×10^{3}	205	0.632	10×10^{-2}	35×10^{-5}	63×10^{3}	248
♂	Mantle/body	0.990	64×10^{2}	20×10^{2}	48×10^{1}	42	0.988	37×10^{-1}	22×10^{2}	29×10^{1}	36
	Mantle/(body-mantle)	0.985	12×10^{1}	26×10^{-2}	61×10^{-1}	42	0.981	52×10^{1}	28×10^{2}	37×10^{1}	36
	Arms/body	0.991	-97×10^{-1}	52×10^{-2}	11	41	0.995	-12×10^{-1}	47×10^{2}	39×10^{1}	36
	Arms/(body-arms)	0.963	−13	10×10^{1}	24	41	0.983	-85×10^{-2}	87×10^{-2}	74×10^{1}	36
	Brain/body	0.796	63×10^{-4}	28×10^{5}	35×10^{-3}	40	0.614	86×10^{3}	40×10^{5}	44×10^{3}	36

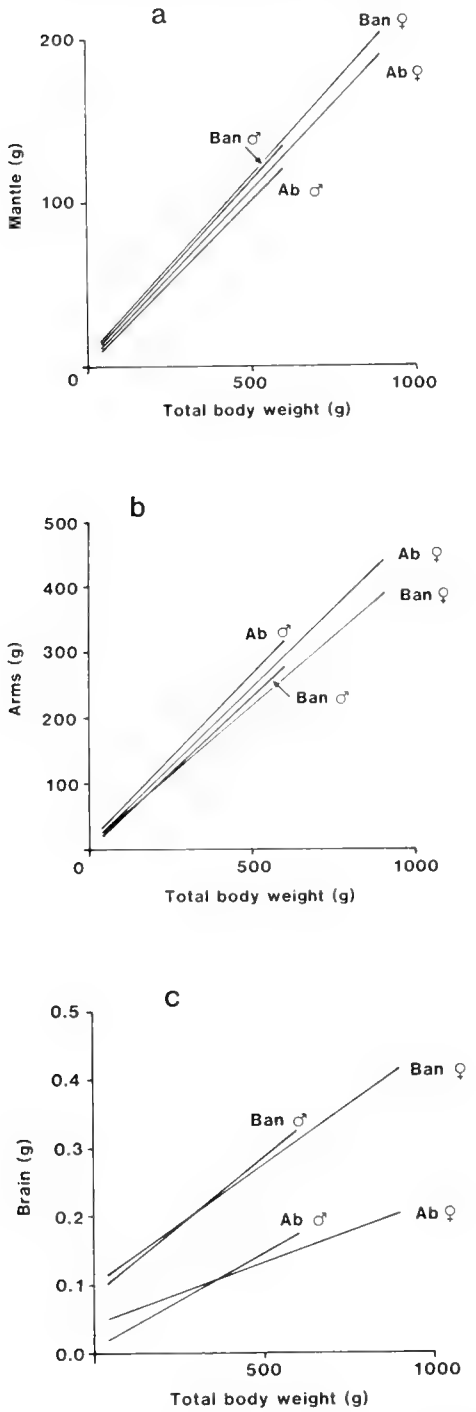

FIG. 3. Linear regression of mantle (a), arms (b) and brain (c) on total body weight for Aberdeen and Banyuls animals. Males and females are treated separately and the Aberdeen sample selected by size so that nearly coincident size ranges are compared.

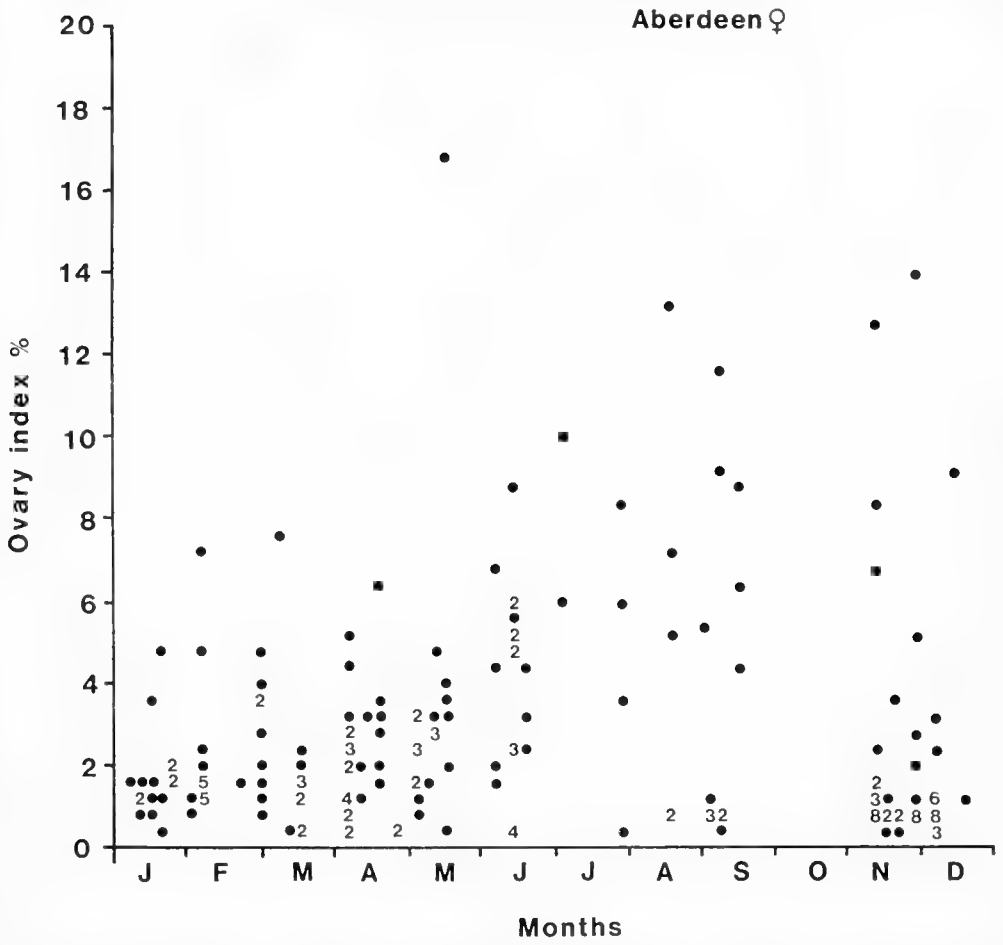

FIG. 4. Annual distribution of values for ovary index (ovary weight/total body weight) × 100, for Aberdeen animals (n = 207). Scatter diagram showing superimposed data points as a single number, thus a 9 indicates that nine or more points are present at the particular coordinates.

sample) it had still not recovered to the mean values of the early part of the year.

Fecundity

An estimate of the total number of eggs contained within the ovary was made gravimetrically from the weight of the counted sample of eggs and the total weight of eggs contained in the ovisac. The estimates ranged very widely but are summarized in Table 3. The mean number of eggs estimated for Aberdeen females (8948) was much larger than at Banyuls (5551), but when related to body size the estimates were much closer. This result implies that larger animals produce a greater number of eggs, but when estimated egg number is correlated directly with total body weight the relationship is not strong and is masked by the variation in egg estimate. Neither estimate of fecundity was significantly different between the two populations (T test). A similar procedure produced estimates of total number of spermatophores for the males in the sample (Table 3).

DISCUSSION

Although discussion of these results is limited by the relatively small sample size and

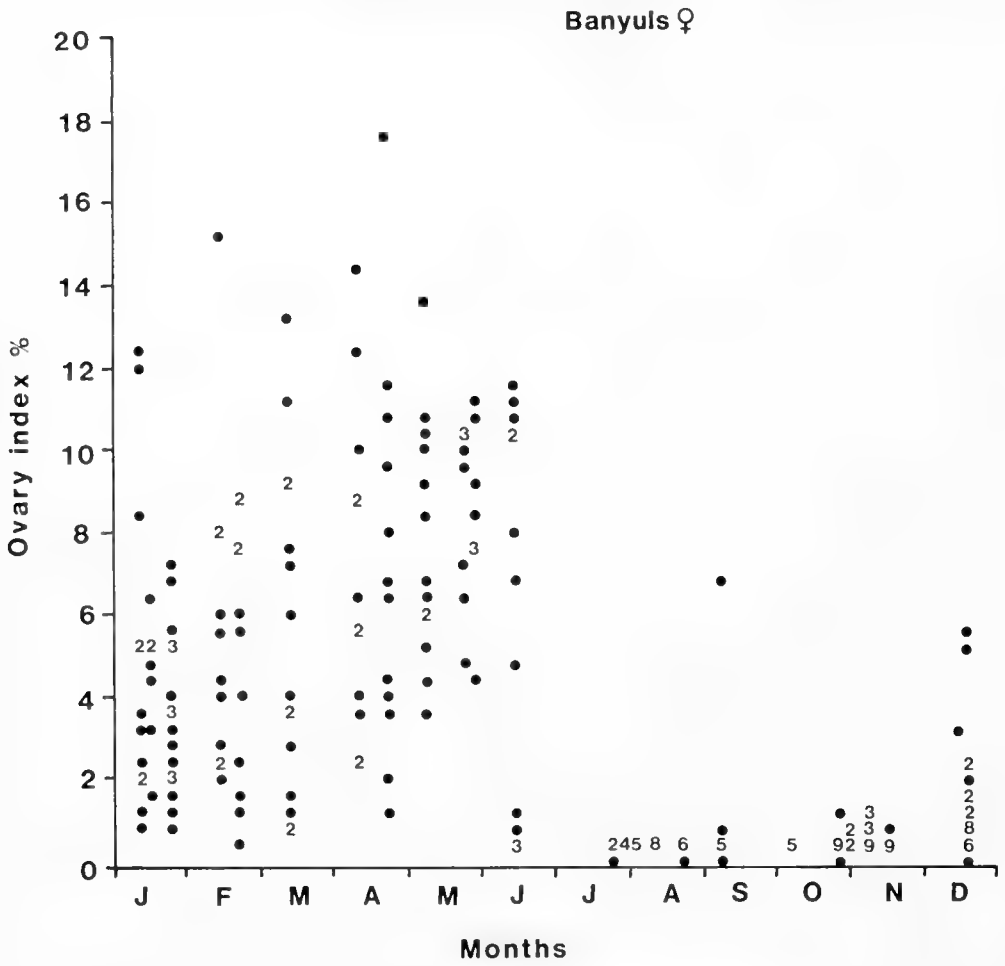

FIG. 5. Annual distribution of values for ovary index (ovary weight/total body weight) × 100, for Banyuls animals (n = 248). Scatter diagram showing superimposed data points as a single number, thus a 9 indicates that nine or more points are present at the particular coordinates.

short sampling period, for the first time it is possible to compare temporal aspects of two cephalopod populations as well as purely morphometric data.

Sexual dimorphism in the form of a substantial size differential between male and female is a characteristic of both populations. Since the samples have been obtained by trawling at both centres throughout an annual cycle, it is unlikely that these differences result from selective sampling. In this species, then, females normally grow to almost twice (1.6 – 2) the size of males.

In these samples the sex ratio is strongly unbalanced, females predominate by about 7:1. The bias towards females has been reported earlier for the North Sea (Boyle & Knobloch, 1982a) and Mediterranean (Mangold-Wirz, 1963; Moriyasu, 1981). The smaller size of males would naturally cause them to be under-represented in trawl-caught samples. For the Mediterranean, previous evidence is available for the segregation of the sexes by depth, the females moving into shallower water to spawn (Mangold-Wirz, 1963). It is notable that in these samples the sex ratio at the two sites was similar (6.9 : 1, Banyuls; 9.4 : 1, Aberdeen total sample) and closer to the figure of 7 : 1 previously reported for the North Sea (Boyle & Knobloch, 1982a)

TABLE 3. Estimates of fecundity for male and female *E. cirrhosa* expressed as the total number of eggs or spermatophores per individual and as the number per gram of wet tissue

	Aberdeen	Banyuls
Sample n	86	150
Mean egg no. ± SE	8948 ± 585	5551 ± 745
Range	2077 – 23915	537 – 85032
Mean eggs/gm ± SE	10.5 ± 0.7	13.7 ± 1.2
Range	2.8 – 30.5	1.6 – 135.7
Sample n	17	7
Mean spermatophore no. ± SE	160 ± 33	79 ± 20
Range	8 – 600	35 – 193

rather than the overall Mediterranean statistic of 1.2 : 1 (Mangold-Wirz, 1963). Since the animals in the present Banyuls sample were trawled from relatively shallow water (60–90 m), it is most probable that the bias to females is due to their shoreward movement and is not representative of the population in deeper water (100–200 m) (Wirz, 1958; Mangold-Wirz, 1963). No such sex segregation is known for the North Sea.

Comparison between sites shows that both males and females were relatively much larger at Aberdeen. Sampling bias, due to differences in fishing gear and technique, may be involved but it is highly unlikely that it can account for the scale and consistency of these differences. In general, the Banyuls sample, resulting from the activity of a single boat fishing regularly in the same area, is more homogeneous than that from Aberdeen where several boats and a wider geographical area were involved. Aberdeen females had a mean weight more than twice that of those from Banyuls and males were over 60% larger. For both sexes the total size range of individuals caught at Aberdeen exceeded the size range caught at Banyuls even though only one type of gear was used at both centres. This is not likely to result from minor variations in net mesh size that may have been present. We conclude, therefore, that there is also a real difference in individual size for this species taken from these two sites.

The small, but statistically significant differences found between slopes and elevations of the regressions of some of the body components on total body weight suggests further morphometric differences between the two populations. The few bivariate analyses presented here are not sufficient to confirm such a conclusion. Multivariate analysis of a larger number of characters has not yet been undertaken. It should also be noted that the "brain" character that showed the largest differences between the two populations (being much larger relative to body weight in the Banyuls sample) is also the character most vulnerable to differences in dissection technique. Every care was taken to achieve consistency both within and between the samples, but before the differences in brain weight between the populations can be accepted, we believe that the measurements should be repeated under strictly comparable conditions.

Taking ovary index as a crude measure of the state of progress towards reproductive maturity, the data in Figs. 4 & 5 reveal interesting similarities and differences between the two populations. The relatively wide range of maturity states over much of the annual cycle is obviously a characteristic. Very few were captured in the final stages of maturity at either site. The Aberdeen data suggest a gradual increase in the incidence of more mature animals throughout the year with the appearance of an immature cohort in November–December. For Banyuls the maturing population present at the beginning of the year disappears almost completely between June and July. These differences suggest a one year life cycle in Banyuls with little overlap between succeeding generations, whereas in Aberdeen the trend is towards a two-year life cycle with a wider temporal spread of breeding activity, growth and maturation.

These results are thus compatible with the 1- or 2-year life cycle proposed for this species (Boyle, 1983), and for the sampling year dealt with here the Mediterranean animals are tending to a 1-year cycle in contrast with the

northern 2 year. Ideally it is necessary to determine the age of individual animals by an independent method so that the size and maturity of different cohorts can be treated separately. It is also unfortunate that we have no means of directly relating the occurrence of mature stages in the population with spawning since there are no useful records of the appearance of egg deposits in the field. A third factor missing is a reliable estimate for the duration of the juvenile phase, between hatching and recruitment into the fished populations. At Banyuls juveniles are abundant in the period February to May, together with "adults."

The absence of field data on egg deposition also prevents a comparison between the fecundity estimates made gravimetrically from egg samples with the numbers of eggs actually laid. Since our method includes eggs at all stages of maturity it attempts to estimate the maximum possible number and therefore may be an overestimate of true fecundity.

More information about the bottom ecology at the Aberdeen and Banyuls sites would be very helpful in the interpretation of these results. Water temperature is one factor that differs significantly. At Banyuls the bottom temperature was rather constant throughout the sampling period at 13 to 14°C. One haul only, which fished into water of 20 m, reached water of 17°C. At Aberdeen we do not have temperature data for the individual hauls, but the average bottom temperature in the region where most of the fishing took place was about 8°C, with a range of 5 to 12.5°C. Here, the change in mean maturity state did not correlate with the seasonal temperature fluctuation (Boyle & Knobloch, 1982b) and at Banyuls there was no systematic variation. The biological differences between the two populations are, therefore, unlikely to be related simply to seawater temperature regime.

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MALACOLOGIA, 1988, 29(1): 89–100

A CONTRIBUTION TO THE REPRODUCTIVE BIOLOGY AND GEOGRAPHICAL DISTRIBUTION OF ANTARCTIC OCTOPODIDAE (CEPHALOPODA).

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ABSTRACT

Benthic octopods were collected during a bottom trawl survey on the western shelf of Elephant Island (South Shetland Islands, Antarctica) in mid-March 1981. Twelve hauls between 68 and 470 m yielded five species; most abundant was *Pareledone charcoti* (n = 114 or 50.2% of individuals) followed by *P. polymorpha* (n = 55 or 24.2%) and *P. turqueti* (n = 47 or 20.7%). Another species of the genus *Pareledone* not yet identified and one species of the genus *Benthoctopus* were present with 7 (3.1%) and 4 (1.8%) individuals, respectively. In *Pareledone charcoti*, wet weight ranged from 1.8 to 136.1 g in specimens of 2.1 to 8.2 cm mantle length. Wet weight ranged from 9.8 to 164.6 g in *Pareledone polymorpha* of 3.1 to 9.7 cm ML. *Pareledone turqueti* weighed 5.3 to 275.4 g wet weight and were 2.9 to 14.1 cm in ML. The largest specimen recorded was a female *P. turqueti* of 6907 g wet weight and 22.5 cm ML. In general, fecundity was low and egg size large when compared to other octopodid species from temperate and warmer seas. Fecundity of females was highest in one of the smaller species, *P. polymorpha*. From the large variation of the gonad index and the size-frequency distribution of ova as well as from the morphology of gonads, there was evidence that spawning in mid March had already commenced.

Keywords: Octopoda; Antarctica; zoogeography; distribution; reproductive biology.

INTRODUCTION

Cephalopods are believed to form major elements of the Antarctic benthic and pelagic ecosystems (Everson, 1977; Roper, 1981). Closer reviews of the existing literature indicate that the significance of squids, e.g. as krill predators, has been overestimated (Kock, 1985), and knowledge of the role of benthic cephalopods is still rather poor. Most studies have focussed on the taxonomy and geographical distribution of octopods (e.g. Robson, 1932; Taki, 1961; Voss, 1976). Of the about 10 species described from S of the Antarctic Convergence, only two have a circumantarctic distribution (Dell, 1972), but this figure is likely to increase once the taxonomy of antarctic octopods undergoes detailed revision (Voss, in preparation). Moreover, only one attempt has been made to describe the octopod fauna of a certain area, namely the Kerguelen Province of the Indian Ocean (Lu & Mangold, 1979).

What little information is available on biology and ecology is limited to occasional reports on food or gonad development (e.g. Massy, 1916; Taki, 1961; Voss, 1968). Investigations on the stomach contents of Weddell Seals and Elephant Seals in the zone of seasonal ice cover indicate that benthic octopods may form significant portions in the diets of these warm-blooded top predators (Clarke & Macleod, 1982a, b). Benthic octopods themselves are likely to represent important top predators of the benthic food webs, along with fish.

The antarctic environment is characterized by high seasonality of the annual light cycle and ice cover, to a large extent governing biological processes. Thus, cyclic phenomena like reproduction might be expected to be related to these seasonal events. The present paper contributes to the geographical distribution and reproductive biology of octopodids based on material collected in the Antarctic in mid-March 1981.

MATERIALS AND METHODS

Between March 17 and 20, 1981, 227 specimens of benthic octopodids were sorted from the by-catch of a bottom trawl survey off

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Elephant Island (South Shetland Islands) by the FRV *Walther Herwig* in the Atlantic Sector of the Southern Ocean. The original intention of the survey was to do a stratified random sampling program for fish stock assessment on the shelf of the island (Hempel *et al.*, 1982), i.e. to sample the depth strata adequately to define their extent and area. Unfortunately, the survey had to be abandoned due to problems with the ship's engine. Therefore, only 13 bottom-trawl stations were carried out on the north-western shelf between 68 and 480 m (Fig. 1). The gear was a 67 m bottom trawl with a narrow mesh cod end. Specimens were deep-frozen at −30°C immediately after removal from the catch. In the laboratory, various morphometric measurements were taken from specimens after thawing, and radula as well as statoliths and beaks were removed, examined and measured. Species were identified following part of an unpublished manuscript by G.L. Voss (Rosenstiel School of Marine and Atmospheric Science, Miami, Florida) and information given by Joubin (1905), Massy (1916), Berry (1917), Robson (1932), Taki (1961), Voss (1976) and Palacio (1978).

Before dissection, total wet weight was determined to the nearest g. Total length (TL) and dorsal mantle length (ML) (see Roper & Voss, 1983) were measured to the nearest mm, statolith length to the nearest 0.1 mm. Specimens were sexed and gonads inspected to determine the state of sexual maturity. In females, ovaries with oviducts and oviducal glands were weighed and the colour of the oviducal glands as well as the appearance of eggs through the wall of the ovary (see Mangold-Wirz, 1963; Boyle & Knobloch, 1982) were recorded; in males, the genital bag (penis, Needham's sac, spermatophoral glands and testis) was weighed. Ovaries and Needham's sac were opened to count and measure (nearest 0.1 mm) the ova and spermatophores, respectively.

RESULTS

Vertical distribution

Benthic octopodids occurred in 12 of 13 hauls; one tow was heavily biased by huge numbers of pelagic organisms and therefore the by-catch was only inspected briefly. Four species of the genus *Pareledone* and one species of the genus *Benthoctopus* were identified; four specimens of *Pareledone* and the *Benthoctopus* specimens could not be determined to species and possibly represent new species that are being investigated. The hauls were grouped into two strata: 50 to 200 m and 200 to 500 m, represented by six tows each and the results are set out in Table 1. The water temperatures at fishing depths were around 0°C and salinities were in the 34.5 to 34.8% range. The *Pareledone* species were present over the whole depth range fished, whereas *Benthoctopus* sp. was restricted to the deeper shelf below 200 m. *P. charcoti* was most abundant, with more than 50% of individuals, followed by *P. polymorpha* and *P. turqueti.* The *Pareledone* species were equally abundant in both depth strata, although *P. charcoti* appeared to be more numerous at the shallower part of the shelf.

Size and sex composition

The mantle length frequencies of the three most abundant species are given in Fig. 2. In *P. charcoti* and *P. polymorpha* the distribution shows only one maximum at 4 and 5 cm ML, respectively, whereas at least two peaks can be recognized in *P. turqueti,* with distinct modes at 4 or 5 to 10 cm ML, the latter exclusively represented by males. The largest specimens were females in all three species. The absence of small octopods less than 1 or 2 cm ML may either be due to the mesh size of the net bag or to the cursory inspection of the by-catch, i.e. due to inadequate sampling or sorting. The total wet weight of specimens ranged between 1.8 and 136.1 g in *P. charcoti,* between 9.8 and 164.6 g in *P. polymorpha* and the bulk of *P. turqueti* weighed 5.3 to 275.4 g, with one large specimen attaining 6907 g (22.5 cm ML). The latter values represent the largest benthic octopodid so far recorded S of the Antarctic Convergence (Nesis & Propp, 1968 cited in Dell, 1972). The sex ratio was close to unity in *P. charcoti* and *P. polymorpha* but far more males than females were present in *P. turqueti,* particularly in specimens larger than 9 cm ML. Differences in sex ratios with regard to depth were not observed.

Maturity and fecundity

The morphology and size of ova as well as the morphology and the relative size of gonads provide possible estimates of maturity (Voss, 1983). Mangold-Wirz (1963) and Boyle

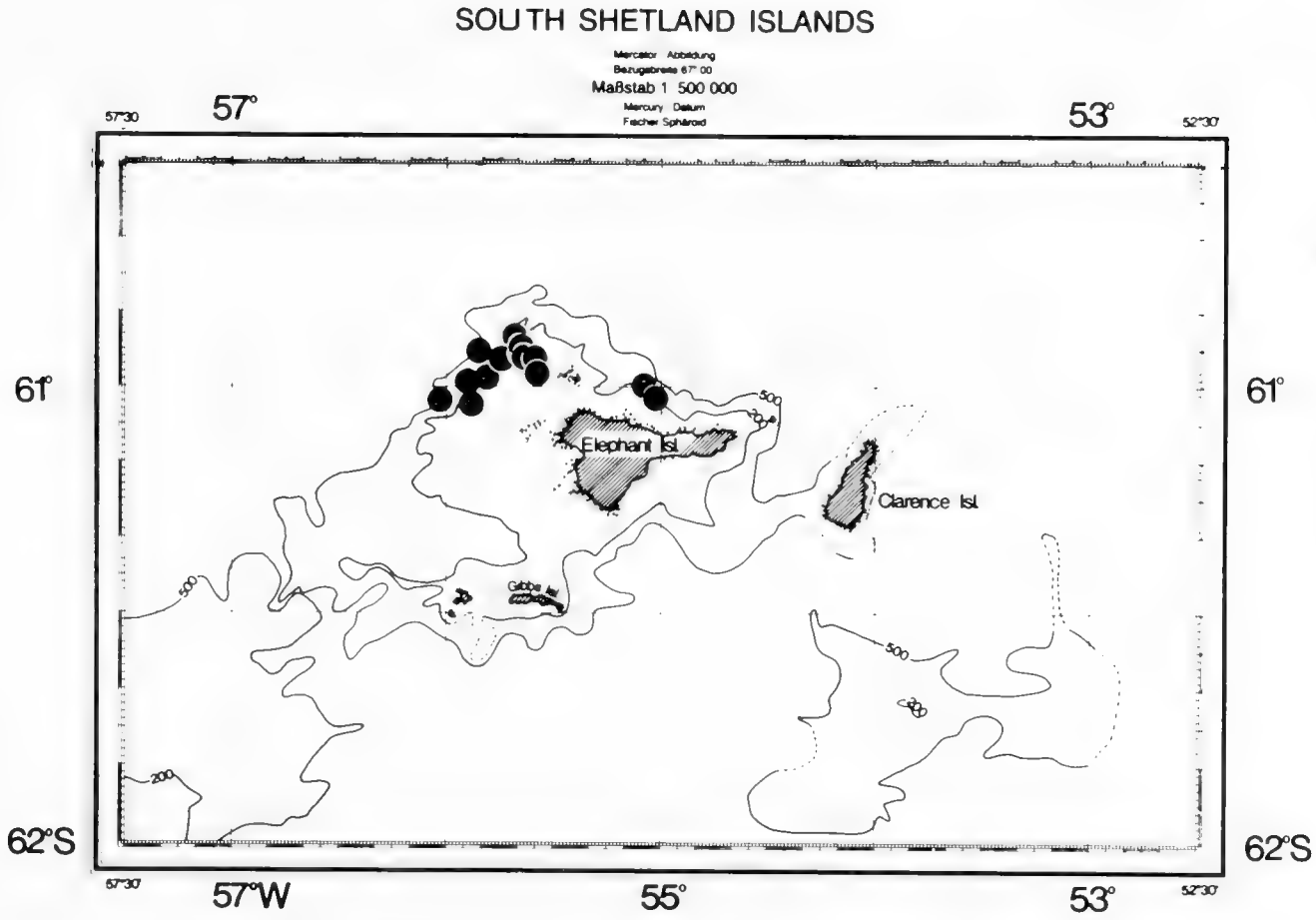

FIG. 1. Map of the study area with locations of the bottom trawl stations of FRV "Walther Herwig."

TABLE 1. Vertical distribution of the *Pareledone* species and *Benthoctopus* sp. off Elephant Island in mid-March, 1981. N denotes abundance in the two depth strata, 50–200 m and 200–500 m, and in the total depth range; D is the percentage abundance.

	Depth strata					
	50–200 m (n = 6 hauls)		200–500 m (n = 6 hauls)		50–500 m (n = 12 hauls)	
Species	N	D (%)	N	D (%)	N	D (%)
Pareledone charcoti (Joubin, 1905)	63	56.8	51	44.0	114	50.2
Pareledone turqueti (Joubin, 1905)	21	18.9	26	22.4	47	20.7
Pareledone polymorpha (Robson, 1930)	26	23.4	29	25.0	55	24.2
Pareledone sp.	1	0.9	3	2.6	4	1.8
Benthoctopus sp.	0	0	7	6.0	7	3.1
Total	111	100	116	100	227	100

& Knobloch (1983) observed in several octopod species that the stage of egg maturation is indicated by the appearance of striations on the surface of the ova. Boyle & Knobloch (1983) described the formation of this striation as follows: each growing egg is surrounded by a layer of follicle cells that proliferate faster than the growing egg can accommodate them as a single lamina, and so the layer becomes folded. These folds give a longitudinally striped appearance to each egg, obviously externally. In the final phase of egg expansion, the follicle cells are lost and the eggs are smooth and loose in the ovisac. At the same time that the striated ova were observed in the *Pareledone* species, they showed swollen, yellowish ovaries with the oviducts attached closely. Therefore, the striation was used to group female octopods into specimens with only small immature ova and those with large maturing, striated ova. The number of ova of each length class was then pooled for the two groups separately and the percentage frequencies of the ova length classes were calculated from pooled data. The results are given in Fig. 3.

In *P. charcoti,* the ova length distribution of females with immature ova was characterized by only one distinct peak, at 2 mm, with only a few ovaries containing large eggs up to 11 mm long. In females with maturing ova, the majority of ova varied between 6 and 18 mm long. Only a few females were found with smaller ova present. In *P. polymorpha,* among females with immature ova only, the highest frequency was recorded at 2 mm but ova up to 10 mm long were also found. The ova length in ovaries bearing maturing eggs ranged mainly from 7 to 14 mm. In the first group of *P. turqueti,* the length of ova varied between 1 and 17 mm with modes at 2 and 10 mm, the latter mode apparently representing a generation of developing ova. A few of these were striated, but the ovaries were not swollen and coloured. The largest maturing eggs (19mm) were observed in *P. turqueti,* but only one female with maturing eggs was investigated. There is evidence that in *P. charcoti* and *P. polymorpha* eggs of more or less equal size begin to mature, whereas in the larger species, *P. turqueti,* egg size at the onset of maturation is also larger (see Fig. 3).

Another estimate of maturity is the gonad index, i.e. the wet weight of the ovary including ducts and glands, or the genital bag expressed as percentage of the total body weight. This index is shown as a function of total length of female *P. charcoti* in Fig. 4; here females were also split into two groups. A total of 29% of females had maturing eggs. They were observed from 5.5 cm ML onwards, corresponding to a wet weight of 43 g. Their gonad index varied between 3 and 20%, indicating that at least partly spent females were present. Indeed, the lowest figures at 3 and 5% represent two females, the ovaries of which contained 60 small and 4 large ova and

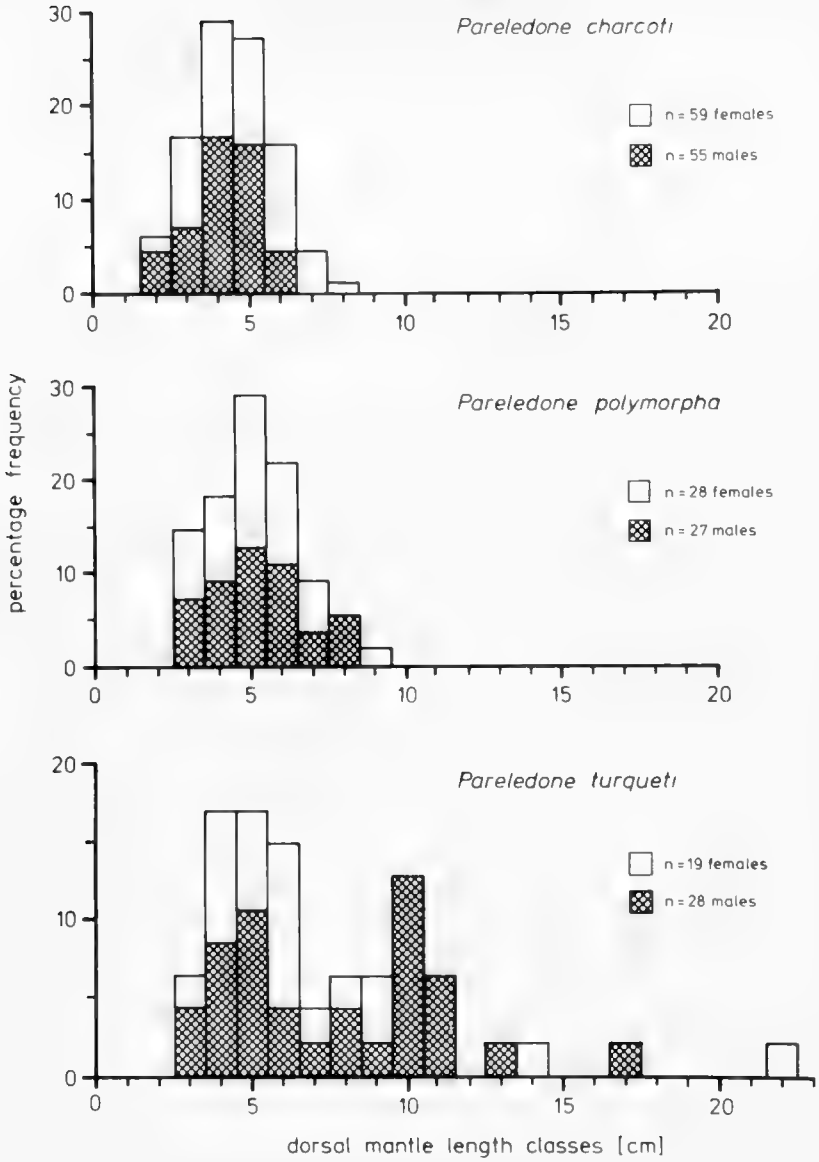

FIG. 2. Length-frequency distributions of *Pareledone charcoti*, *P. polymorpha* and *P. turqueti*.

21 large ova, respectively. As in *P. charcoti*, the gonad index varied considerably in female *P. polymorpha*, ranging from 0.8 to 15% in specimens bearing maturing ova (Fig. 5). These occurred from 5 cm ML upward; 9 out of a total of 25 females had maturing eggs (36%). In *P. turqueti*, the available number of specimens was too low to present results adequately and would not even permit a preliminary analysis, because the size range was much broader in this species. The one female with striated ova had a mantle length of 6.6 cm at a wet weight of 68.9 g.

The developmental stage of maturation was not investigated in males. Only the presence or absence of spermatophores in

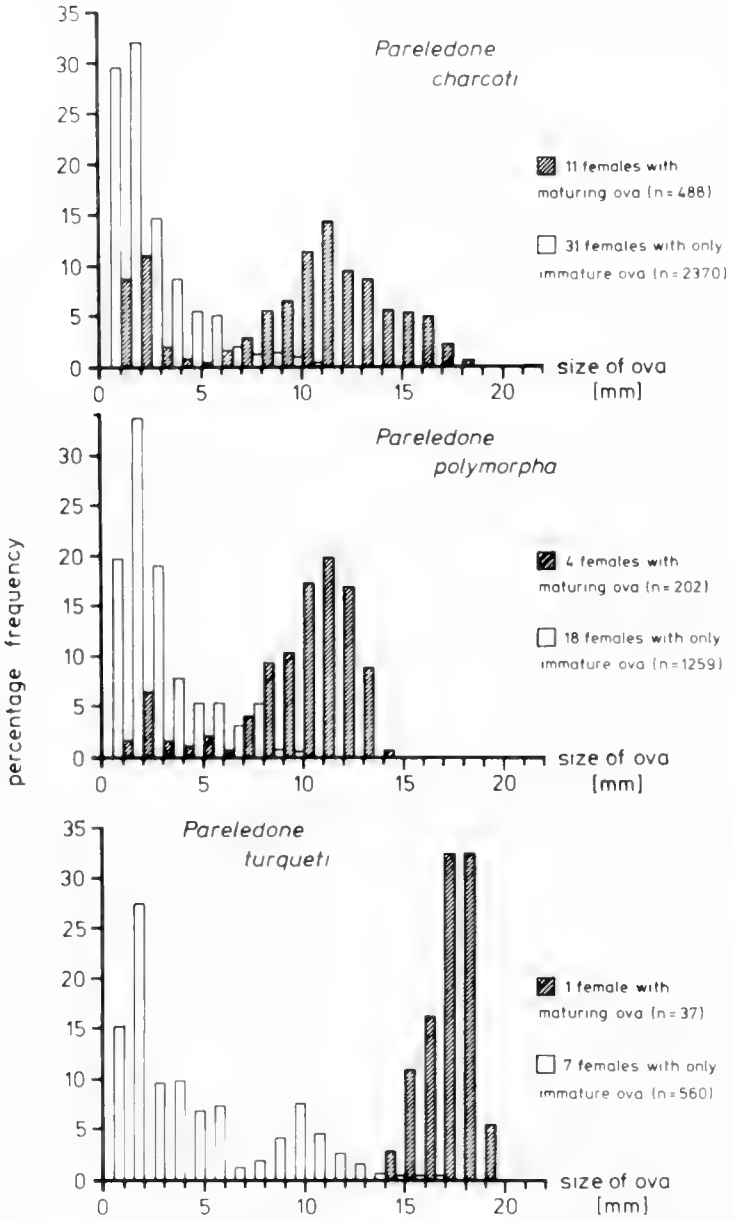

FIG. 3. Frequency distributions of ova lengths in *Pareledone charcoti, P. polymorpha* and *P. turqueti,* grouped by females with maturing ova and females with immature ova only. Pooled data (see text).

Needham's sac was taken as indicative of maturity; in some cases in *P. charcoti,* spermatophores were encountered in the penis and diverticulum. The portion of mature males was 76% of the total population. Spermatophores were recorded in specimens from 3 cm ML upwards, corresponding to a wet weight of 9 g. Also in males, the high variation of the gonad index suggests that spawning had already commenced (Fig. 6). However, the number of spermatophores is not related directly to spawning since they can be stored

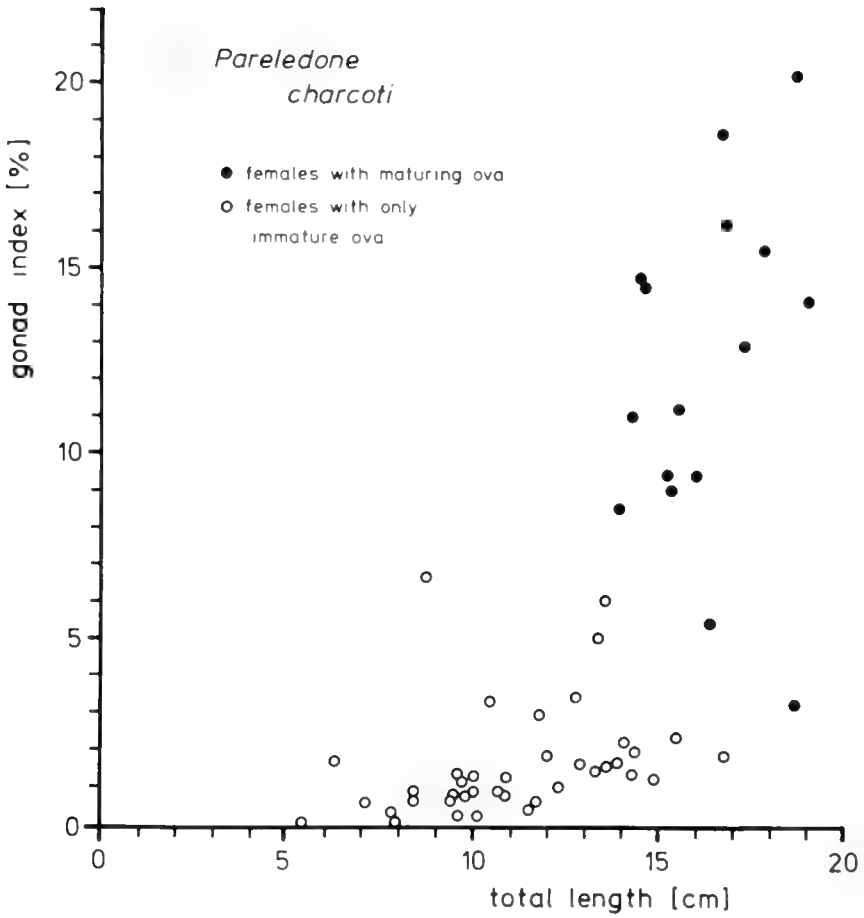

FIG. 4. *Pareledone charcoti.* Gonad index of females as a function of total length.

in Needham's sac and new ones are likely to be produced. This is reflected by the number of spermatophores counted in *P. charcoti* over the total wet weight range (Fig. 7). Although a rather consistent tendency of increase in number with wet weight is apparent, there is a considerable overlap of weight ranges at a given number of spermatophores. This means also that males of equal size may have one to five spermatophores. In male *P. polymorpha,* spermatophores were observed from 4 cm ML upward. They were present in 20 of 27 males (74.1%). The smallest male *P. turqueti* in which spermatophores were observed was 4.3 cm ML, weighing 21.4 g. The maximum recorded number and length of spermatophores differed considerably among species. In *P. charcoti* (max. number = 7), they were up to 8.5 cm, while in *P. polymorpha* (max. number = 5), maximum length was 6.9 cm. Spermatophores were largest in *P. turqueti* (max. number = 9) with a maximum of 16.5 cm.

To obtain an estimate of fecundity, the number of eggs was considered from females with maturing ova. It is assumed that, with the possible exception of *P. turqueti,* the immature ova will not have been spawned in the same season. This means that in *P. charcoti* only ova between 6 and 18 mm length were included, and in *P. polymorpha* between 7 and 14 mm. Then the absolute fecundity

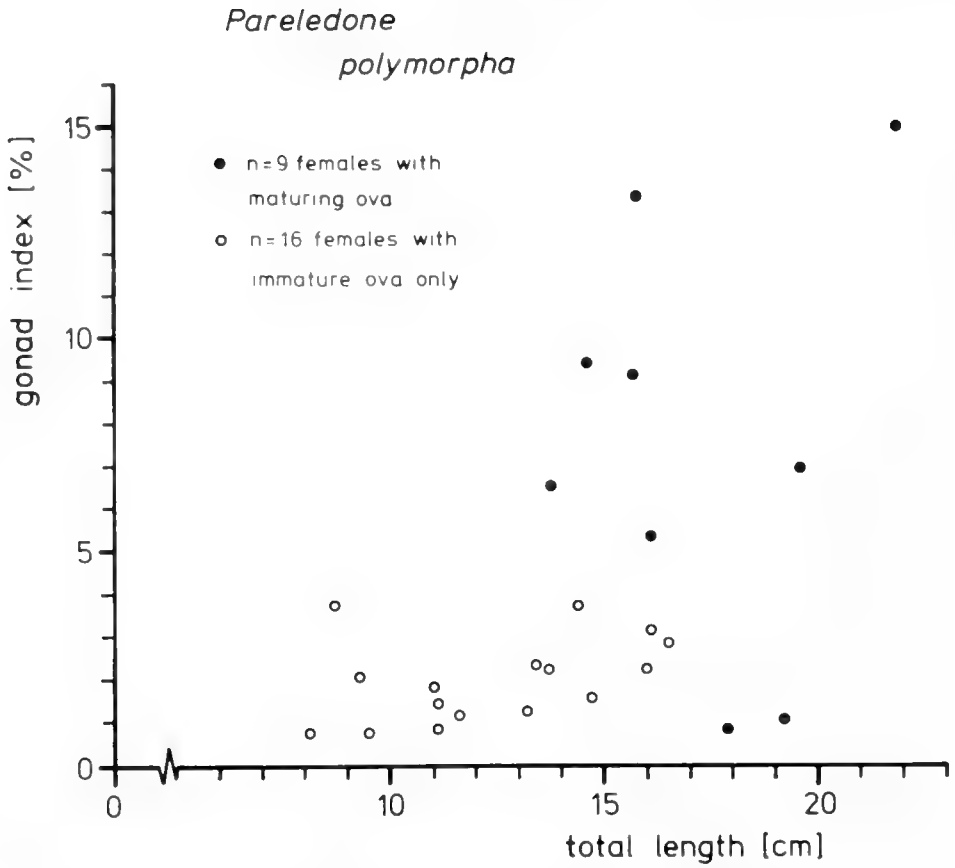

FIG. 5. *Pareledone polymorpha*. Gonad index of females as a function of total length.

varied between 21 and 58 in *P. charcoti* of wet weights 49.2 and 136.1 g. The relative fecundity, i.e. the number of ova per g total body weight, was 0.4 to 0.8 (mean 0.5 ± 0.2) for this species. In *P. polymorpha* of 44.6 to 164.6 g wet weight, the number of ova ranged from 34 to 65 with a relative fecundity of 0.4 to 0.9 (mean 0.7 ± 0.2). The one female of *P. turqueti* contained 37 ova, of 0.5 per g wet weight.

DISCUSSION

Two of the species recorded off Elephant Island are known to have a circumantarctic distribution: *Pareledone charcoti* and *P. turqueti* (Dell, 1972). The first has been found previously at depths from the upper eulittoral zone down to 1500 m, whereas *P. turqueti* has been reported from shallow waters to the upper continental slope at about 550 m (Joubin, 1905, Palacio, 1978). This is corroborated by the present findings. However, for *P. polymorpha,* both the vertical and geographical distributions can be extended widely through our samples. This species has so far only been observed off South Georgia at a depth of 273 m (Robson, 1930; Palacio, 1978). However, the present results show that off Elephant Island, *P. polymorpha* has the same vertical range of occurrence as the other two species, and hence can also be termed eurybathic. The geographical distribution of *P. polymorpha* is not limited to South Georgia. During the German Antarctic Expedition in 1985, all three species were recorded in the Weddell Sea (Kuehl, in preparation), suggesting that the geographical distribution of *P. polymorpha* may extend even farther along the continental coast.

Males and females were present in equal

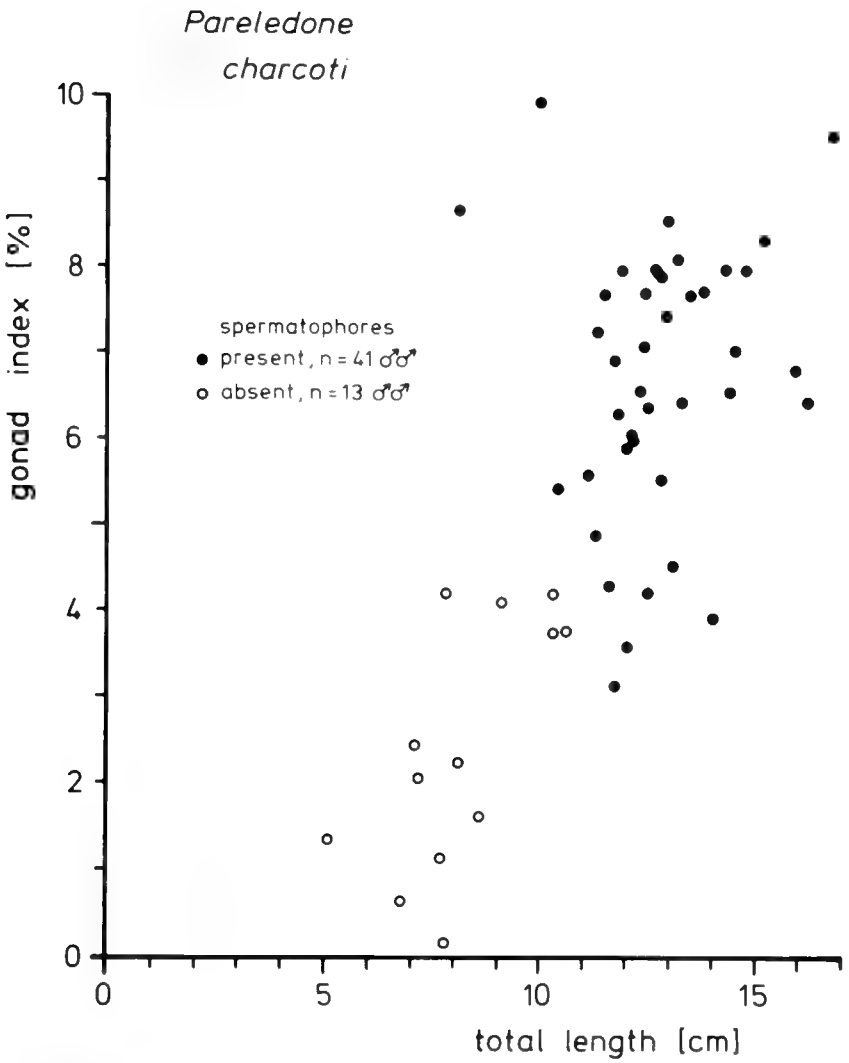

FIG. 6. *Pareledone charcoti.* Gonad index of males as a function of total length.

numbers in *P. charcoti* and *P. polymorpha;* none of the two depth strata was preferred. In these two species, there was strong evidence that spawning had already commenced. In contrast, the portion of males was much higher in *P. turqueti.* If the striking lack of large females is because they occur usually in deeper waters, then this could indicate a spawning migration into the shelf area, with males preceding females. Such a migration pattern was observed in *Octopus vulgaris* from the Mediterranean, although the vertical depth range there was much less extended (Mangold & Boletzky, 1973).

Fecundity is extraordinarily low and egg size is large in these antarctic species when compared with figures from other regions. Comparable values of number of eggs are reported for many octopods (cf. Boyle, 1983), including *Bathypolypus sponsalis* (Mangold-Wirz, 1963) and *Bathypolypus arcticus* (O'Dor & Macalaster, 1983). O'Dor & Macalaster (1983) observed 20 to 80 eggs per female of 9–14 mm in length in *Bathypolypus arcticus,*

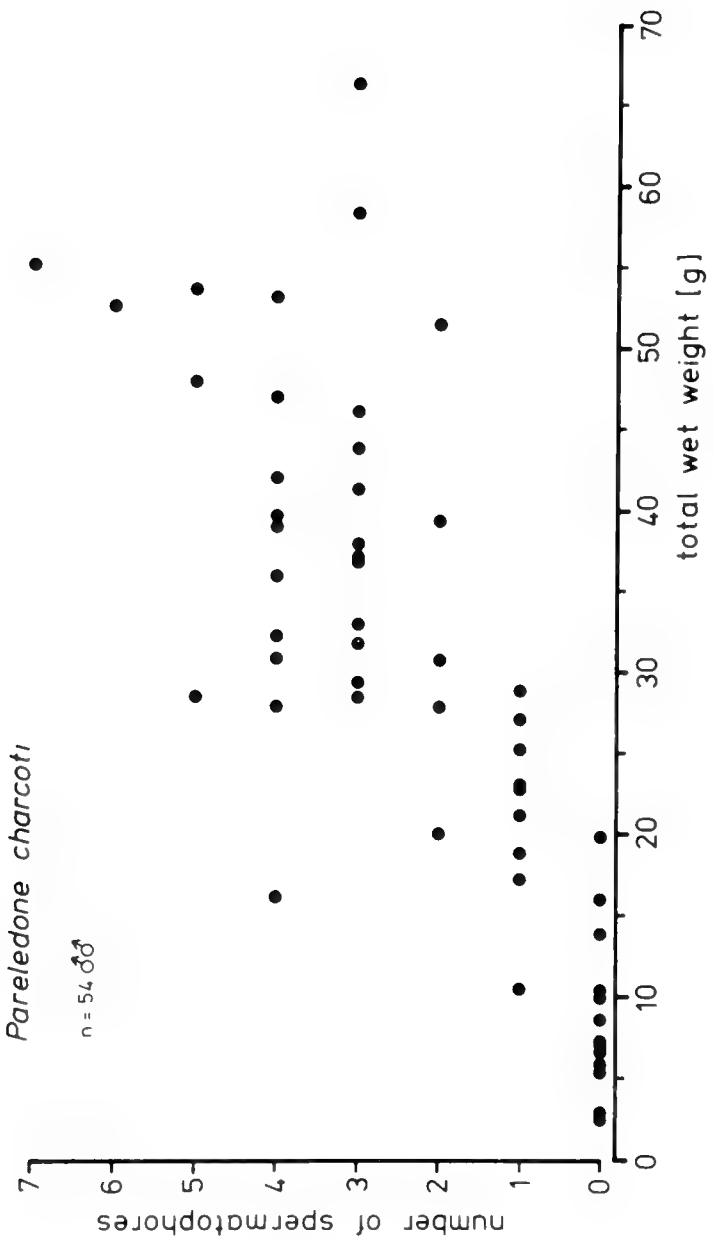

FIG. 7. *Pareledone charcoti*. Numbers of spermatophores plotted versus total body wet weight.

whose geographical distribution extends into arctic waters. Among the *Pareledone* species, differences in fecundity and egg size were obvious; the number of eggs was higher (both absolute and relative) and eggs smaller in *P. polymorpha* than in *P. charcoti.* Largest eggs were observed in *P. turqueti;* since there is an inverse relationship between egg size and number (Voss, 1983), *P. turqueti* can be expected to have the lowest fecundity of the three species.

The length distribution of ova and variation of the gonad indices suggest that the spawning season extends over a considerable period of time. The long spawning period is due to the variation of gametogenesis in individual females as manifested by the wide range of maturing ova in *P. charcoti,* and possibly to repeated spawning events in individual females. This was indicated by the peak of small ova co-occurring with a few large ripening ones in *P. charcoti* and *P. polymorpha.* However, resorption or degeneration of ova after spawning is completed may occur (see Van Heukelem, 1973). Multiple spawning within one season would mean that fecundity is underestimated in the two species. The question is still open as to whether the females with only immature ova arrive at the spawning stage during that spawning season. In other words: how much time does it take the eggs to develop? As can be seen from Figs. 4 and 5, most females are much smaller than females with maturing ova. Hence it seems unlikely that they would have attained spawning condition in the same season. However, the overlap in size distribution of ova between the two groups of females indicates that at least the larger immature females may do so. Thus, there are two possibilities. The first is that spawning occurs more or less continuously due to smaller females subsequently entering maturity; thus there would be no distinct seasonality in the spawning period. The second is that the immature females attain sexual maturity not until the following year and this would mean that gametogenesis takes longer than one year and that spawning is seasonal. In *Bathypolypus arcticus,* no indication of a seasonal reproductive cycle was noted (O'Dor & Macalaster, 1983).

The period of this investigation was in March, i.e. austral autumn. At this time of year, pack ice drifting out of the Weddell Sea appears in the region and sea ice starts forming. Due to their large size, incubation time of eggs is probably long and extends over the winter months. In *Bathypolypus arcticus,* eggs developed over eleven months at temperatures between 10 and 3°C (O'Dor & Macalaster, 1983). However, *B. arcticus* is not endemic to arctic waters, and only extends its distribution that far N. The *Pareledone* species are endemic and hence are probably stenothermal. Therefore, incubation times are probably on the same order of magnitude or even less, despite the lower environmental temperatures. Many antarctic benthic invertebrates show a seasonal reproductive cycle, timed so that the offspring are released during spring and summer at the time of maximum food availability (White, 1977). Thus, the adaptive significance of the octopod spawning season in austral autumn might be to provide maximum chances of survival for the brood, e.g. when young crustaceans or molluscs of suitable sizes are abundant.

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SQUID LIFE HISTORIES

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The shortness of this session was appropriate, since it reflects the fact that we understand less about the lives of squids than we do about almost any other major animal group. However, the mixture of reports in this session illustrated well the importance of squids in marine ecosystems, the ease with which simple observations can still change what we think we "know" about them, and the difficulty of interpreting the activities of such complex animals, even those few that can be studied in the controlled environment of the laboratory. One published report (J. Shears) is an elegant experimental study establishing that predator avoidance is a more important influence on the life style of a benthic squid than food acquisition, perhaps suggesting why the high energy cost of pelagic life is worthwhile. A second paper (M. Vecchione) deals with an accidental observation that may change our whole view of the reproductive biology of a major species in a relatively well-studied marine ecosystem, as well as our approach to such studies in the future. There is also a description (N. Balch, A. Sirois & G.V. Hurley) of a technique that may allow us to patch together reasonably accurate descriptions of growth-age relations for oceanic squids from the limited samples that are actually available and, finally, an extrapolation (R.K. O'Dor) from studies of the energetics of squid locomotion that suggests that their life histories may take place on geographic scales larger than most of us like to contemplate. In addition to the works here, the oral presentations at the Symposium included an analysis of *Illex illecebrosus* population structure that emphasized the interaction rather than the isolation of seasonal breeding groups (M.L. Coelho), an exciting preliminary report on most of the life stages of the hitherto mysterious *Moroteuthis* (W.F. Gilly, F. Horrigan & N. Fraley), a detailed field and laboratory study of the life cycle of *Sepioteuthis lessoniana* in Japan (S. Segawa) and a clear demonstration that oceanic squids are the major components in the diets of some salmonids and other economically important marine species (W.G. Pearcy & K. Jefferts).

Squids are still not easy to study, but they are becoming easier all the time. Who would have believed a decade ago that a remote-controlled submarine might be the key to assessing the reproductive potential of a squid species without ever catching a single specimen, or that every young squid was keeping a record of its growth rate for us in its head—and that we could read it! The gap in our knowledge of how these complex, high-mobile animals function in the sea is rapidly closing as we get better at keeping them alive in captivity and develop new techniques to learn about their activities in nature.

The world-at-large is beginning to recognize these advances—and actually seems to care—as we learned in after-hours sessions at the Symposium. The AMU meeting brought squid researchers from around the world together with members of the Intergovernmental Oceanographic Commission-FAO Guiding Group of Experts on the Programme of Ocean Sciences in Relation to Living Resources for a continuation of discussions of the development of an International Recruitment Program (IREP) on squids. These discussions began at the 1984 squid symposium in Halifax sponsored by Fisheries and Oceans, Canada, continued at CIAC's 1985 symposium in Banyuls-sur-Mer, France, and seem finally to have reached a consensus in Monterey. The Group has requested presentation of a proposal, probably at its 1987 meeting, for a ten-year global program aimed at understanding the interactions of biological and physical factors that produce the incredible variations in the number of young squids recruited to fisheries in different years. The focus would be on physical discontinuities in the oceans that create nursery areas for squid early life stages and the ultimate goal is to understand and possibly influence or predict recruitment to allow more stable exploitation of squid resources as one of the world's few remaining underutilized protein sources. This goal may be unattainable with even a decade

of work, but the approach should at least determine whether adult potential or larval survival is the limiting factor and cannot fail to improve our understanding of the role of squids in the ecosystem. A number of specific projects appropriate to the program have been discussed by CIAC's IREP subcommittee, but the agenda is still open and either general or specific comments on the future course of the program are welcome and may be addressed to me.

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GROWTH INCREMENTS IN STATOLITHS FROM PARALARVAE OF THE OMMASTREPHID SQUID *ILLEX* (CEPHALOPODA: TEUTHOIDEA)

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ABSTRACT

Statoliths of ommastrephid squid paralarvae, mainly *Illex* spp., were examined for growth increments using transmitted light microscopy. Thirteen paralarvae were sampled on the shoreward edge of the Gulf Stream off Florida in mid-January and two from a laboratory rearing experiment. Immediate post-hatch paralarvae from the laboratory, with dorsal mantle length (ML) of 1.0 mm, had one or no increments. Field samples with 1.8 to 2.9 mm ML had 16 to 25 increments. These data are the first to provide age estimates for *Illex* spp. paralarvae. The method provides an easily obtained time base with which to assess factors such as spawning time and locations as well as paralarval growth rates.

Key words: rhynchoteuthions; statoliths; ommastrephid paralarvae; age determinations; *Illex* spp.

INTRODUCTION

The use of statolith growth increments as a means of aging squids has increased in recent years, covering several species and life history stages, both wild-caught and laboratory-reared (see Lipinski, 1986, for references). Some recent studies have validated the hypothesis that increments are deposited on a daily basis (Dawe *et al.*, 1985; Hurley *et al.*, 1985; Lipinski, 1986; Yang *et al.*, 1986). However, there have been few reported observations of increment counts in statoliths from early life stages. Lipinski (1986) studied statoliths of paralarval and juvenile *Alloteuthis subulata* but published no increment counts. Morris & Aldrich (1985) published increment counts of wild-caught juvenile *Illex illecebrosus*, with the smallest having a dorsal mantle length (ML) of approximately 15 mm and an estimated age of 60 days. Hurley & Beck (1979) showed increment counts for two juvenile *I. illecebrosus* of 10 mm ML and an estimated age of 60 days. Dawe *et al.* (1985) observed no increments in a single statolith from a 3-day-old *I. illecebrosus* reared in the laboratory and showed a statolith from a juvenile (11 mm ML). Spratt (1979) observed growth increments in laboratory-reared *Loligo opalescens*, starting at age 28 days. Yang *et al.* (1986) counted increments in statoliths from laboratory-reared *L. opalescens*, but only commencing at an age of 21 days. Kristensen (1980) reported statolith growth increments in *Gonatus fabricii*, with the smallest specimen having 19 increments. Thus, only a few papers have provided direct age data on paralarval forms, and only one of these is on *Illex*. As a result, although many field surveys have collected *Illex* paralarvae (e.g. Rowell *et al.*, 1985; Hatanaka, *et al.*, 1985), there has been no means of assigning ages to the specimens collected. This has meant that no conclusions could be drawn regarding fishery-related concerns such as exact spawning times and locations, as well as paralarval growth rates. The present paper reports growth increments in statoliths from wild-caught rhynchoteuthion paralarvae, mainly *Illex* spp. (1.8–3.3 mm ML), as well as from laboratory-reared *I. illecebrosus* (1.0 mm ML) soon after hatching.

MATERIALS AND METHODS

As proposed by Young & Harman (1987, this volume), we use the term "paralarva" for the developmental stage from hatching to juvenile, with the latter occurring at ML of 8 to 10 mm in *I. illecebrosus* (O'Dor, 1983).

Paralarvae were collected with oblique

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bongo net tows to a depth of 150 m with a 505 μm mesh at Stations 69 and 72 on the inshore edge of the Gulf Stream off Cape Canaveral, Florida, on 16 January 1985 (Rowell & Trites, 1985). Rhynchoteuthion paralarvae were sorted onboard and a small subsample was preserved in 70% ethanol. Because of the small number of paralarvae collected and because the routine survey protocol required most specimens to be preserved in formalin for optimum identification, only 13 specimens were preserved in ethanol for statolith analysis.

Identification of the specimens was carried out during the Cephalopod International Advisory Council workshop in France, 1985. The present state of taxonomy of rhynchoteuthions does not allow wild-caught *Illex* to be assigned to species, especially in locations where the adults of more than one species are known to co-occur. The non-*Illex* specimens were identified as Type B′ rhynchoteuthions, considered currently to be *Ornithoteuthis antillarum* or *Ommastrephes pteropus*. Several juvenile *Illex* were obtained from a collection made S of Newfoundland (40° 37.6′N, 56°00.0′W) on 23 February 1982. Because of their geographical location they can be identified as *I. illecebrosus*.

The laboratory-reared squids were obtained from egg masses spawned by *I. illecebrosus* and incubated in the Aquatron Laboratory (Balch *et al.*, 1985). Because of severe constraints imposed by the incubation of the large egg masses and the poor survival of hatchlings, only statoliths from two immediate post-hatch squids were extracted.

Most statolith preparation and increment counting methods are relatively complicated, involving mounting, grinding and polishing, acid etching and light or scanning electron microscopy (e.g. Dawe *et al.*, 1985; Morris & Aldrich, 1985). However, due to the small size and transparency of the statoliths studied here, increment counts could be made using transmitted light microscopy on untreated statoliths. Extraction of paralarval statoliths was relatively simple, although painstaking due to their small size (e.g. those from laboratory-reared hatchlings were as small as 23 μm in diameter); pre-hatch statoliths could be seen in fresh animals but could not be extracted successfully from preserved specimens. Statoliths were separated from the animal with fine dissecting needles on a microscope slide under a stereomicroscope. A drop of distilled water on each statolith prevented opacity and formation of salt crystals due to desiccation while being photographed. The slide was transferred to a photomicroscope where photographs were taken under a 32× long-working-distance objective, using Tech Pan film. Because of the extreme thinness of the paralarval statoliths, adequate increment definition could normally be obtained by focusing on a single optical plane. In the few cases where this was not possible, two optical planes were photographed so that subsequent counts could be made by combining the most clearly defined areas on the two photographs. Photographic prints (20.3 × 25.4 cm) were produced to a standard final magnification of 1200× on Ilfospeed Multigrade II paper (MG.1M) with a maximum contrast filter.

Increment counts were made from the photographs independently by two authors, both making counts of each statolith at five separate reading sessions. An increment was defined as a light lamella plus the immediately following dark lamella, as proposed by Pannella (1980). Statolith length was defined as the maximum measurable dimension. Mean increment widths for the distal 10 increments were calculated from measurements made along the axis from the center of the primordium to the extreme distal tip of the statolith.

RESULT

Increment counting

Increment counts vs ML are plotted in Fig. 1, with the curve derived from the data. Table 1 gives dimensions of the paralarvae and statoliths, as well as mean increment counts for each statolith obtained from ten replicate counts, five by each reader. A paired t-test showed a significant difference between the counts of the two readers ($p < .05$). The mean difference was 1.4 increments, which provides an indication of reader bias. No significant difference was found between counts of paired statoliths of the six paralarvae from which it was possible to extract statolith pairs (one-way ANOVA, $p > .05$). The largest variations were with the few photographs where increments were indistinct in parts of the statolith, particularly in the area immediately distal to the primordium. The method depends upon maximum care being taken to obtain optimum increment definition during the photographic process.

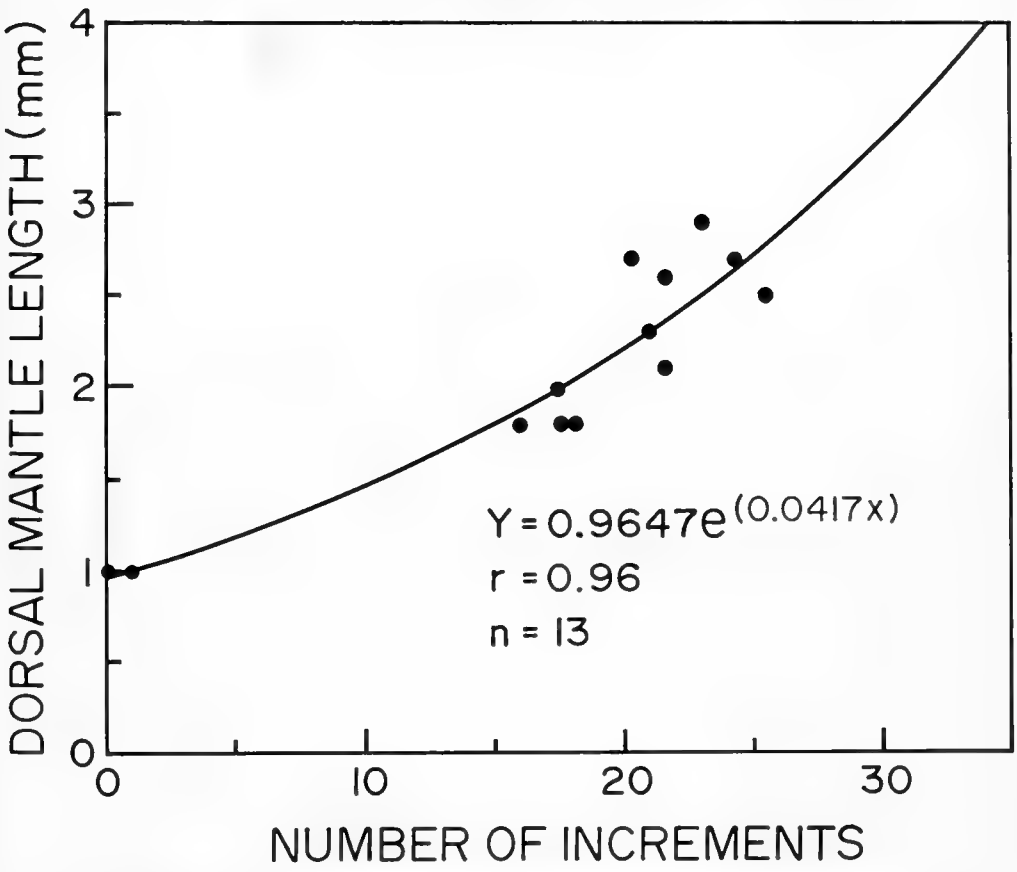

FIG. 1. Relationship of dorsal mantle length to statolith increment counts for *Illex* spp. paralarvae.

To determine whether the method is applicable to counting growth increments on statoliths from larger animals, statoliths were extracted from three juveniles (22–29 mm ML) taken S of Newfoundland. Because of the thickness of these statoliths it was not possible to obtain photographs with sufficient definition to permit reliable counts. Counts made directly on the microscope required multiple optical sectioning and the results were extremely variable, e.g. increment counts in a statolith from a 22.0 mm ML juvenile varied from 55 to 64. Thus there is a maximum size within the range 3 to 22 mm ML at which the increased thickness of the statolith renders the present method insufficiently accurate to be acceptable. Grinding and polishing is required for these larger statoliths (e.g. Dawe *et al.*, 1985; Morris & Aldrich, 1985).

Statoliths from laboratory-reared hatchlings

Statoliths from two immediate post-hatch paralarvae were observed to test the hypothesis of Morris & Aldrich (1985) that there could be as many as 40 increments present at hatching. Due to the large size of the tanks required to incubate the egg masses, it was not possible to observe exact hatching times of individual paralarvae and thus establish the ages of the 1.0 mm ML hatchlings from which statoliths were extracted more precisely than from 0 to 3 days. Fig. 2 shows statoliths from two such hatchlings, with diameters of 23 and 42 μm. The smaller statolith is similar both in shape and size to the primordia that can be seen as the central dark areas in Figs. 3–5. It is also similar in size and shape to the primordia shown in SEM photographs by

TABLE 1. Paralarvae and statolith dimensions, and increment counts from wild-caught rhynchoteuthions. Mean increment width is of the outer 10 increments.

Paralarvae		Statoliths				
No.	ML (mm)	Pair	Mean no. of increments (n = 10)	S.D.	Total length (μm)	Mean increment width (μm)
Illex spp.						
1	1.8		15.9	1.0	109	3.3
2	1.8	A	17.4	1.4	108	3.2
		B	18.6	1.1	108	2.9
3	2.1		21.5	1.4	154	3.1
4	1.8	A	17.2	1.7	123	3.6
		B	17.7	1.4	134	3.3
5	2.7	A	19.5	1.1	181	3.7
		B	20.8	1.3	170	3.6
6	2.6		21.5	1.2	141	3.3
7	2.9		22.9	0.8	158	3.6
8	2.0	A	17.6	1.9	134	3.2
		B	17.2	1.0	138	3.2
9	2.7	A	24.1	1.0	158	3.2
		B	24.2	1.0	174	3.3
10	2.5		25.4	1.9	146	3.6
11	2.3	A	21.1	1.5	142	3.5
		B	20.4	1.5	150	3.1
Type B′						
12	3.1		27.9	3.4	162	2.4
13	3.3		49.4	1.8	147	1.7

Radtke (1983) and Dawe *et al.* (1985). The apparent narrow outermost rings in both statoliths are refractive artifacts, similar to those seen near the outer edge of several of the larger statoliths, e.g. Fig. 3. In addition, the larger of the two hatchling statoliths shows a broad light band around the dark inner core. Since the core is similar in size and structure to the smaller statolith, it can be assumed reasonably that it is the primordium. The first broad increment distal to the primordium is similar in width to the initial increments in the older paralarval statoliths (e.g. Fig. 3), as well as to those in photographs published by Radtke (1983) and Dawe *et al.* (1985). Because both the primordium and the area immediately distal to it were sometimes indistinct in larger statoliths, the primordium in Fig. 1 was used as an overlay to establish the position from which to initiate counts. Although this constitutes a form of extrapolation, it is not an unreasonable approach since the size of the primordium appears to remain relatively constant between specimens.

Statoliths from wild-caught Illex *paralarvae*

Figs. 3–5 show statoliths from *Illex* paralarvae in the size range 1.8 to 2.9 mm ML. Table 1 gives dimensions of paralarvae and statoliths, as well as increment counts and mean widths. The scatter in the plot of these data (Fig. 1) reflects a combination of growth variability, the error inherent in ML measurements on paralarvae (which because of their fragility are normally damaged by net capture) and the error in the increment counting method. Nonetheless, the correlation between increment counts and ML is significant ($y = 0.9647\ e^{(0.0417x)}$; $r = 0.96$; where y = ML, x = increment count).

Evidence that the increments were formed daily can be found in comparisons of the mean increment width data with data published by other workers, particularly where they were validated as daily markers. Because the increments near the primordium are significantly wider than the more distal ones, mean increment values from paralarval

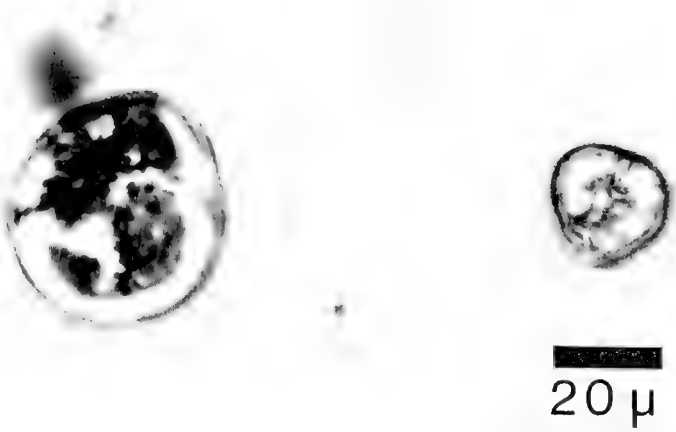

FIG. 2. Statoliths from 2 laboratory-reared *Illex illecebrosus* hatchlings (1.0 mm ML), 0 to 3 days old.

Fig. 3. Statolith A from wild-caught *Illex* sp. paralarva #4 (1.8 mm ML).

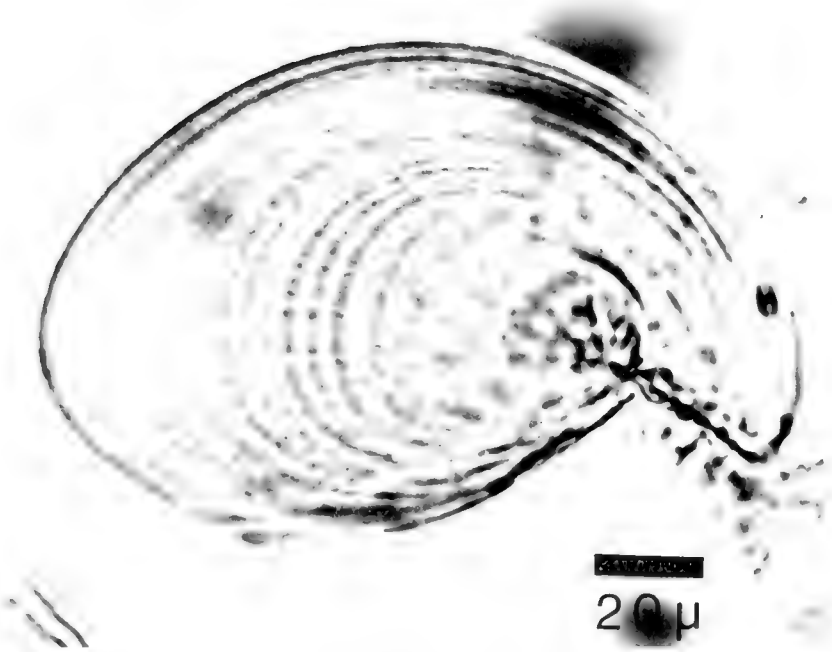

FIG. 4. Statolith from wild-caught *Illex* sp. paralarva #6 (2.6 mm ML).

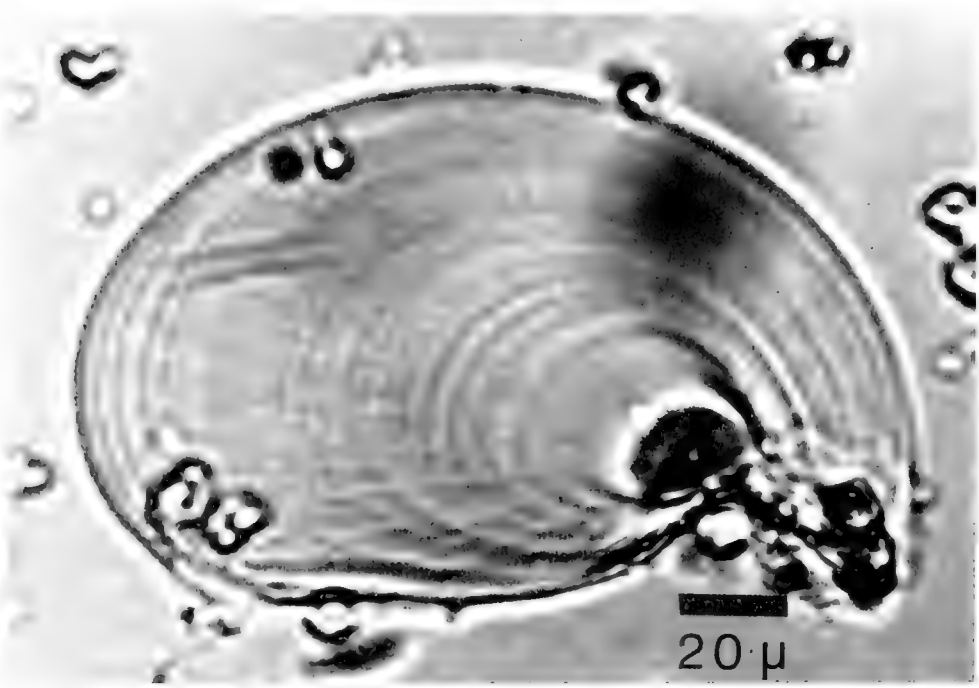

FIG. 5. Statolith from wild-caught *Illex* sp. paralarva #7 (2.9 mm ML).

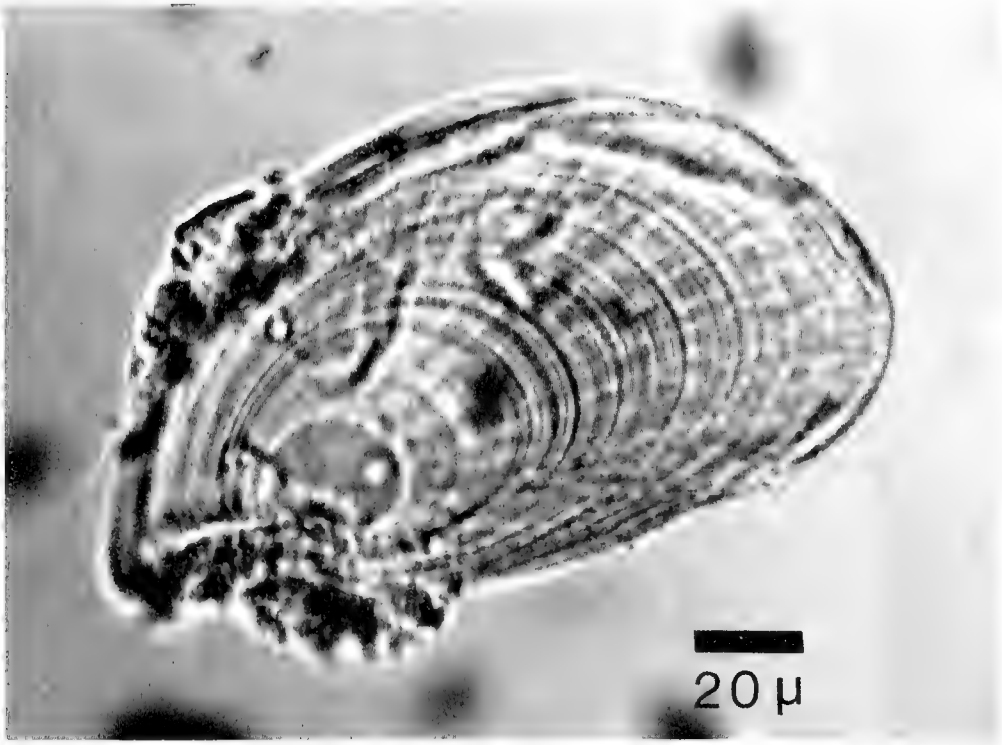

FIG. 6. Statolith from wild-caught Type B′ rhynchoteuthion paralarva #13 (3.3 mm ML).

statoliths with few increments would be significantly biased in comparison with values obtained from older animals. For this reason, values for mean increment widths were calculated for the outer 10 increments. The resulting means ranged from 2.9 to 3.7 μm, with a total sample mean of 3.3 μm. These compare well with the range 1.4 to 3.6 μm for *I. illecebrosus* daily increments validated by strontium marking by Dawe *et al.* (1985). The similarity of increment widths with those validated as being daily provides reasonable evidence that the paralarval increments reported here are daily. This is in agreement with the growing body of evidence that several squid species exhibit daily growth increments, e.g. the observations of Yang *et al.* (1986) with laboratory-reared *Loligo opalescens.*

Statoliths of type B′ rhynchoteuthion paralarvae

Paralarvae #12 and #13 in Table 1 were identified as Type B′ rhynchoteuthions and are therefore not included in the statistical analysis of ML vs increment counts. Since the mean increment widths of statoliths of the two Type B′ specimens (1.7 and 2.4 μm) are less than those of the *Illex* specimens (range 2.9 to 3.7 μm), there would appear to be a difference in this parameter between the statoliths of the two groups. Further comparisons of such species-specific parameters could assist in clarifying the systematics of these poorly known rhynchoteuthions.

DISCUSSION

The statolith at hatching

Based upon indirect evidence, several workers have proposed that only the primordium is present at hatching: Kristensen (1980) with *Gonatus fabricii,* Rosenberg *et al.* (1981) with *Todarodes sagittatus* and Dawe *et al.* (1985) with *Illex illecebrosus.* However, Morris & Aldrich (1985) proposed that in *I. illecebrosus*

the central 40 increments in the region designated as R1 are formed prior to hatching. Our preliminary evidence from the laboratory-reared hatchlings in this study shows that this is not the case, but that the statolith at hatching is the primordium.

The wide increments laid down immediately distal to the primordium have been observed commonly, although it is not certain whether they are broad daily increments or undifferentiated regions possibly laid down prior to entrainment of a daily deposition cycle. More detailed analyses of a time-series of laboratory-reared paralarvae immediately after hatching could answer this question, although this will depend upon improvements in culture methodology, since there has been limited success to date with rearing rhynchoteuthions (Balch *et al.*, 1985).

Early life history growth patterns

Growth data for *Illex* obtained by previous workers have been based mainly upon adults, with only a few observations made on juveniles down to 10 mm ML. Therefore, extrapolations have had to be made from these data to the paralarval period, and thus to hatching. These have been generally unsatisfactory; e.g. the data of Morris & Aldrich (1985) predict 61 increments for a 1 mm ML, the size at hatching. A more successful extrapolation was made by Hurley & Beck (1979), even though their regression for combined male and female data predicted 14 increments at 1 mm ML. The fit of the present paralarval data to their curve was tested using a "lack of fit test," and no significant departure was observed ($p < .05$), indicating that their curve does provide a reasonable growth curve for the early life history of *Illex.*

The question of a transition from paralarval to juvenile growth and behavioral patterns, and of a related change in statolith growth patterns, has been raised by Kristensen (1980) and Morris & Aldrich (1985). Although field data indicate that splitting of the *Illex* proboscis is completed in the range of 8 to 10 mm ML (O'Dor, 1983), it has not been possible to establish the age range associated with this process. The only data bearing on the matter can be found in the few increment counts made in juvenile statoliths. The smallest juvenile (14 mm ML) reported by Morris & Aldrich (1985) had an estimated age of 58 to 60 days. Hurley & Beck (1979) estimated the ages of two 10 mm ML juveniles as 60 days. In addition, the rough counts we have made of juvenile statolith increments indicate ages of approximately 55 to 67 days for a ML range of 22 to 29 mm. The transition from paralarval to juvenile forms must therefore occur prior to an age of approximately 60 days. In addition, having shown that the statolith transition between zones R1 and R2 (at 31 to 51 increments) cannot be related to hatching, as stated by Morris & Aldrich (1985), it would be reasonable to associate it with some other major developmental and/or behavioral shift, the most obvious one being the shift from paralarva to juvenile. Changes related to this shift could be the final splitting of the proboscis, a change from a paralarval feeding mode, such as the one proposed by O'Dor *et al.* (1985), to raptorial feeding by juveniles, and a change from planktonic to nektonic swimming. One might expect that these latter two behavioral shifts would be associated intimately with statocyst function, and thus be reflected in statolith form.

Spawning period

One aim of this research was to understand the timing and location of spawning by *Illex.* To what extent do the paralarval aging data provide insights into this aspect of *Illex* biology? The ages of the *Illex* paralarvae reported here would indicate a narrow hatching period between December 22 and 31. However, the statoliths were obtained from only a narrow size range (1.8 to 2.9 mm ML), whereas a broader total range (0.8 to 6.0 mm ML) was sampled during the cruise (Rowell & Trites, 1985). Furthermore, juveniles sampled on the same transect were in the size range 12 to 28 mm ML, which, from the discussion in the previous section, could be at least 60 days old. In addition, the regression of Hurley & Beck (1979) predicts an age of 64 days for a 28 mm ML. Thus the overall hatching period could have spanned at least 45 days, from mid-November to late December. Using estimates for development time published by O'Dor *et al.* (1982), spawning would have occurred from 6 to 16 days earlier, near the beginning of November. It must be noted that these approximate dates are based only upon the squids captured; they are not proposed to delimit the entire spawning period.

Since identification can only be to genus, and since the study area is very dynamic oceanographically, with the potential for intermixing originally discrete spawning stocks,

the observed age variations could be explained either by the presence of several species of *Illex*, or by a protracted spawning period, or a combination of both. It is clear that the former is highly probable, considering the evidence that at least three *Illex* spp. co-occur in the area (Voss & Brakoniecki, 1985); and there is a growing body of evidence that spawning is protracted, perhaps even over the entire year (Hatanaka *et al.*, 1985; Rowell *et al.*, 1985; Coelho, 1986). The results of the present study cannot resolve this uncertainty. But they do indicate the value of a relatively simple technique for establishing a time base for use in future early-life-history studies, not only of ommastrephids but of other cephalopods as well.

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THE ENERGETIC LIMITS ON SQUID DISTRIBUTIONS

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ABSTRACT

Limited data are now available on the cost of locomotion for squid, suggesting that they swim with optimal efficiency at about 2 mantle lengths per second. The cost (C) is high, 8.6 kJ $kg^{-1}km^{-1}$ for a 0.23 m ML squid (equivalent to 9% body weight per day food consumption) but decreases with size, $C(kJ\ kg^{-1}km^{-1}) = 3.43\ ML(m)^{-0.67}$. Comparing estimates of migration speeds and ranges for squids of different sizes from these observations to the results of tag/recapture studies indicates that squids do not swim continuously and cannot migrate on energy reserves. The assumption that they swim half of the time at optimal speeds during the period of maturation fits the available data and suggests that many of the larger oegopsids can range over and perhaps between the oceans. A trans-Pacific migration of 6500 km is shown to be plausible for *Ommastrephes bartrami*, and *Architeuthis* spp. could potentially travel from the Arctic to the Antarctic on a seasonal basis. There are no energetic barriers to prevent global distributions of the larger squids.

Keywords: migration; squids; bioenergetics; distribution; speciation; optimum speeds; cost of transport.

INTRODUCTION

The degree of speciation within cephalopod genera is highly variable, ranging from *Octopus* and *Sepia*, which each include more than a hundred species, to genera such as *Onychoteuthis*, in which one species nearly covers the globe. This may result from taxonomic conventions or real differences in the biological or physical constraints on the rate at which speciation occurs. This report examines the energetic constraints on squid migration distances as a step toward distinguishing between these two possibilities and presents a model for using maximum adult size to predict the ranges and life cycles of poorly known species.

The origin of speciation is presumably reproductive isolation that can reflect genetic, anatomical, physiological or behavioral incompatibilities. However, such differences usually only become fixed after long periods of temporal or spatial separation of populations or stocks, and exchanges between populations of as little as 1% are enough to avoid genetic differentiation (Harden Jones, 1980). Benthic species with planktonic stages such as *Octopus vulgaris*, which has a nearly global distribution, apparently depend upon currents to distribute juveniles that then provide sufficient exchange between populations to prevent speciation. Juveniles of nektonic forms also use currents. Harden Jones (1980) suggests that such populations have a "Grand Strategy of ensuring a sufficient number of viable offspring to maintain the population up to the limit that can be fed." Their swimming abilities allow juveniles of such species to follow the movements of the rich food supplies provided by upwelling and seasonal blooms in big current systems. As adults, they must migrate back to locations that provide their offspring with the same advantages they had. The problem in maintaining such mobile and widely dispersed species is probably ensuring sufficient concentrations for reproduction; there are almost certainly enough stragglers to provide exchange.

Recent quantitative studies of squid locomotion (O'Dor, 1982; Freadman, Hernandez & Scharold, 1984; Webber & O'Dor, 1985 and 1986) provide the basis for predicting the speed and energetic costs of such migrations. The number of species studied to date is small, but a consistent pattern seems to be emerging that allows generalizations. The life cycle and range of a particular species, of course, involves a complex interaction of biological and oceanographic factors, but some limits can be set and tested by comparing predictions to real records of better known species.

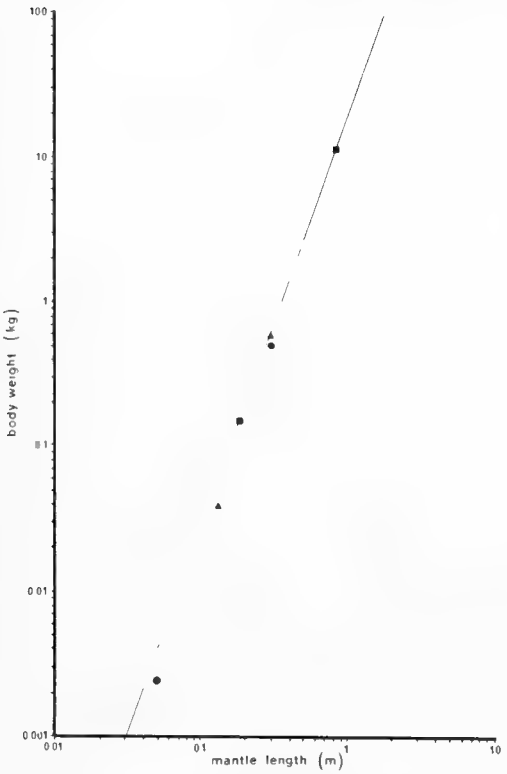

FIG. 1. A general relationship between dorsal mantle length (ML) and total body weight (W) for squids. Symbols indicate the range of squid sizes used in determining regression for individual species (given in text). Circles are for *Illex illecebrosus;* triangles, *Todarodes pacificus* and squares *Dosidicus gigas.* The solid line is the overall relationship, W (kg) = 20 ML $(m)^3$.

MATERIALS AND METHODS

Cost of transport

Swim-tunnel respirometry has been used extensively to study locomotion in fish (Brett & Groves, 1980) and the use of the same equipment to study squids has been described in detail elsewhere (O'Dor, 1982; Webber & O'Dor, 1985). Briefly, squids were placed in a screened chamber inside a hollow, seawater-filled annulus and exposed to increasing currents at speeds up to 1 ms^{-1} produced by a recirculating pump. Water speed was increased in stages, and the oxygen required by the squid to maintain position at each speed was measured using an oxygen electrode. Oxygen consumption was converted into energy equivalents using values for fish metabolism (Brett & Groves, 1980; 1 L O_2 = 4.6 kcal = 19.3 kJ). The gross cost of transport at each speed was calculated by dividing the weight specific energy consumption in kJ $kg^{-1}h^{-1}$ by the speed in km h^{-1}. Energy expenditures determined in the swim-tunnel in micro-turbulent water have been shown to be comparable to those of free-swimming squids in static water (Webber & O'Dor, 1986). Good estimates of the optimum cruising speed, which requires the least energy to move a unit of squid mass over unit distance, are available from regression analysis for *Loligo opalescens* (O'Dor, 1982) and *Illex illecebrosus* (Webber & O'Dor, 1985), and there is an approximate value for *Loligo pealei* (Freadman *et al.,* 1984 and personal communication).

Actual individual ranges

Only tag/recapture studies provide reliable data on how far individuals travel, and such studies are rare for squids. In a few cases it is also possible to be reasonably confident of distances traveled where distributions and spawning grounds are known.

RESULTS

Basic assumptions

For this approach to succeed, some generalizations about squids must be established. Fig. 1 compares the overlapping mantle length (ML)-weight (W) relations for three "typical" squids for weights from 1 g to 10 kg: *Illex illecebrosus,* W(g) = 0.0481 ML $(cm)^{2.72}$ (Lange & Johnson, 1979; O'Dor, 1983); *Todarodes pacificus,* W(g) = 0.0091 ML $(cm)^{3.2472}$ (Murata, 1978); *Dosidicus gigas,* W(g) = 0.02646165 ML $(cm)^{2.989379}$ (Ehrhardt *et al.,* 1983). Despite the authors' sometimes overzealous use of decimal places, Fig. 1 shows that:

$$W(kg) = 20\ ML(m)^3 \qquad (1)$$

is an adequate representation of squids, in general, for present purposes.

Clarke *et al.* (1985) recommend the use of 20 kJ g^{-1} dry weight as a universal conversion factor for the energy (caloric) value of squids. The assumption that dry weight is 20% of wet weight is less universal, but 4 kJ

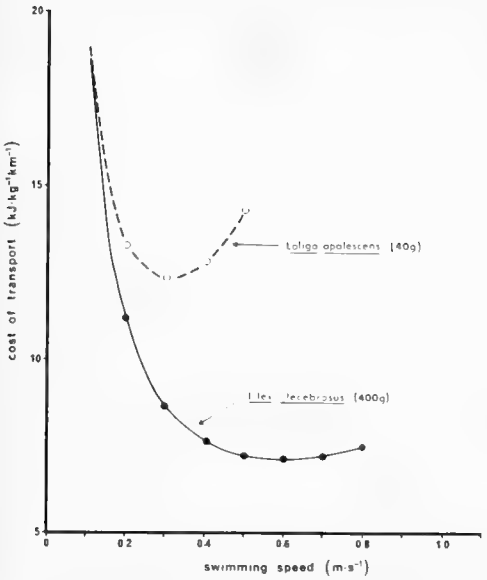

FIG. 2. Changes in the gross cost of transport with swimming speed for the squids *Loligo opalescens* (0.13 m ML; O'Dor, 1982) and *Illex illecebrosus* (0.27 m ML; Webber & O'Dor, 1985). Minimum costs occur at about 2 ML s^{-1} which should be the most efficient speed for migration.

g^{-1} wet weight will serve for most of the non-ammoniacal squids to be discussed here. There are no good data on the proportion of their body energy that squids can mobilize, but octopuses can tolerate the loss of up to 50% of their body *weight* during starvation (O'Dor & Wells, 1978). The assumption that squids can do likewise is probably an exaggeration, but they may tolerate the loss of 50% of their body *energy,* since many of them do have larger lipid reserves than octopuses (O'Dor & Wells, 1987). In any case, as will be shown below, it is essential to overestimate rather than underestimate on this point. From these values, the available energy for migration (E_a) can be estimated:

$$E_a = 2000 \text{ kJ kg}^{-1}. \quad (2)$$

Fig. 2 shows the gross cost of transport (C) for *Loligo opalescens* of 0.04 kg and 0.13 m ML (O'Dor, 1982) and *Illex illecebrosus* of 0.4 kg and 0.27 m ML (Webber & O'Dor, 1985) plotted against swimming speed. These curves show optima (minimum transportation costs) because of the high resting metabolism of squids and the exponential increase in the cost of swimming with speed. In each case, the optimum speed (S_o) is about 1.3 body lengths s^{-1}, and, since ML is approximately 65% of body length (excluding tentacles):

$$S_o = 2 \text{ ML (m s}^{-1}). \quad (3)$$

Although the same detail is not available, metabolic costs at various speeds are known for *Loligo pealei* of 0.1 kg and 0.16 m ML, and the situation is very similar (Freadman *et al.,* 1984 and personal communication). Calculated minimum C's for the three species are plotted against ML in Fig. 3. The regression of this relationship is:

$$C(\text{kJ kg}^{-1}\text{km}^{-1}) = 3.43 \text{ ML(m)}^{-0.67}. \quad (4)$$

Prudence would normally prevent extrapolation from such a limited data set to three orders of magnitude, as shown in Fig. 3, but similar extrapolations over six orders of magnitude proved reasonably accurate for undulatory swimmers from large salmon to bull spermatozoa (Schmidt-Neilsen, 1972), so there is a precedent in this field. There is also indirect evidence that the cost of transport scales similarly for newly hatched *I. illecebrosus* and *Loligo opalescens* (O'Dor *et al.,* 1986; O'Dor & Webber, 1986).

Extrapolations

The distances squids can travel, either actively or in currents, determine the ranges over which individuals can remain part of a breeding population. The equations above allow reasonable projections of active ranges knowing only the squid's ML. Additional assumptions can be tested for better-known species.

The simplest assumption to test is that squids migrate on their stored reserves as fish commonly do. Dividing Equation 2 by Equation 4 gives the reserve range, R_r(km), as:

$$R_r = 583 \text{ ML(m)}^{0.67} \quad (5)$$

which is plotted in Fig. 4. A typical *Todarodes pacificus* of 0.23 m ML would have a range of 215 km. Since dozens of tagged *T. pacificus* have been recaptured at greater distances (see below), often after only a few weeks and traveling in directions for which there are no currents to assist, this assumption cannot apply.

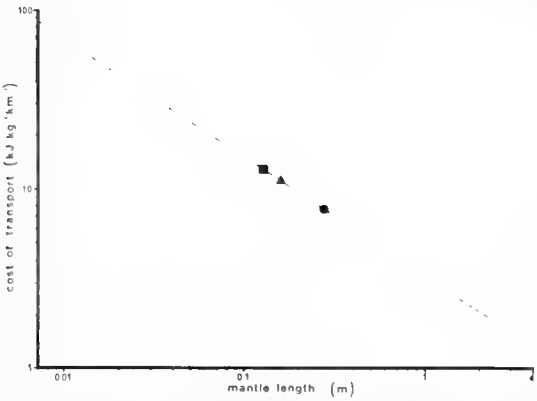

FIG. 3. The proposed relationship between dorsal mantle length and the cost of transport for squids based on three species: circle, *Illex illecebrosus;* triangle, *Loligo pealei;* square, *Loligo opalescens.* The equation for the line is $C(kJ\ kg^{-1}\ km^{-1}) = 3.43\ ML\ (m)^{-0.67}$.

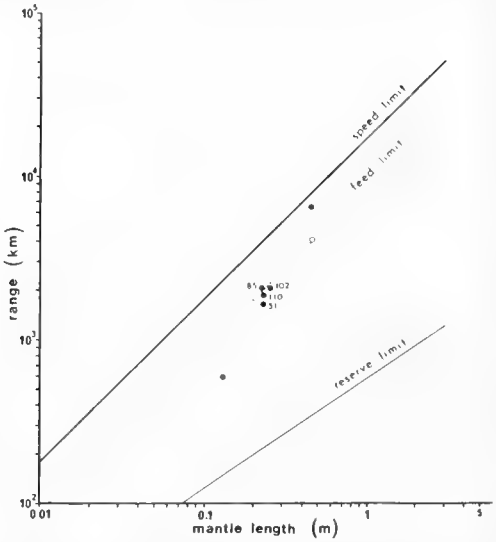

FIG. 4. Some possible limits on migration ranges for squids of various sizes. See text for details. Actual migration distances for tagged squid, with travel times in days, are plotted at 0.23 m (*Todarodes pacificus*) and 0.25 m (*Illex illecebrosus*). Estimated migration distances are plotted at 0.13 m and 0.45 m for *Loligo opalescens* and *Ommastrephes bartrami,* respectively. The open symbol is correct for current.

The most extreme assumption that seems remotely plausible would be that migrating squids swim continuously at their optimum speeds. A 0.23 m *T. pacificus* travels at 0.46 m s^{-1} or 40 km d^{-1}, weighs 312 g and requires 8.6 kJ $kg^{-1}km^{-1}$. This is the energy equivalent of 9% of its body weight per day, which would require a rather high feeding rate, but it might not be impossible for a cannibalistic squid traveling with a school of potential prey. The fastest long-distance travel rate for *T. pacificus* known to the author is 0.37 m s^{-1} (Murata *et al.*, 1971). Shevtsov (1973) recorded speeds up to 0.60 m s^{-1} for shorter trips, and Sasaki (1929) reported a 2 h trip at 1.8 m s^{-1}. The latter was probably current-assisted since, under experimental conditions, similar squids can only maintain such speeds for a few seconds. The longer trips were all against the prevailing surface currents but could potentially have involved some use of slower, less-well-described deep currents. There is also a record for a similar-sized *I. illecebrosus* traveling at 0.37 m s^{-1} in a place and direction where currents could make only a minor contribution (Hurley & Dawe, 1980). These records represent only one squid in a billion, and these squids are probably handicapped by drag caused by the tags they carry. Considering that squids also are unlikely to travel in straight lines, these results do not rule out the extreme assumption and are, in fact, tantalizingly close to predictions. However, they do not prove them either, and it seems more reasonable to assume that the squids stop for lunch.

Intermediate assumptions are more difficult to test. Fig. 4 contains two additional lines; one showing the range for 100 d of continuous travel and the second for 100 d allowing a 12 h lunch break each day. One hundred days is approximately the period of the growth plateau seen in most squid species, which presumably begins with the onset of sexual maturation (see Boyle, 1983). The same cue that triggers maturation may initiate the spawning migration (O'Dor *et al.*, 1977). These assumptions not only simplify the calculations, they also fit almost perfectly with the best long-distance records for tagged squids (Dawe *et al.*, 1981) that are plotted with travel times in Fig. 4. Unfortunately, both *T. pacificus* and *I. illecebrosus* are of similar size, and, while these points suggest that allowing 50% travel time gives about the right range, they provide no information about slope of the line and the predictions of size-dependent ranges. Data for larger and smaller squids are needed.

The largest squid for which there are reasonable data of a migratory pattern is *Ommastrephes bartrami* (Osako & Murata, 1983). There are some tag returns for these squids, but none over long distances. As yet, there are no published values for average speeds, but there are some data consistent with the sort of life cycle this study proposes. These squids are thought to spawn south of Japan west of 150°E. The juveniles are carried N and E by the Kuroshio and North Pacific Currents, and many specimens up to 0.45 m ML have been taken in the area around 40°N, 135°W. To return to the spawning grounds and contribute to restarting the cycle, these squids would have to travel about 6500 km. As Fig. 4 shows, this puts them close to the upper speed limit for continuous travel at an S_o of 0.9 m s^{-1} or 78 km d^{-1}. However, evidence suggests that maturing squids move S of the Subtropical Convergence where the North Equatorial Current is moving east at 20–25 km d^{-1} (Pickard & Emory, 1982). Over 100 d this would reduce the net travel distance to about 4000 km, very close to the 50% travel time prediction.

The smallest squids for which there is some evidence of spawning migrations is *Loligo opalescens* at 0.13 m ML, which is known to congregate in several areas for mass spawnings (Hixon, 1983). The species is found from Baja California to southern Alaska over a total range of about 3500 km, but Kashiwada & Recksiek (1978) suggest that there are three morphologically distinct stocks in Baja, North and Central California and Puget Sound. If the total range is divided into three equal segments and squids from each segment travel to a central spawning ground, this suggests an average range of about 600 km. This clearly very speculative figure is only half the 50% travel time prediction. *L. opalescens* certainly can swim at the required speed since it was one of the tested species, but there are many reasons why it might fall off the line. The current regime affecting this species is very complex (Coelho, 1985) and adults may actually have to swim against currents. Alternatively, it could be that these relatively smaller squids spend less of their life traveling or more of their time feeding. Karpov & Cailliet (1978) report that cannibalism is rare in *L. opalescens,* unlike most other species discussed, and without this buffer against starvation feeding may be more critical.

DISCUSSION

It is clear that many squids in the size range of 0.1 to 0.5 m ML make extensive spawning migrations, and that they do not make them on energy reserves alone. None of the evidence suggests that squids spend much more than 100 d making such migrations, and none requires that they swim at more than their optimum speed for any length of time. Most records suggest that squids could spend up to half of their time resting or feeding. They could, alternatively, swim slower, but there is no economic advantage in this. The evidence from natural migrations does little to strengthen the calculated scaling of optimum speeds with squid size from studies on captive squids, but it is not in conflict either. Traveling 2000 km is not a particularly remarkable feat for an *I. illecebrosus* of 0.25 m ML, and traveling 8000 km (the total known distribution of the species; Nesis, 1983) would be no more remarkable for a *Dosidicus gigas* of 1.0 m ML.

The discussion of migratory range in relation to distribution areas is limited to oegopsids, which are known to be capable of mid-water spawning (O'Dor & Balch, 1985) and are not restricted to continental margins like the demersally spawning myopsids. It should, then, be relatively conservative to suggest that there is no reason to expect reproductive isolation to develop within a circle with a diameter equal to the estimated maximum range of a single individual, since:

(1) A mating pair could potentially have started out twice this far apart.

(2) Larger squids may live longer and spend longer traveling.

(3) Currents could add to the range, as seen for *O. bartrami.* (Admittedly, they could also reduce it.)

(4) It is not even necessary for interbreeding to occur in each generation.

Clarke's (1966) summary of the available data on oegopsid maximum adult sizes is shown in Fig. 5 with the estimated maximum ranges. Clarke points out that these maxima are generally underestimates, since big squids are much harder to catch than small ones. Thus, conservatively, 20% of all oegopsid species should be able to cross the Atlantic (5000 km) and 5%, the Pacific (10,000 km).

If the scaling remains the same, a 3.0 m ML *Architeuthis* could go around the world in 80 days (under the North Pole?). Why it would

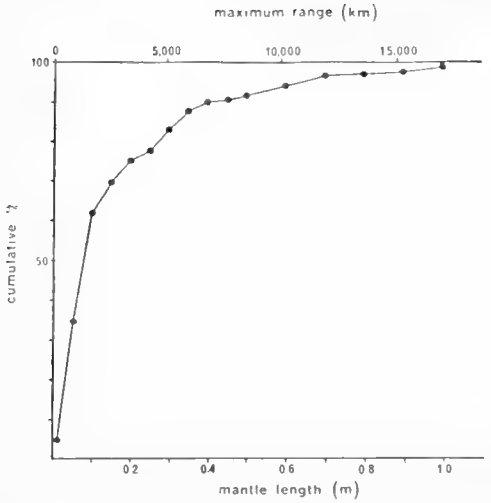

FIG. 5. Cumulative percentage curve of oegopsid species with known maximum mantle lengths not exceeding the indicated values (modified from Clarke, 1966) with estimated maximum ranges for comparison.

want to is unclear, but traveling from the northern bloom to the southern bloom annually is energetically feasible for some whales and could take *Architeuthis* less than a month. Such a pattern is consistent with its distribution (Roper & Boss, 1982). It is now popular to say that *Architeuthis* is not a strong swimmer, but the evidence is only that it is ammoniacal and not as muscular, relatively, as smaller squids. This may not be critical, as the speeds under discussion here are cruising speeds, not escape jets. At cruising speeds, squids use only 10% of the power (and presumably 10% of the muscle) available for an escape jet (Webber & O'Dor, 1986). Perhaps *Architeuthis* only needs cruising muscle, since there cannot be too many things it needs to escape from.

The intention of this report, however, is not to synonymize species of *Architeuthis* on the basis of its swimming ability or to suggest that there cannot be three species of *Ommastrephes* in the Atlantic. Land masses, historically, and even current systems presently in existence could have produced reproductive isolation and separate species. On the other hand, there is reasonable evidence that there are no physical restrictions on the larger oegopsids that would prevent a single species from maintaining gene flow on a global scale. There is certainly no reason to assume, *a priori,* that specimens from widely separated areas are, or should even be likely to be, separate species. Resolution of such questions would be aided greatly by more tag/recapture studies where feasible, but more information on swimming energetics over a wider size range and for other types of squids would also be valuable.

ACKNOWLEDGEMENTS

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THE USE OF A SAND-COAT IN RELATION TO FEEDING AND DIEL ACTIVITY IN THE SEPIOLID SQUID *EUPRYMNA SCOLOPES*

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ABSTRACT

The sepiolid *Euprymna scolopes* Berry, 1913, buries in the bottom during the day. When this animal emerges from the bottom it often has a covering of sand (a sand-coat) on the dorsal surface of its body. Camouflage during the day has been suggested as the function for the sand-coat but this has not been investigated.

In the laboratory, animals did not use a sand-coat at night. They only used a sand-coat when forced to emerge from the bottom to feed during daylight. Squids carried a sand-coat more frequently when they attacked a slow-moving prey than when they attacked a fast-moving prey. When carrying a sand-coat squids took longer, and had to make a greater number of attempts, to capture prey.

Laboratory animals were active mainly at night, under two different light and two different feeding regimes. Thirty-six of 44 squids collected at night in the field had food in their stomachs, and digestion rates determined in the laboratory indicated that most of the 36 field animals had begun feeding after sunset. Errant polychaetes represented over 90% of the food items in the 36 stomachs, suggesting further that feeding had occurred at night.

Key words: sepiolid; squid; camouflage; feeding; behavior; activity.

INTRODUCTION

The family Sepiolidae (Cephalopoda: Sepioidea) is primarily benthic-neritic in habitat (Berry, 1914; Naef, 1923; Voss & Williamson, 1972; Boletzky, 1983) and its members are noted especially for burying in the bottom (Boletzky & Boletzky, 1970; Boletzky *et al.*, 1971). *Euprymna scolopes* Berry, 1913, has an epithelial secretory system that produces a mucous coat and gives it the unique ability to adhere a continuous coating of bottom materials to the dorsal surface of its body (i.e. carry a "sand coat") when emerged from the sand (Singley, 1982). The squid (*E. scolopes* is hereafter referred to as a "squid" although it is not a true teuthoid squid) also has the ability to instantaneously drop this sand-coat as a unit. Singley (1982) and Moynihan (1982) have suggested that in *E. scolopes* the sand-coat is used as daytime camouflage when emerged. However, studies have yet to determine if the sand-coat is used only during the day or whether it serves to camouflage the animals against either their predators or prey. This is the first study to examine the use of the sand-coat. To investigate the camouflage hypothesis, the relationship between the diel activity pattern of *E. scolopes* and the use of the sand-coat had to be established first.

Field studies of activity patterns in cephalopods are difficult to conduct because of their mastery of cryptic coloration, and because many travel long distances in a day. Activity studies conducted in the laboratory can avoid some of the problems inherent in field studies and have the advantage of being mechanized easily. While laboratory studies may introduce some artifacts in behavior, insight into this possibility can be obtained through limited field work.

Activity was monitored in the laboratory to determine if *Euprymna scolopes* has a pattern of diel activity. Stomach contents of squids collected in the field were examined also, to provide insight into the timing of feeding activity (and therefore presumably general activity) in its natural habitat. In the laboratory, the occurrence of the use of the sand-coat behavior was compared between day-active and night-active squids. To examine the function of the sand-gluing behavior, squids were forced to be active during day-

light by limiting their access to food. The squids' response to two different prey species was then investigated.

MATERIALS, METHODS AND RESULTS

Collection and maintenance of animals

Euprymna scolopes were collected at night on the reef flats of Kaneohe Bay, at the island of Oahu, Hawaii, between September 1983 and December 1984. Animals were sighted using a gas lantern for illumination, and captured with dip nets as they swam near the surface. Animals were transferred immediately to plastic bags filled with fresh sea water and transported to the laboratory within 2 hr. Each animal was held separately in a 50 liter compartment until the experiment. Water temperature was maintained between 19° and 21°C. The floor of each tank was covered with a 4.0-cm-thick layer of coarse coral sand (average particle diameter ~ 3 mm) and was equipped with a subsand filter. The squids fed *ad libitum* while in the holding tanks.

In the laboratory, an artificial light regime of 12 h light : 12 hr dark was used. The daytime light source was a set of three 100 watt incandescent bulbs (with reflectors) that produced a maximum irradiance about one twentieth the measured irradiance of the sun overhead on Oahu at midday (i.e. the irradiance of light at a depth of 3 m in clear tropical waters at midday; McFarland & Munz, 1975). Light levels were measured using an uncalibrated United Detector Technologies ® Model 11A Photometer with a Model 221 Sensor Head and cosine collector. The light phase was controlled by an automatic timer that turned the lights on or off once every 12 hours. During the 12 hour dark period (artificial night) a 75 watt incandescent light suspended above the water surface served as artificial moonlight. A variable transformer allowed adjustment of the intensity of the overhead light to an irradiance, at the water surface, 10^4 lower than that used during the daylight hours (the measured irradiance of a full moon in clear skies over Oahu at night).

A 200 liter aquarium placed inside an enclosure, located inside a light-tight room, served as the observation tank in this study. The aquarium was divided into two compartments: one for the squid whose activity was to be monitored (50 cm wide by 35 cm long; the water level was at a depth of 35 cm), and an adjacent compartment used to hold live shrimps for food. The holding compartment allowed shrimps to be placed in the observation tank without disturbing the squid during an experiment. Perforations in the divider between the food compartment and the squid's compartment allowed the shrimp to move freely throughout the tank. Experiments were run on one squid at a time.

A TV camera, connected to a TV monitor, and a video-cassette recorder (VCR) located outside the light-tight room, were used to observe the squid's activity. A red light (Wratten ® 89B filter; bandpass at wavelengths >700 nm) with a diffuser was placed behind the compartment in which the squid was held. During the artificial night the red light silhouetted the squid for the camera. Cephalopods appear to have a low visual sensitivity to red light (Hubbard & St. George, 1958; Hamasaki, 1968; Boston, 1976; Muntz & Johnson, 1978) and *Sepiola,* a close relative of *Euprymna,* has a peak spectral sensitivity of 492 nm (Brown & Brown, 1958). Thus the camera could detect movement while the behavior of the squid appeared unaffected. A digital clock placed in front of the aquarium was used to determine the time of day the squid was active. The activity and timing of any locomotor event could thereby be recorded for detailed analysis at a later time. This system also allowed the animals to be monitored visually without disturbing them during experiments.

Activity patterns were determined by recording the number of activity events that occurred during each hour of an experiment. Based upon preliminary results, an activity event was scored arbitrarily each time the squid swam off the bottom of the tank for 30 sec or less. If the squid swam off the bottom for longer durations, its activity was scored as events that were the nearest whole multiple of 30 sec. Videotapes were scanned quickly until activity was detected. Each event was then reviewed carefully to determine its duration and the time of day the event occurred. Squids were placed in the observation tank at least one day prior to the beginning of an experiment.

Activity of animals under a natural phase light cycle

Experiments: In this set of experiments the artificial light cycle followed the phase of

TABLE 1. The activity of four squids under a natural phase light cycle and *ad libitum* feeding. D_{max} the maximum difference (using the K-S test) between observed activity patterns of the squid and a theoretical even diel activity pattern. p = significance level of D_{max}

Expt. no. (duration in hrs)	Squid no.	Total diel activity events	Total diurnal activity events	Total nocturnal activity events	Percent of diel activity that was nocturnal	D_{max} for diel vs even diel activity	p for diel vs even diel activity
1 (24)	1	70	8	62	88.6	0.458	<0.01
2 (24)	2	35	8	27	77.1	0.376	<0.05
3 (48)	1	470	0	470	100.0	0.500	<0.01
4 (24)	3	8	0	8	100.0	0.500	<0.01
5 (24)	3	40	1	39	97.5	0.475	<0.01
6 (48)	3	147	24	123	83.7	0.411	<0.01
7 (48)	4	67	0	67	100.0	0.500	<0.01
8 (24)	4	22	0	22	100.0	0.500	<0.01
All (264)	—	1042	41	1001	96.1	0.461	<0.01

the naturally occurring cycle. Recording periods were either 24 or 48 hr long, due to limitations on the availability of the VCR equipment. Two hundred and sixty-four hours of observations were made on four squids during eight experiments. All animals were sexually mature and weighed from 2.70 to 7.03 g wet wt (17.0 to 23.0 mm dorsal mantle length: ML). Squids had been in captivity 9 to 57 days before an experiment. Squids fed *ad libitum* on *Palaemon pacificus*, *Palaemon debilis* and *Halocaridina rubra* during the experiments.

To examine if the pattern of activity was truly nocturnal, the squid's diel activity was tested against the null hypothesis that the squid showed equal amounts of activity during each hour of both the day and night (an even diel activity distribution). Comparisons were made using the Kolmogorov-Smirnov test (K-S test) with the extrinsic null hypothesis that no difference existed between the squid's diel activity pattern and the hypothetical even diel activity pattern. Enright's (1965) periodogram analysis was used to calculate the periodicity of the squid's activity (data sets from each of these experiments were too small to be analyzed separately using fourier analysis). As a check on the periodogram analysis, the periodicity of the squids' activity was determined using fourier analysis to calculate a raw power spectrum for the combined data from the eight experiments (Jenkins & Watt, 1968; Bloomfield, 1976). A computer program was used to calculate the discrete fourier transform of the data from the activity records after the data had been detrended linearly.

Results: In all cases animals showed a clear difference in locomotor activity between day and night. Only occasionally did squids emerge from the sand during the day (and only when they tried to catch a shrimp). After sunset, they emerged very slowly from the bottom and always had a sand-coat. The squids would then shed the sand-coat before moving away from the bottom. They did not rebury until sunrise. Most feeding occurred in the dark. Approximately 96% of the total diel activity was nocturnal (range = 77.1 to 100.0% for experiments 1 to 8; Table 1), with especially elevated activity just after sunset and just before sunrise. The distribution of activity in each experiment was significantly different from the even diel activity distribution (K-S test, p's ranged from <0.05 to <0.01). The periodogram analysis showed the pattern of activity in each experiment to follow a 24 hr periodicity. Fourier analysis of the combined data also confirmed that the dominant periodicity was 24 hr.

Activity when the light cycle was shifted 5.5 hours

Experiments: Squids were run with the phase of the light cycle shifted 5.5 hr to determine if they were entrained to the light cycle rather than some coincident entraining cue (such as the tidal cycle prior to being collected, or to the regular daily activity in the building). In experiment 9, the locomotor ac-

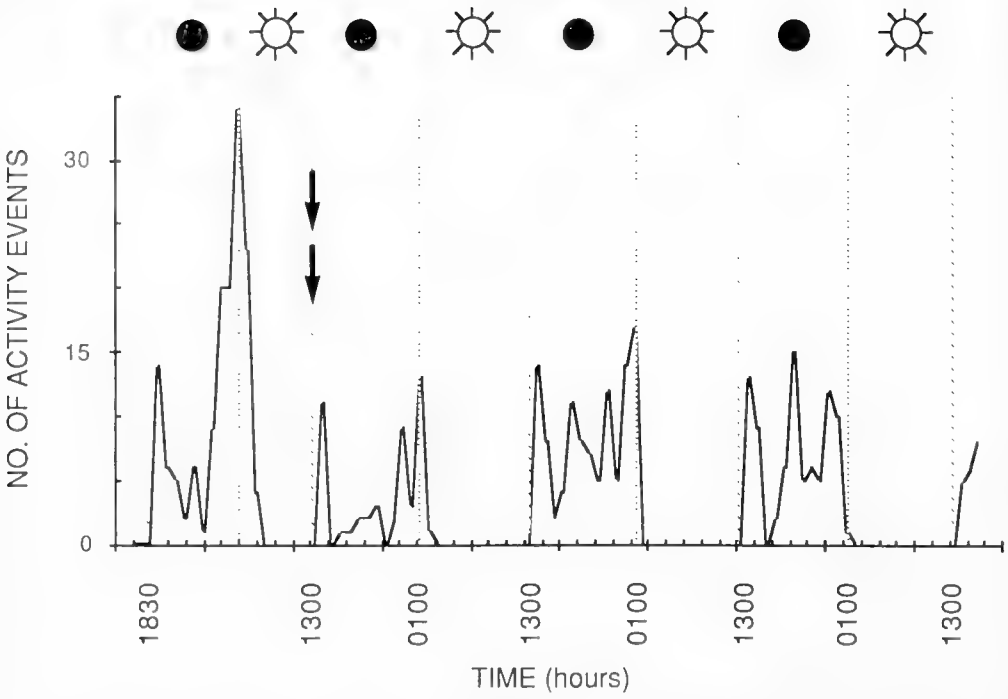


FIG. 1. The raw activity record of squid no. 4 during Experiment 9. The arrows indicate the time of the shift in the phase of the light cycle. Each value represents the sum of the activity for the preceding hour. Dashed lines represent sunset and dotted lines sunrise, in the laboratory. Note that beginning with the second sunset the dark period begins at 1300 rather than 1830 h.

tivity of squid 4 was measured for 24 hr under the natural phase light cycle. Sunset was then artificially set 5.5 hr early and the squid monitored for the next 76 hr under the new phase of the light cycle. Squid 5 was maintained simultaneously with squid 4 under the light cycle (but was in a separate tank) and had been on the phase-shifted light cycle for 14 days before it was monitored in experiment 10. Squid 5 was observed for 108 hr. Both squids were sexually mature, weighing 5.13 and 4.90 g (21.0 and 20.0 mm ML) and had been in captivity 86 and 97 days, respectively. To determine if the pattern of activity was nocturnal, the squids' diel activity was tested as described above and the periodicity of the activity checked using fourier analysis.

Results: Squid 4's activity pattern, before the shift in the phase of the light cycle, showed peaks corresponding to the times of light change (sunset and sunrise; Fig. 1). The pattern shifted phase exactly with the change in the phase of the light cycle. Activity was reduced during the middle of the first 12-hr dark period under the new phase of the light cycle, but a third activity peak appeared during the subsequent dark periods. The difference in the midnight activity period before versus after the phase-shift is especially apparent in the averaged data (Fig. 2). The diel activity pattern after the 5.5 hr phase shift was significantly different from an even diel activity distribution (K-S test, $p < 0.01$; Table 2) with 99.6% of the activity occurring at night. Fourier analysis of the activity pattern after the phase-shift showed a dominant period of 24 hr.

Squid 5 showed a small peak in activity just after sunset, which then increased through the dark period to a maximum level just before sunrise (Fig. 3). This squid's average activity for each hr of the light cycle shows that there was no peak during the middle of the dark period. The diel activity pattern was significantly different from an even diel activity pattern (K-S test, $p < 0.01$), with 98% of the activity occurring at night. Fourier analysis of

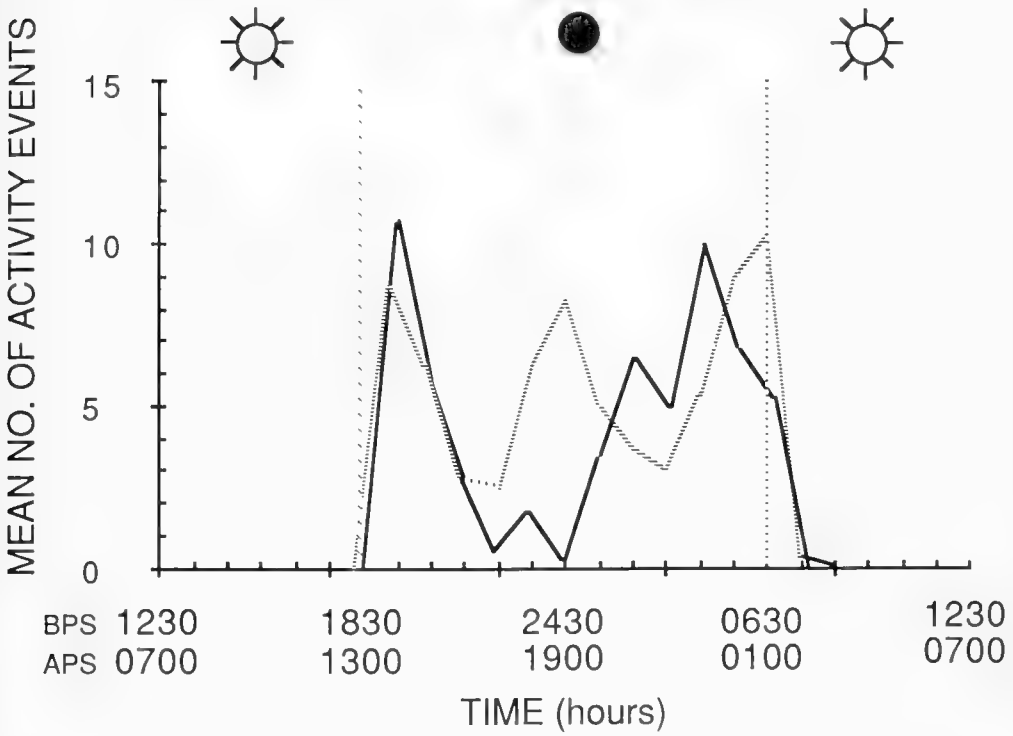


FIG. 2. The average hourly activity shown by squid no. 4 during a natural phase light cycle (solid line; data are from Experiments 7 and 8 and the first 24 hr of Experiment 9 combined; N = 96 hourly values), and the first 76 hr of the light cycle after it was shifted 5.5 hr (hatched line). Each value represents the mean activity for the preceding hr. The abscissas have been shifted so that the corresponding times for sunset and sunrise for both light cycles are superimposed (BPS: before phase-shift, APS: after phase-shift).

the data showed the activity to have a dominant period of 24 hours.

Time of last feeding in the field

Experiment: To determine if activity patterns observed in the laboratory were like those of *Euprymna scolopes* in nature, a field sampling program was conducted. However, since *E. scolopes* is difficult to sample during the day (over 12 studies conducted in Kaneohe Bay with large nets have failed to capture any; T.A. Clarke, personal communication), a study was undertaken only to determine if nocturnal activity occurred, as indicated by feeding.

Animals for stomach content analysis were collected and preserved in a 7.0% buffered formalin-seawater solution immediately upon capture. Since a lantern was used during collection, the light could have allowed the squids to feed abnormally just prior to capture. However, squids either appeared momentarily stunned or tried to swim away rapidly (often ejecting ink several times) when encountered during collecting. Since I walked around the reef-flats while sampling, the possibility that the squids might be attracted to the light to feed was reduced.

Morphometric measurements and determination of sex were made, and stomach fullness was determined subjectively as being either (1) distended (stomach completely filled and its wall completely stretched), (2) full (stomach filled with food but wall only slightly stretched), (3) partially filled (food present in the stomach but also much fluid), or (4) empty (stomach with only fluid and/or nondigestible items such as crustacean exoskeleton, fish scales, statoliths, etc. present).

Results: Forty-four squids (23 females and

TABLE 2. The activity of squids 4 and 5 during Experiments 9 and 10. The activity values for squid 4 before the phase-shift are a combination of data from Experiments 7, 8 and the first 24 hr of Experiment 9.

Squid no.	Expt. no. (duration in hrs)	Total diel activity events	Total diurnal activity events	Total nocturnal activity events	Percent of diel activity that was nocturnal	D_{max} for diel vs even diel activity
4	Before phase-shift (96 h)	229	0	229	100.0	0.500
4	9 (After phase-shift, 76 h)	239	1	238	99.6	0.496
5	10 (108.5 hr)	1174	27	1147	97.7	0.475

21 males) were collected over a period of 6 weeks (April to June). Sampling was concentrated between 2000–2030, 2130–0200, and 0400–0600 hr (sunset occurred around 1930 hr and sunrise around 0530 hr). The squids weighed 0.110 to 9.058 g (5.5 to 25.0 mm ML). Twenty squids had full stomachs, 14 squids had distended stomachs, 2 squids had partially filled stomachs and 8 squids had empty stomachs.

Errant polychaetes of the family Nereidae (some were *Perinereis* spp.; Hartman, 1966) represented over 90% of the items in the 36 stomachs that contained food. Large numbers of eggs were sometimes found associated with the bodies of the worms. One squid had a small intact mysid (possibly *Anisomysis* sp.) and four others had the remains of unidentified shrimp in their stomachs. Digestion rates determined in the laboratory using shrimp as prey (Shears, 1986) suggested that material in the squids' stomachs was ingested between 4 and 8 hr before the time of capture (Fig. 4).

Reaction of animals to diurnally limited feeding

Experiments: Squids were forced to be diurnally active by controlling their access to food. They were fed *ad libitum* on live shrimp between experiments but were starved for two days before an experiment. Six sexually mature *Euprymna scolopes* weighing 1.70 to 3.60 g (12.0 to 18.0 mm ML) were used during 10 experiments (nos. 11 to 20). In each experiment the squids were presented with shrimp only during the day. Squids were in captivity 5 to 48 days before each experiment.

Palaemon pacificus and *Palaemon debilis*, found in *Euprymna scolopes*' natural habitat, were used as prey. Preliminary observations indicated that the squids responded differently to the two shrimp. *P. pacificus* was slow-moving and almost always stayed on the bottom, while *P. debilis* was active, fast-swimming and spent much time off the bottom. Squids did not always have a sand-coat when they attacked prey. Attacks were therefore recorded as (1) attacks with a sand-coat or (2) attacks without a sand-coat. Each time a squid emerged, made any attempts to capture a shrimp and then reburied, was considered to be a single attack sequence. Squids often made several attempts to catch a shrimp during each attack sequence. Each attempt to capture a shrimp (whether successful or not) was considered a single attack. The number of attack sequences, the number of attacks made within each attack sequence, the number of prey successfully captured and the duration of each attack sequence were recorded also.

One hundred and three shrimp (72 *P. pacificus*, 31 *P. debilis*) were presented to the six squids. Three to four shrimp were presented (time of presentation determined using a random numbers table) during each daylight period. A prey was not placed in the compartment if a previously presented shrimp was still visible in the compartment, or if the squid was still emergent after having captured a prey. To prevent squids from feeding during the night, any shrimp remaining were removed from the aquarium before artificial sunset.

Results: Fifty-three of the *P. pacificus* and 24 of the *P. debilis* were attacked. Squids with a sand-coat attacked twenty-eight of the slow-moving *P. pacificus* but only three of the fast-moving *P. debilis*. When squids detected prey they usually emerged slowly from the bottom and always had a sand-coat. When retaining the sand-coat for an attack, they often then made short hops along the bottom in an attempt to either approach or attack a

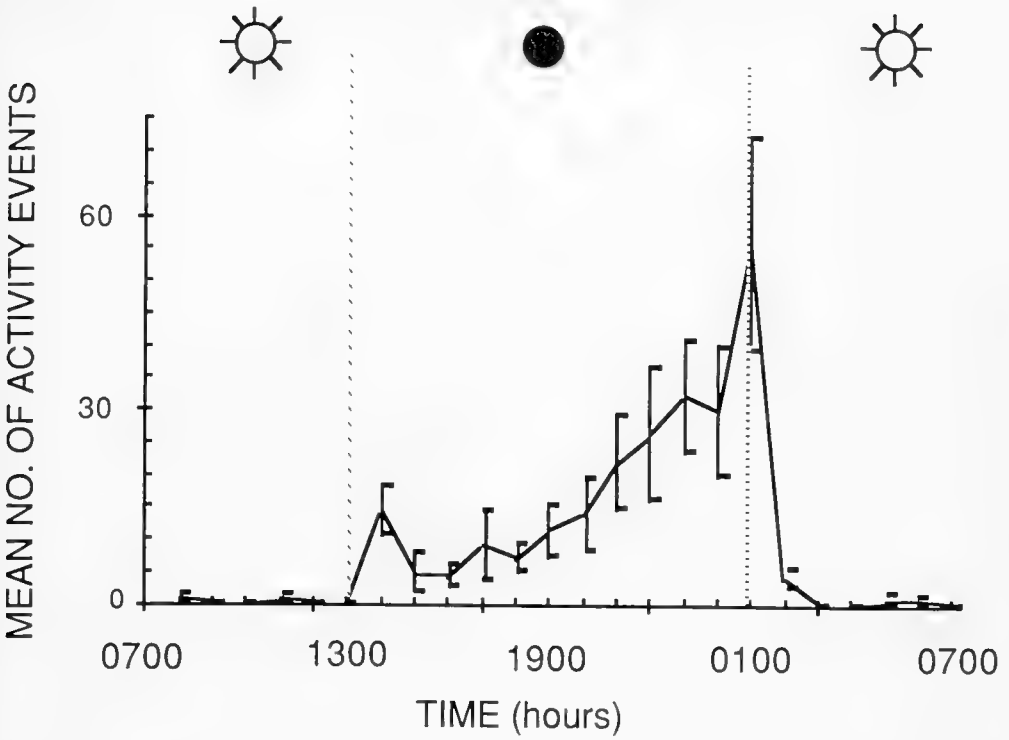


FIG. 3. The average hourly activity of squid no. 5 during a light cycle shifted by 5.5 hr. Error bars are for the standard error of the mean (N = 108 hourly values).

prey. If squids attacked without a sand-coat, they usually shed the sand after emerging and before swimming off the bottom. The squids almost always stayed within two body lengths of the bottom, regardless of whether they had a sand-coat or not. Only once during all of the attack sequences did a squid chase a shrimp that had swum away from the bottom (actually chasing the shrimp to the surface). This squid kept its sand-coat during the entire attack sequence.

When all attacks on *P. pacificus* were compared to all attacks on *P. debilis,* there was no difference between either the number of attacks within an attack sequence (Mann-Whitney U-test; $t_s = 1.920$, $p > 0.5$) or the duration of the attack sequences ($t_s = 1.389$, $p > 0.1$). When attempting to capture an individual *P. pacificus,* squids with a sand-coat made significantly more attacks within an attack sequence ($t_s = 2.28$, $0.05 > p > 0.02$) and had significantly longer attack sequences ($t_s = 4.97$, $p < 0.01$) than squids without a sand-coat. Although the attack data for *P. debilis* indicated that there may be a difference between the number of attacks within an attack sequence and the duration of attack sequences made with a sand-coat as compared to attacks without a sand-coat, the differences were not significant (for the number of attacks, $t_s = 0.490$, $p > 0.5$; for the attack sequence duration, $t_s = 0.356$, $p > 0.5$). This result is attributable presumably to the small data set for attacks made by squids with a sand-coat on *P. debilis* (only 3 attacks). The small number of such attacks would require an extremely large difference between attacks made with and without a sand-coat to produce a significant statistic with the Mann-Whitney U-test.

Based upon a success index (calculated as the number of shrimps captured divided by the number of shrimps attacked), squids captured similar proportions of *P. pacificus* and *P. debilis* (chi-square = 1.338, d. f. = 1, $0.25 > p > 0.10$) regardless of whether they attacked with or without a sand coat (Table 3). However, an attack index (number of shrimp

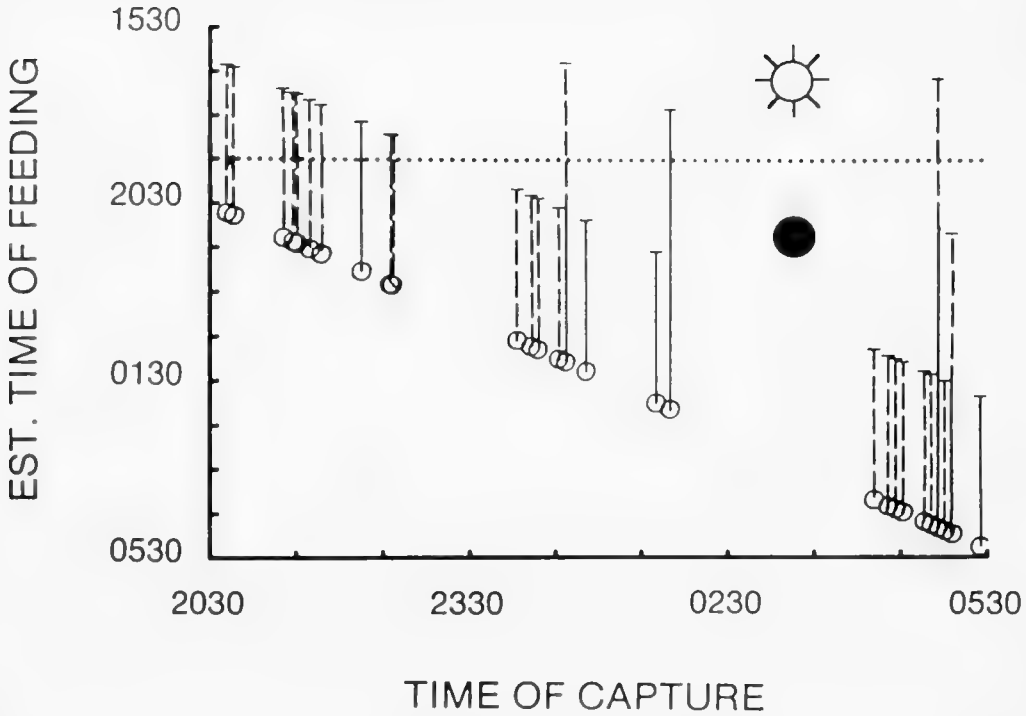


FIG. 4. The estimated time of feeding for 36 squids collected in the field. Estimates could only be made as meals ingested within intervals of 4, 8, 12 or 16 hr before capture. Solid bars represent the interval before the time of capture (open circles) within which squids could confidently be estimated to have fed. Dashed bars indicate the maximum interval within which a squid could have fed. The approximate time of sunset was 1930 h (dotted line).

caught divided by the number of attacks needed to capture the shrimp) indicated that squids making attacks with a sand-coat made significantly more attempts to catch a shrimp than when they attacked without a sand-coat (chi-square = 6.014, d. f. = 1, $p < 0.01$).

Activity of animals during diurnally limited feeding

Experiments: One thousand and three hours of observation were made on activity during experiments 11 to 20. Squids were monitored usually for 96 hr during each experiment to see if forcing them to feed during the day affected their basic pattern of activity relative to squids fed *ad libitum*. To determine if the squids were still predominantly nocturnal, their pattern of hourly diel activity was tested and the periodicity of the activity determined as outlined previously for experiments 1 to 10.

Results: Nocturnal activity accounted for approximately 85% of the activity (range: 55.5 to 99.3%) and the observed diel activity patterns were significantly different from the hypothetical even diel activity pattern (K-S test, p's $<<0.01$; Fig. 5 and Table 4). Animals showed peaks of activity just after sunset and just before sunrise. The basic pattern of activity did not differ from that of squids under *ad libitum* conditions. Only squid 6 (experiment 13) exhibited nearly equal amounts of activity during both the day and night (101 total diurnal vs 126 total nocturnal activity events). However, this squid still had strong activity peaks just after sunset and just before sunrise (Fig. 6). Fourier analysis showed that squid 6 had dominant periods of activity at 28, 24 and 19 hour frequencies. All other squids had a clearly dominant period of activity with a frequency of 24 hr.

All diurnal activity was associated with attacks on shrimp. Only during experiments 14,

TABLE 3. The number of shrimps attacked and the number of attacks made (regardless of whether the attacks were successful) when six *Euprymna scolopes* were forced to feed during daylight hours.

Type of attack	No. of shrimps attacked	No. of shrimps caught	No. of attacks made	Success index	Attack index
P. pacificus Attacked by squids with sand-coat	28	19	83	0.679	0.229
P. pacificus Attacked by squids without sand-coat	25	19	45	0.760	0.442
P. debilis Attacked by squid with sand-coat	3	2	16	0.667	0.125
P. debilis Attacked by squid without sand-coat	21	21	32	1.000	0.656

15 and 18 (the squids had only been in captivity for five or six days before the experiment) did squids not readily attack shrimps. One attack was recorded during these three experiments despite 22 shrimps being offered during 13 days (308 hr) of observation. If the 2 days without food before each experiment are included, squid 9 ate only one *P. pacificus* during a 5 day period, squid 8 went for 7 days and squid 10 went for 6 days without feeding.

DISCUSSION

These experiments demonstrated that *Euprymna scolopes* has a 24-hr cycle of locomotor activity under two different phases of an artificial light cycle and under the conditions of *ad libitum* feeding and feeding limited to the daylight period. Activity was greatest during the dark phase of the light cycle, especially just after dusk and before dawn.

The finding that both squid 4 and squid 5's activity followed the light cycle closely and that squid 4's activity shifted its pattern immediately in response to a 5.5 hour phase-shift, indicates that light was the entraining cue for the squids' activity in this study. The peaks of elevated activity shown by all squids at dawn and dusk (regardless of whether they were on the natural or phase-shifted light cycles, or on either of the two feeding regimes) are also evidence that light is the entraining cue for the squids' activity patterns. While the increased activity at dusk might be argued to be a laboratory artifact, resulting from the abrupt change in light intensity at sunset, this interpretation is not supported by the activity records. Squid 4's development of a strong peak of activity during the middle of the dark period, after the change in the phase of the light cycle, corresponds to the time of sunset to which this squid had been exposed for 86 days before the phase-shift. Apparently the pattern of elevated activity just after sunset is controlled by an endogenous circadian rhythm entrained by the light cycle (Aschoff, 1960). Furthermore, the increased activity just prior to sunrise could only be produced if the squids were anticipating the artificial dawn. This also could occur only if the squids had an endogenous circadian rhythm entrained by the light cycle (Aschoff, 1960; Pittendrigh, 1960).

Since 36 of the 44 squids collected at night had fresh food in their stomachs, most of the squids collected in the field had apparently fed after sunset. The large number of errant polychaetes found in the stomachs suggests further that the squids had fed at night. The polychaetes (some with eggs) were probably swarming at the surface, as is typical of some species when spawning (Dales, 1967). Thus, the feeding studies support the conclusions from the laboratory studies: *E. scolopes* is a nocturnally active animal in its natural habitat.

Few squids used a sand-coat when attacking the fast-moving *Palaemon debilis*. When attacking the slow-moving *Palaemon pacificus*, squids with a sand-coat made significantly more attacks per attack sequence

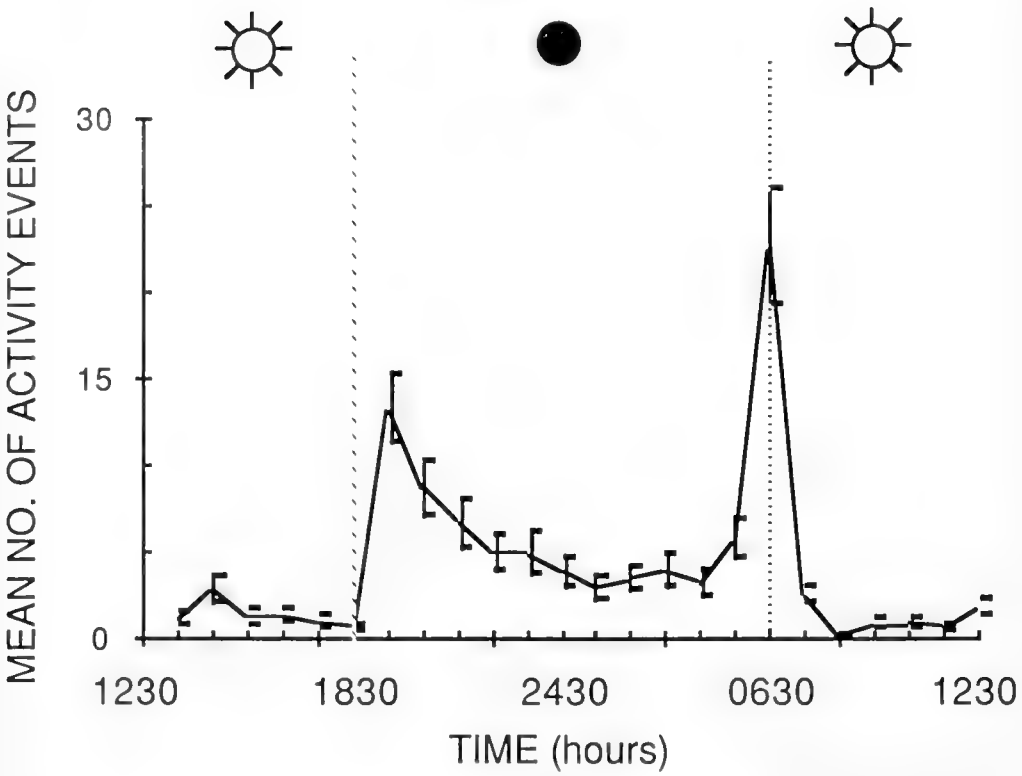


FIG. 5. The average hourly activity exhibited by six squids forced to feed during daylight (Experiments 11–20; N = 1003 hourly values). Bars represent standard error of the mean.

TABLE 4. The activity of six squids under a natural phase light cycle and diurnally limited feeding (for terms see caption to TABLE 1).

Expt. no. (duration in hrs)	Squid no.	Total diel activity events	Total diurnal activity events	Total nocturnal activity events	Percent of diel activity that was nocturnal	D_{max} for diel vs even diel activity
11 (103)	7	562	131	431	76.7	0.280
12 (100)	7	775	118	657	84.8	0.348
13 (97)	6	227	101	126	55.5	0.493
14 (138)	8	351	8	343	97.7	0.477
15 (73)	9	571	4	567	99.3	0.493
16 (97)	9	319	53	266	83.4	0.334
17 (99)	9	242	55	187	77.3	0.260
18 (97)	10	233	25	208	89.3	0.393
19 (99)	10	289	33	256	88.6	0.348
20 (100)	11	395	60	335	84.8	0.314
11–20 (1003)	—	3964	588	3376	85.2	0.352

and had significantly longer attack sequences than squids without a sand-coat. The attack index indicated that regardless of whether squids were attacking *P. pacificus* or *P. debilis*, the sand-coat made it more difficult to catch a shrimp. In this study, *Euprymna*

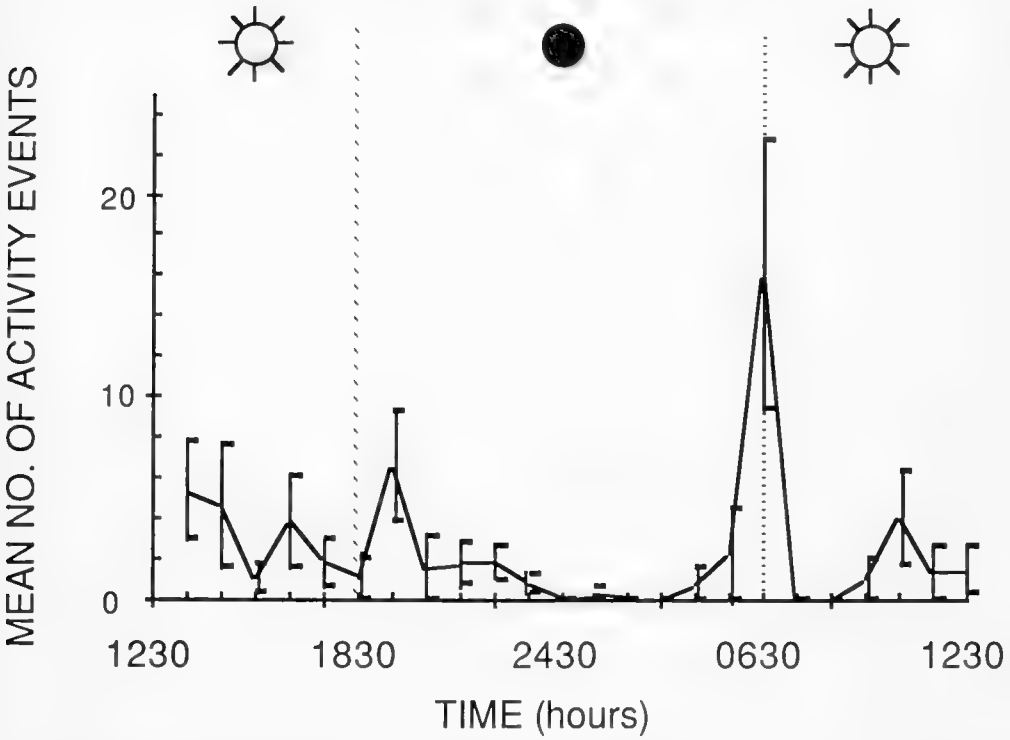
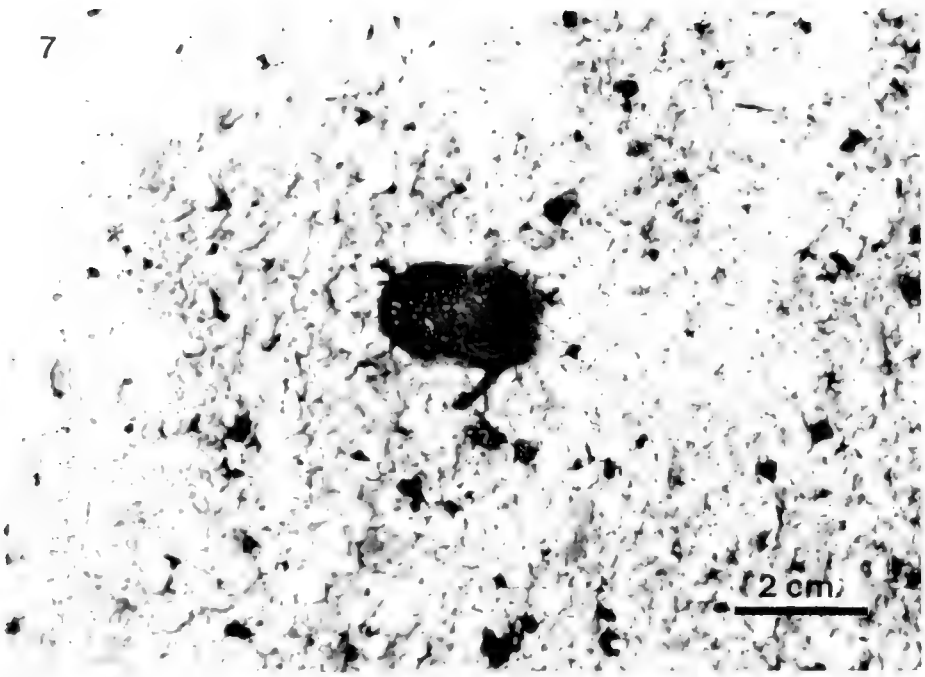


FIG. 6. The average hourly activity of the squid exhibiting the greatest diurnal activity (squid 6, Experiment 13; N = 97 hourly values).

scolopes' sand-gluing behavior interfered with prey capture, rather than aiding in prey capture by making the approaching squid difficult to detect.

The sand-coat makes the squid difficult to detect visually from above (Figs. 7, 8) and functions presumably to prevent *E. scolopes* from being seen by predators. Two aspects of the squids' diurnal behavior support this conclusion: (1) the sand-coat was used only during the day and (2) squids almost always stayed within two body lengths of the bottom where camouflage offered by their sand-coat would be most effective. The weight of the sand-coat did not prevent the squids from leaving the bottom, since on the one occasion that a squid did swim well off the bottom while pursuing a shrimp it had a very prominent sand-coat. The squids in experiments 14, 15 and 18 (animals run within one week of capture) apparently had not been in captivity long enough to acclimate completely to the laboratory conditions and were behaving probably much like they would under similar food-limited conditions in the field. The behavior of the squids in these three experiments suggests that animals in the field will not attack prey readily during the day. These squids had almost gone to the point of starvation, without emerging during the day to feed. In contrast, squids that had been in captivity for more than two weeks would emerge readily during the day when presented with prey. Presumably the squids that had been in captivity for some time learned that predators posed less of a threat in their new habitat.

When *Euprymna scolopes* emerges from the bottom (whether for the first time during the night or to attack prey during the day) it always emerges with the sand-coat. This observation suggests that the sand-gluing behavior in *E. scolopes* may serve to consolidate the sediment when the squid is buried (the function of this would be to prevent bottom debris from entering the mantle cavity when the squid is breathing when submerged in the sand). The ability in *E. scolopes* to use



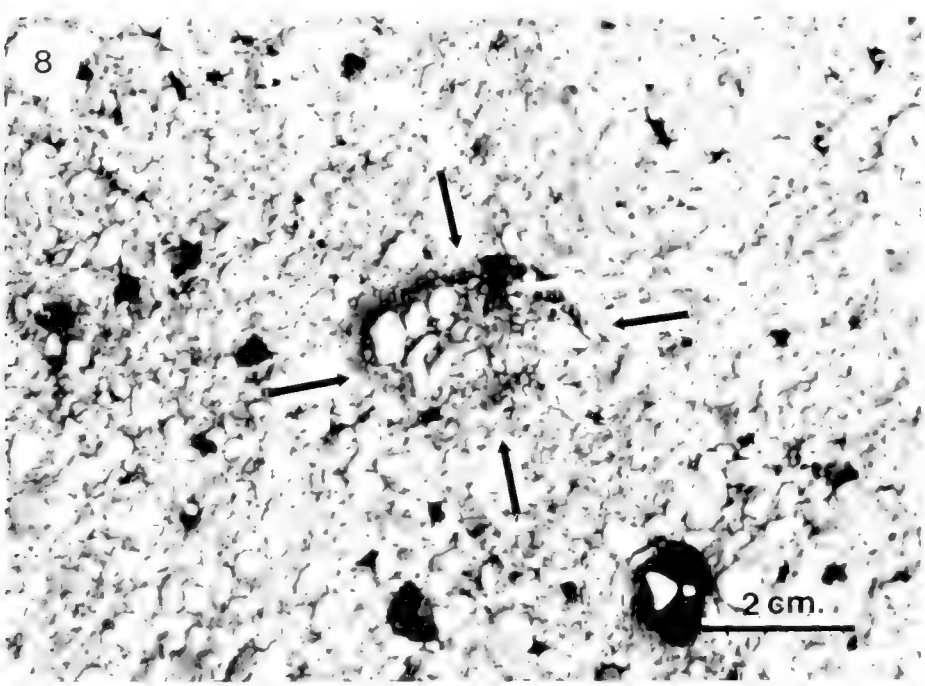


FIG. 7. Dorsal view of a captive *Euprymna scolopes* emerged from the bottom without a sand-coat during daylight.
FIG. 8. Dorsal view of the same animal as in Fig. 7, emerged from the bottom and carrying a full sand-coat during daylight (arrows indicate location).

a sand-coat for camouflage may have evolved from the initial use of the behavior for sand consolidation.

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IN-SITU OBSERVATIONS ON A LARGE SQUID-SPAWNING BED IN THE EASTERN GULF OF MEXICO

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ABSTRACT

A loliginid (probably *Loligo plei*) spawning bed was traversed by a remote-controlled submersible during a survey off Charlotte Harbor, Florida, in June, 1985. This large area of sand bottom was covered with small clusters of egg capsules. Such a pattern may indicate that large groups of squids concentrate in spawning aggregations over patches of suitable substrate. Preserved hatchlings from retrieved egg capsules bring into question the use of chromatophore patterns for specific identification of preserved specimens of early juvenile *Loligo* spp. that are sympatric and similar morphologically.

Key words: spawning; squid; *Loligo;* Gulf of Mexico.

INTRODUCTION

Along with the increasing commercial exploitation of squid stocks on the east coast of the United States (Lange & Sissenwine, 1980), there has also been increasing interest in development of commercial fisheries for squids in the Gulf of Mexico (Hixon *et al.*, 1980; Voss & Brakoniecki, 1985). The species that are of greatest commercial interest include the loliginids *Loligo pealei* and *L. plei* and an ommastrephid *Illex* spp.

Questions about the biology and reproductive ecology are of particular importance in determining the population dynamics of short-lived, fast-growing species like loliginid and ommastrephid squids. Little is known about the basic biology and population ecology of neritic squids in the Gulf of Mexico. In the Atlantic, *Illex* spp. are known to spawn pelagic egg masses (O'Dor & Balch, 1985) that are very difficult to collect. Some observations have been made, though, on the loliginids, which are bottom spawners. However, it is clear that substantial gaps exist in our knowledge of reproduction by *Loligo* spp. in the Gulf of Mexico.

Reproduction and early life history of *L. pealei* have been studied for many years in northern waters. Laboratory studies have described mating, egg laying and embryonic development (Arnold, 1962; McMahon & Summers, 1971). These studies have been complemented by field studies of reproductive biology, behavior, egg laying and early life history (Griswold & Prezioso, 1981; Macy, 1980; Vecchione, 1981). Recently, progress has been made in studying *L. pealei* in the Gulf of Mexico (Hixon, 1980; Hanlon *et al.*, 1983), but what is known of this species, even in the well-studied waters around Woods Hole, seems inadequate when compared with current knowledge of some other commercially exploited species such as *Loligo opalescens* (e.g. Fields, 1965; McGowan, 1954; Okutani & McGowan, 1969; Recksiek & Frey, 1978; Shimek *et al.*, 1984; and others reviewed by Hixon, 1983). Furthermore, the behavior and biology of *L. pealei* may differ in many ways between populations in the Gulf of Mexico and the well-studied Middle Atlantic Bight (e.g. depth distribution is certainly different between the two areas for this species; thus light cues inducing migration or maturation must also be different). Spawning of Gulf specimens has been observed in the laboratory (McConathy *et al.*, 1980), but field studies of reproductive ecology are scarce (e.g. Hixon, 1980).

Our knowledge of *Loligo plei* is even more limited. Mating and egg deposition have been described from studies in the laboratory (LaRoe, 1971; Roper, 1965; Hanlon *et al.*, 1983) and in the field (Waller & Wicklund,

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1968); however, these observations on squid behavior in the field, from a manned submersible sitting stationary on the bottom, may have been influenced strongly by the lights of the submersible. Laboratory observations, on the other hand, have all been on individuals that were limited in number at any one time and kept in a confined space. Thus, these observations may not be indicative of natural behavior.

I present observations on a loliginid spawning bed in the Gulf of Mexico. These observations, made during a survey with an unmanned submersible, may indicate behavior unlike that described previously. I also present evidence on the specific identity of the squid eggs.

MATERIALS AND METHODS

A remote-controlled submersible was used in June 1985 to survey the sea bottom on the central continental shelf at approximately 60 m depth, 171 km west of Charlotte Harbor, Florida. The submersible was tethered by power and data cables to the M/V *Universal Surveyor* and was controlled by an operator aboard the host vessel using a joystick and video display terminal. A color video camera on the submersible provided continuous viewing of the bottom. Time and location of the submersible were superimposed on the video display. The location of the submersible was determined by a computer on the host vessel that interfaced a precision radio-triangulation system, indicating the host vessel's position, and an acoustic system used to determine the position of the submersible relative to the host vessel (Vecchione & Gaston, 1985). The 20 hp submersible was equipped with a "claw"-type manipulator, 360° scanning sonar, floodlights and a 35 mm still camera.

This system was used to survey ca. 70 km (linear track) of sea bottom along predetermined transect lines (Fig. 1). High-precision navigation fixes were recorded every 152 m along these transects. The bottom substrate consisted primarily of sand or sand and shell but was covered largely by coralline algae. Areas of emergent rock were seen also, some of which were covered with sponge-coral communities. Clear sand bottom was limited.

OBSERVATIONS

Within about 760 m of the end of the final transect, the submersible came across an area of sand bottom that was covered largely (50–80%) with *Loligo* egg capsules. The transect direction was NE–SW, but the submersible doubled back over the spawning bed several times to estimate its size (Fig. 2). At one point, the submersible was stopped for about 2 min and its claw was used to pick up some capsules; because there was no sample basket or other such device, the capsules were held in the claw until the submersible was retrieved at the end of the transect. While the submersible was stationary on the bottom, changes in its computed position were noted to determine variability in the navigation readout. Based upon these observations, I estimate that the NE–SW size of the spawning bed was ca. 40 m ± 7 m. Other dimensions could not be determined because the submersible had to abandon the spawning bed and return to surveying the transect before sunset resulted in nighttime interference with the radio navigation system.

The egg capsules were arranged in groups of about 10–40 with their bases collocated. These groups appeared to be spaced randomly about one capsule-length apart. Thus, there was considerable overlap and entanglement among groups of capsules, making precise counting difficult. In contrast to most of the surveyed bottom, there was little or no coralline algae and no emergent hard substrate in this area. The bases of the capsules were inserted directly into the sand.

None of the egg capsules collected by the claw were complete by the time they were brought aboard the host vessel. However, when one fragment was placed in a cupful of sea water, three squids hatched within minutes. One of these hatchlings lived for 1½ days before it was sacrificed at the end of the cruise. There were no microscopes aboard the survey vessel; thus, detailed examination of the live hatchlings was not possible. Subsequent microscopic examination of the preserved hatchlings ashore showed that these hatchlings, which had been preserved in 50% isopropanol, were about 1.8 mm in dorsal mantle length (ML). Assuming shrinkage from the preservative, the squids probably hatched at about 2 mm ML. The external yolk sac had been absorbed or broken off in all specimens, including one that was sacrificed shortly after hatching. The locations of dark chromatophores (reddish in preservation) on the hatchlings are presented in Fig. 3. Dark chromatophore locations were the same in all three specimens. The yellow chromatophores

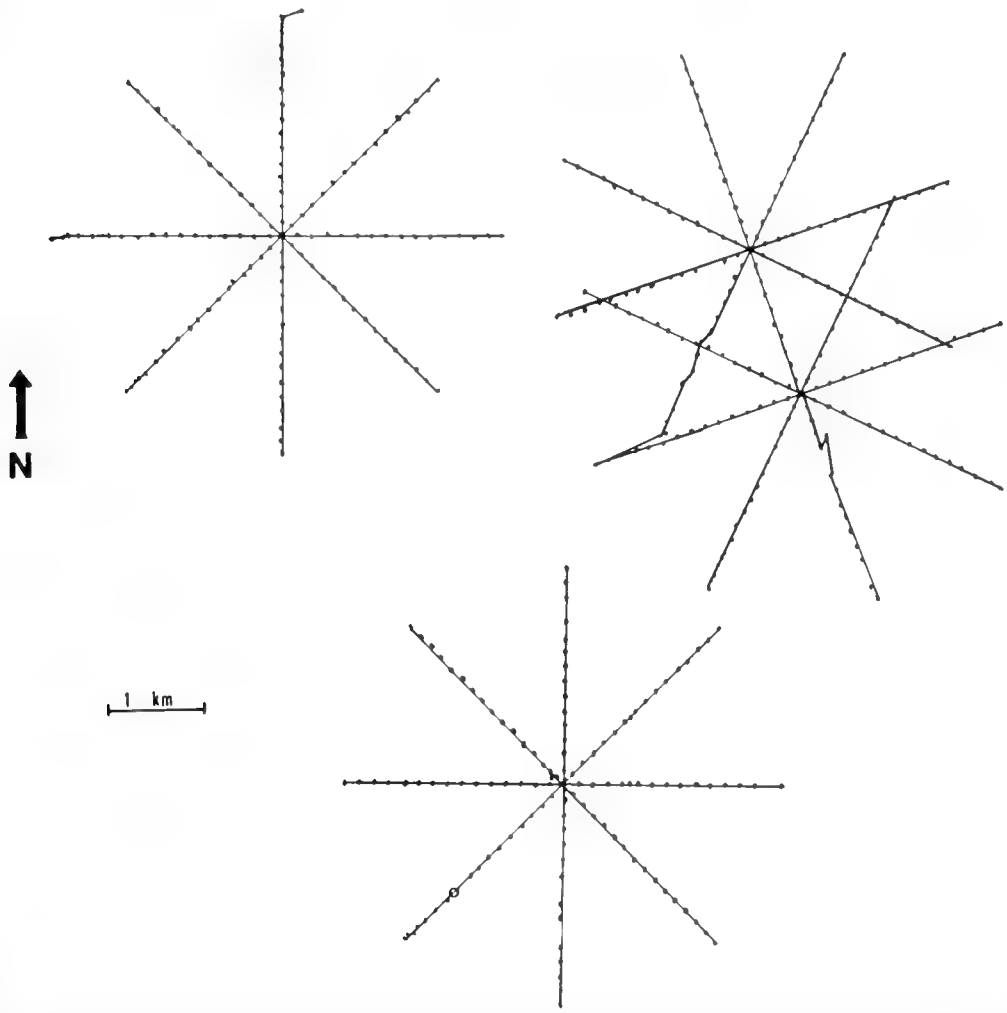

FIG. 1. Survey track followed by the remote-controlled submersible. Dots indicate high-resolution navigation fixes. Circle at SW corner of southern pinwheel indicates the location of the squid-spawning bed.

(McConathy *et al.*, 1980) were very bright but their exact locations were quite difficult to determine because of overlap with the darker (red) chromatophores (see Vecchione, 1982, for a discussion of this problem).

The egg capsules were two egg-strings thick, each chorion measuring about 2 mm wide by about 3.5 mm long. Six embryos were removed and examined; they were very similar to the hatchlings in overall appearance and their chromatophore distribution was indistinguishable from that of the hatchlings, indicating that they were probably very close to hatching. These embryos differed from the hatchlings only in the presence of an external yolk sac extending beyond the tips of the tentacles.

Three species of fishes were seen in the spawning bed (filefish, *Monacanthus* sp.; blackedge moray, *Gymnothorax nigromarginatus*, and sand perch, *Diplectrum formosum*); none were observed to be feeding on squid eggs or capsules.

DISCUSSION

The structure of the egg masses and the morphology of the hatchlings and embryos

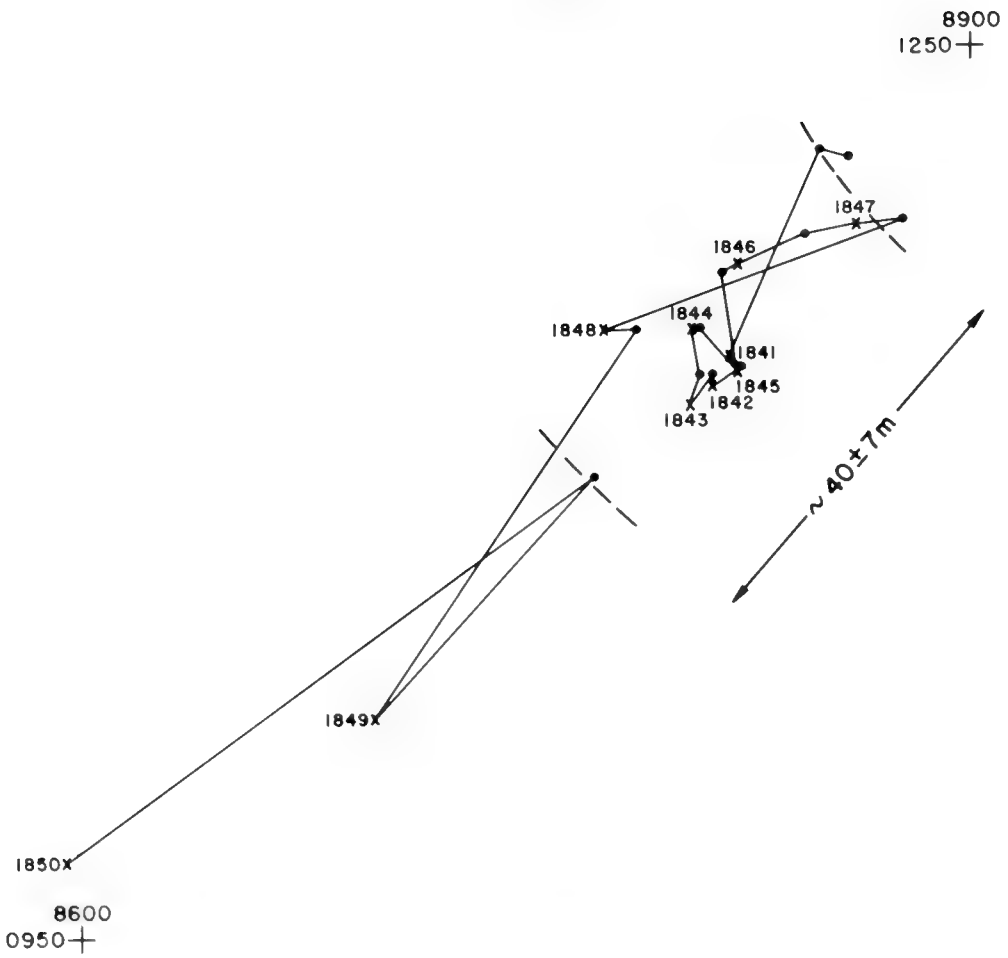

FIG. 2. Path of the submersible in the vicinity of the spawning bed. Location of the submersible at 1 min intervals is indicated by X's with time given. The submersible was actually stationary from 1841–1843; changes in its computed location during this time indicate variability in the combined radio/acoustic navigation system. Reference marks are based upon the host vessel's radio-navigation system. Dashed lines indicate the extent of the spawning bed.

indicate clearly that this is a spawning bed of a species of loliginid squid. In addition to *Loligo pealei* and *L. plei,* loliginids that may be present in this area include *Lolliguncula brevis* and, although unlikely, *Sepioteuthis sepioidea.* These last two species can be eliminated from consideration both because of the depth of the spawning bed (Arnold, 1965; Hixon, 1980) and because of the morphology of the hatchlings (Hanlon *et al.,* in press; Vecchione, 1982).

Ample evidence exists that *L. pealei* normally attaches its egg capsules to a hard substrate (e.g. Arnold, 1962; Griswold & Prezioso, 1981). It is possible that in the absence of adequate hard substrate, *L. pealei* would begin establishing egg clusters by attaching the first few strands to small shells, giving the appearance of spawning on a sandy bottom. Hanlon *et al.* (1983) illustrated *L. pealei* planting egg capsules into gravel substrate in the laboratory. However, because we found several substantial areas of hard substrate, both with and without attached "live-bottom" communities, in the surveyed area, it seems unlikely that *L. pealei*

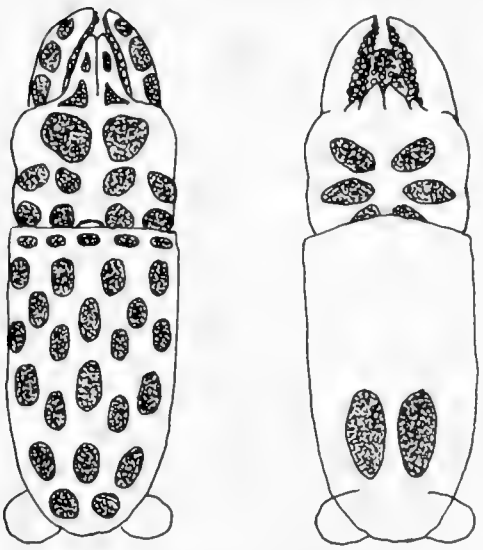

FIG. 3. Hatchling from egg capsules retrieved from the spawning bed. Left: ventral aspect; right: dorsal aspect. Positions of dark (red) chromatophores are shown.

would have chosen to spawn on a bare sand bed rather than on available hard substrate. Furthermore, *L. pealei* tends to remain in deeper waters, both in the western Gulf of Mexico (Hixon, 1980) and in the northeastern Gulf (Vecchione, unpublished). *Loligo plei* is the most abundant loliginid at 60 m depth in the western Gulf of Mexico (Hixon, 1980) and has been shown to lay its eggs on sandy substrates in the laboratory (Roper, 1965; R.T. Hanlon, personal communcation, 1985). Thus, *L. plei* is the most likely species to have laid the eggs described here.

The importance of visual stimuli for both the initiation of egg laying and the formation of large communal clusters of egg capsules has been stressed for *L. plei*'s congeners *L. pealei* (Arnold, 1962) and *L. opalescens* (McGowan, 1954; Yang *et al.,* 1986). Both species respond to previously laid egg capsules or, in the absence of such capsules, any structure that resembles a cluster of egg capsules (e.g. "artificial mops"). However, Roper (1965) observed that such behavior may not be typical of *L. plei.* His squid laid eggs on two occasions in complete darkness in a tank devoid of egglike structures. Contradictory evidence was provided by the *in-situ* observations of Waller & Wicklund (1968). They found that, in the lights of their submersible at night, *L. plei* oriented visually on previously deposited egg capsules and formed a huge communal cluster of egg capsules. Furthermore, *L. plei* in captivity will lay their eggs around artificial egg mops (R.T. Hanlon, personal communication, 1986).

The spawning bed that I have described is very different from that of Waller & Wicklund (1968), although both belonged presumably to the same species. The presence of very many small clusters of egg capsules, dispersed and covering a large area of the bottom, contrasts with Waller & Wicklund's (1968) observations of "a cluster or mop about 3 feet in diameter with a narrower band of capsules extending out another 3 to 4 feet. The main cluster was located at the point of maximum light intensity where the beams from the two underwater lights intersected. A smaller mop was formed under the vehicle's side-mounted floodlight."

I propose that the pattern described by Waller & Wicklund (1968) was an artifact of the intense illumination by the submersible's floodlights. It may be that the squids respond naturally to the brightest area of sea bottom as might be expected from a patch of bare sand illuminated by a full moon. This could result in substantial aggregations of squid mating and spawning in the vicinity of patches of adequate substrate, similar to those seen in the Pacific species, *L. opalescens.* Anecdotal reports from shrimpers off the southwest coast of Florida (G.L. Voss, personal communication, 1986; W. Rathjen, personal communication, 1986; A. Kemmerer, personal communication, 1986) indicate that they occasionally fill their nets with squids. This may be another indication of large spawning aggregations.

Unlike *L. opalescens,* there is evidence that *L. plei* does not die immediately after spawning (Roper, 1965; Waller & Wicklund, 1968; R.T. Hanlon, personal communication, 1986). I saw no dead squids during this survey, whereas *Loligo* (probably *L. plei*) were abundant in the ship's lights every night during the survey. Because all embryos examined were well developed and some may have been competent to hatch, this spawning bed was at least several days (or weeks) old at the time of the survey. Thus it is possible that the adults had died and been devoured by scavengers or swept away by currents.

The hatchlings described above are the first "paralarvae" (see Young & Harman,

1988 this volume) collected in the field that could be assigned reasonably to *L. plei*. McConathy, Hanlon & Hixon (1980) presented diagnostic chromatophore patterns to separate *L. plei* from *L. pealei* (red chromatophores on the ventral mantle: *L. plei*, 15–23 usually in 5 rows; *L. pealei*, 21–32 usually in 6 rows) based upon tens of hatchlings spawned and hatched in the laboratory. These authors (*op.cit.*) stressed the strong similarity in patterning of the hatchlings of *L. plei* and *L. pealei*. Whereas I have inferred that my specimens are *L. plei*, they probably would have been identified as *L. pealei* based upon chromatophore patterns. Furthermore, McConathy, Hanlon & Hixon (1980) showed no red (or dark) chromatophores on the dorsal surface of the head of either species. My specimens definitely had six dark chromatophores on the dorsal head in a pattern similar to that described for *Loligo opalescens* (*op. cit.*), an eastern Pacific species not found in the Gulf of Mexico. One possible explanation for this would be that six of the nine yellow chromatophores found on the head of both species at hatching may have turned dark sooner in this brood or population than the hatchlings studied by McConathy, Hanlon & Hixon (1980). All yellow chromatophores of *Loligo* hatchlings turn dark eventually (R.T. Hanlon, unpublished data).

McConathy, Hanlon & Hixon (1980) were hopeful that use of chromatophore patterns could "ultimately lead to a key for live squid hatchlings." Their characters, though, are being adopted by investigators as if the characters are definitive for the specific identification of preserved planktonic specimens. The low variability in patterns of dark chromatophores among my specimens (which came from two capsules out of a single clump and therefore were likely to have been laid by a single female), and the contrast in dark head chromatophores between my specimens and those of McConathy, Hanlon & Hixon (1980), indicate that not only interspecific but also intraspecific variability in hatchling morphology may be large compared to variability within a brood. Hatchlings from a large number of females may be required before a complete description contrasting the species of *Loligo* in the Gulf of Mexico can be developed. The systematics of *Loligo* in this part of the world are not finalized and it is possible that other species occur in the Gulf of Mexico; for example, *L. roperi* and *L. ocula* are reported in the Caribbean Sea (Cohen, 1976). Although chromatophore patterns are definitely useful for generic determination of hatchling loliginids in the Gulf of Mexico, I question their use for separation of closely related species of *Loligo* when preserved specimens are used.

In conclusion, the spawning bed described here is possibly that of *Loligo plei*. If so, *L. plei* does not lay large communal clumps of egg capsules. Instead, many small clusters of egg capsules are dispersed over a large communal spawning bed consisting of sand substrate devoid of algal cover. Such a spawning pattern may result in large numbers of squids being concentrated over patches of suitable substrate. Whereas chromatophore patterns are useful in squid larval taxonomy, they are of questionable use for separation of *Loligo plei* from *Loligo pealei*.

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GENERAL LIFE HISTORIES OF CEPHALOPODS

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This session was notable for the diverse approaches to the study of cephalopods exhibited by the speakers and the variety of topics presented. Stillman Berry would have been pleased. About half of the papers presented are reproduced here.

The paper by Boucher-Rodoni & Mangold examines ammonia excretion in cephalopods by measuring the ammonia content in the sea water surrounding the animals. They examined species of *Loligo, Sepia* and *Octopus* and found that the ammonia excretion story is very complex. The linear increase in ammonia they observed with time suggests to the authors that they are measuring primarily extra-renal excretion. If this is so, then the relative importance of renal ammonia excretion must be re-examined. The authors found ammonia excretion to vary with species, size, nutritional state, metabolic level and "condition" of the animal. They suggest that circadian variation may also occur in *Loligo.* Examination of 0 : N ratios confirmed the high dependence on protein for metabolic energy sources but, again, considerable variation exists among species examined.

Eventually when ammonia excretion is more fully understood, the apparently simple and non-intrusive measurement technique used by the authors could prove extremely valuable in examining various problems relating to cephalopod life history. Along with O_2 measurements, this technique may provide an accessible "window" to the physiological state of the animal. Especially intriguing is the possible use of the technique to measure the apportioning of energy to growth vs. other metabolic requirements in cephalopods of various ages exposed to various conditions.

The paper by Roper & Hochberg dealt more with systematics and zoogeography than with life histories. Their contribution was still appreciated. In their paper, the authors relate natural history observations on a variety of cephalopods, some of which previously had never been observed alive. The "ambling" behavior they observed in *Metasepia pfefferi* is remarkable. They also document all the behavioral "patterning components" that they were able to observe. This paper, like several others in the Symposium, demonstrates the value that observations on living cephalopods have to cephalopod taxonomy. This is demonstrated especially in the clear distinctions they found in the color patterns of three species of the octopus *Hapalochlaena*, which they document fully with illustrations and photographs.

The paper by Toll & Strain indicated that several types of cephalopods can survive for up to 166 days on a variety of exotic foods. Even for researchers located near the ocean, providing cephalopods with an adequate food supply can be a problem. For inland researchers this problem can be prohibitive. The authors' success at an inland institution is certainly encouraging, even though the sublethal effects of an exotic food source on an animal's health, physiology and behavior are unknown. A more suitable solution would be for someone to produce a cheap, nutritionally balanced pellet that would be accepted by cephalopods in spite of its "dead" nature. Until this is found (if it ever is), the authors have demonstrated that feeding problems are no longer a major barrier for land-locked cephalopod culturists.

The paper by Young and Harman is a plea for the use of definable terms for describing life history stages in cephalopods. They redefine "juvenile" and "subadult." They point out that "larva" as a general term in cephalopods has questionable validity and is operationally undefinable. Rather than replacing the term with another undefinable term, they side-step the problem by introducing the somewhat parallel term "paralarva," which can be defined since it is based upon ecological as well as developmental criteria. Expanding the number of names used in cephalopod life-history terminology seems to be a necessary evil at this point. Hopefully it will not lead to the plethora of terms found in fish life-history terminology.

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COMPARATIVE ASPECTS OF AMMONIA EXCRETION IN CEPHALOPODS

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ABSTRACT

Ammonia excretion was investigated in mature adults of three species of cephalopods, the pelagic *Loligo forbesi*, the nectobenthonic *Sepia officinalis* and the benthic *Octopus vulgaris*. The accumulation of ammonia in the sea water reflected renal and extrarenal excretion. A continuous increase in the total concentration indicated that diffusion through the gill epithelium (and possibly other epithelia) was an important source of ambient ammonia.

The highest excretory rate was recorded in the squid *Loligo forbesi*. No striking sex-related difference was observed between males and females of the same species, except for one hyper-mature female squid where ammonia excretion rate was increased. In *Sepia officinalis*, size-related differences were observed, the smaller individuals excreting relatively less ammonia than the larger.

The response of mature animals to experimental starvation depends upon the nutritional condition and metabolic level of the animal at the beginning of food deprivation. During short periods of fasting, the rate of ammonia release is decreased, the animal using protein and lipid as a metabolic substrate, before shifting to an exclusively protein metabolism source for energetic needs.

Key words: Ammonia; excretion; *Sepia; Octopus; Loligo.*

INTRODUCTION

Cephalopods can maintain a relatively constant inner milieu by active regulation of osmolarity, pH, ionic concentration and excretion, due to the joint or independent action of several organs or tissues (Schipp *et al.*, 1975). As far as excretion is concerned, the organs involved are the renal sacs, branchial hearts and their appendages or pericardial glands, the renopericardial canals, the digestive duct appendages, the digestive gland and the white bodies. One of the most widely recognized functions of excretory organs is the removal of nitrogenous waste. Nitrogen end-products are produced mainly by the catabolism of amino acids and nucleic acids. They can be eliminated under different forms, mainly soluble ammonia, urea, amino acids and uric acid. However, in many aquatic molluscs, the kidney contains also concretions of uric acid and/or purines, which contribute to the total loss of nitrogenous material (Potts, 1967). Mucus secretions by the skin and digestive duct also contribute to the elimination of nitrogenous compounds (Delaunay, 1931).

Cephalopods excrete more than 50% of the nitrogen metabolism end products as ammonia, and are thus ammonotelic organisms (Delaunay, 1931; Potts, 1965, 1967; Martin, 1983; Boucher-Rodoni & Mangold, 1985, in preparation). Different factors are known to influence ammonia excretion rates. Among the biotic factors, trophic level and growth appear to be of particular importance in many species of marine invertebrates (Needham, 1957; Potts, 1967; Bayne *et al.*, 1976; Regnault, 1986). Ammonia excretion can also be used as an indicator of adaptation to environmental fluctuations, since abiotic parameters such as temperature and salinity can influence its rate of production (Regnault, 1984). Carnivorous animals are known to have an enhanced nitrogenous excretion, when compared to herbivores. Cephalopods are carnivorous and most of them have mainly protein reserves, and only limited amounts of lipids and even fewer carbohydrates (Giese, 1969; Boucher-Rodoni, 1973; O'Dor *et al.*, 1984). Among molluscs, Potts (1967) reported the highest ammonia concentration to be found in the blood of carnivorous cephalopods.

The similarities between mollusc and vertebrate kidneys have often been mentioned. In both groups, urine is first produced by ultrafiltration and then modified by secretion and resorption. In many aquatic ammonotelic

organisms (teleost fish, molluscs, crustaceans), a large part of the ammonia loss may take place extra-renally, mainly by diffusion through the gill epithelium (Potts, 1967; Bayne *et al.,* 1976; Forster & Goldstein, 1969; Regnault, 1986). Urine production thus provides only an incomplete image of the real excretory rates. The branchial epithelium (and other epithelia) is permeable to ammonia and is concerned with its excretion in cephalopods (Schipp *et al.,* 1979). Thus to have a real image of total ammonia excretion, we measured its concentration in the surrounding sea water, where urine and diffusion ammonia accumulate. Some data on ammonia production by the benthic *Octopus vulgaris* were obtained previously (Boucher-Rodoni & Mangold, 1985). They will be compared with the excretory rates of the necto-benthonic *Sepia officinalis* and the pelagic *Loligo forbesi,* in relation to trophic level of the animals and at different periods of their life cycles.

MATERIALS AND METHODS

Ammonia excretion was investigated in three species of cephalopods, representing three different modes of life. The benthic octopod *Octopus vulgaris* was studied in Banyuls-sur-mer (2 animals); the experiments concerning the necto-benthonic sepoid *Sepia officinalis* (9 animals) and the pelagic teuthoid squid *Loligo forbesi* (4 animals) were undertaken in Roscoff. All the experimental animals were studied in post-digestive condition, i.e. fed for the last time the evening before experimentation (after a period of regular feeding or after recent capture from nature). The effect of starvation was investigated in *Sepia* and compared with *Octopus:* the day of the last meal was noted and the animals were thereafter kept without food for some days (8 days for *Octopus* and 4 days for *Sepia*), while ammonia excretion was measured regularly.

The animals were placed individually in experimental tanks (15–20 liters for *Octopus* and *Sepia,* and 53 liters for *Loligo*), covered with an air-tight lid. They were allowed to acclimate for 1 to 2 hr before experimentation. At T_0, the sea water input was stopped and incubation was started. A closed-circuit circulation of sea water was maintained with a Eheim pump to insure good mixing of the medium. The water temperature was similar to the temperature of the sea water *in situ* at the season of experimentation (15.5°C for *Octopus,* 15°C for *Sepia,* 11°C for *Loligo*). The shortest total duration of one single incubation was 30 min, and the longest 120 min, but most of the experiments lasted 50–70 min.

At regular intervals (10 min usually), one sample of sea water was taken from the experimental tank. Ammonia was determined by the colorimetric indophenol method (Solórzano, 1969). In a few incubations with *Sepia,* only the starting and final ammonia concentrations in the sea water were recorded. Oxygen consumption was also measured in some of the experiments (Winkler method).

RESULTS

All three species are essentially ammonotelic organisms. Ammonia release into the sea water is a continuous linear process over short periods of time (Fig. 1). The mean relative excretion rate was highest in *Loligo* and lowest in *Sepia* (Table 1).

Ammonia excretion and trophic level

The consequence on ammonia excretion of short experimental periods of starvation was tested in adult *Sepia officinalis* and compared with *Octopus vulgaris.* In both species, the absence of regular feeding slowed down ammonia release (Table 2). The preliminary results concerning the answer to starvation in terms of oxygen consumption in *Sepia* allow us to calculate the O : N ratio (oxygen consumption : ammonia-nitrogen excretion). The O : N ratio is known to vary depending upon the type of substrate being oxidized for energy in marine invertebrates (Conover & Corner, 1968; Bayne & Scullard, 1977; Stickle & Bayne, 1982). Pure protein could yield a ratio around 8, higher values meaning that lipids and/or carbohydrates are used also. Cephalopods are known to mobilize some lipids during fasting, although most of the demand is met from protein reserves (O'Dor *et al.,* 1984). Thus, in *Sepia* as in *Octopus,* an increased O : N ratio suggests the use of lipid and protein reserves as metabolic substrate for energetic needs during the first days of starvation.

In *Sepia,* replicates with different animals showed that when the ammonia excretion

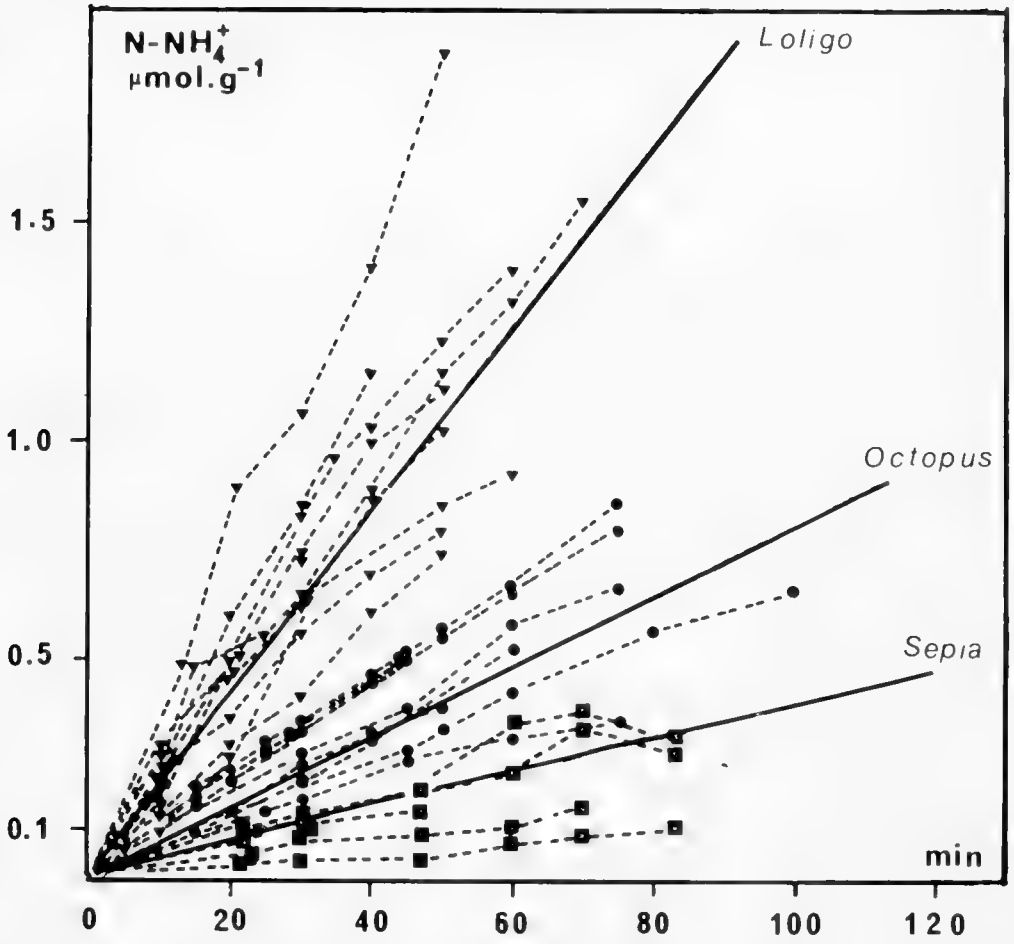

FIG. 1. Ammonia-nitrogen excretion (µmol. g $^{-1}$), in the three species during short-term incubations (min). The points coming from each experiment are linked (dashed lines). The regression lines (continuous lines) are presented as: Y = 0.021X + 0.018(r = 0.91; n = 73) for *Loligo*, Y = 0.007X + 0.083 (r = 0.89; n = 75) for *Octopus*, and Y = 0.004X + 0.011 (r = 0.96; n = 36) for *Sepia* (Y = V N-NH_4 and X = body weight).

rate was high in post digestion, its drop was more important during starvation than when the post digestive rate was lower than the average, which was the case in two individuals. Thus the response to starvation depends in fact on the condition of the animal at the beginning of the test period.

Ammonia excretion and life cycle

Ammonia excretion was measured in mature animals of the three species and specific differences were observed (Fig. 1). Within one species, the results concerning *Loligo forbesi* and *Sepia officinalis* showed that generally there were no significant differences between mature males and females of the same species (Table 3). In one case, a female *Loligo* started to lay eggs 90 min after the end of the experimental incubation. This animal showed an increased ammonia excretory rate.

For *Sepia officinalis*, ammonia excretion was measured at different periods of the life cycle of the animal: small immature and large immature individuals, and large mature ones

TABLE 1. Mean ammonia excretion rates (μmol. g $^{-1}$. day $^{-1}$ ± standard error of the mean) in the adults of the three species, in post digestive conditions. The mean oxygen consumption/ammonia excretion rates (O : N) are indicated also.

Species	Mean ammonia excretion rate	*n*	O : N	Reference
Octopus vulgaris	13.45 ± 1.48	15	10.7	Boucher-Rodoni & Mangold, 1985
Loligo forbesi	31.88 ± 2.08	14	15.1	present paper
Sepia officinalis	7.71 ± 0.71	21	23.1	present paper

TABLE 2. Mean ammonia excretion rate during feeding and starvation (μmol. g $^{-1}$. day $^{-1}$ ± standard error of the mean) in *Octopus vulgaris* and *Sepia officinalis*.

	Mean ammonia excretion rate				O : N	
	Fed	*n*	Unfed	*n*	Fed	Unfed
Octopus vulgaris	13.45 ± 1.48	15	7.37 ± 0.89	15	10.7	17.2
Sepia officinalis	7.29 ± 1.07	37	4.58 ± 1.18	6	22.5	26.8

TABLE 3. Mean ammonia excretion rates (μmol. g $^{-1}$. day $^{-1}$ + standard error of the mean), in mature males and females *Loligo forbesi* and *Sepia officinalis*.

Loligo forbesi		*Sepia officinalis*	
Males	Females	Males	Females
28.54 ± 3.36	31.01 ± 2.26 39.67 ± 4.54[1]	7.46 ± 0.57	7.93±0.92

[1]Female that started to lay eggs 90 min. after experimentation

(Fig. 2). The mean ammonia excretion rate depended principally upon the size of the animal rather than on the maturation stage. In large animals the excretion rate was similar in all animals, whatever their sexual maturation stage. If oxygen consumption in cephalopods is generally higher in smaller individuals, the ammonia excretion rate follows the reverse tendency in *Sepia officinalis*, where the larger animals excrete relatively more than smaller ones.

The relationship between the rate of ammonia excretion and body weight was described by the allometric equation $Y = b^*X$, where Y is the metabolic rate, X the body weight, a and log b the slope and intercept of the linear regression log X/log Y (Stickle & Bayne, 1982). The relation between ammonia excretion ($\mu M.h^{-1}$ animal^{-1}) and body weight (g) in *Sepia officinalis* was expressed by: $V\ N\text{-}NH_4 = 0.093\ BW^{1.17}$ (Fig. 2).

DISCUSSION AND CONCLUSIONS

Ammonia excretion is a linear continuous process in the three species studied. The pelagic *Loligo* showed the highest excretory rate, associated with a high oxygen consumption rate (Boucher-Rodoni & Mangold, in preparation). Potts (1965) suggested that ammonia loss through the gill epithelium might be an important process, since he recorded a decreased ammonia blood concentration in the efferent vessels of *Octopus dofleini* gills. The gills of cephalopods serve both respiratory and excretory functions. The continuous increase in ammonia concentration measured in the surrounding sea water means presumably that the extra-renal excretion, e.g. diffusion through the branchial epithelium, is an important means of removal of nitrogenous waste. This is known to be the case in many other marine invertebrates and in teleost fish

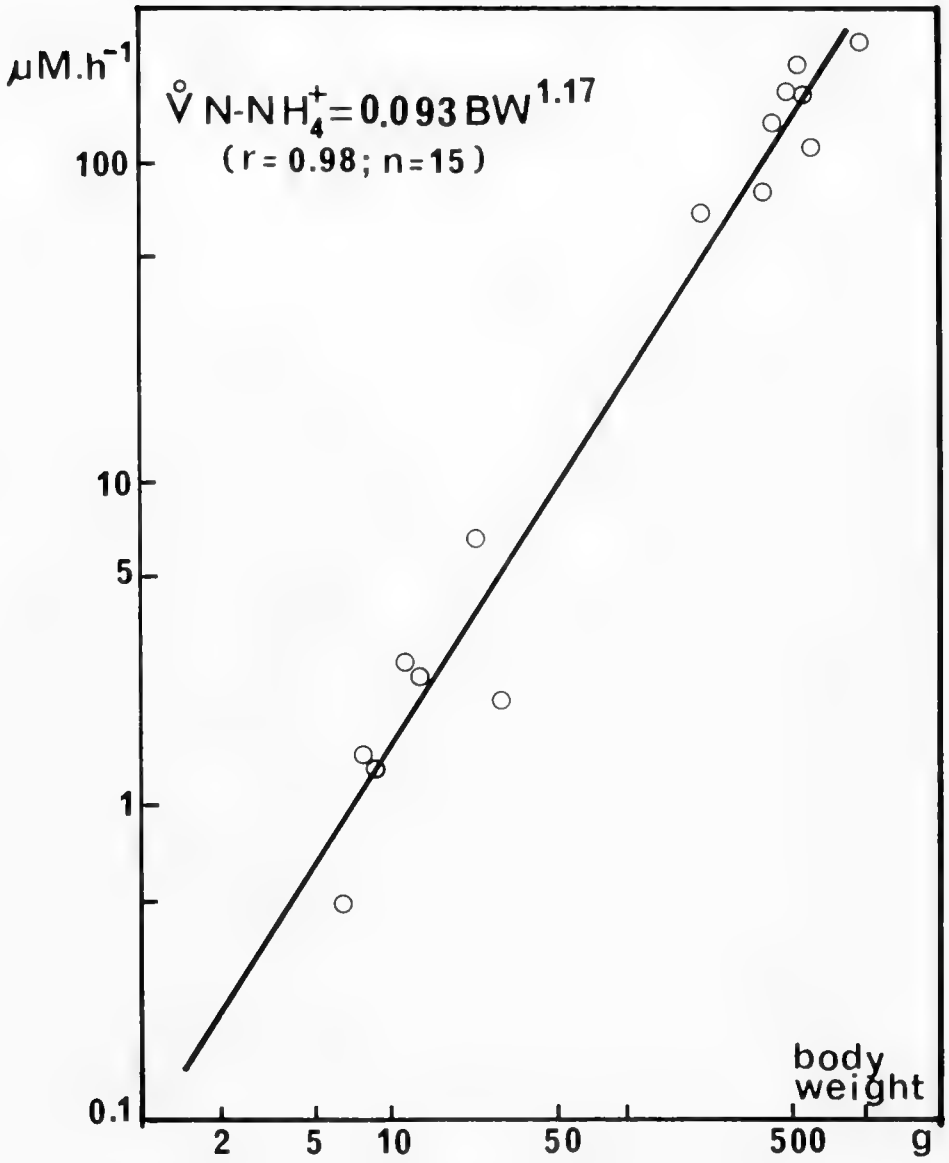

FIG. 2. *Sepia officinalis.* Ammonia-nitrogen excretion (μmol. h^{-1}) in relation to body weight.

(Potts, 1967; Bayne *et al.*, 1976; Forster & Goldstein, 1969; Regnault, 1986). In fish and crustacea, ammonia elimination can take place either by passive diffusion through the branchial epithelium, or by an active ionic exchange system of NH^+_4 against Na^+. Schipp *et al.* (1979) suggested that the permeability of the gill epithelium in *Sepia officinalis* might be ruled by an active ionic exchange system of NH^+_4 against K^+.

The atomic O : N ratio indicates that proteins are the main metabolic substrate for energetic needs in *Octopus vulgaris* and *Loligo forbesi.* O'Dor *et al.* (1984) demonstrated that protein is a high proportion of the diet of *Octopus,* and is both the most highly

conserved nutrient and the one used most extensively as fuel. The authors conclude that the basic energy requirements of most tissues are met through amino acid catabolism. In *Sepia* a low rate of ammonia production and a high metabolic rate (higher than in *Octopus* but lower than in *Loligo*) lead to a higher value for the atomic O : N ratio, which seems to indicate that lipids and/or carbohydrates from the diet are also used to some extent to meet the energetic needs of the animal.

The response to experimental starvation in *Sepia* depends upon nutritional status and metabolic level at the beginning of fasting. Initially, a decreased excretion rate was observed, in *Sepia* as in *Octopus*. An increased O : N atomic ratio during the first days means that proteins and lipids and/or carbohydrates are used as fuel in *Octopus* and *Sepia*. O'Dor *et al.* (1984) showed a drop in the lipid content of the digestive gland of *Octopus* after a six day fast. If the experimental starvation is maintained, a drop in the atomic O : N ratio shows that most of the demand is met again from protein reserves (Boucher-Rodoni & Mangold, 1985).

Mature males and females of the same species have similar ammonia excretion rates. Modifications in ammonia production are correlated with body weight, larger animals excreting relatively more than smaller ones. A relationship between ammonia excretion rate and body size exists in other molluscs (Emerson, 1969; Ansell & Sivada, 1973; Bayne *et al.*, 1975). However, Bayne *et al.*, (1976) suggest that in bivalves this relationship may be explained partly by seasonal changes in glycogenic reserves and in the synthesis and utilization of nitrogenous compounds as substrate for energy metabolism. In cephalopods, there are no such season-related changes in metabolic reserves, since proteins are always the main fuel. The animals of different body weight used for the present experiments (*Sepia*) were all captured together. The increased relative ammonia excretion rate in adults might be related to decreased needs of proteins for growth, cephalopods' strategy being to lay down protein reserves in rapid growth and then to convert part of them into gametes (O'Dor *et al.*, 1984). So, small *Sepia*, although feeding on the same diet as larger animals, waste less of the nitrogenous compounds, which are efficiently utilized for rapid growth, i.e. protein deposition (O'Dor *et al.*, 1984).

In addition to variations in ammonia excretion rates that occur during the life cycle, short-term variations seem also to exist. Circadian rhythms of ammonia excretion (and oxygen consumption) are suspected in *Loligo forbesi*, in relation with the crepuscular habits of the species (Boucher-Rodoni & Mangold, in preparation).

Nitrogen metabolism is overwhelmingly important in cephalopods, and in some cases even end-products like ammonia can be used, for instance for buoyancy. Many oceanic squids achieve nearly neutral buoyancy by accumulating large amounts of ammonium in some tissues, where its concentration can approach half molar (Clarke *et al.*, 1979). Ammonia excretion studies in such species would be of great interest. Another noteworthy feature related to ammonia production in cephalopods is that the primary role of GDH (glutamate dehydrogenase) in squid mantle tissue is to regulate the catabolism of amino acids for energy (and ammonia) production (Storey *et al.*, 1978). Recent literature focusing on other invertebrates suggest that GDH can function as glutamate oxidase to a significant extent (Batrel & Regnault, 1985). However, an activity ratio of 1 : 1 (glutamate oxidation *versus* glutamate synthesis) reported in the squid mantle (Storey *et al.*, 1978) is to our knowledge one of the highest found in the literature.

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BEHAVIOR AND SYSTEMATICS OF CEPHALOPODS FROM LIZARD ISLAND, AUSTRALIA, BASED ON COLOR AND BODY PATTERNS

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ABSTRACT

Cephalopods were observed *in situ* and under laboratory conditions at Lizard Island, Great Barrier Reef, Australia. Observations on habitat, foraging and activity patterns are included. The major chromatic components and body patterns are described for *Octopus cyanea, O. ornatus, Hapalochlaena* spp., *Metasepia pfefferi* and *Sepia papuensis*. Components of body pattern include color, texture, posture and locomotion. A remarkable new type of locomotion, "ambling," is described for *M. pfefferi*. This is the first description of living *M. pfefferi* and *S. papuensis*. On the basis of body patterns, behavior and morphology, the elevation of the subgenus *Metasepia* to generic status is confirmed. Observations of live *Hapalochlaena* at Lizard Island and in Sydney and color photographs of live animals from several other localities confirm the existence of a widespread complex consisting of at least three species and support the validity of the genus. Based on observations of live animals and a systematic evaluation of preserved specimens, the presence of *Octopus ornatus* is reported in Australian waters for the first time.

Key words: *Octopus; Sepia; Metasepia; Hapalochlaena;* cephalopods; color patterns; behavior; systematics; field observations; Great Barrier Reef.

INTRODUCTION

An International Workshop on Molluscs was conducted on Lizard Island, Australia, from 2 to 14 December, 1975 (Ponder, 1979).[3] The Workshop was sponsored by the Australian Museum, Sydney, which operates the Lizard Island Research Station located near the northern end of the Great Barrier Reef, Queensland, at 14°40′S, 145°28′E (Fig. 1). Lizard Island is a continental island about 2.9 square km in area composed primarily of granite; it lies about 30 km off the coast and is 17 km from the outermost barrier reefs (Ponder, 1979). Participants included malacologists from Australia, Great Britain, Hong Kong and the United States. One of us (C.F.E.R.) participated in the Workshop with the objective of surveying the cephalopod fauna around Lizard Island. Cephalopods were collected in various habitats and live animals were observed in their natural habitat and in aquaria. A preliminary checklist with collection and habitat data for the 27 species of cephalopods collected at Lizard Island was published separately (Roper & Hochberg, 1987).

This paper presents observations on behavior and body patterning made in the field and in laboratory aquaria on five species of cephalopods: *Octopus cyanea* Gray, 1849; *O. ornatus* Gould, 1852; *Hapalochlaena* cf. *maculosa* (Hoyle, 1883); *Metasepia pfefferi* Hoyle, 1885, and *Sepia papuensis* Hoyle, 1885. One *Hapalochlaena* cf. *fasciata* (Hoyle, 1886) was maintained in an aquarium at the Australian Museum, Sydney, subsequent to the Workshop. For the purpose of future identification, a synopsis is provided in which species characters are diagnosed and information on distributions and life histories are summarized. In addition to color and body patterning, observations are included on foraging, resource partitioning and activity patterns.

We regard these as preliminary observations that were made in 1975 in a fortuitous and opportunistic manner prior to the formulation of a classification of behavior that characterizes more recent studies of color and

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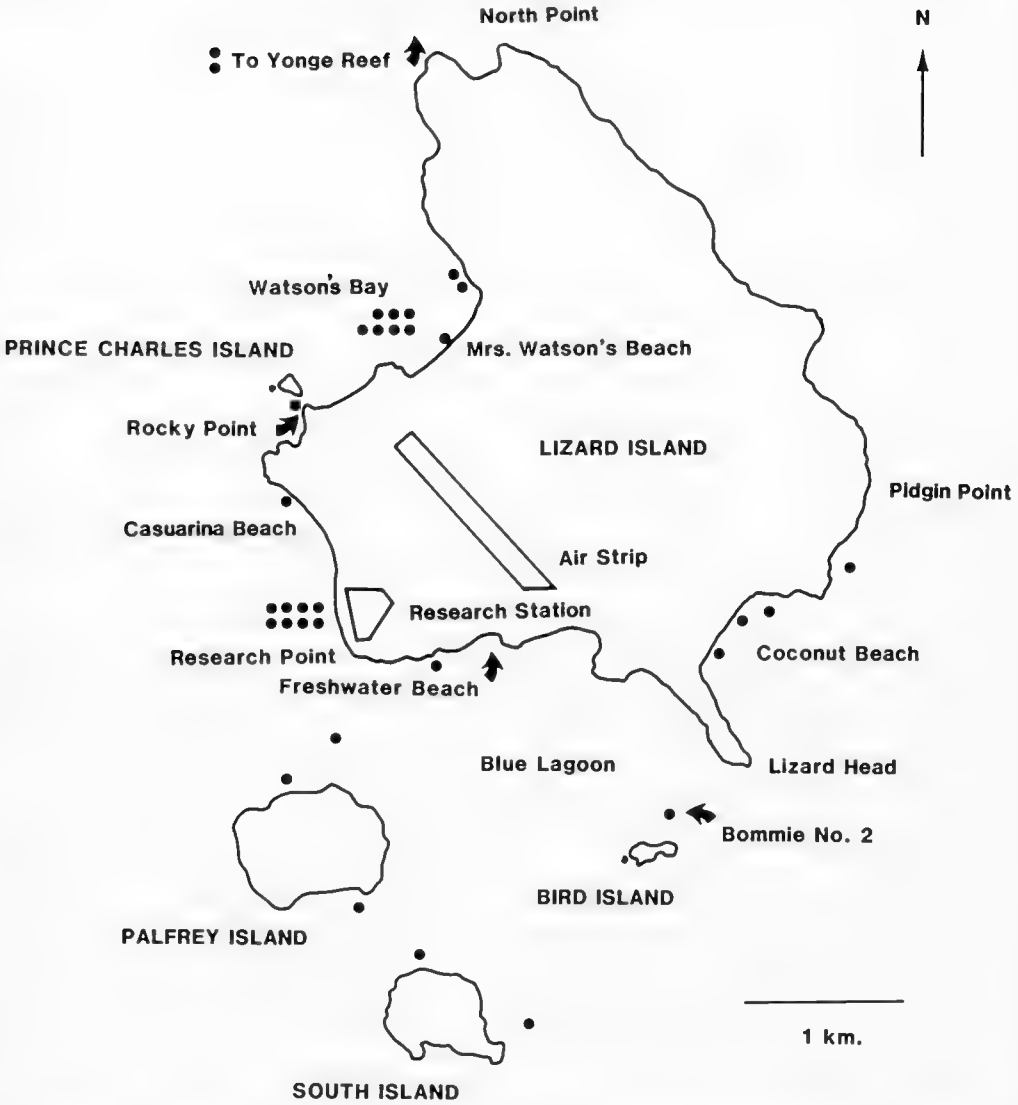


FIG. 1. Map of Lizard Island, Australia (14° 40′S, 145° 28′E) showing adjacent islands and locations of collecting stations (solid dots). See Roper & Hochberg (1987) for station and habitat data.

body patterning in squids, octopuses and cuttlefishes; see especially Holmes (1940), Packard & Sanders (1969, 1971), Moynihan (1975, 1985), Moynihan & Rodaniche (1977, 1982), Packard & Hochberg(1977), Hanlon & Hixon (1980), Boyle & Dubas (1981), Hanlon (1982), and Hanlon & Messenger (1988).

Two papers are especially significant in relation to our work. Packard & Hochberg (1977) defined and summarized the hierarchy of anatomical and behavioral systems that lead to the generation of patterns in *Octopus* and other genera. Hanlon & Messenger (1988) present a comprehensive and detailed study of body patterns and behavior in *Sepia officinalis*. To the extent possible we have attempted to follow the concepts and terminology presented in these two papers, which we consider the standards for modern work. Both papers emphasize that these magnificent and complex animals are capable of providing an almost infinite number of combi-

nations and gradations of colors, textures, postures and body patterns. Two of the genera we worked with, *Hapalochlaena* and *Metasepia,* are so different that we could not always fit our observations into existing terminology and hence have introduced several new terms.

The majority of detailed research on color and body patterning in cephalopods has been done on species from the Mediterranean and Europe, the Caribbean and Gulf of Mexico, the west coast of North America, Panama, Hawaii, Palau and Guam. Other than photographs of blue-ringed octopuses and occasionally of other octopuses and cuttlefishes that have appeared in popular magazine articles, little information is available on the biology, behavior and body patterns of Australian cephalopods.

This paper describes for the first time color and body patterns and other aspects of behavior of living *Hapalochlaena* spp., *Metasepia pfefferi* and *Sepia papuensis.* It also expands the observations made on *Octopus cyanea* and *O. ornatus* in Hawaii (e.g. van Huekelem, 1966, 1973, 1983; Wells & Wells, 1970) and on *H.* cf. *fasciatus* in Australia (e.g. Tranter & Augustine, 1973, as *H. maculosa*). In addition, we describe for the first time "ambling" in *M. pfefferi,* a newly recognized mode of locomotion for sepiid cephalopods.

MATERIALS AND METHODS

Observations and collections were made in the intertidal zone (primarily during low tides at night) and on the patch, fringing and barrier reefs by skin and SCUBA diving. Thirty-seven stations were occupied at Lizard Island for collection and observation during the Workshop. See Fig. 1 for the location of collecting sites. In the text, stations occupied by the senior author are indicated by the abbreviation CFER. For a complete list of stations and for a checklist of all cephalopods recorded from Lizard Island, see Roper & Hochberg (1987). Station data, diving logs, notes on field and laboratory observations are contained in a notebook on file at the National Museum of Natural History, Washington, D.C.

Cephalopods collected for observation were returned to the Lizard Island Research Station where they were maintained in aquaria supplied with running seawater. Glass-walled aquaria varied from 20–30 liter capacity tanks for small individuals of *Hapalochlaena* cf. *maculosa, Metasepia pfefferi* and *Sepia papuensis* to a large 150–200 liter tank that housed *Octopus cyanea* and *O. ornatus.* Aquaria contained fine, light-colored sand, coral rubble or other material to provide a resemblance of habitat for each species. Activity and behavior of all animals were observed both during the day and at night. Results of observations were recorded or sketched in a notebook (day) or tape recorded (night) for later transcription. Photographs, using Kodachrome 64 film, were taken with a Nikon F 35 mm camera, 55 mm macro lens and one or two Braun electronic flash units. Observations on a live *Hapalochlaena* cf. *fasciata* were made following the Workshop at the Australian Museum, Sydney. The animal was kept for two weeks in a 20 liter aquarium where it was studied and photographed.

Following observation, cephalopods were fixed in 8% buffered sea water formalin. Voucher specimens of all species discussed in this paper are deposited in the Department of Invertebrate Zoology—Mollusks, National Museum of Natural History, Washington, D.C. or in the Department of Malacology at the Australian Museum, Sydney.

The species, number of individuals and museum catalog numbers of these vouchers are: *Octopus cyanea* (2 specimens), USNM 816646 and 816647; *Octopus ornatus* (2 specimens), USNM 816649 and 816650; *Hapalochlaena* cf. *maculosa* (4 specimens), USNM 730598, 730599 and 816623; *Hapalochlaena* cf. *fasciata* (1 specimen), Australian Museum; *Metasepia pfefferi* (14 specimens), USNM 816620 and 816621; *Sepia papuensis* (2 specimens), USNM 816619.

While most of the terminology we use is adapted from other works (see especially Packard & Hochberg, 1977, Hanlon & Messenger, 1988) some terms need definition here. Chronic patterns are long-term (hours) patterns that allow an undisturbed animal to blend in with the substrate or background (crypsis or concealment). Acute patterns are short-term (only seconds or a few minutes) patterns produced in many cases in response to a disturbance. These patterns are striking or vivid in expression and typically stand out in bold contrast to the background. Acute patterns may take a number of forms, among which we define the following:

1) *passing cloud(s)*—conspicuous, pulsat-

ing flushes of dark color that pass in an amorphous front, unidirectionally across the body (see Packard & Hochberg, 1977).

2) *passing wave(s)*—distinct sequence of well-defined bands that pass like a set of wave fronts over the dorsal surface of the body. We introduce this term to distinguish this pattern from passing clouds, since the wave sets move both anteriorly and posteriorly at the same time.

3) *play(s) of color*—small flushes of dark color that can appear randomly anywhere on the body and radiate out from a point source (see van Heukelem, 1966).

4) *flash*—instantaneous expansion of chromatophores that highlights or darkens specific components of body patterns such as the ocelli, maculae of *Hapalochlaena* and mating stripes of *Octopus cyanea*. When expressed these components often appear to pulsate or flash.

5) *flush*—instantaneous expansion of chromatophores that can uniformly darken the entire body or when directed toward an interacting animal or disturbance can darken the body unilaterally or just dorsally (see Fig. 61).

6) *blanch*—instantaneous retraction of chromatophores that uniformly pales or whitens the entire body as in the deimatic (the "dymantic display" of Packard & Sanders, 1969) and mating patterns (see photographs in van Heukelem, 1970); the opposite of flush.

The above acute patterns may be single events (as in a flush or blanch) or multiple (as in passing waves or flashes).

Throughout we define continuous, unbroken lines that are oriented transversely as *bands* and those that run longitudinally as *stripes*. *Bars* are broken or interrupted bands and *streaks* are broken or interrupted stripes. *Diagonals* are short lines that are oriented at angles oblique to bars and streaks. *Maculae* are spots of dark chromatophores that surround the iridescent blue rings of *Hapalochlaena*. Unlike ocelli or eye spots in some *Octopus* species, the diameters of the maculae are not fixed but are capable of expanding or contracting.

Primary papillae are the largest and most conspicuous of the papillae on the body and often are erected for long periods of time. The distribution of primary papillae is fixed morphogenetically and can be used as a diagnostic feature at the genus level. A variety of characteristic shapes may be expressed such as simple conical, compound bifid, compound papillate ridge, flat-truncated flaps, etc. *Secondary papillae* are smaller and expressed only intermittently. They generally are all simple and conical in shape.

Cephalopods, in particular cuttlefishes, *swim* by means of fin undulations, *float* when buoyancy is controlled by the cuttlebone, *hover* when they gently pump water through the funnel and *jet* when they forcibly pump water through the funnel. Octopuses *scuttle* when they move across the bottom using their arms.

KEY TO ABBREVIATIONS

adep, anterodorsal eye papilla
admp, anterodorsal mantle papilla
ads, arm dark stripe
af, ambulatory flap
apw1, anterior passing wave, first wave
apw2, anterior passing wave, second wave
avep, anteroventral eye papilla
aws, arm white spot
dep, dorsal eye patch
dhdf, dorsal head dark field
dhws, dorsal head white spot
dhwt, dorsal head white triangle
dmdf, dorsal mantle dark field
dmds, dorsal mantle dark spot
dmlf, dorsal mantle light field
dmp, dorsal mantle papilla
dmwb, dorsal mantle white bar
dmws, dorsal mid-mantle white spot
flp, finline papilla
flws, finline white stripe
fws, frontal white spot
fwsp, frontal white spot papilla
ldmp, laterodorsal mantle papilla
lhlf, lateral head light field
lmws, lateral mantle white spot
mdmf, mid-dorsal mantle flap
mtpr, mantle tip papillate ridge
mwbf, mantle white bar flap
mwsp, mantle white spot papilla
pdep, posterodorsal eye papilla
pdmf, posterodorsal mantle flap
pdmp, posterodorsal mantle papilla
pdmpr, posterodorsal mantle papillate ridge
phws, posterior head white spot
pmds, posterior mantle dark spot
pmws, posterior mantle white spot
ppw1, posterior passing wave, first wave
ppw2, posterior passing wave, second wave
ppw3, posterior passing wave, third wave

vepr, ventral eye papillate ridge
wws, web white spot

I, dorsal arm
II and III, lateral arms
IV, ventral arm

1, primary
2, secondary

OBSERVATIONS, SYNTHESIS AND DISCUSSION

1. *Octopus cyanea* Gray, 1849.

Common name: Cyane's octopus. "Big blue octopus," the common name normally used, is a misnomer resulting from an incorrect interpretation of the specific name. Gray (1849: 15) named the species *"Cyanea,"* the capital "C" denoting a patronym referring to Cyane in Greek mythology, a nymph of Persephone who was turned into a fountain. Had Gray intended to refer to the color blue, he would have had to use the word "cyaneus," uncapitalized. Hence, the correct common name should be "Cyane's octopus." This is often called the "day octopus" in Hawaii.

A. Synopsis

Diagnosis: Body globose, muscular, mantle length to 100 mm, total length to 1200 mm, total weight to over 5 kg; skin smooth to heavily papillate, with two conspicuous web ocelli; eyes large; arms medium length, subequal, 4–5 times mantle length, thick and muscular; arm formula typically IV.I.II.III; enlarged suckers on all arms of males, especially conspicuous on arms I and II; gills with 9–10 lamellae per demibranch; right arm III hectocotylized, length 75–80% of left arm III; end organ minute, 0.5–1.5% of hectocotylized arm length; ligula bluntly pointed, open with low inrolled edge, groove with faint ridges, calamus small; eggs small, 2.5–3.0 mm long; hatchlings planktonic.

Distribution: Widespread in tropical waters of the Indo-Pacific from Hawaii through the Pacific Islands to Australia, through the Indian Ocean to East Africa and the Red Sea. One of the most common shallow-water octopuses in New South Wales and Queensland, Australia. Found in rocky or coral reef habitats from the intertidal zone to 45 m.

Life history: Reviewed in van Heukelem (1983). This large octopus is commercially important in Hawaii and elsewhere in the South Pacific.

References: Berry, 1914; Le Souef & Allen, 1933, 1937; Boone, 1938; Dew, 1959; Vevers, 1961; Young, 1962; van Heukelem, 1966, 1970, 1973, 1979, 1983; Maginniss & Wells, 1969; Wells & Wells, 1969, 1970, 1972a, b; Yarnall, 1969; Houck, 1982; and Young, Harman & Hochberg, in preparation.

B. Field observations

Two animals of *O. cyanea* were encountered during the study period; both inhabited dens in cemented coralline rock on the shallow reef flat directly offshore from the Research Station on Casuarina Beach. The reef flat habitat is described under the section on *O. ornatus*. Animal 1 was captured at night during low tide (CFER-18; refer to Roper & Hochberg (1987) for station data). It was placed in an aquarium and observed for 12 days. Animal 2 was discovered during a daytime dive on the reef flat at high tide (CFER-27). *In situ* observations on animal 2 continued for nine days.

All *in situ* observations on animal 2 were made during daytime within three hours before or after high tide. The den, located in a pile of rubble and cemented coralline rock, was elevated about 60 cm above the surrounding reef flat. The entrance was littered with a midden of mollusk shells, crustacean parts and coralline pebbles. When examined three days later the den was empty. An intense search along the contour of the reef flat revealed an occupied den about 20 m N of the first den. Although the occupant from den 1 had not been tagged and no unusual markings had been noted, the animal in den 2 was very similar in size and appearance.

On the last day of the Workshop, den 1 was still vacant. Den 2 also was empty, although a pile of fresh shells littered the front entrance. Further searching located a third den 30 m along the reef flat N of den 2. It was occupied by a large *O. cyanea* that appeared to be the same as the individual observed during the preceding week.

Octopus cyanea is a transient den dweller that moves periodically from den to den along the reef in search of food or in response to disturbance (van Heukelem, 1966, 1983). Yarnall (1969) and van Heukelem (1966) reported that *O. cyanea* may use its den for up

to one month or more. In this regard it resembles the behavior of *O. vulgaris* (Hochberg & Couch, 1971) and *O. bimaculatus* (Ambrose, 1982) (see Table 3). *Octopus cyanea* is active during morning and early evening hours and remains quiescent in its den during the day and at night (Houck, 1982). Loch (1980) discussed the hole-drilling technique used possibly by *O. cyanea* to feed on three species of *Cypraea.* Mating, egg laying and brooding have been reported by van Heukelem (1970, 1983) and Le Souef & Allan (1933, 1937).

Although a large number of dives was made by the Workshop participants in the reef flat area adjacent to the Research Station, only two *O. cyanea* were observed. In contrast, over 30 individuals of *O. ornatus* were observed in the same area during only three excursions onto the reef flat at night during low tide. *O. ornatus,* like *O. macropus,* apparently is a more free-ranging species that is active at night (van Heukelem, 1966; Houck, 1982) (see Table 3).

C. Components of body patterns

Octopus cyanea is a robust, medium-sized species similar to *O. vulgaris* or *O. bimaculatus.* The body is heavy set and the arms relatively short and muscular. When occupying its den, *O. cyanea* assumes one of two body postures that we term the "lookout" (Figs. 2–4) and the "guard" (Fig. 5) postures. In the lookout position the head and eyes are raised out of the entrance of the den with the eyes greatly protruded, so the animal can monitor activities around the den (Figs. 2–4). In this posture mantle, head, web and arms are covered densely with both flat-truncate and conical, cream-colored papillae that give the animal a very rugose appearance that blends well with the surrounding habitat. A single, large compound papilla generally is erected over each eye (Figs. 2, 3); three or four additional simple dorsal eye papillae also may be present. The entire body is covered with a light mottle of reddish purple/maroon on a beige/cream background. Two large ovate ocelli ("eye spots") often are visible on the web just below (anteroventral to) the eyes (Fig. 31). The aboral and lateral surfaces of the arms are banded with dark patches of red interrupted by small to medium-sized white spots. The papillate skin and coloration match the irregularly textured and mottled appearance of the substrate. When approached closely or disturbed the animal abandons the lookout posture, flushes a dark mottled maroon/red with alternating dark maroon and cream lines or rays radiating around the eyes (Fig. 5) and then retreats into the den.

In the "guard" posture the octopus is withdrawn into the den and sits sideways just inside the entrance. The eye (either right or left) is erect and peers out over the second and third arms that are extended across the entrance and turned outward so that the largest suckers are exposed and visible (see Fig. 5). In this position the octopus can effectively guard the entrance visually; it also can grasp and test with the exposed suckers anything that approaches the entrance to the den. In the guard position the entire animal is covered with primary cream-colored papillae, both simple, conical as well as flat-truncate papillae. The primary papilla over each eye is leafy and flat and may bear three or four secondary papillae (Fig. 2); primary compound papillae also occur anterior to the eyes, in the mid-line at the junction of the web and head, and one each at the base of the arms. Typical cryptic coloration is a light mottle of red/brown on a cream/beige background. A dark black band extends along the head and through the eye effectively masking it. When disturbed the animal flushes a dark mottle and the eye mask changes to the radiating pattern of light cream and dark red lines mentioned above. Upon repeated disturbance the animal flushes a uniform dark red. The dark flush starts at the head and radiates down the arms and mantle until the animal is a uniform dark color.

When seen in the open or when disturbed enough to leave the den, the animal jets across the bottom. When jetting, the body texture is smooth to granular without conspicuously erect papillae, and the color is light cream mottled with red patches (Fig. 6).

When approached in the aquarium by *O. ornatus,* the captive *O. cyanea* exhibited a typical response in which the body reared up and the arms flared out to present the enlarged suckers. This is equivalent to the "fighting display" of Packard & Sanders (1971). The animal changed from a cryptic color pattern to a uniform dark flush. Such posturing effectively drove off the *O. ornatus.*

An inventory of the currently recognized components exhibited by *O. cyanea* is presented in Table 1. The data are based on our observations of live animals at Lizard Island and on published reports, figures and photographs.

FIGS. 2–5. *Octopus cyanea*. Figs. 2, 3: Frontal view (2) and left lateral view (3); lookout posture with raised flat, truncate dorsal eye papillae. Fig. 4. Frontal, lateral view, left side; lookout posture without raised papillae. Fig. 5. Left lateral view; guard posture with radiating dark and light lines around eye, and exposed suckers. Photographs by W. F. van Heukelem (Honolulu, Hawaii).

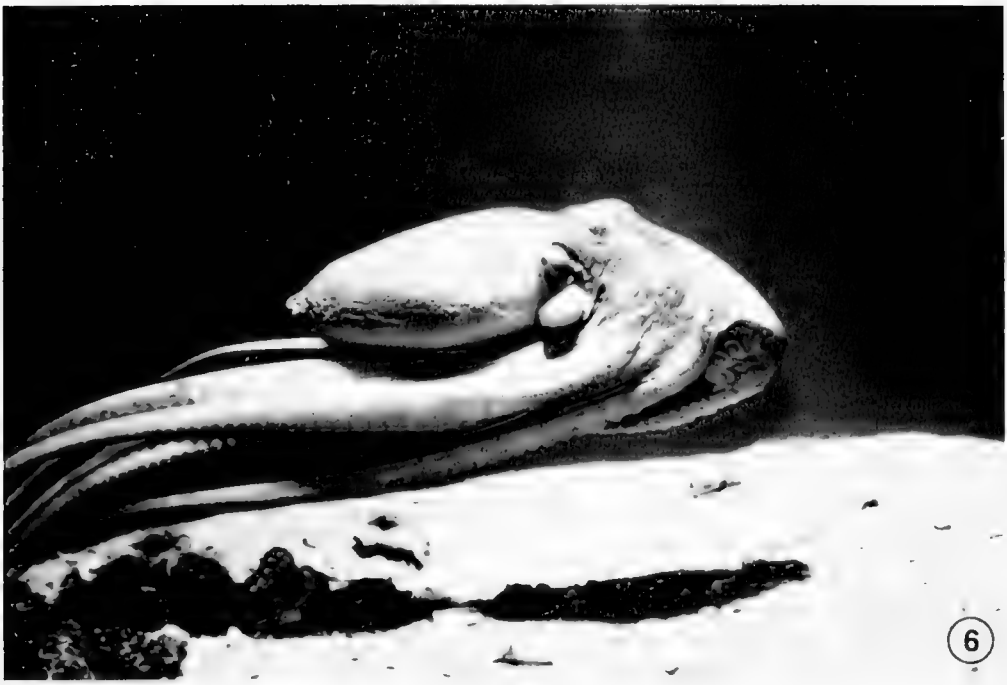


FIG. 6. *Octopus cyanea.* Right lateral view, jetting in forward direction over the bottom in light color phase without erect papillae. Photograph by W. F. van Heukelem (Honolulu, Hawaii).

D. Discussion

The observations on *O. cyanea* at Lizard Island basically confirm those of other workers (especially van Heukelem, 1966, 1983) in that the species is active during crepuscular periods and quiescent during both night and day. A widely-spaced, transient den-dweller, *O. cyanea* on Lizard Island ranges along the reef flat, occupying one den for a few days to several weeks before moving on to the next. Niche and food resource partitioning are discussed in the following section on *O. ornatus.*

Van Heukelem (1983) stressed that *O. cyanea* is capable of showing a large variety of color patterns, textures and postures but that a detailed inventory had not been formulated. Illustrations and brief notes on color and body patterns are in the literature: sexually mature adults (van Heukelem, 1966, 1983; Wells & Wells, 1972b), brooding females (Le Souef & Allan, 1933, 1937), hatchlings (Le Souef & Allan, 1937; Dew, 1959) and newly settled juveniles (Wells & Wells, 1970). Hawaiian specimens of *O. cyanea* were described and figured by Hoyle (1885a, 1886, see also Berry, 1914) under the name *O. marmoratus,* which refers to the color pattern of "ochreous red maculated with purple" and to the series of "intercotyledonary color bands down the surface of the arms."

Taki (1964) provided additional notes on color of a species he described as *Callistoctopus magnocellatus* but this is now known to be a synonym of *O. cyanea.* Taki's species name refers to the presence of a large ocellus that he described as having three parts: a black center 22 mm in diameter, a pale ring and an outer black ring 3–4 mm wide. The total diameter measured 40 mm. In other octopuses the ocellus also is known to contain species specific patterns of chromatophores and iridophores (Hochberg, in preparation); this character needs further detailed documentation.

The best records of the color and patterns of this species are photographs such as those in van Heukelem (1970, 1983), Voss (1971), Roessler (1977) and Travieso (1978). Of all the patterns of *O. cyanea,* perhaps the most colorful and interesting are the dramatic courtship and mating patterns. Although not

observed at Lizard Island these have been photographed and well documented in Hawaii by van Heukelem (1966, 1970, 1983) and by Wells & Wells (1972b).

To prepare a complete inventory of patterns in *O. cyanea,* extensive field and laboratory observations of young and adult animals are needed. In addition, further comparisons of patterns need to be made in widely separated geographic populations.

2. *Octopus ornatus* Gould, 1852.

Common name: white striped octopus. This common name is used to emphasize the pattern of conspicuous white markings on the mantle. This is often called the "night octopus" in Hawaii.

A. Synopsis

Diagnosis: Mantle globular to elongate, muscular, mantle length to 120 mm, total length to 1000 mm, total weight to 500 g; skin granular to rough and warty, purplish red, with conspicuous pattern of white markings on the mantle and elongate oval or round white spots on arms; eyes large; arms very long, attenuate at tips, thick and muscular proximally, 6–8 times mantle length; arm formula I.II.III.IV; enlarged suckers on arms I of males; gills with 12–14 lamellae per demibranch; right arm III hectocotylized, length 60–75% of left arm III; end organ medium sized, 4.5–8% of hectocotylized arm length; ligula elongate, pointed, inrolled edges, groove smooth to faintly striated, calamus small; eggs small, 2–4 mm long; hatchlings planktonic.

Distribution: Widely distributed in tropical waters; Indo-Pacific from Hawaii through the Pacific islands to Australia, into the Indian Ocean to East Africa; common shallow water species, free ranging through reef flat areas, from intertidal to 15 m. This is the first confirmed report of the species in Australia. Known currently only from the Great Barrier Reef, northern Queensland.

Life history: Life span not known. Large numbers of small eggs are laid. Larvae are planktonic upon hatching. Feeds principally on small crustaceans. Active at night. Free ranging predator, without fixed dens.

References: Gould, 1852; Berry, 1914; Boone, 1938; Taki, 1964; van Heukelem, 1966; Yarnall, 1969; Voss, 1981; Houck, 1982; and Young, Harman & Hochberg, in preparation.

B. Field observations

Thirty individuals of *O. ornatus* were found in a 20 × 100 m area of the reef flat immediately offshore from Casuarina Beach and were observed during three low tide surveys conducted on the reef on moonless nights.

At low tide much of the reef flat was exposed leaving a network of tide pools that varied in size from small shallow puddles to pools several meters across with depths to 15–20 cm. The bottom of the tide pools consisted of coarse coralline sand and small pieces of coral rubble. The reef flat habitat consisted of scattered heads of dead coral, coralline algae and rubble, and it appeared to be ideal for *O. ornatus.* The many holes and crevices provided numerous places of protection for resting octopuses, as well as refuge for prey animals, particularly crabs.

Three individuals were observed scuttling over open sandy bottom immediately shoreward of the reef flat, but all others were associated closely with the reef flat pools. One individual hunted by scuttling slowly over the reef flat while the long, sinuous arms were engaged simultaneously in search of prey. The arms were fanned out in all directions, exploring every nook and crevice in the pool. Several arms investigated deep into holes, while others swept under rocks. During the observation period this individual captured two small crabs (unidentified) that immediately were transferred to the web and later devoured.

Although frequent dives were made in the reef flat area during the 12-day period of the Workshop, *O. ornatus* never was observed during the daytime, nor were any found at rest in dens. We assume the animals either hide deep in the reef complex or bury themselves in the sand where they remain quiescent all day. This is in sharp contrast to *O. cyanea,* a crepuscular den dweller, and is similar to the behavior of *O. macropus* (Hochberg & Couch, 1971) (see Table 3).

C. Components of body patterns.

The mantle of *O. ornatus* is elongate and pointed posteriorly. The arms are extremely long, slender and attenuate. The typical cryptic body pattern in the field is a low-intensity, light, brownish-red mottle. The mantle, head, web and arms are covered with light cream to white colored markings (Fig. 31), the vividness of which is controlled by overlying dark

TABLE 1. The components of body patterns in *Octopus cyanea*.

I. Chromatic components		
A. Light	B. Dark	C. Other
arm white spots[2]	dark arm bands	ocelli
dorsal head white stripe	dark arm stripes[1]	
dorsal mantle white stripe[2]	dark arm tips[1]	
white mantle (blanch)[1]	dark uniform mantle[1]	
white web (blanch)	dark anterodorsal mantle patch[1]	
light mantle bands	dark head[1]	
	dark eye stripe	
	dark eye region[1]	
	radiating eye lines	
II. Textural components		
A. Primary papillae	B. Secondary papillae	C. Other
(compound, flat)	(simple, conical)	granular
dorsal eye papillae (1/eye)	dorsal eye papillae (3 or 4/eye)	smooth
mantle papillae	uniform body papillae	
arm base papillae (1/arm)		
III. Postural components		
radiating arms	standing[1]	
curled arms	lookout (= alert[1])	
coned arms	guard	
flared arms	submissive:	
raised head & eyes	male/male[1]	
enlarged sucker presentation	male/female[1]	
sucker shield[1]	flared web	
IV. Locomotor components and maneuvers		
sleeping	prey drilling	
resting/sitting	inking	
scuttling	ritualized fighting[1] (= territorial defense)	
jetting	distance copulation[1,4]	
escape (body first)	courtship strut[1]	
stalking (arms first)[1]	bobbing[1]	
hunting/prey capture	escape (body first)	
speculative pounce[1,3]	grooming[1]	
attack jump	burying	
food gathering under web		
V. Body patterns		
A. Chronic	B. Acute	
light cryptic mottle (grey, light brown)	dark conflict mottle	
light uniform	dark uniform (red or brown flush)	
sleeping[1]	light uniform (blanch):	
	deimatic (white w/ocelli[1])	
	copulatory (white w/o ocelli[1])	
	courtship stripes (male)[1,4]	
	dominant male mantle stripes[1]	
	flamboyant[1]	
	plays of color[1]	

[1]Van Heukelem (1966, 1970, 1983)
[2]Roessler (1977)
[3]Yarnall (1969)
[4]Wells & Wells (1972b)

TABLE 2. The components of body patterns in *Octopus ornatus*.

I. Chromatic components		
A. Light	B. Dark	C. Other
dorsal mantle white stripes or streaks	dark uniform	bluish-green iridescence
dorsal mantle white spots		
head white spots		
frontal white spots		
arm white spots (2 rows/arm)		
II. Textural components		
A. Primary papillae	B. Secondary papillae	C. Other
compound bifid mantle tip papillae	Simple uniform body papillae	granular
simple lateral mantle tip papillae		smooth
compound bifid dorsal eye papillae (1 or 2)		
simple frontal white spot papillae (1/white spot)		
simple arm base papillae (1/arm)		
III. Postural components		
radiating arms		
coned arms		
elongate pointed mantle		
IV. Locomotor components and maneuvers		
resting/sitting		
scuttling		
jetting		
inking		
hunting w/arms		
V. Body patterns		
A. Chronic	B. Acute	
light cryptic mottle	dark conflict mottle	
	dark uniform (flush)	
	deimatic	

chromatophores. White spots on the arms are squarish with rounded corners and arranged in two regular rows; they decrease in size to the tips of the arms. Spots on the web and head are oval and they become elongate interrupted stripes or streaks on the mantle. When a light is shone on the animal at night the white spots, stripes and light patches on the lateral surfaces of the suckers and the circumference of the suckers are visible as a vivid bluish-green iridescence (see comments by Roper & Young in Voss, 1981: 533). Body patterns observed in the field appeared identical to those seen in the aquaria and are described below. All laboratory observations were on animals captured at CFER stations 18 and 20.

During the night, inactive or resting animals sit in the typical octopus posture with the head and mantle lying horizontally over the relaxed, radiating arms and web. The mantle is finely granular, while the head and arms are smooth. The posterior tip of the mantle has several papillae. A large, bifid papilla is erect at the very tip of the mantle during jetting, while the rest of the body is smooth. Two or three simple, secondary papillae occur along each side of the lateral tip of the mantle. Above each eye is one or two low, broad, bifid papillae. A papilla also is present anterior to the eyes in the center of each white spot on the web at the base of each arm. Resting animals have a light to medium dark cryptic mottle with large subdued white spots on the head, web and arms. White markings are not visible on the dorsal mantle. When disturbed

TABLE 3. Comparison of factors for coexistence of *Octopus cyanea* and *O. ornatus*.

Factor/species	*O. cyanea*	*O. ornatus*
Body form	medium sized, robust	small, sleek
Arms	robust, normal length	very long, attenuate
Activity period	crepuscular, dawn and dusk	nocturnal
Dwelling	"territorial" in fixed den sites, periodic transient	temporary shelters, changed daily, "non-territorial"
Hunting sites	in immediate home range	over broad range
Prey	mollusks (secondarily crabs)	crabs
Mode of life	reclusive, solitary	nomadic
Relative abundance	low	high
Comparable species in other areas	*O. vulgaris* (Mediterranean, Caribbean) *O. bimaculatus* (California, Mexico)	*O. macropus* (Mediterranean, Caribbean) *O. alecto* (Mexico)

the animal first darkens the mottle and then produces a uniform dark flush that covers the entire body.

All arms are engaged in locomotion when the animal scuttles rapidly across the bottom at night. During this type of locomotion the mantle is elongate and pointed posteriorly. The entire body is smooth except in the posterior region where several primary papillae are erect. The animal shows a darkened mottle in which the white markings are visible.

While the animal is at rest during daytime, the coloration is a deep brownish red reticulation with subdued white markings. Occasionally three small white spots may show on the dorsal mantle, one in the midline just posterior to the junction of the mantle and head and two (the dorsal mantle white spots) lateral to the midline and in the anterior third of the mantle.

When disturbed, *O. ornatus* jets mantle-end first across the bottom. The mantle is elongate and pointed and the arms are brought together in a cone. The body is streamlined with the skin entirely smooth except for the above-described primary papillae erected on the posterior tip of the mantle. Color is a light to dark mottle to nearly uniform dark with vivid white markings.

Table 2 presents the currently recognized components of body patterns of *O. ornatus* based upon our observations and a compilation from published reports and photographs.

D. Discussion

Octopus ornatus has been studied primarily in Hawaii (van Heukelem, 1966; Houck, 1982). The Lizard Island animals represent the first records of this species in Australian waters, and our observations confirm those made in Hawaii. On Lizard Island, animals are conspicuously abundant at night as they range across the reef flats actively hunting cryptic prey, primarily crabs, with very long, attenuate arms. During the day the animals retreat to temporary shelters of convenience, rather than to any permanent den site, or they may burrow into the sand.

The observations on *O. ornatus* and *O. cyanea* living sympatrically on a small area of patch reef suggest a system of niche and food resource partitioning. Table 3 presents the currently recognized morphological and ecological characteristics that permit these two species to coexist in the same habitat. The existence of ecomorphs has been demonstrated in other groups. We suggest that this phenomenon occurs between *O. cyanea* and *O. ornatus* and may occur in a number of sympatric octopod species in various parts of the world.

Little information is available on the color, texture and body patterns of *O. ornatus*. A color plate by J. Drayton appeared in the original description by Gould (1852) along with notes on the color of the living animal. Taki (1964) illustrated his description of *Callistoctopus asakawai* (now regarded as a synonym of *O. ornatus*—see Voss, 1981) with a color painting of a preserved specimen and several black and white photographs of live animals. Additional brief references to color and texture are noted in Berry (1914), Boone (1938), van Heukelem (1966) and Voss (1981). The majority of these papers concentrate on the striking pattern of white spots or stripes but do not provide other color or pattern observations. Van Heukelem (personal communication) reported differences in

basic ground color (from deep orange to dark purplish brown to deep reddish brown) that appear to have a geographic or populational basis. This may be an artifact of observation and preservation or an indication that a complex of cryptic species may be involved.

Van Heukelem's photograph (1970) of *O. ornatus* in Hawaii is reproduced here (Fig. 31) for reference to the conspicuous pattern of white markings of this species. *O. ornatus* closely resembles *O. macropus* (see Hanlon, this volume, for comparison photograph), a species that has white spots instead of stripes on the mantle. *O. macropus* has been reported in Australia (Lu & Phillips, 1985). Careful study and comparison of *O. ornatus* and *O. macropus* should be made, since in many parts of the world their distributions appear to overlap.

3. *Hapalochlaena* cf. *maculosa* (Hoyle, 1883)

Common name: Lesser blue-ringed octopus. We introduce this name to draw attention to the very small blue rings (1–2 mm in diameter) on the mantle, head, web and arms.

A. Synopsis

Diagnosis: Mantle small, elongate, ovoid, pointed posteriorly; mantle length to 30 mm; total length to 80 mm; total weight to 40 g; skin wrinkled or densely covered with papillae, with conspicuous pattern of dark maculae and small iridescent blue rings (1–2 mm in diameter) on dorsal mantle, head, web, and arms, ventral head and mantle without rings; eyes large and prominent; arms short, subequal, 1.5 to 2 times mantle length; typical arm formula III.II = IV.I; suckers small, numerous, none enlarged; gills with 6 or 7 lamellae per demibranch; right arm III hectocotylized, length 70 to 80% of left arm III; end organ medium size, 4 to 6% of hectocotylized arm length; ligula smooth; calamus small; egg size unknown.

Distribution: This species appears to be widespread, perhaps as a complex of species, all around Queensland, Victoria, Tasmania, South Australia and Western Australia. In tropical waters it occurs close to coral reefs in shallow water to 25 m, on sandy silt bottom, apparently in association with attached green algae. In temperate waters it occurs in rock reefs and rock rubble areas, tide pools, mollusk shells, bottles and cans.

Life history: Unknown.

References: See *H.* cf. *fasciata.*

B. Field observations

Four individuals of *Hapalochlaena* cf. *maculosa* were collected during the day in Mrs. Watson's Bay. Animal 1 was discovered in 14 m (CFER-25) and animal 2 in 20–22 m (CFER-28). Both were found on open sand/silt bottom devoid of rocks but scattered with coral rubble and living solitary corals. Conspicuous fauna and flora included numerous black holothurians, probably *Holothuria atra* or *H. edulis,* scattered patches of attached green algae, *Caulerpa cupressoides* and *Halimeda* sp. When first sighted, animal 1 looked like a piece of alga.

Animals 3 and 4 were captured at 12 m (CFER-42) on a mixed sand and silt bottom dominated by a dense stand of *Halimeda* sp., scattered *Caulerpa cupressoides,* many large black holothurians and a few brown-yellow holothurians (probably species of *Holothuria, Pentacta* or *Stichopus*). Both animals were observed in close association with *Halimeda* plants. Animal 3 was clinging with the tips of its outstretched arms to the upright branches of the alga about 3 cm above the bottom. The skin showed cryptic coloration of mottled olive-green/brown and was finely papillate; this patterning very closely matched the color and texture of the *Halimeda.* No spots or blue rings were evident. When capture was attempted with a small net, the animal escaped and flashed a vivid pattern of dark patches (maculae) surrounding bright, iridescent blue rings. The animal immediately dropped to the bottom and resumed a pale cryptic pattern to match the grey/beige color of the substrate. All rings and patches disappeared. When finally captured, it again flashed its dark chromatophore patches and iridescent blue rings. This behavior also was elicited in the aquarium and is described in detail below.

When animal 4 was first approached by the diver, it was hovering in a motionless position immediately above a *Halimeda* plant. The animal was oriented with the body at a 45° angle to the bottom and the arms drooping downward. A cryptic color pattern and papillate texture covered the entire body. When the diver was sighted the animal instantly dropped to the bottom, as if in a "free fall," and assumed the characteristic deimatic pat-

tern with arms spread out, web expanded and a pale color. Upon capture the animal flashed its vivid blue rings and surrounding dark maculae, then flushed dark reddish brown.

The *Halimeda* plant to which animal 3 was clinging was inhabited by a rich fauna of epiphytes that included small gastropods, scallops and other bivalves, a small stomatopod and several hermit crabs and swimming crabs. This would seem a rich source of food for a small octopus like *Hapalochlaena*. In the laboratory the animals were maintained on small *Ocypode* crabs. *Hapalochlaena* cf. *maculosa* also has been observed clinging to *Caulerpa cupressoides,* another green alga that provides habitat for a diversity of epiphytic organisms, including the gastropods *Engina* and *Anachis* visible in Fig. 14.

C. Components of body patterns

The Lizard Island *Hapalochlaena* have a distinct and fixed pattern of small rings 1–2 mm in diameter (Figs. 7, 8) that differ from the rings and lines of the other species. In *H. lunulata* they are fewer in number and larger, 7–8 mm in diameter (Fig. 13), whereas in *H.* cf. *fasciata* the mantle has a pattern of blue lines (streaks and diagonals) instead of rings (Figs. 11, 12). The configuration, dimensions and distribution of these characters often are obscured or distorted in preserved specimens, but they are very distinct and prominent in living animals when they are disturbed.

The ring/macula complex is similar in appearance and function to the ocelli of 2-spotted octopuses such as *O. bimaculoides* (see Packard & Hochberg, 1977). The center and periphery of each ring are raised and densely invested with dark chromatophores. In the region of the ring itself, the skin overlying the blue iridophores is transparent or translucent and indented. When *Hapalochlaena* cf. *maculosa* is resting, a cryptic mottle pattern is assumed. In this pattern the blue rings essentially are invisible and the dark maculae are subdued to invisible (Fig. 18). As the chromatophores are expanded and the maculae darken, the vividness of the blue rings intensifies in a fashion similar to the expression of the blue rings in ocelli (Figs. 16, 17, 26–28). When the chromatophores are partially expanded the centers of the rings are lighter than the maculae surrounding the rings, but when the chromatophores are maximally expanded over the entire body, the centers are very dark and the blue rings may be obscured (Fig. 15). A single, small, conical papilla often is erected in the center of each ring.

During the day when the animals are inactive and resting on a sandy bottom in the aquarium the eyes are erect. The arms either are held straight out with the tips curled aborally, or they are curled and tucked up under the body. Often the animal sits partially buried in the sandy substrate. Small conical papillae are erected over the entire body. Four large, primary, light-colored, conical papillae are present on the dorsal mantle and two large compound papillae are located on the head above the eyes. A prominent light-colored, papillate ridge is located ventral to each eye. A large, flat, papillate ridge is located in the midline on the posterior dorsal mantle. The posterior tip of the mantle often is drawn out into a point with an elongate, flat, papillate ridge (Figs. 8, 9).

Resting, inactive animals all exhibit cryptic color patterns that match the background. The body and arms are either uniform light beige or a beige mottled with light brown that corresponds to the mantle and arm maculae. The iridescent blue rings are only faintly visible on the mantle, but they are slightly more conspicuous on the arms. A broad light beige stripe, the dorsal mantle light stripe, extends along the dorsal midline from the head to the posterior tip of the mantle (Fig. 15). In the mid-region of the dorsal mantle there are two conspicuous, irregularly shaped, white spots at the center of which is a single, large, mantle papilla (Figs. 7, 8, 16). A third white spot is present on the posterior mantle midline. A posterior head white spot is present on the head just posterior to the eyes. In this species it is in the shape of a curved bar or crescent. A frontal white spot is located on the head just anterior to the eyes, and a single conical papilla is present in the center of this spot. All five white spot regions appear to be underlain by dense patches of leucophores (see Figs. 7, 8, 16).

At night *Hapalochlaena* cf. *maculosa* rests with the arms curled under the body. The skin texture is granular to minutely papillate and the color a darker or more intense mottle. The frontal white spot is especially conspicuous at night in the beam of a flashlight.

Animals in aquaria were observed in a few instances to bury themselves partially in

the sand. Burying behavior is initiated as the ventral arms push sand aside laterally. As the excavation continues, arms III help to enlarge and deepen the depression. Jets of water from the funnel are not used. Only the ventral half of the mantle, head and arms III and IV are buried. The skin is uniformly papillate and the color is a light cryptic mottle that matches the substrate perfectly. This behavior was not observed in the field; however, field observations were quite limited.

Occasionally animals jet about the aquarium without being stimulated by the observer. The body is smooth except for the large, primary, mantle papillae, which are erect. The color is a uniform pale beige, and maculae and rings are not visible. In contrast, animals that are disturbed jet away in an escape reaction; the body is entirely smooth with papillae retracted. When animals are disturbed the color flushes from an intense yellowish beige mottled with dusky brown maculae to a very dark mottle. Next, the dark maculae enlarge and coalesce, coloring the octopus a uniform dark reddish brown. Often ripples of color, in the form of "passing clouds" move over the body surface. A dark stripe extends along each side of the mantle and head and through the eye, then tapers along the lateral edge of the dorsal arms nearly to the tips (Figs. 9, 10). The other arms are an intense yellow. The blue rings and associated dark maculae pulsate in synchrony, which gives the appearance of flashing rings. Ring/macula flashes initially are directed unilaterally toward a disturbance (Fig. 26). If the disturbance continues, the ring/macula flashing pattern becomes bilateral as it darkens and intensifies, then spreads wave-like over the entire dorsal and lateral surfaces of the mantle, head, web and arms (Fig. 27).

When a disturbance persists or is very intense, young *Hapalochlaena* cf. *maculosa* discharge a cloud of thin, reddish brown ink. The ink rapidly dissipates and does not congeal or "hang" in the water as a pseudomorph, as is typical in other *Octopus* species. Up to 10 clouds of ink were discharged during a period of repeated disturbances. The ink sac degenerates with growth, so that full-grown adults lack the ability to produce ink.

Table 4 presents the currently recognized components of body patterns of *H.* cf. *maculosa* based on observations and photographs at Lizard Island.

D. Discussion

A discussion of *Hapalochlaena* species appears at the end of the section on *H. lunulata.*

4. *Hapalochlaena* cf. *fasciata* (Hoyle, 1886)

Common name: Blue-lined octopus. We introduce this name to draw attention to the distinct blue lines on the mantle of this species. Because of taxonomic confusion, this species frequently has been called the "blue-ringed octopus," but we recommend that use of this common name be restricted henceforth to the true "ringed" species.

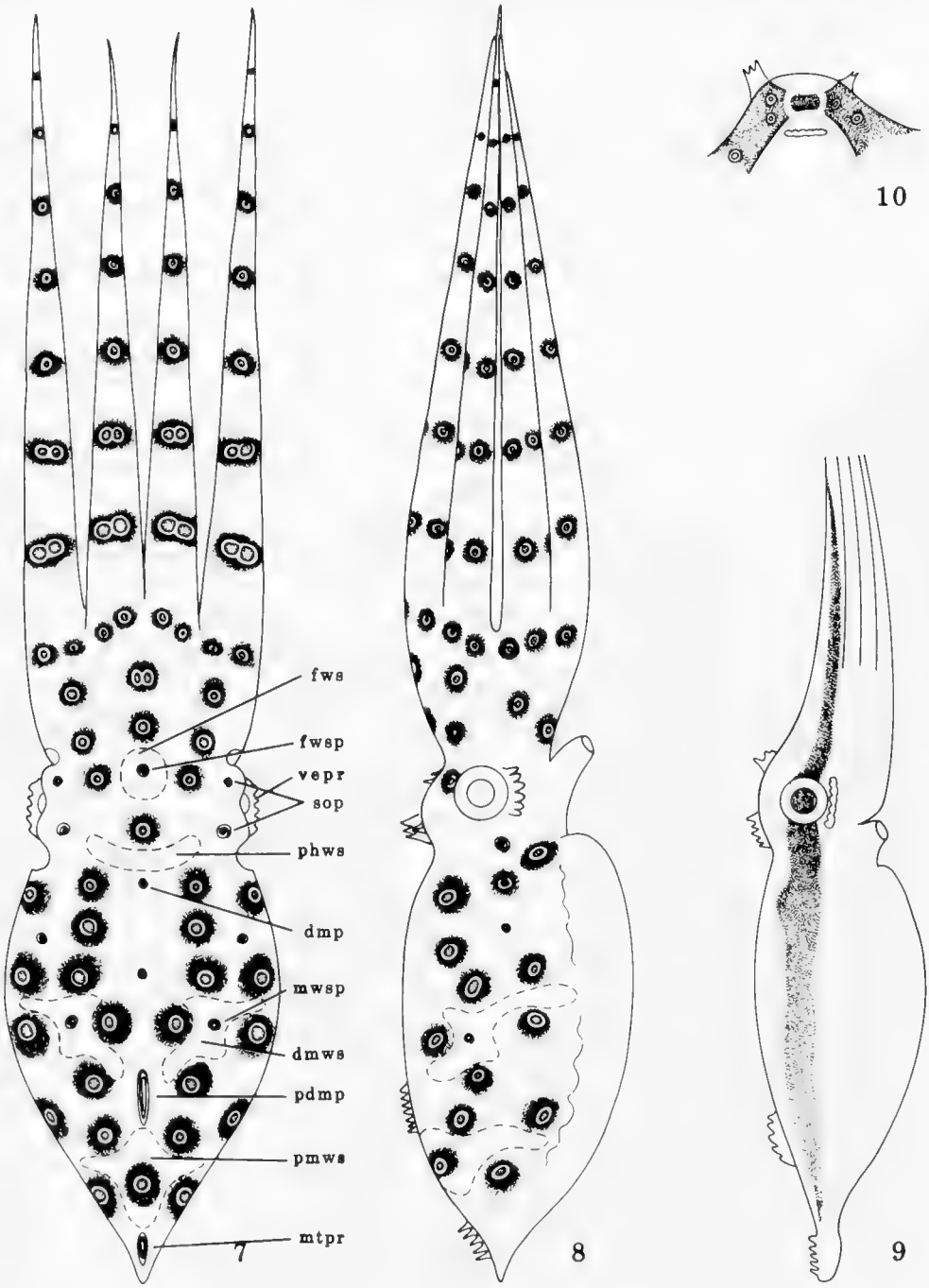
A. Synopsis

Diagnosis: Mantle small, elongate, ovoid, pointed posteriorly; mantle length to 40 mm; total length to 110 mm; total weight 40–50 g; skin papillate, with conspicuous pattern of dark elongate maculae and iridescent blue lines on mantle and dark maculae with small iridescent blue rings on head, web and arms; eyes small; arms short, 1.5 to 2.5 times mantle length, subequal; typical arm formula IV.III.II.I; suckers small, numerous, none enlarged; gills with 4 or 5 lamellae per demibranch; right arm III hectocotylized, length 95% of left arm III; end organ medium to large, 7–9–12% of hectocotylized arm length; ligula flat and smooth, calamus large; eggs 7–9 mm long.

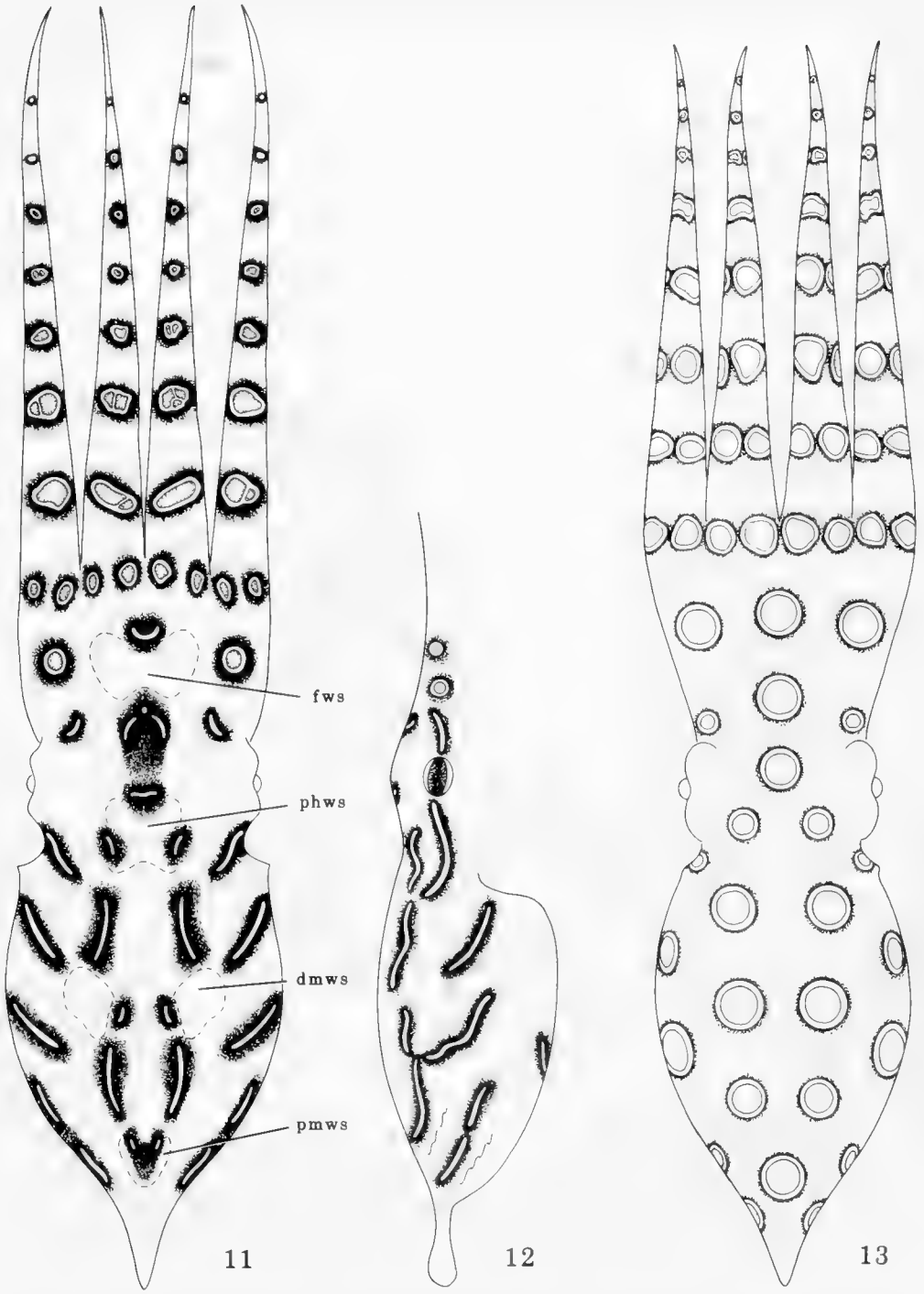
Distribution: Australian endemic. Temperate to subtropical, apparently restricted to New South Wales. Widespread in shallow, sheltered coastal waters from the intertidal zone to depths of 10 to 30 m. Typically found along rocky shores where it lives in crevices, rock pools and underwater caves. Also found in bays, living under rocks, in grass beds and in debris such as cans, bottles and empty bivalve shells (scallops, oysters and mussels).

Life history: Life span 7 to 9 months. Sexually mature at 4 months. In the Sydney area, spawning occurs in March and from September to December. 100 to 200 large eggs (7 to 9 mm) laid by female and brooded loosely in web and arms. Embryonic development takes about 60 days. Development direct; young benthic upon hatching. Food primarily crabs, but bivalves also may be eaten.

References: Anon., N.D.; McMichael, 1957, 1958, 1964, 1971; Dew, 1959; Hopkins, 1964; Lane & Sutherland, 1967; Sutherland &



FIGS. 7–10. *Hapalochlaena* cf. *maculosa*. Figs. 7, 8: Stylized dorsal (7) and right lateral (8) views to show size and distribution pattern of blue rings (clear circles) surrounded by dark maculae (black stipple), primary papillae (concentric, spiral and peaked lines) and white spots (encompassed by dashed lines). Fig. 9. Right lateral view, jetting octopus with dark lateral stripe along arms, head and mantle. Fig. 10. Right lateral view of head and eye (anterior to right) with dark lateral stripe.



FIGS. 11–13. *Hapalochlaena* cf. *fasciata*. Figs. 11, 12 and *H. lunulata*, Fig. 13: Stylized dorsal (11, 13) and right lateral (12) views to show size and distribution pattern of blue lines and rings (clear lines and circles) surrounded by dark maculae (black stipple) and white spots (encompassed by dashed lines).

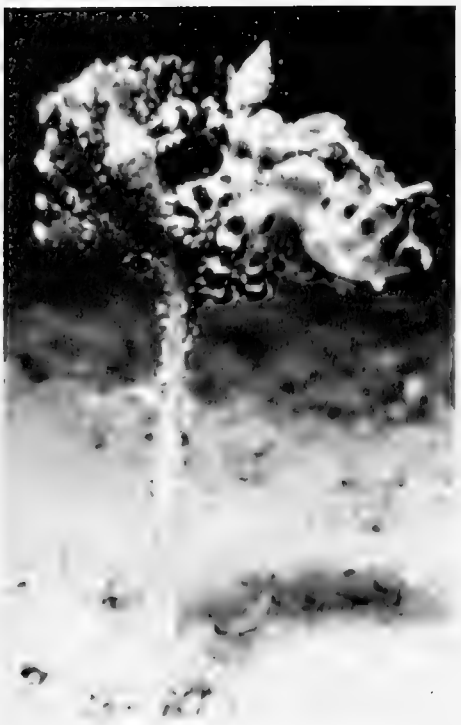


FIG. 14. *Hapalochlaena* cf. *maculosa*. Attached to the green alga *Caulerpa cupressoides*, in Watson's Bay, Lizard Island. Left lateral view; note prominently erected papillae on mantle and ventral to the eye. Epiphytic gastropods are *Engina* and *Anachis*. Photograph by N. Coleman.

Lane, 1969; Deas, 1970; Freeman & Turner, 1970; Croft & Howden, 1972; Cropp, 1972; Friese, 1972; Tranter & Augustine, 1973; Reynolds, 1983; Keith, 1986; Marsh & Slack-Smith, 1986. (Note: Unless illustrations or photographs are provided, identifications in the literature cannot be relied upon with regard to *H.* cf. *maculosa* and *H.* cf. *fasciata*, hence both are combined here.)

B. Field observations

None.

C. Components of body patterns

A single individual of *H.* cf. *fasciata* (Hoyle, 1886) was observed for 14 days in an aquarium at the Australian Museum in Sydney. This species is distinct from those observed at Lizard Island (*H.* cf. *maculosa*) and the species traditionally identified as *H. lunulata*. We consider the species commonly collected in New South Wales to be *H.* cf. *fasciata*. For comparison, we present information on the color and body patterns of the New South Wales specimen (Figs. 19–25). Body proportions differ in *H.* cf. *fasciata* from those in *H.* cf. *maculosa*. The arms are longer and more attenuate and the mantle is slightly larger and more robust, although still distinctly elongate and tapered posteriorly (Figs. 19–21). The normal background coloration is a deep orange/yellow. The dorsal and lateral surfaces of the mantle are covered with a regular pattern of iridescent blue lines, not rings (Figs. 11, 12, 19–25). The lines are oriented on the body as streaks or diagonals. The ventral surface of the mantle lacks lines or rings. Small blue rings are present on the head, web and arms. Those on the arms coalesce proximally into large irregularly shaped rings or transverse figure-8's and distally into dots of blue (Fig. 11). The body can be covered with small conical and large flat papillae or papillate ridges. The morphogenetic fields or patterns of distribution of papillae appear to be similar to the Lizard Island species, *H.* cf. *maculosa*. Rows of small papillae occur in the light areas between the dark lines on the lateral mantle but not within the maculae. In *H.* cf. *fasciata* three to four low, connected papillae lie ventral to the eye rather than a strong, raised papillate ridge, as in *H.* cf. *maculosa*.

Behavioral responses manifest in color and body patterns are very similar to the species at Lizard Island. At rest, the cryptic pattern is a uniform light grey/beige (Fig. 22). The iridescent blue mantle lines and the head, web, and arm blue rings are visible but subdued, the maculae are absent, and the texture is finely granular with minute papillae. With increasing disturbance (Figs. 20, 23–25) the animal flushes to a uniform dark charcoal or slate grey and the iridescent blue lines and rings become very intense surrounded by maculae of very dark chromatophores. In this pattern the primary papillae may be erect and prominent.

Table 5 presents an inventory of components of body patterns currently recognized for *H.* cf. *fasciata* based upon observations and photographs of a living animal in Sydney and on the literature.

D. Discussion

A discussion of *Hapalochlaena* species appears at the end of the section on *H. lunulata*.

TABLE 4. Components of body patterns in *Hapalochlaena* cf. *maculosa*.

I. Chromatic components		
A. Light	B. Dark	C. Other
light-colored papillae	dark maculae (spots on mantle, web and arms)	iridescent blue rings
dorsal mantle stripe	dark lateral stripe	
dorsal mid-mantle white spots (dmws, 2)		
posterodorsal mantle white spot (pmws)		
posterior head white spot (phws)		
frontal white spot (fws)		
transverse white streak (tws)		
II. Textural components		
A. Primary papillae	B. Secondary papillae	C. Other
simple dorsal mantle papillae (dmp, 4)	minute simple uniform body papillae	granular
compound dorsal eye papillae (adep, pdep, 2/eye)	simple ring papillae (1 in center of each ring)	smooth
compound ventral eye papillate ridge (vepr, 1/eye)	simple intermacular lateral mantle papillae	
compound flat posterior dorsal mantle papillate ridge (pdmpr, 1)		
compound flat posterior mantle tip papillate ridge (mtpr, 1)		
simple dorsal mantle white spot papillae (mwsp, 1/spot)		
simple frontal white spot papilla (fwsp, 1)		
extended posterior mantle tip		
III. Postural components		
radiating arms		
curled arms		
coned arms		
drooping arms		
flared web		
elongate pointed mantle		
raised head and eyes		
IV. Locomotor components and maneuvers		
resting/sitting		
hovering		
scuttling		
jetting		
inking		
free fall		
burying		
V. Body patterns		
A. Chronic	B. Acute	
light cryptic mottle	dark conflict mottle	
light uniform	dark uniform (flush)	
	deimatic (white blanch)	
	flashing maculae and blue rings	
	flamboyant	

5. *Hapalochlaena lunulata* (Quoy & Gaimard, 1832)

Common name: Greater blue-ringed octopus. We introduce this name to draw attention to the large blue rings (7–8 mm in diameter) on the dorsal mantle, head, web and arms.

A. Synopsis

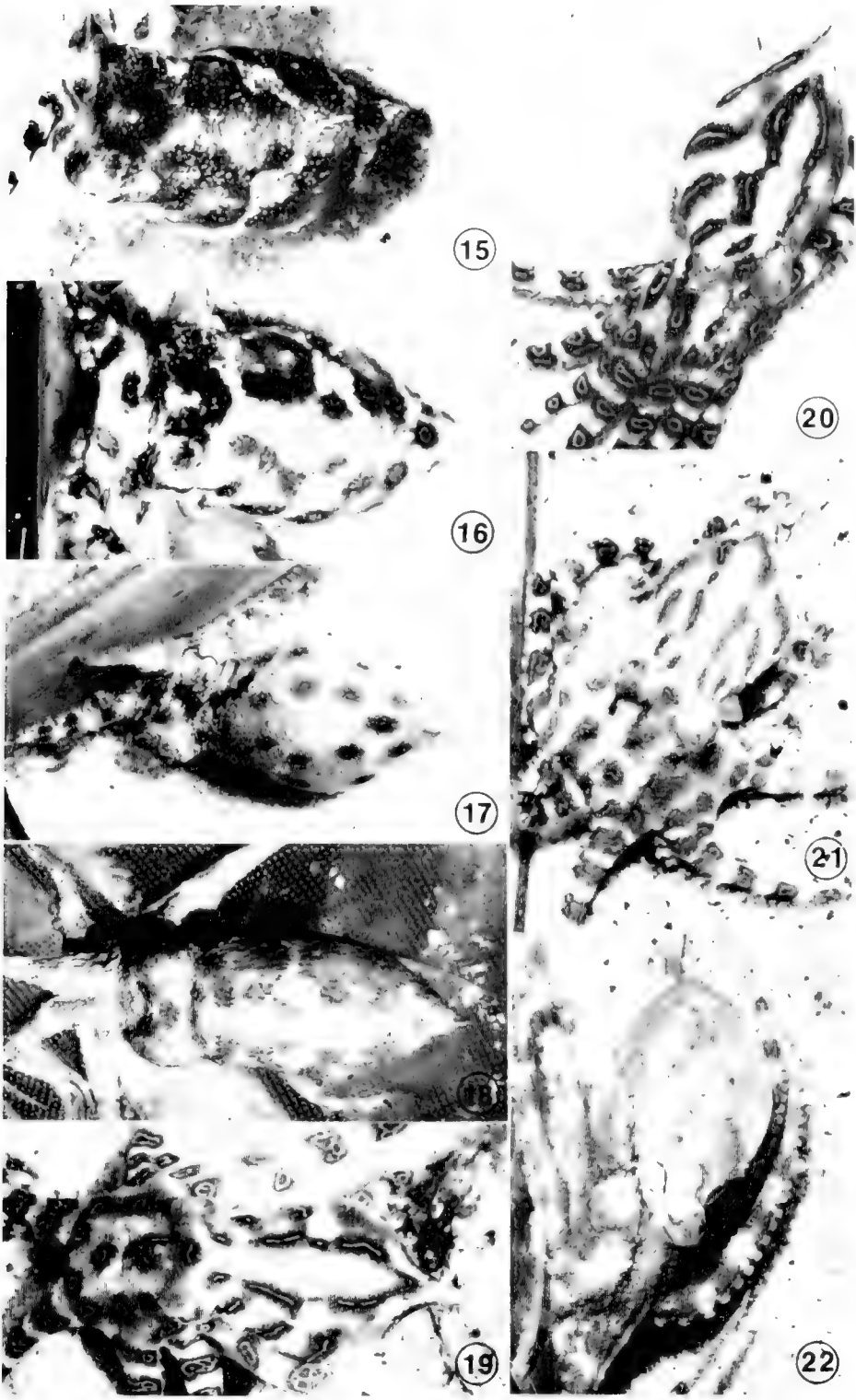
Diagnosis: Mantle ovoid, pointed posteriorly, slightly flattened dorsoventrally; mantle length to 55 mm; total length to over 100 mm; total weight to 80 g; skin soft and semi-gelatinous, mantle, head, web and arms with

TABLE 5. The components of body patterns in *Hapalochlaena* cf. *fasciata*.

I. Chromatic components		
A. Light	B. Dark	C. Other
dorsal mantle stripe	dark maculae (spots on web and arms)	irridescent blue lines and rings
dorsal mid-mantle white spots (dmws, 2)	dark lines (on mantle)	
posterodorsal mantle white spots (pmws)	dark orange/yellow	
posterior head white spot (phws)		
frontal white spot (fws)		
II. Textural components		
A. Primary papillae	B. Secondary papillae	C. Other
simple dorsal mantle papillae (4)	minute simple uniform body papillae	granular
compound dorsal eye papillae (2/eye)	simple intermacular lateral mantle papillae	smooth
compound ventral eye papillate ridge (1/eye)		
compound flat posterior dorsal mantle papillate ridge		
compound flat posterior mantle tip papillate ridge		
simple dorsal mantle white spot papillae (1/spot)		
simple frontal mantle white spot papilla		
extended posterior mantle tip		
III. Postural components		
radiating arms		
curled arms		
coned arms		
erect head & eyes		
elongate pointed mantle		
copulatory embrace[1]		
IV. Locomotor components and maneuvers		
resting/sitting		
scuttling		
jetting		
inking		
mating[1]		
V. Body patterns		
A. Chronic	B. Acute	
light cryptic mottle	dark conflict mottle	
light uniform	dark uniform (flush)	
	flashing maculae, blue lines and rings	
	flamboyant	

[1]Tranter & Augustine (1973).

FIGS. 15–22. *Hapalochlaena* cf. *maculosa*. Figs. 15–18, dorsal views of resting animals and *H.* cf. *fasciata*, FIGS. 19–22, dorsal views. Fig. 15. Dark mottle; blue rings completely subdued; maculae dark, expanded and coalesced; mantle and head white spots and dorsal mantle white stripe expressed but subdued. Fig. 16. Light mottle; blue rings and maculae expressed, especially on right side (top) facing disturbance; all mantle and head white spots expressed. Fig. 17. Light uniform; blue rings expressed; maculae subdued, separate. Fig. 18. Dark uniform; blue rings greatly subdued; maculae expanded and coalesced. Fig. 19. Scuttling locomotion while disturbed; blue lines and rings and maculae dark, expressed moderately; mantle, head and frontal white spots evident. Fig. 20. Warning pattern; blue lines and rings expressed vividly; maculae dark, white spots subdued to absent. Fig. 21. Resting after disturbance; blue lines and rings expressed moderately; maculae subdued; white spots subdued. Fig. 22. Resting position; light uniform; blue lines and rings very subdued; maculae absent; no white spots expressed except subdued frontal spot; mantle tip rounded.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conspicuous pattern of large iridescent blue rings (7–8 mm in diameter) with broad dark maculae around the outer periphery and clear centers; eyes small; arms short, 1.5 to 2 times mantle length, subequal; typical arm formula IV.III.II.I; suckers few, level with oral surface of arms; gills with 7 to 9 lamellae per demibranch; right arm III hectocotylized, length 80 to 90% of left arm III; end organ medium-sized, 7 to 9% of hectocotylized arm length; ligula flat, smooth, with slightly elevated edges; calamus small, open; eggs 2.5 to 3.5 mm long; hatchlings planktonic.

Distribution: Widely distributed throughout the Indo-West-Pacific and Indian Oceans. Australia, New Guinea, Philippines, Sri Lanka, Vanuatu Is., Solomon Is., Misal Is., Andaman Is. In Australia the species has been recorded in Queensland, Northern Territory, and Western Australia. Little information is available on vertical distribution and habitat.

Life history: Life span not known. In the Philippines, spawning occurs in March and April. 60 to 100 small eggs laid by female and attached to substrate in festoons of about 20 eggs each. Embryonic development takes about 25 to 35 days. Hatchlings are briefly planktonic prior to becoming benthic. Species feeds actively on crabs and bivalves.

References: Adam, 1954; Flecker & Cotton, 1955; McMichael, 1957, 1971; Overath, 1973; Overath & Boletzky, 1974; Marsh & Slack-Smith, 1986; Wells & Bryce, 1986.

B. Field observations

None.

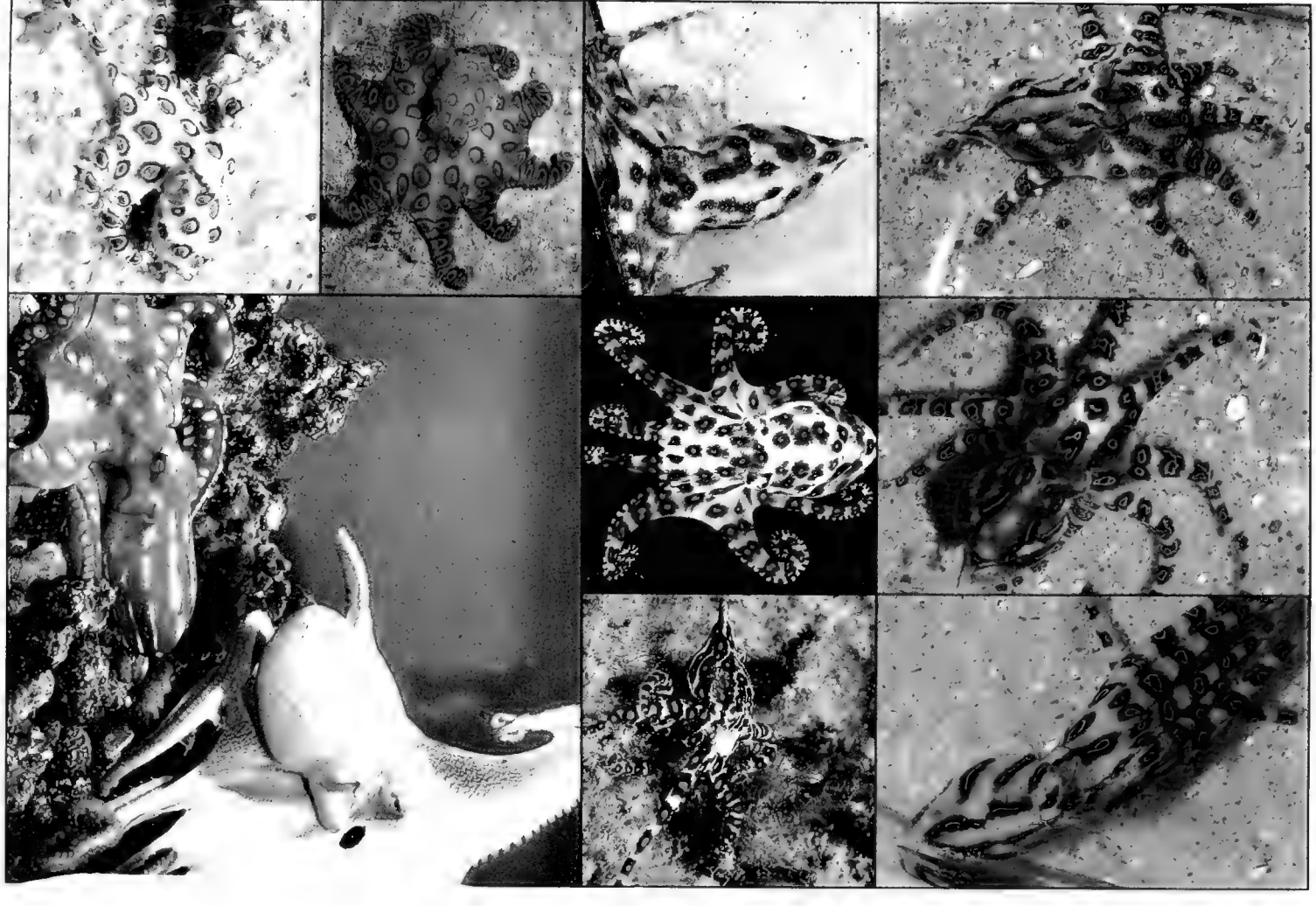
C. Components of body patterns

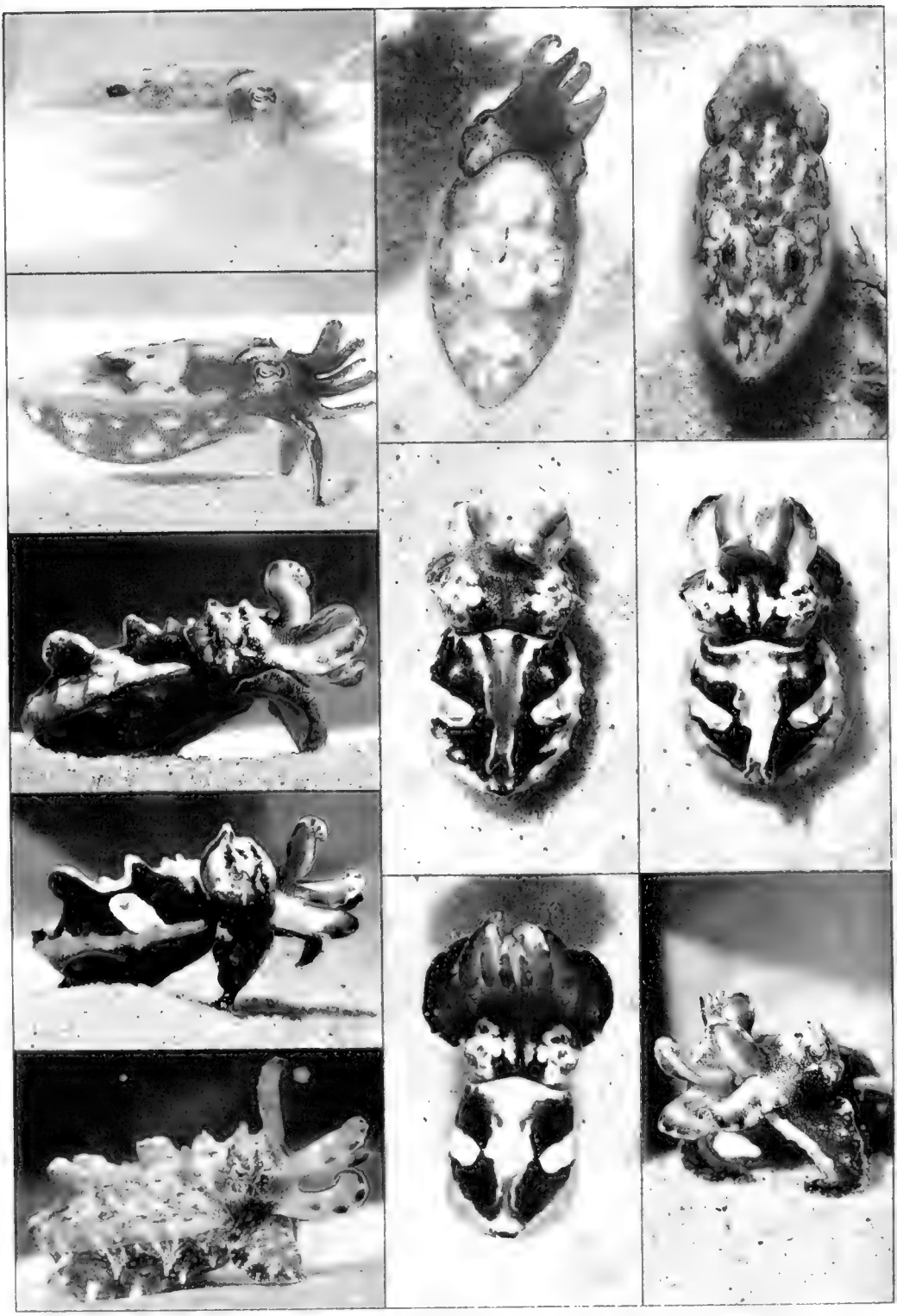
Not observed in life. This very distinct species has been studied by us only from color transparencies of live animals provided by C. Bryce and A. Kertstich (Figs. 29, 30). The drawing (Fig. 13) was developed from a photograph by Kertstich to contrast the general body pattern of *H. lunulata* with the patterns of the two species we observed alive. Photographs of live animals appear in Wells & Bryce (1986) and in Marsh & Slack-Smith (1986).

Discussion of *Hapalochlaena* spp.

The genus *Hapalochlaena* is a complex of octopuses with small bodies, short arms and shallow webs. The genus is further characterized by the unique presence of iridescent blue rings or lines set in macula of dark chromatophores. Observations were made on live animals of two very distinct species of *Hapalochlaena*, one from Lizard Island, the other from near Sydney. When observed alive or from color photographs, these two species are very easy to distinguish, whereas preserved specimens may be less distinctive. In fact, the study of living animals and of color photographs of living animals has enabled us to recognize species differences and thus to

FIGS. 23–31. *Hapalochlaena* cf. *fasciata*, Figs. 23–25, dorsal views; *H.* cf. *maculosa*, Figs. 26–28, dorsal and left lateral views; *H. lunulata*, Figs. 29–30, dorsal and left lateral views, and *Octopus cyanea O. ornatus*, Fig. 31, lateral views. Fig. 23. [top row, left] Light mottled (grey phase); blue lines and rings expressed; maculae expressed lightly; mantle, head and frontal white spots expressed. Fig. 24. [top row, center] Dark mottle, blue lines and rings expressed vividly; maculae dark, expanded and coalesced; frontal white spot visible, other white spots suppressed. Fig. 25. [top row, right] Light mottle (beige.yellow phase); blue lines and bars expressed; maculae dark and separate; white spots suppressed. Fig. 26. [middle row, left] Moderate warning pattern of Lizard Island animal; blue rings expressed; maculae expanded dark and coalesced on right side facing disturbance (left of image), otherwise subdued; mantle white spots visible. Fig. 27 [middle row, center] Deimatic pattern; blue rings and black maculae expressed vividly; texture finely papillate Photograph by C. Bryce (Shark Bay, Western Australia). Fig. 28. [middle row, right] Scuttling locomotion, warning pattern; blue rings expressed vividly; black maculae expanded very dark and coalesced. Photograph by C. Bryce (Albany, Western Australia). Fig. 29. [third row, left] Deimatic pattern; blue rings vivid; maculae expressed minimally as black rings outside of blue rings, centers light. Photograph by C. Bryce (Exmouth Gulf, Western Australia). Fig. 30. [bottom row, left] Deimatic pattern; blue rings vivid; dark macular rings expanded around outside of blue rings, centers dark. Photograph by A. Kerstitch (Vanuatu Islands). Fig. 31. [bottom row, right] *Octopus cyanea* (right) in light uniform pattern (deimatic) with vivid ocellus and smooth texture. *Octopus ornatus* (left) with white streaks on mantle and white spots on arms against maroon background. Photograph by W. F. van Heukelem (Honolulu, Hawaii).





begin resolving problems that have puzzled investigators since the original descriptions of the nominal species over 100 years ago.

While a great deal of work remains to be done, we have been able to distinguish at least three species based upon the size and configuration of their iridescent blue markings. Many living and preserved specimens from localities over the entire geographic range of the genus must be examined to make final judgments and decisions. At present, we concur with the identifications provided by S. S. Berry (in Halstead, 1965) and recognize the following distinct species:

1. *Hapalochlaena* cf. *maculosa* (Figs. 7–10) is characterized principally by small blue rings, 1–2 mm in diameter, on the dorsal and lateral (but not ventral) surfaces of the mantle, head, web and arms. This species occurs at Lizard Island and similar small-ringed forms have been recorded from numerous localities in Australian and Indonesian waters. Small-ringed forms may represent a widely distributed species, but more likely a species complex is involved, since in at least one area the ventral mantle of specimens examined is partially covered with a number of blue rings. This is the *maculosa* type of blue-ringed octopus originally described by Hoyle in 1883.

2. *Hapalochlaena* cf. *fasciata* (Figs. 11, 12) is characterized by short, blue lines (streaks and diagonals) on the mantle and by very small blue rings (single circles, figure-8's or complex circular designs) on the head, web and arms. The configuration and distribution of blue lines and rings is comparable in general terms to that of the lesser blue-ringed octopus. This is the species we studied from waters near Sydney. It has been referred to repeatedly as *H. maculosa* in the literature. However, all the specimens we have examined from New South Wales have blue lines not rings and, hence, the specific name *fasciata* Hoyle, 1886 seems applicable. The extent of its distribution is unknown because it has been frequently confused with the "*maculosa*" species.

3. *Hapalochlaena lunulata* (Fig. 13) is distinguished by relatively large blue rings up to 7 or 8 mm in diameter on the dorsal and lateral surfaces of the mantle, head, web and arms. This species also seems to be widely distributed in Australian and central Indo-Pacific waters. A complex of species or subspecies may be involved that can be resolved only by further critical study.

What could be the function of the brilliant, iridescent blue displays of *Hapalochlaena*? Judging from the behavioral responses to disturbance or threat, these seem to be a form of warning coloration with which these small octopuses signal their unpleasant taste or poisonous bite. Interestingly, there are several other vividly marked small octopuses (e.g. *Octopus zonatus, O. chierchiae*), but it is not known whether they also are poisonous.

Surprisingly, reference to color and body patterns in the *Hapalochlaena* complex is very limited. The majority of reports detail the blue rings and dark spots or maculae on the body and arms. Although several photographs show animals with distinct lines on the mantle, the animals still are referred to as the "blue-ringed octopus." On the basis of photographs in published reports we can identify the following: *H.* cf. *maculosa*—Reynolds, 1983; Marsh & Slack-Smith, 1986; *H.* cf. *fasciata*—Sutherland & Lane, 1969; Deas, 1970; Hal-

FIGS. 32–42. *Sepia papuensis*, Figs. 32–35, right lateral and dorsal views; *Metasepia pfefferi*, Figs. 36–41, right lateral and dorsal views, and *Metasepia tullbergi*, Fig. 42, right lateral view. Fig. 32. [top row, left] Bipod position; dark mottle on dorsal mantle, light mottle on ventral mantle; dorsal mantle dark spot prominent; lateral mantle white spots expressed against light background. Fig. 33. [second row, left] Bipod position, dark mottle on dorsal and ventral mantle; head, mantle and finline white spots conspicuous. Fig. 34. [top row, center] Light mottle approaching light uniform; unilateral dorsal mantle dark spot directed toward source of disturbance; dorsal mantle white spots expressed. Fig. 35. [top row, right] Dark mottle. Fig. 36. [third row, left] Tripod posture; raised arms I; diagonal mantle white bars, finline white stripe, lateral mantle white spots expressed; head and arm light components expressed; yellow and magenta absent. Fig. 37. [fourth row, left] Ambling locomotion on ambulatory flaps and arms IV, right arm IV down, left arm IV raised to take next step; flamboyant body pattern; yellow and magenta expressed. Fig. 38. [middle row, center] Dark uniform with diagonal mantle white bars expressed; yellow and magenta absent. Fig. 39. [middle row, right] Passing wave pattern, especially on left side of mantle; first anterior wave in mid-cycle, second anterior wave originating at anterior mantle margin; posterior wave originating at posterolateral margins of mantle. Fig. 40. [bottom row, center] Prone position with flanged fins and flattened arms; yellow and magenta expressed. Fig. 41. [bottom row, right] Head-on view of flamboyant arm pattern; suckers enveloped by yellow protective membranes. Fig. 42. [bottom row, left] Flamboyant pattern of color, texture and posture; note ambulatory flap. Photograph of Japanese animal by T. Koyama, courtesy of T. Okutani.

TABLE 6. The components of body patterns in *Metasepia pfefferi*.

I. Chromatic components

A. Light
- arm IV white spots (aws IV)
- arm III white spots (aws III)
- arm I white spots (aws I)
- web white spots (wws)
- dorsal head white spots (dhws)
- dorsal head white triangles (dhwt)
- lateral head light field (lhlf)
- dorsal mantle white bars (dmwb)
- dorsal mantle light field (dmlf)
- finline white stripe (flws)
- lateral mantle white spots (lmws, ventral to fin)
- ventral mantle white spots

B. Dark
- dorsal head dark field (dhdf)
- lateral mantle dark fields
- posterior mantle dark spot (pmds)
- dorsal mantle dark spots (dmds) (eye spots of deimatic pattern)
- arm IV dark stripe (ads)

II. Textural components

A. Primary papillae and flaps
- anteroventral eye papilla (avep, 1/eye)
- anterodorsal eye papilla (adep, 1/eye)
- posterodorsal eye papilla (pdep, 1/eye)
- finline papillae (flp 1, 5–7/side)
- laterodorsal mantle papillae (ldmp, 2 pairs)
- anterodorsal mantle papilla (admp, 3)
- posterodorsal mantle papillae (pdmp, 1 pair)
- posterodorsal mantle flaps (pdmf, 1 pair)
- mid-dorsal mantle flaps (mdmf, 1 pair)
- mantle white bar flaps (mwbf, 1 pair)

B. Secondary papillae
- dorsal head papillae
- dorsal mantle papillae
- finline papillae (flp2, 5/side)
- lateral mantle white spot papillae

III. Postural components
- arms I raised
- arms IV lowered (tips on substrate)
- flamboyant arm splay
- drooping arms (while hovering)
- raised head
- flanged fin (folded down)
- flattened/flared arms
- bent arms IV (while ambling)
- bipod (body off bottom)
- tripod (posterior mantle on bottom)
- prone (body flat on bottom)

IV. Locomotor components and maneuvers
- resting
- floating
- hovering
- swimming
- jetting
- ambling
- inking

V. Body patterns

A. Chronic
- light cryptic mottle
- light uniform

B. Acute
- dark conflict mottle
- dark uniform (flush)
- flamboyant
- passing wave (apw, ppw, 1,2)
- deimatic

stead & Danielson, 1970; Voss, 1971; Cropp, 1972; Tranter & Augustine, 1973; Keith, 1986; *H. lunulata*—Robson, 1929 (pl. 4, fig. 1); Flecker & Cotton, 1955; Anon., 1968; Marsh & Slack-Smith, 1986; Wells & Bryce, 1986.

Brief descriptions of color in adults and hatchlings of *H.* cf. *fasciata* were provided by Dew (1959) and Tranter & Augustine (1973), who found that the characteristic blue lines and rings of *H.* cf. *fasciata* appeared 6 weeks after hatching, although the macula pattern developed at an age of 3 or 4 weeks. The postures and activities associated with mating were described by Tranter & Augustine (1973) but patterns of color specific to courtship and mating were not mentioned.

Species of the subfamily Octopodinae are all very similar morphologically and anatomically. Hence, few supraspecific taxa have been erected to help subdivide an enormous number of seemingly uniform species. The fixed and characteristic markings observed in *Hapalochlaena* lead us to conclude that this species complex should be recognized as distinct at the generic level.

Morphogenetic patterns, especially of color and texture, are fixed, conservative characters within families and genera. Although more research is needed, this phenomenon will add a whole new suite of genetically stable characters upon which to base systematic analyses, diagnoses of species and genera, etc. The elucidation of these morphogenetic patterns, through study of living animals or color photographs of living animals, will provide valuable information for understanding both the systematics and behavior of cephalopods, and for identification of species in the field.

6. *Metasepia pfefferi* Hoyle, 1885

Common name: Pfeffer's flamboyant cuttlefish. We name this species after the German teuthologist Georg Pfeffer. "Flamboyant" emphasizes the striking, flowery body patterns of the genus *Metasepia*.

A. Synopsis

Diagnosis: Mantle very broad, oval; mantle length to about 80 mm; total length to about 160 mm; dorsal mantle with 3 pairs large, primary flat, flap-like papillae and 1 pair prominent mantle white bars; head with primary papillae over eyes; arms broad, blade-like; fins broad, transparent; tentacular club short, with swimming keel twice as long as club; dorsal and ventral protective membranes separate on tentacular stalk; club suckers very few in number, in about 5 transverse rows, with 3 or 4 median suckers enormously enlarged; cuttlebone broad, rhomboidal, shorter than mantle; dorsal surface completely chitinized, without median rib or spine.

Distribution: Tropical Australian waters: Arafura Sea (type locality), Queensland (Capricorn group, Moreton Bay—Alan Jones, personal communication) to Western Australia (to about 33°S). Shallow-water species on sand/silt bottom to about 50 m.

Life history: Unknown. This paper is the first report of observations on living animals.

References: Hoyle, 1885b, 1886; Adam & Rees, 1966; Adam, 1979; Lu & Phillips, 1985; Wells & Bryce, 1986.

B. Field observations

Two juvenile *M. pfefferi* were captured in 10 to 13 m at separate locations during daytime dives in Watson's Bay (CFER-22 and CFER-42). In both localities, the flat bottom was composed of a mixture of sand and silt, devoid of rocks and rubble. The bottom was inhabited by a large number of black holothurians, *Holothuria* sp., a few brown-yellow holothurians, and dense patches of attached green algae: a calcareous species of *Halimeda* and scattered *Caulerpa cupressoides*. In its natural habitat, *M. pfefferi* looks like anything but a cuttlefish. When first observed and prior to capture, animal 1 was variously identified by divers as a small frilly crab, a crayfish, a pufferfish or a piece of alga slowly moving or drifting along the bottom. Even when the divers knew what to look for during subsequent dives, recognition of animal 2 was difficult. When first observed it was hovering in a stationary position 10 cm above the bottom, motionless except for gentle mantle undulations. Upon sighting the diver, it swam to the bottom and assumed a cryptic pattern that matched the beige/grey color of the sandy silt substrate. When pursued and captured, the animal changed to a vivid black and yellow color pattern and a distinctly papillate texture. *Metasepia pfefferi*'s ability to conceal itself is due both to the vivid and exaggerated color and textural patterns as well as the unusual configuration of the arms (described below). Observations of behavior and body patterns in aquaria were consistent with those observed in the field.

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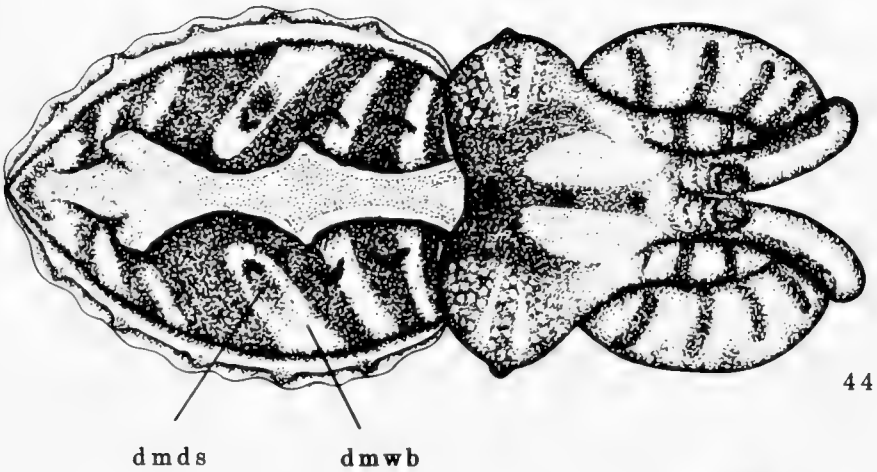

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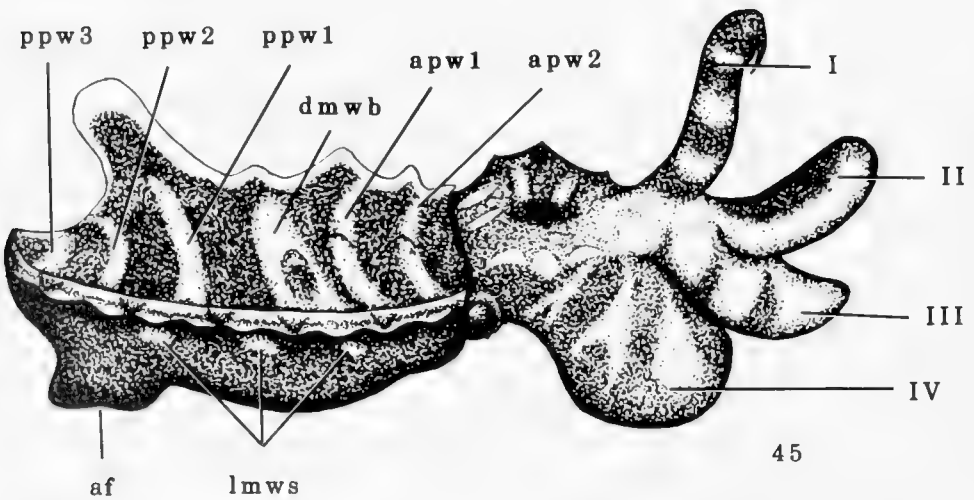

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FIGS. 43–45. *Metasepia pfefferi*. Field drawings to show texture, flamboyant configuration of arms and color patterns on mantle during passing wave pattern. Fig. 43. Head-on view; arm configuration observed while in bipod, tripod and prone postures. Fig. 44. Dorsal view. Fig. 45. Right lateral view; prone posture; note ambulatory flaps (shown shorter than normal). Drawings by B. Morton of Lizard Island animal in aquarium.

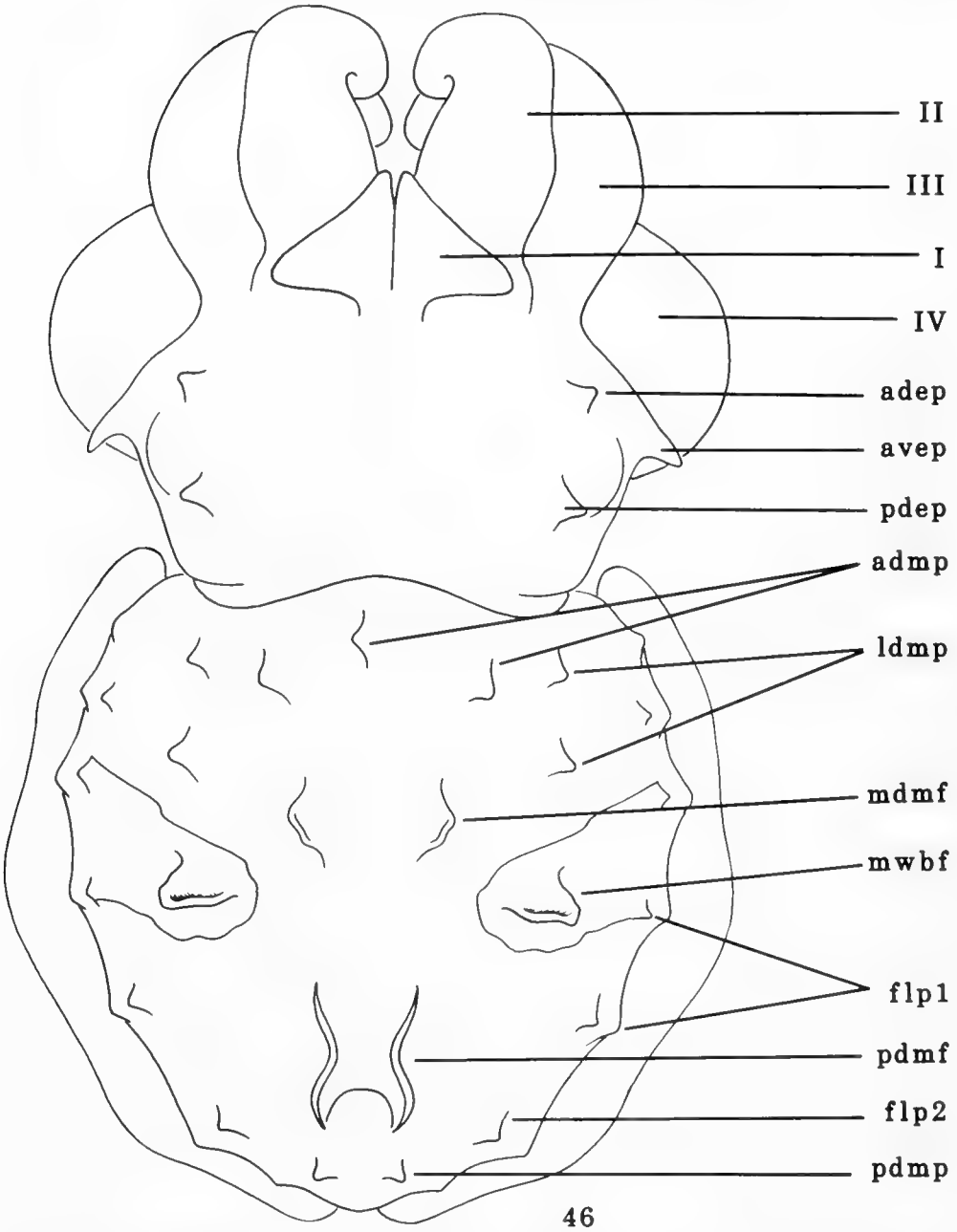
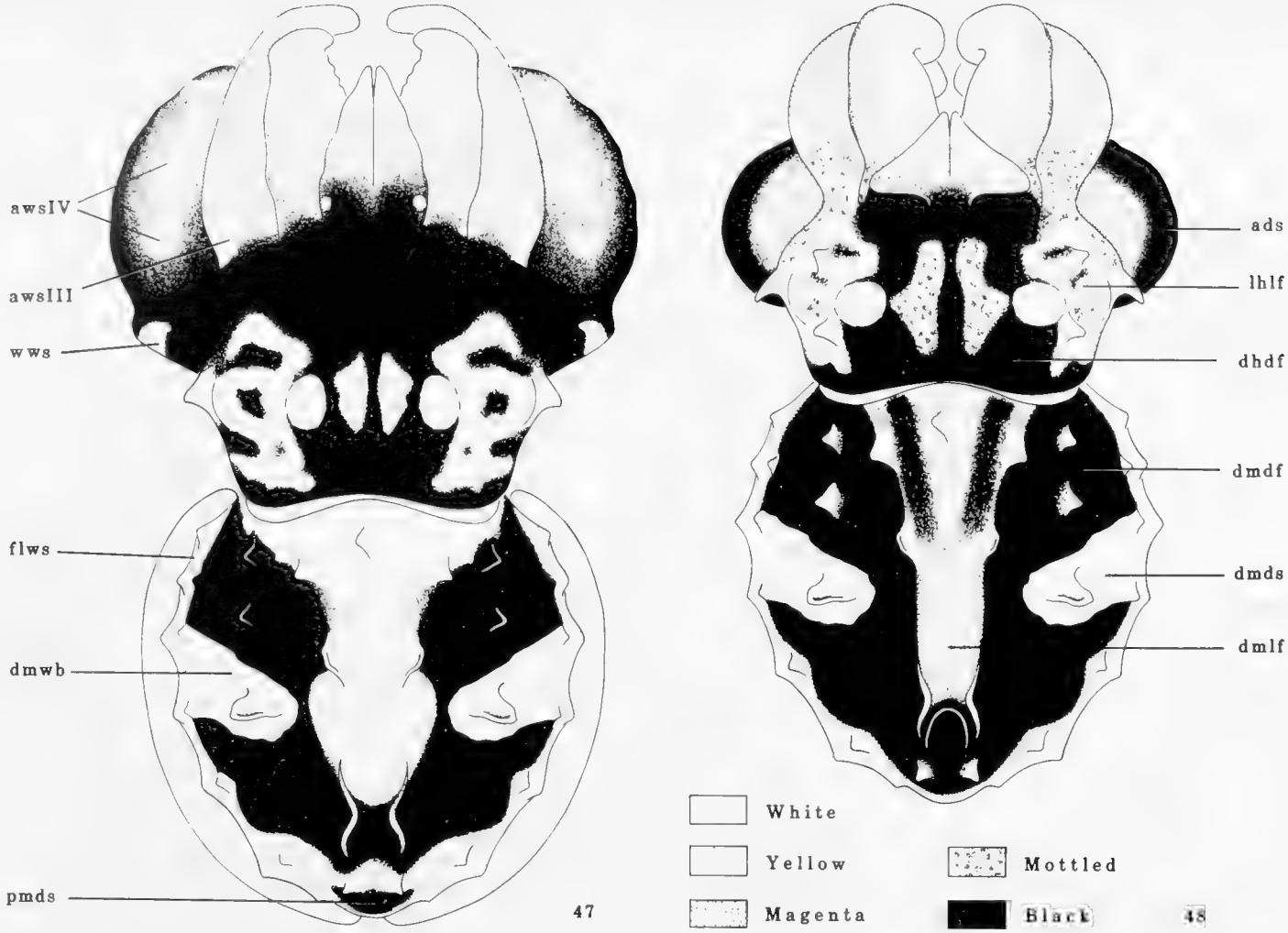


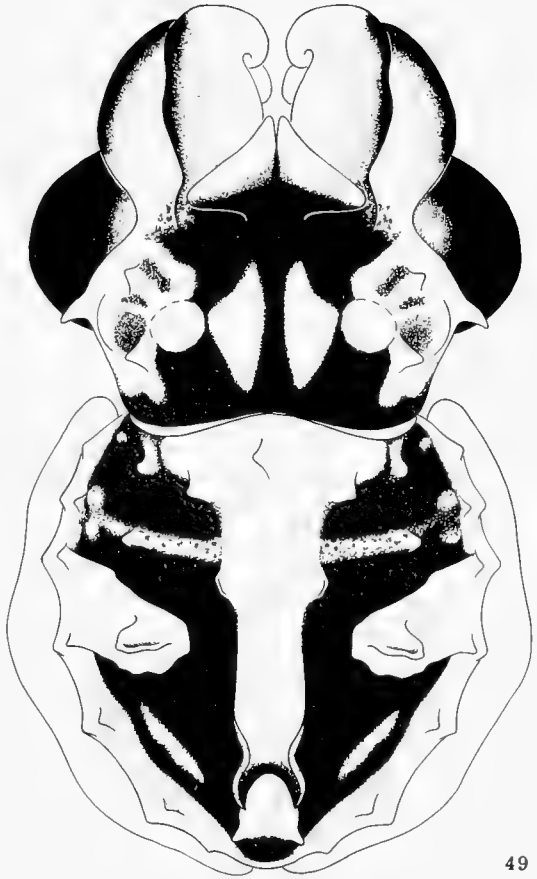
FIG. 46. *Metasepia pfefferi:* Stylized dorsal view showing location of large, primary papillae and flaps and smaller secondary papillae.

C. Locomotion

In addition to floating, hovering, swimming and jetting, *M. pfefferi* moves along the bottom by means of a remarkable new type of locomotion we term "ambling." This mode of locomotion is aided by a pair of muscular flaps along the margin of the posterior third of



FIGS. 47–48. *Metasepia pfefferi*. Stylized dorsal views to show color and body patterns in arms flared (47) and flanged fin (48) patterns.



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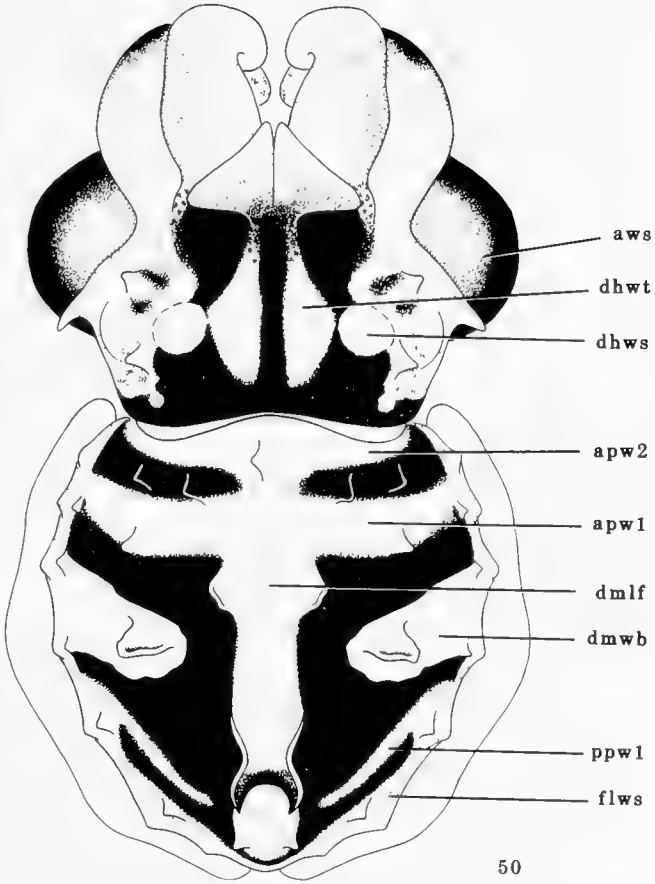

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FIGS. 49–50. *Metasepia pfefferi*. Stylized dorsal views to show color and body patterns during two phases of the passing wave pattern.


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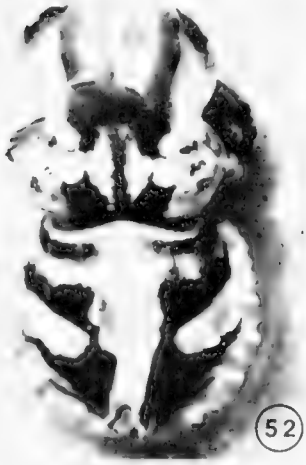
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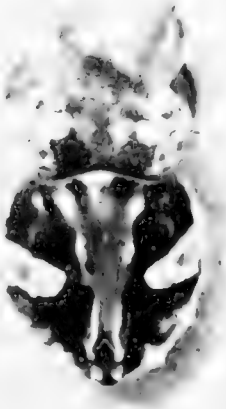
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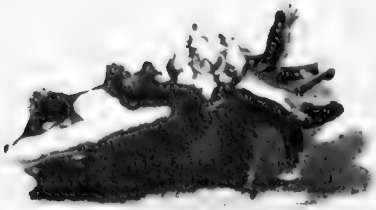
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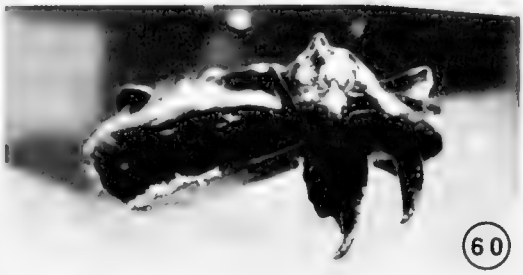
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the ventro-lateral mantle (Figs. 37, 45, 58, 59). The flaps, which look like a pair of elongate, ventrally-directed fins, border the edge of the ventral "suction" disk. They are grey to beige in overall color with two indistinct pale yellow vertical bands. We term these structures "ambulatory flaps," to denote their function in this unusual mode of locomotion.

The ambulatory flaps are erect most of the time, whether the animals are resting, swimming, hovering or ambling. At rest the animals sit tripod-fashion (Fig. 36) on the erected ambulatory flaps and on the ventrally directed ventral arms. The tips of the ventral arms typically are flattened and curled medially giving the appearance that the animals are resting on their "forearms" (Fig. 41). In this position the head and mantle opening are elevated well above the bottom.

The animals amble in a "slow walking" motion along the bottom using the ventral arms as "legs." The ambulatory flaps participate by sliding or shuffling forward alternately in sequence with the ventral arms (Figs. 37, 58, 59). This locomotion pattern is similar to the gait of a quadruped vertebrate in that the right ventral arm in front and the left ambulatory flap in the rear move forward, followed by the left front arm, right rear flap. The movement is slow and deliberate, resembling an amble or a shuffle. By reversing the sequence the animals are able to move backwards. When approached from the head end, the animals amble backward rather than jetting away as is typical of most other cuttlefishes.

Finally, *M. pfefferi* can amble sideways when threatened from the side. Side stepping also is quite slow and deliberate. In moving to the right, for example, the body is supported on the right ventral arm and the right ambulatory flap. The left ventral arm and flap are swung toward the midline and set down in place. The weight then is shifted to the left arm and flap and the "appendages" on the right side are swung out to the right and placed on the substrate.

Many details of the fascinating new "ambling" behavior of *M. pfefferi* still remain to be worked out, but these original observations leave no doubt that this type of locomotion is normal for this species. In fact, it seems to be preferred, as the animals were seldom observed to swim.

D. Components of body patterns

Figs. 43 to 50 and Table 6 indicate the location and terminology of the various color, textural and postural components discussed below.

When resting or sitting on the bottom, the animals assume one of three basic postures. (1) As an animal moves into the resting position it hovers just above the bottom (Fig. 60), lowers the ventral arms to a vertical position, then settles into a "bipod" position with the weight supported on the tips of the two lowered ventral arms; it may remain in the bipod position for some time. (2) In the "tripod" position, the body is supported by the ventral arms and the posterior mantle, which rests on the ambulatory flaps in contact with the bottom (Figs. 36, 37, 57). The head and anterior mantle are elevated, arms II and III are splayed apart and arms I are raised dorsally and held together with the tips curled ventrally (Figs. 36, 37, 57). Arms IV may be either straight or curled at the tips. When the arms are all splayed out, and variously flattened and curled, the resemblance to a plant is striking (Fig. 41). (3) In the "prone" resting position, the entire ventral surface of the mantle is in contact with the bottom, and the ventral arms are horizontal with the bottom. In this position the head and anterior mantle are not elevated.

In these resting positions, the skin is sculptured with a complex pattern of primary and secondary papillae characteristic of the genus. Primary papillae and flaps are large and tonically erect (Figs. 36, 37). They may be colored solid white or yellow or tipped in white or yellow. The primary papillae include the dorsal and anteroventral eye papillae, the five to seven lateral mantle papillae on each side

FIGS. 51–60. *Metasepia pfefferi*, dorsal and right lateral views. Figs. 51–54. Passing waves, various stages; note papillate and chromatic pattern on head and arms. Fig. 55. Dark chromatic phase with subdued dorsal head white triangles and dorsal mantle white stripe; flanged fins. Fig. 56. Flamboyant color, texture and posture; dorsal mantle dark spots prominent. Fig. 57. Tripod position, flamboyant arm splay. Fig. 58. Ambling locomotion, both arms IV on bottom. Fig. 59. Ambling locomotion, left arm IV set, right arm IV raised to take step. Note ambulatory flaps in figs. 57–59. Fig. 60. Moving off bottom with drooping arms IV; ambulatory flaps reduced, edges covered with sand.

just dorsal to the insertion of the fin, two pairs of flat dorsal mantle flaps, a field of seven conical papillae on the anterior dorsal mantle and a pair of cup-shaped flaps located in the center of the mantle white bars. A discrete series of smaller conical secondary papillae is located in parallel with the primary papillae especially along the finline (Fig. 39).

The overall color of undisturbed animals is a uniform pale grey-white that matches the sandy silt background. The protective membranes on the arms are closed over the sucker rows and are striped in pale yellow (Fig. 41). When animals are disturbed slightly the head and dorsal mantle change to a highly contrasting black and white body pattern with pale yellow along the finline (e.g. Figs. 39, 47, 51–54). The dorsal head white triangles and the broad dorsal midline white stripe may be masked with black chromatophores (Figs. 38, 55). Yellow or magenta are not expressed except along the protective membranes. "Passing waves" may move over the dorsal mantle (see details below). The fins lack chromatophores and always appear transparent. When animals are disturbed repeatedly the lateral stripe above the fins, the finline stripe, turns bright yellow and the yellow of the protective membranes and the oral surfaces of the arms is intensified (Figs. 39, 40, 56).

The dorsal mantle is black and partitioned distinctly by the white midline stripe and the intense mantle white bars; mantle dark spots may be expressed (Fig. 56). The lateral mantle, ventral to the fins, is black with yellow/orange spots outlined in darker orange (Fig. 36). The ventral mantle is a uniform pale grey with a single white spot posteriorly. The dorsal head white spots are intense white; the dorsal head white triangles may be yellow or white and the dorsal eye patches are white mottled with black (Figs. 39, 40). The arms often are flattened. The aboral edges of arms I–III are magenta, the mid-regions are white and the oral edges bright yellow. The tips are pale yellow. Arms IV are dark brown/purple fringed and mottled with white. A distinct patch of yellow occurs on the aboral edge of arms III. This appears to be related to the flamboyant pattern described in juveniles of other genera of cuttlefishes and octopuses.

Often while the disturbed animals are in this color and body configuration, a series of "passing waves" washes over the surface of the dorsal mantle (Figs. 39, 49–54). This is seen as a band-like wave of white that moves through the dark field of the mantle dorsal to the fins. The white midline stripe is not affected. As one transverse wave originates at the anterior mantle margin and moves posteriorly, another wave originates on the sides of the posterior mantle and moves anteriorly until the two waves meet and disappear in the region of the mantle white bars. A second set of waves typically is generated before the first set is extinguished (Figs. 39, 50), so it is possible to see for an instant four passing waves, two moving posteriorly and two moving anteriorly.

When the fins are used to hover in a stationary position the body is oriented horizontally to the bottom and the arms either are splayed out as described above or held together in a ventrally drooping position (Fig. 60). At times the arms are extremely flattened dorso-ventrally and flared out laterally (Figs. 40, 47). In this posture the color pattern may be either a high contrast black and white or a pattern of black, white, yellow and magenta.

Metasepia pfefferi typically rests on or hovers just above the bottom. When disturbed the animals generally "amble" away and were only rarely observed to swim or jet through the water when prodded repeatedly with a rod or finger. When they do swim or jet the primary papillae and dorsal mantle flaps are erect over the entire body and the color is either a pale uniform beige/white or a vivid black, white, yellow and magenta as described above.

Some variations on these basic patterns are seen in the photographs. It is important to stress that our observations were brief and hence represent only a preliminary inventory of the chromatic, textural and postural components shown by this species. To develop a more complete body pattern inventory that can be used for comparison with other genera and species of cuttlefishes, additional effort in the laboratory and field is needed.

E. Discussion

The studies on *M. pfefferi* at Lizard Island represent the first and only detailed observations of live animals published since its original description in 1885. A photograph of a live animal is published in Wells & Bryce (1986). Working from the framework provided by Hanlon & Messenger (1988) for young *Sepia officinalis,* we have been able to recognize over 50 chromatic, textural, postural and locomotor components based upon observa-

TABLE 7. The components of body patterns in *Sepia papuensis*.

I. Chromatic components

A. Light
- dorsal mantle white spots
- dorsal mantle white stripe
- finline white spots
- finline white stripe
- lateral mantle white spots (ventral to fin)
- dorsal head white patch
- dorsal head white bar
- lateral head white stripe

B. Dark
- dorsal mantle dark spots (eye spots of deimatic pattern)
- dark uniform
- dark mottle
- lateral mantle dark fields
- dorsal head dark field

II. Textural components

A. Primary papillae
- mantle white spot papillae (1/spot)
- dorsal eye papillae (2/eye)
- anterodorsal mantle papillae
- finline papillae (outer row)
- dorsal mantle flaps (1 pair)

B. Secondary papillae
- dorsal head papillae
- dorsal mantle papillae
- finline papillae (inner row)
- lateral mantle white spot papillae
- arm papillae
- dorsal eye lid papillae (1/eye)

C. Other
- smooth

III. Postural components
- bipod (body off bottom)
- tripod (posterior ventral mantle on bottom)
- prone (body flat on bottom)
- arms I raised
- arms IV lowered
- splayed arms
- flanged fin (folded down)

IV. Locomotor components and maneuvers
- resting
- floating
- hovering
- swimming
- jetting
- burying
- inking

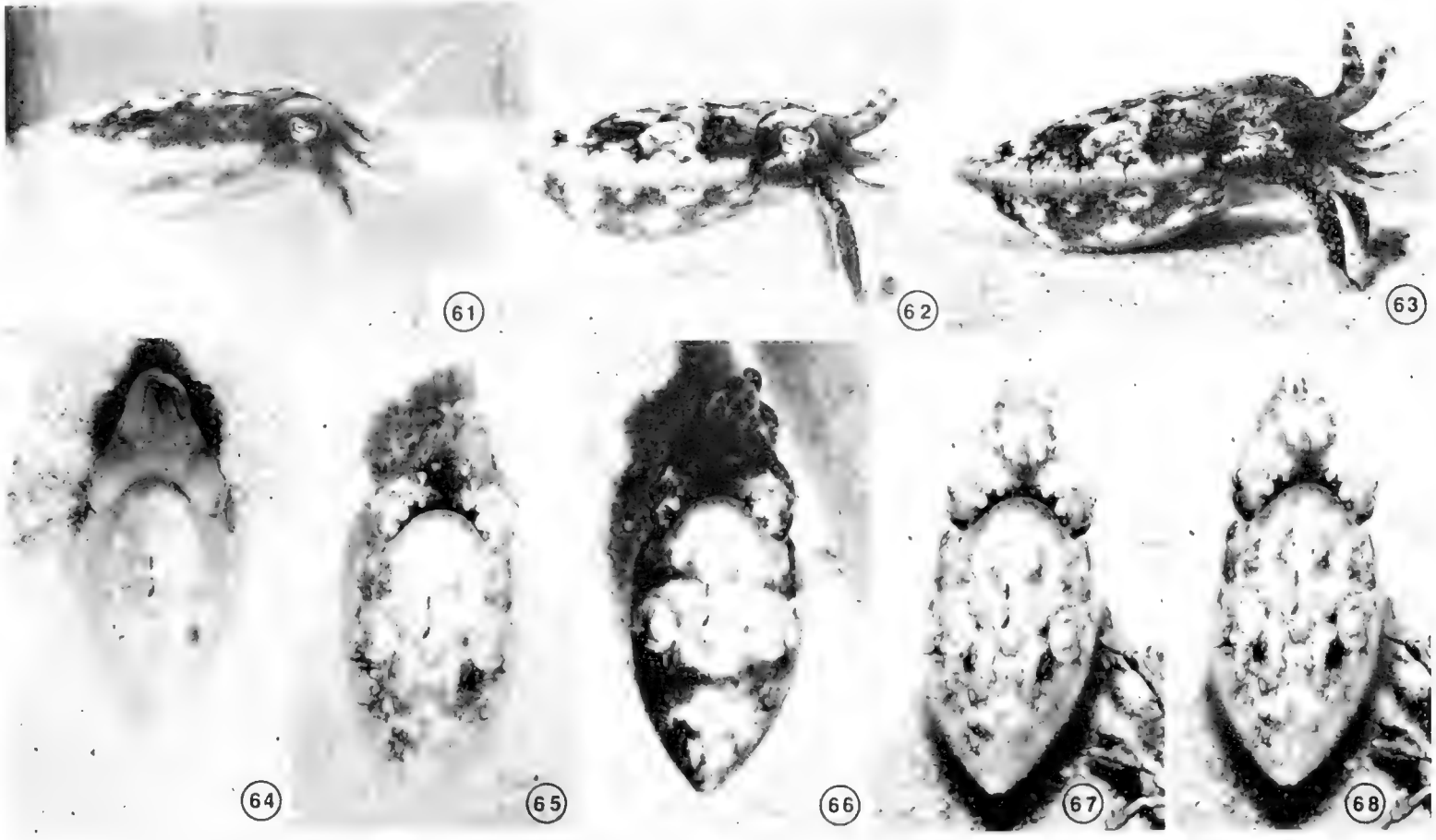
V. Body patterns

A. Chronic
- light cryptic mottle
- dark cryptic mottle
- light uniform

B. Acute
- dark conflict mottle
- dark dorsal mantle (flush)
- dark uniform (flush)
- deimatic (blanch)
- passing wave

tions and photographs on two juvenile specimens studied separately for only a few days. *Metasepia pfefferi* thus revealed an extremely rich repertoire of components. Observations of interacting and sexually mature adults will add still further to the already impressive behavioral repertoire of this species.

Traditionally, most authors have stated that the species *Sepia pfefferi* and its congener from Japanese waters, *S. tullbergi* Appellof (1886), belong in the subgenus *Metasepia* erected by Hoyle (1885b) to contain *pfefferi* (e.g. Adam & Rees, 1966; Adam, 1979; Natsukari, 1979; Lu & Phillips, 1985). However, some authors, such as Iredale (1954), Okutani (1973) and Okutani & Habe (1975) have interpreted *Metasepia* as a genus. The primary characters that distinguish *Metasepia* from *Sepia* are: (1) the short, round, dorsoventrally thickened body; (2) the very unusual morphology of the cuttlebone that is rhomboidal in shape, very broad, covered dorsally with a chitinous layer, and lacks the posterior spine and a dorsal midline rib and



FIGS. 61–68. *Sepia papuensis*, dorsal and right lateral views. Fig. 61. Bipod posture; arms splayed; dark mottle on dorsal mantle and light mottle on ventral mantle. Fig. 62. Bipod posture; mantle, head and fin light components and dark fields prominent. Fig. 63. Tripod posture; dark mottle on dorsal and ventral mantle. Fig. 64. Dorsal view; light uniform, cryptic coloration; dorsal mantle dark spot slightly expressed on right side. Fig. 65. Light mottle; dorsal light components expressed. Fig. 66. Dark mottle, dorsal mantle white components expressed prominently; dorsal head white patches moderately expressed. Figs. 67–68. Two views of light mottle.

(3) the subspherical shape of the egg capsules.

On the basis of these morphological differences and our observations on the chromatic and textural body components and ambulatory flaps, as well as the behavioral modifications associated with the distinctive body patterning and ambulatory locomotion, we believe that *Metasepia* is correctly referred to as a genus distinct from *Sepia.* Photographs (see Fig. 42) and observations (T. Okutani & Y. Natsukari, personal communication; see also fig. 37b in Packard & Hochberg, 1977) on *M. tullbergi* indicate that it is as striking and colorful as *M. pfefferi.*

Roeleveld & Liltved (1985) described fleshy keels on the ventral surface of the mantle in *Sepia pulchra* and its close relatives *S. robsoni, S. faurei, S. typica* and *S. dubia.* These keels, together with the swollen undersurfaces of the ventral arms, form a "sole" by which the animal adheres to vertical rock surfaces, presumably by forming a differential in pressure. The two keels appear to extend the entire length of the ventral surface of the mantle, one on each side (see figs. 2 and 12 in Roeleveld & Liltved, 1985). *Sepia officinalis* also is reported to have a ventral sole (Hanlon & Messenger, 1988). Thus the form and the function of the ventral keels in the genus *Sepia* are distinct from those of the ambulatory flaps in the genus *Metasepia.* In *Sepia* they serve to hold the animal stationary, while in *Metasepia* they provide an additional means of locomotion.

7. *Sepia papuensis* Hoyle, 1885

Common name: Papuan cuttlefish. This is named here for Papua New Guinea, the locality of original capture.

A. Synopsis

Diagnosis: Mantle elongate, narrow; mantle length to 110 mm; total length to about 200 mm; surface of dorsal and ventral mantle and dorsal and lateral head covered with small papillae; fin narrow and transparent; swimming membranes of arms I, II, and III bear a series of semicircular, elongate lappets; protective membranes of tentacular club separate at base of club in young, fused in adults, the dorsal one much longer; cuttlebone with wide, well-defined mid-dorsal rib and thick chitinous ledge formed by posterior part of fused outer and inner cones; cuttlebone extends anteriorly to between eyes.

Distribution: Central Indo-West Pacific Ocean; Arafura Sea (type locality); Philippines; Bali; Ternate; Australia, from Fremantle, Western Australia, northward around to Queensland (Low Isles). Animals have been captured to about 150 m on sand/silt to mud bottoms.

Life history: Poorly known. To our knowledge, this work represents the first observations on living animals.

References: Hoyle, 1885b; Adam & Rees, 1966; Adam, 1979; Lu & Phillips, 1985.

B. Field observations

A single immature animal (CFER-28) was captured during the day at a depth of 20 m in Watson's Bay. The habitat consisted of flat, open, sand/silt bottom with scattered patches of *Halimeda* sp., *Caulerpa cupressoides* and solitary corals.

The cuttlefish was first observed sitting or resting in the open on the bottom not in association with the *Halimeda.* The surface of the dorsal mantle, head and arms was covered with a light mottled brown and yellow color pattern that closely matched the background color of the sand/silt bottom on which it rested. The animal was captured and placed in an aquarium for observation.

C. Locomotion

During the day the animal often assumed a resting position with the head and the dorsal and lateral arms raised off the bottom, the body supported by the ventral arms and the posterior ventral mantle (tripod posture, Fig. 63). At night the undisturbed animal often was observed floating or hovering motionless in the tank. When floating or hovering, the fins are stationary and folded down against the mantle. Arms I are raised dorsally and arms IV hang down ventrally. When disturbed the animal exhibits a strong escape response and jets away. The arms are flattened and brought together in a cone. When the animal slows down it swims around the tank until it once again begins to float or hover, or it lowers the ventral arms to rest on the bottom in a bipod posture (Figs. 32, 33, 61, 62).

D. Components of body patterns

When the animal rests with the head raised and the body supported on the tips of the

elongate ventral arms, all the arms are strongly compressed or flattened and the protective membranes are closed over the suckers. In this position, arms I are raised and the distal ends are tightly coiled. Arms II also may be raised and curved dorsally and medially. Arms III are extended horizontal to the bottom with the distal ends curved medially. Tiny papillae are raised along the aboral ridge (keel) of all arms.

The mantle of *S. papuensis,* unlike *M. pfefferi,* is elongate and pointed posteriorly. The outline of the cuttlebone is clearly visible through the skin of the dorsal mantle. The head is broad and short, and the eyes are prominent. The fins are very narrow, thin and transparent, which enhances the elongate appearance of the mantle. The finline (fin/mantle fusion line) lies in the upper 1/3 to 1/4 of the body, about in line with the lower margin of the eyelid.

The dorsal mantle is rarely smooth, but normally is uniformly covered with widely scattered papillae in two series: (1) A conspicuous series that consists of a pair of large triangular, flattened primary papillae that emerge from the mantle white spots; (2) Just posterior and lateral to the mantle white spots lies a pair of papillate ridges located along the boundary between the white midline stripe and the darker lateral fields. Additionaly, laterally along the junction of the fins and the dorsal mantle is a series of finline papillae, several of which are longer than the rest. Dorsally on the head, two pairs of large papillae are located over the anterior and posterior ends of each eye. Secondary papillae are smaller and more numerous and hence, less conspicuous than the larger but fewer primary papillae. A small rounded papilla occurs in the center of each dorsal eyelid. The dorsal head between the eyes may be textured with tiny papillae. Ventral to the finline, along the lateral mantle, a series of conspicuous white spots is dotted with numerous primary and secondary papillae.

During the morning (0800-0930), when at rest in contact with the light-colored sandy bottom, the animal assumed a uniform light olive color (Fig. 64) or a very pale beige mottled pattern (Figs. 32, 65) or a reticulated pattern of tiny light brown chromatophores. At night (2200–2315) the animal assumed a darker mottled color consisting of a light yellow overall ground color reticulated with dark reddish-brown (Figs. 33, 63). In both cases the primary papillae are raised moderately and colored beige to white. In these chronic patterns the animal typically matches the color, intensity and texture of the substrate and thus blends cryptically into its surroundings.

While at rest a pair of distinct crescent-shaped mantle white spots is visible, one on each side of the dorsal mantle in the midregion of the mantle (Figs. 34, 35, 65–68). Each white or pale yellow spot has a large papilla in the center. Directly posterior to the mantle white spots is a pair of large mantle dark spots (Figs. 34, 35, 64–68). These are the "eye spots" expressed in the deimatic pattern, and often also visible when the animal assumes light mottled or uniform patterns. A broad midline region that extends the length of the dorsal mantle appears more lightly pigmented than the lateral fields. Dorsal to and along each finline lies a series of white spots that may be expanded into a continuous finline white stripe (Figs. 35, 67, 68). The dorsal mantle is densely covered with tiny red-brown chromatophores that, when expanded, effectively screen all the light areas of the mantle (Fig. 63).

Laterally the mantle is yellow with 6 to 10 large white spots ventral to the finline (Figs. 32, 33). The chromatophores on the lateral mantle are larger than on the dorsal mantle and fewer in number. The flattened ventral mantle is pale with a few large, widely scattered brown chromatophores. The dorsal head is predominantly red overlying or intermixed with yellow. Very fine bluish white lines indicate the outline of the curved anterior edge of the cuttlebone under the surface of the mantle (Fig. 64). Dorsally on the head, medial and anterior to the eyes, are irregular white head patches (Figs. 66–68) that are stippled with minute red-brown chromatopheres. The white head patches can be expanded and merged into a single white head bar. When coalesced they have the appearance of a cauliflower. Laterally, a distinct white stripe extends from the finline through the ventral eyelid where it either terminates just anterior to the eye or continues along the aboral edge of arms III (Figs. 33, 62, 63).

At night the broad curved tips of the flattened arms I are white, contrasting vividly with the red of the remainder of the arm. The proximal papillae along the arm keel are red, the middle ones are white against red, and the distal ones are white. Arms II and III are red with tiny white papillae. Arms III also may be entirely white while all the others are red.

Arms IV are small and red with white papillae. The aboral edge of the arm keel of the ventral arms and the oral surface with the protective membranes normally are white. During the day the ventral arms usually are much lighter, showing little red-brown color.

When the animal is undisturbed and hovering off the bottom the dorsal mantle is a uniform pale olive color with a light beige lateral stripe along the finline. When disturbed by prodding, the animal jets or swims away. The now mottled color is darkened or intensified until a uniform dark brownish-red color is shown. The initial pattern is directed unilaterally toward the side approached. The entire animal darkens only when continually prodded or pursued. The mantle dark spots and white spots are expressed maximally and papillae are extended maximally.

E. Discussion

Even though only one immature animal was available for study, a great deal of information was obtained from it, largely because of the availability of the comprehensive manuscript of Hanlon & Messenger (1988) on *S. officinalis,* which provided the foundation for recognizing and delineating the many components of body patterns, texture and posture.

Sepia papuensis has many of the same components as *S. officinalis,* but the details are species-specific. Due to the cross-sectional nature of our study and the immaturity of the single animal observed, it was not possible to formulate a profile of all the potential patterns and chromatic components (Table 7). However, the basic light and dark components observed are similar to those seen in *S. officinalis* (Hanlon & Messenger, 1988).

The cuttlefishes of Australia are poorly known from the standpoint of observations on living animals. A growing inventory of observations and photographs is being accumulated by biologists and diver/naturalists that eventually should allow a species to be identified on the basis of color and patterns. Watson-Russell (1981) provided photographs of live *S. apama, S. mestus* and *S. plangon* that also occur off Lizard Island. Detailed field observations on *S. latimanus,* another species reported from Lizard Island, are presented in Corner & Moore (1980) and later discussed in Moynihan & Rodaniche (1982). Additional photographs of a cuttlefish tentatively identified as *S. latimanus* are presented in Catala (1986). Our observations on *S. papuensis* indicate that this species, in terms of generic patterns, is similar to the other Australian species indicated above.

Undoubtedly every cuttlefish species has its own repertoire of body pattern components, some more, some fewer than *S. officinalis,* depending upon the selective pressures and conditions imposed during evolution. We can expect a number of variations and permutations on the basic plan that Hanlon & Messenger have revealed. Studies such as are reported here represent the coalescence of behavior and systematics in the sense that more basic, broad-based components represent higher level taxa, e.g. genera or families, while the variations and permutations are species level character traits.

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NOTE ADDED IN PROOF: Color photographs of live animals or references to behavior of cephalopods discussed in this paper are contained in the following recent publications:

CONIFF, R., 1988, The ten most venomous animals. International Wildlife, 18(2): 18–25. [*Hapalochlaena* cf. *lunulata,* p. 20–21].

DANIELS, P., 1984, Watercolors. *International Wildlife,* 14(4): 29–30. [*Haplochlaena lunulata,* p. 30].

ELLIS, R. & DOUBILET, D., 1987, Australia's southern seas. *National Geographic,* 173(3): 286–319. [*H.* cf. *maculosa,* p. 302–303].

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NESIS, K. N., 1987, *Cephalopods of the World.* T. F. H. Publications, Neptune City, 351 p. [Octopus cyanea, p. 71; *O. ornatus,* p. 74; *H.* cf. *fasciata,* p. 78; *Metasepia pfefferi* p. 54].

VAN PEL, P., 1987, An encounter with a deadly octopus. *Hawaiian Shell News,* 35(2): 4. [*H.* cf. *maculosa,* p. 4].

Although relevant to this paper, we did not have access to the final draft manuscript of Hanlon's paper in this volume and hence were not able to cite it in our discussion. Further, we were provided only with the descriptive section of the Hanlon & Messenger (1988) manuscript. Hence, although we cited it extensively, as a note of caution, we cannot verify that the citations are appropriate in every case.

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FRESHWATER AND TERRESTRIAL FOOD ORGANISMS AS AN ALTERNATIVE DIET FOR LABORATORY CULTURE OF CEPHALOPODS

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ABSTRACT

Preliminary results based upon four octopodids and one sepiid held in small, recirculating seawater systems suggest that rearing and long-term maintenance can be supported on a diet based exclusively upon live freshwater and terrestrial prey items. Crayfish, fishes, clams, snails, earthworms, insect larvae and salamanders were accepted. Crayfish were most readily accepted and appeared to cause best growth. Use of these exotic diets can extend the availability of live cephalopods for research purposes, especially to laboratories removed from coastal marine environs.

Key words: *Octopus; Sepia;* freshwater diets; maintenance; rearing; mariculture.

INTRODUCTION

Recent advances in technology related to small-scale, recirculating seawater systems for holding cephalopods, particularly octopodids and sepiids, have expanded the availability of these organisms for descriptive and experimental research applications (see Spotte, 1979; Forsythe & Hanlon, 1980; Boletzky & Hanlon, 1983). Additional studies on water quality and disease prevention pertaining to cephalopods in culture have enhanced these applications further (see Hanlon *et al.*, 1984).

Feeding of laboratory animals has followed the traditional practice of offering natural prey items, predominately live marine crabs, shrimps, fishes, worms, clams and snails. Achievement of maximal growth rates has often been the objective. In our opinion, this traditional approach to the feeding of laboratory-held cephalopods has served as the last major impediment to the wider utilization of live cephalopods in laboratories removed from coastal marine environs.

Boletzky & Hanlon (1983), as part of a comprehensive review of cephalopod culture, included five scattered records of freshwater and terrestrial food items accepted by laboratory-held cephalopods: Vlès (1914), chicken eggs (*Octopus vulgaris*); Haven (1972), pieces of chicken (*Nautilus pompilius*); Matsumoto (1976) and Matsumoto & Shimada (1980), live goldfish (*Doryteuthis bleekeri*); unpublished record, live crayfish (*O. briareus*). In addition, Overath (1975) fed guppies to *Sepia officinalis*.

For ongoing studies on the systematics and classification of octopodines, currently being conducted by one of us (RBT), five species of cephalopods (four octopodids and one sepiid) were reared and/or maintained. Diets consisted exclusively of freshwater and terrestrial prey by necessity of location (south central Tennessee) and to explore the possibility of alternative diets for cephalopods held in laboratories removed from a ready source of live marine prey organisms.

MATERIALS AND METHODS

Two independent, small-scale, refrigerated seawater systems were built and maintained at The University of the South, located at an elevation of 2000 ft above sea level. The University is located on a large tract of land well supplied with creeks, streams and lakes where potential prey could be collected easily.

System 1 (400 liter) is similar to that described by Forsythe & Hanlon (1980) except that power heads are used in place of air lifts. System 2 (800 liter) is similar in design but uses a large (700 liter) floor tank as the filtration tank and a single pump to supply seawater to a manifold fitted with eight supply valves. The valves feed sea water to a total of up to eight holding tanks. A spillover pipe insures high turnover rates through the filtration system at times when holding tank water demand is low. Water from the holding tanks returns to the filtration tank by means of

gravity via a water table collecting the overflow.

Sea water was made using a commercially available sea salts mixture. Water quality was assayed using commercially available salt water test kits. In addition, ammonia levels were determined using the technique described by Spotte (1979). Water quality generally conformed to guidelines suggested by Forsythe & Hanlon (1980). A refrigeration unit was used to maintain water temperature between 15°C and 19°C. Problems relating to ambient room temperature and refrigeration unit load occasionally caused temperatures to reach 22°C for short intervals of time.

Cephalopods were shipped to the laboratory via standard overnight air freight delivery services with the exception of *Sepia* eggs, which were hand-carried by one of us (RBT) from Banyuls, France.

Most prey items were collected from the laboratory environs. A separate, recirculating freshwater system was constructed to facilitate long-term maintenance of live prey. This became essential during winter months when prey availability was reduced. In addition, meal worms (*Tenebrio*) and several fishes (*Gambusia, Poecilia* spp.) were raised in the laboratory to serve as food.

Laboratory-held cephalopods were offered a variety of freshwater and terrestrial prey of various sizes. The composition of the diet of some animals was changed to evaluate relative acceptance of prey. Survivorship was monitored. Weights to the nearest 0.5 g were recorded weekly from some animals.

RESULTS

Octopus bimaculoides, O. maya, O. digueti, O. joubini (see note below) and *Sepia officinalis* were reared or maintained for periods up to 166 days. With the exception of a short acclimatization period for the single *O. bimaculoides* and all *S. officinalis*, during which time live marine foods were offered, animals were fed exclusively on freshwater and terrestrial prey.

Octopus bimaculoides

Four animals have been maintained. Two died from apparent bacterial infections soon after their acquisition. The first survived 25 days. During the first two weeks of laboratory maintenance its weight increased from 31.5 to 45.0 g on a diet of crayfish (*Cambarus* sp.). The second animal survived 29 days. During the first week its weight increased from 22.5 to 26.5 g on a similar diet. Both animals ceased to feed and lost weight in the later stages of the disease.

A third (male) animal was maintained for 93 days. During the first four days of maintenance, marine shrimps, crabs and worms interspersed with crayfish were offered and accepted. Beginning on day 5, freshwater and terrestrial foods were offered exclusively. Crayfish constituted 90% of the diet (Fig. 1). Earthworms, sunfish (*Lepomis* sp.), snails (*Physa heterostropha* and *Helix* ? sp.), clams (*Corbicula fluminea*), insect larvae (Tipulidae, mealworms (*Tenebrio*)) and salamanders (*Necturus* sp.) constituted the remainder. The animal was not weighed for the first 38 days of maintenance but was estimated to have weighed 100 g on day 0. Following 21 days of feeding on this mixed diet, the animal increased in weight from 143.5 to 168.5 g.

On day 62, a salamander (*Triturus viridescens*) was offered and partially eaten. Symptoms consistent with toxicity response were observed for two days. These included general flaccidity, inability to coordinate movement and attach to the aquarium walls, and cessation of feeding with concomitant weight loss. On the third day following ingestion, the animal appeared to be fully recovered and survived for an additional 29 days, during which time it reached a maximum weight of 175.5 g. Death appeared to be due to a bacterial infection that had caused a blackened necrotic swelling of arm tip RII, and small arm lesions; in addition, feeding ceased. Weight loss occurred for about 10 days before death.

A fourth animal was maintained for a total of 111 days (it was still alive as of this writing). For the first seven weeks, the diet consisted almost exclusively of crayfish with sporadic additions of clams (*Corbicula*). Body weight increased from 130.5 to 175 g. Beginning with the eighth week of maintenance, the diet was changed predominantly to sunfish (*Lepomis* sp.) with occasional crayfish. The sunfish

[1]There is a systematic problem concerning the identity of *O. joubini*. Available data suggest that two morphologically similar species have been referred to this taxon, one with large eggs and a second with small eggs. The current study includes animals representing the small egg form. The systematic problems are being investigated currently by R. Toll, S. Hess and G. Voss

were only accepted sporadically and during this period (2 weeks) weight peaked at 178.0 g and then fell to 172.5 g. Beginning with week 11, the diet was changed again to clams exclusively. Weight subsequently remained constant at about 170 g.

Octopus joubini[1]

A mature male and a brooding female were held in a partitioned 5 gal aquarium as part of system 2 and were offered a diet of crayfish and *Corbicula*. The female never fed while guarding her eggs and died after 6 days. The male accepted both prey species sporadically. The male has survived for 92 days as of this writing. Weight has remained near constant at 14 g.

Octopus maya

Fifteen benthic hatchlings (mean ML approximately 6 mm) were held in small (250 ml) cups open to general water circulation and suspended in raft-type culture in system 1 Food consisted entirely of mealworms (*Tenebrio* sp) that were cut into approximately 3 mm segments. Mortality was relatively constant with higher losses occurring in the first several days of rearing and at 45–49 days when food was temporarily unavailable. The longest survival was 70 days.

Five juveniles were placed together in a 10 gal aquarium in system 2. They were offered and sporadically accepted a combination of prey including juvenile crayfish, mealworms and *Corbicula*. Two animals died from presumed bacterial infections shown in part as apical mantle skin lesions. A third animal was cannibalized. No growth data were taken. The longest survival was 81 days.

Octopus digueti

Twelve benthic hatchlings (mean ML approximately 4 mm) were held in raft culture as described above for *O. maya* hatchlings. Diet consisted entirely of mealworms. Mortality was steady throughout but was accelerated when food became temporarily unavailable (days 45–49). Longest survival was 68 days. No growth data were taken.

Sepia officinalis

Approximately 25 eggs of unknown ages but representing at least three different broods were obtained for rearing. Four viable hatchlings were obtained, all from the most advanced egg brood. Mantle lengths of the hatchlings were 7–8 mm. Hatchlings were placed in individual containers held in raft culture. On days 2 through 9 post hatching, *Artemia* nauplii, freshwater plankton, mealworms (*Tenebrio* sp.) and newborn black mollies were offered. There were no signs of acceptance. On day 10, juvenile crayfish were offered to three of the hatchlings and an insect larva (Diptera) to the fourth. All accepted the prey. Beginning on day 11, prey consisted of 95% juvenile crayfish with occasional additions of newborn guppies and *Gambusia* sp. Terrestrial isopods were offered also but were not accepted.

The smallest of the four hatchlings died on day 15 (ML = 7 mm). The next smallest died on day 71 (ML = 16 mm).

On day 135 the smaller of the two remaining animals had skin lesions. The animal was treated with Furanace (10% nifurpirinol), but succumbed to the infection two days later (ML = 26 mm). The fourth animal survived 166 days (ML = 33 mm). Skin lesions were observed for the final six days. These became severe, and feeding ceased three days before death.

DISCUSSION

As a general rule, a preference for crustacean prey among cephalopods is widely known (Boletzky & Hanlon, 1983). The present findings lend further support. Among the various freshwater and terrestrial organisms offered as prey, crayfish were the most widely and readily accepted. They were fed upon most often by a ventral folding of the body at the juncture between the carapace and tail. Flesh was normally removed without disarticulation of the exoskeleton (Fig. 2).

Fishes, clams and snails were accepted sporadically as prey. Clams were pried open, never drilled (Fig. 3). Fishes were most often stripped of flesh along the flanks. The head was eaten rarely with the exception of the eyes, which were consumed occasionally (Fig. 4). Newborn fishes fed to smaller cephalopods were ingested more completely, commonly with the exception of the heads. Snails were removed via the apertures. Only nonoperculate snails were offered.

Salamanders, fed to a single *Octopus bimaculoides*, were most often stripped of

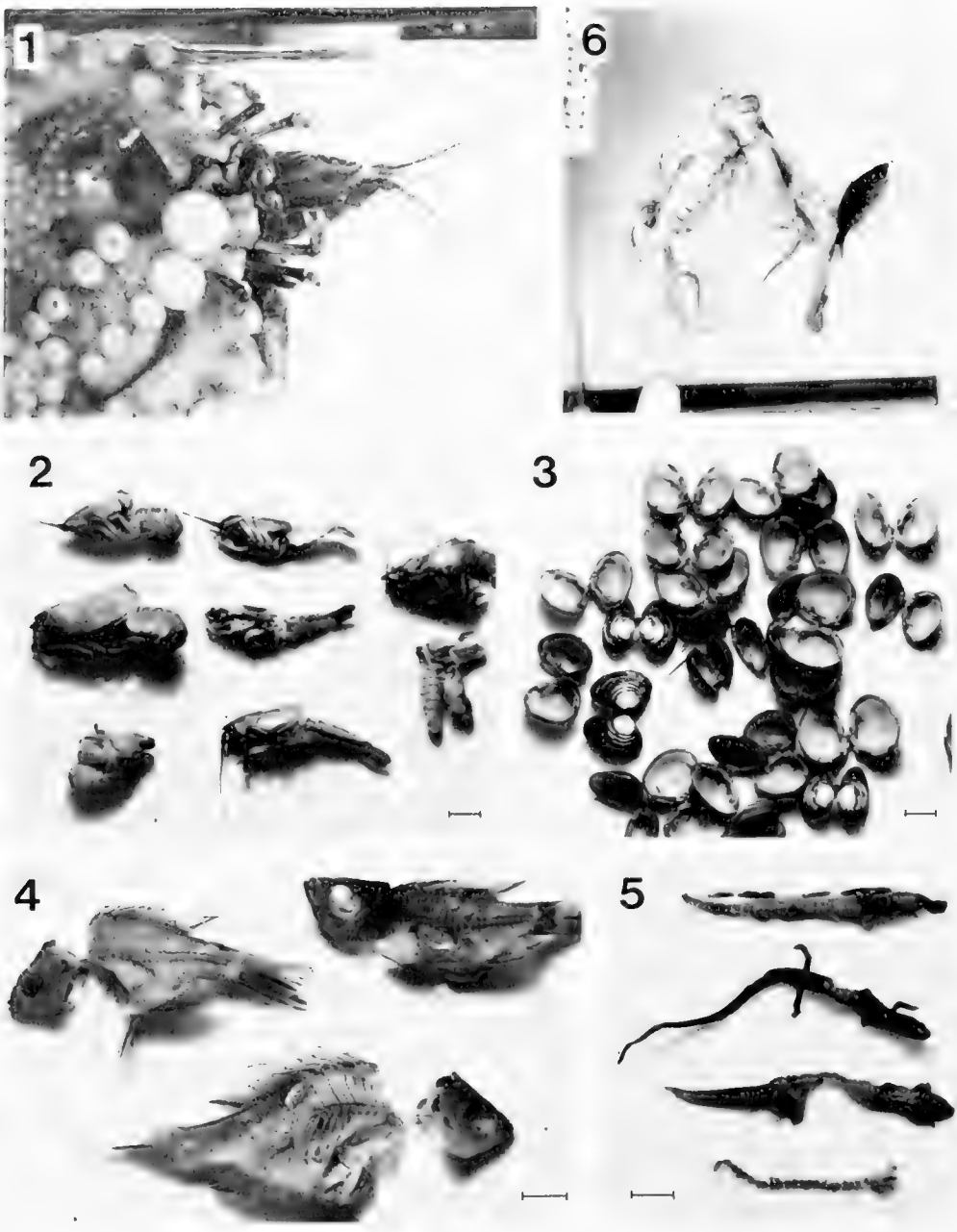

FIG. 1. *Octopus bimaculoides* consuming a large crayfish. Fig. 2. Discarded, vacant exoskeletons of crayfish following predation by *Octopus*. Fig. 3. Discarded shells of *Corbicula fluminea* following predation by *Octopus*. Fig. 4. Remains of sunfish (*Lepomis* sp.) following predation by *Octopus*. Fig. 5. Remains of salamanders following predation by *Octopus*. Fig. 6. Sunfish (*Lepomis* sp.) being offered to *Octopus bimaculoides* on the end of a glass rod in order to simulate life movements. Scale bars equal 1.0 cm.

flesh along the trunk between the forelimbs and hindlimbs. The head and tail were consumed rarely (Fig. 5). Use of salamanders was discontinued because of potential toxicity in some species. *Necturus* sp., however, was accepted commonly without apparent adverse results.

Earthworms, tipulid insect larvae and mealworms were consumed entirely with the exception of mealworm segments fed to hatchling and some juvenile octopodids. In these cases, the flesh was eaten from the cut ends of the worms and the vacant exoskeleton discarded.

Live terrestrial isopods, freshwater amphipods, hatchling tadpoles (*Rana* sp.) and tubifex worms were offered to hatchling octopuses but were never accepted.

Surprisingly, all freshwater and terrestrial prey items survived in full strength sea water for periods of several minutes (e.g. salamanders, earthworms, insect larvae) up to an hour (e.g. sunfishes), and in some cases up to several days (crayfishes). No acclimatization periods in brackish water were used. Sunfish and crayfish that had begun to go into osmotic shock were occasionally offered to octopuses by hand or at the end of a glass rod (Fig. 6). The ability of these organisms to stay alive for even short periods enhanced their receptivity as prey. It is known widely that cephalopods prefer live food (Boletzky & Hanlon, 1983).

Because of inconsistent food supplies and dietary changes, none of the animals reported upon here can be said to have been fed *ad libitum* as is normally the case in the laboratory culture of cephalopods. Therefore, growth rates observed here only begin to suggest normal growth potential on exotic diets. Brief comparisons to growth data obtained from two of the species treated here but cultured on traditional diets were made. Forsythe & Hanlon (unpublished data) have found sustained mean weight gains of about 2 g/day for *Octopus bimaculoides* in the 100 to 200 g size range. This is about double the growth rate seen here with a diet consisting mainly of crayfish. Boletzky (1983) presented growth data for *Sepia officinalis*. Growth and water temperature showed a significant positive correlation. Boletzky's summary for *S. officinalis* suggests a growth rate between two and three times that observed here for the largest individual.

Skin lesions seen on several animals were consistent with reports of bacterially induced lesions described by Hanlon *et al.* (1984). Furanace treatment used here followed the protocol suggested by Hanlon *et al.* (1984). Preliminary microbiological examination of the lesions and the sea water determined several bacteria, including *Vibrio* sp., to be present.

CONCLUSIONS

Small-scale, recirculating seawater systems designed specifically for the culture of octopodid cephalopods can be maintained relatively easily and inexpensively without direct reliance upon facilities and materials available only along the sea coast. Octopodid and sepiid cephalopods studied here accepted a wide variety of live freshwater and terrestrial prey species including crayfish, fishes, clams, snails, earthworms, insect larvae and salamanders. Of these, crayfish were accepted most readily and preliminary data suggest they brought about the best growth. Maximum growth rates for animals reared and maintained in this manner are unknown at present; however, long-term sustained growth has been demonstrated in some species. The use of locally available and relatively inexpensive freshwater and terrestrial prey used as an alternative diet or to supplement marine foods will, we hope, have a positive effect on the wider use of laboratory animals for all types of descriptive and experimental applications of live cephalopods.

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"LARVA," "PARALARVA" AND "SUBADULT" IN CEPHALOPOD TERMINOLOGY

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ABSTRACT

The term "larva" is undefinable as a general term in cephalopods, and its value as a developmental term within specific genera or families is left unresolved.

The term "paralarva" is introduced as a general term for the planktonic young of cephalopods that meet certain ecological, and in some cases, morphological criteria. Because "paralarva" is based at least partially upon ecological criteria, it does not compete with the developmental terms "larva" or "juvenile." Technically, a young cephalopod can be both a larva and a paralarva, or a juvenile and a paralarva. Paralarvae appear to exist only in the Teuthoidea and Octopoda.

The term "juvenile" defines the developmental stage between hatching and the subadult stage, or if "larva" is applied as the post-hatching developmental stage in certain groups, then "juvenile" is defined as the stage between the larval and subadult stages. In the latter case, the criteria that separate these stages are not considered in this paper.

The term "subadult" defines the stage between the juvenile and the adult stages. The subadult stage commences with the full attainment of morphological features used to define the species other than those relating to size and sex. The "adult" stage commences with the attainment of sexual maturity (i.e. presence of complete spermatophores in males and mature ova in females).

Key Words: larvae; paralarvae; juveniles; subadults; cephalopds.

INTRODUCTION

Little consensus exists in the literature on the appropriateness of the term "larva" to designate the early planktonic young of many cephalopod species (e.g., Boletzky, 1974; Nesis, 1979; Fioroni, 1982). Although the term has been widely used, several authors have recently abandoned it altogether (e.g. Okutani & Murata, 1983). The considerable division on this issue that exists among cephalopod workers was apparent during vigorous discussions at the first Cephalopod International Advisory Council workshop in France, 1985, on the early life history stages of cephalopods, but the issue was not resolved.

According to the definition of Geigy & Portmann (1941), a larva goes through a metamorphosis in which, among other things, (1) "larval" parts of the animal are obliterated and in which (2) certain adult parts form from rudiments that remained in an embryonic state. Boletzky (1974) argued that cephalopods, in contrast to other molluscan groups, often have young planktonic stages whose distinctive "larval characters" involve differences in morphometrics and not in basic morphology; therefore, they are not larvae in the sense of Geigy & Portmann. He suggested further that the minor exceptions that do exist (e.g. Kölliker's organs) do not justify use of the term. Nesis (1979), on the other hand, points out the similarity of cephalopod development to that of fishes, where the term "larva" is well accepted although based on a more liberal understanding of the term. He places emphasis on the presence of "larval characters" and on sharp developmental changes in growth coefficients of various structures (i.e. a "metamorphosis"). Without a generally accepted definition of larva, there is no simple solution to this problem.

The term "juvenile," especially as applied to squids, can be so broadly defined that it is of little use. Many cephalopods reach sexual maturity only near the end of their life (see Boyle, 1983) and can be called juvenile from hatching almost to death. As a result, the term "sub-adult" has frequently appeared in the literature to refer to cephalopods that are similar in appearance to adults but are not sexually mature and frequently are smaller than adults (e.g. Young & Roper, 1969; Roper & Young, 1975; Nesis, 1979; Voss, 1980, 1985). Unfortunately, since this term has

never been defined, uncertainty exists in the use of both "juvenile" and "subadult."

In this paper we examine some of the terms used in discussing the early life history stages of cephalopods. While we do not offer a solution to the appropriateness of "larva," we do offer a new term that fills a terminology gap that formerly was filled partially by "larva." We also define the term "subadult" that, in turn, redefines "juvenile."

LARVA/PARALARVA

Fishes have distinct developmental features that provide a consistent operational definition of "larva" (i.e. from hatching to the attainment of complete fin ray counts and the beginning of squamation; Kendall, *et al.*, 1984). With fishes a rationale exists for extending the more traditional use of the term. Not only do cephalopods, in general, lack the degree of larval modification found in fishes (Nesis, 1979), but they lack any universal morphological features that could be applied in the manner found in fishes. Therefore, the term "larva," when applied to cephalopods in general, not only necessitates a further broadening of the concept, but operationally it is undefinable. Nevertheless, the frequent use of "larva" in the cephalopod literature (e.g. 92% of the papers cited in Table 1 use this term) demonstrates the need for a term to designate an early developmental stage that resides in the near-surface plankton and that differs from later stages in habit, habitat and, frequently, morphology. Most pelagic cephalopods and some benthic octopods have stages that fall into this category. While an appropriate term cannot be defined solely on developmental (i.e. morphological) criteria, a term that incorporates both morphological and ecological criteria is feasible. Such a term would not strictly replace "larva" or "early juvenile," which are developmental terms, but would fill a clear gap in our present terminology. Since the term "larva" is used widely in the cephalopod literature, the new term should be sufficiently similar in spelling to be easily recognized as a somewhat comparable term. We propose the term "paralarva" for these young planktonic stages of cephalopods. The prefix "para" is Latin for "almost" or "nearly."

We define paralarva as "a cephalopod of the first post-hatching growth stage that is pelagic in near-surface waters during the day and that has a distinctively different mode of life from that of older conspecific individuals." "Day" is stipulated in the definition because near-surface waters are often occupied by "older conspecific individuals" during the night but not during the day. Differences in habitat, therefore, are more pronounced during the day. "Near-surface" is not defined more precisely because the depth of this zone will vary somewhat with locality. Usually the different "mode of life" will be inferred from: (1) major differences between the daytime habitat of the paralarva and that of older individuals and/or (2) distinct early discontinuities in growth patterns. In general, morphological discontinuities in a developmental series will accompany habitat changes. The definition requires a morphological change only when habitat changes are not obvious (e.g. species that occupy near-surface waters as older individuals such as *Argonauta* spp. or *Thysanotheuthis rhombus*) and requires a habitat change only when morphological changes are not obvious (e.g. the vertically migrating *Pterygioteuthis* spp.).

As an example of the criteria used to specify individuals as paralarvae, we present data of Young & Harman (1986) on squids of the genus *Onychoteuthis*. Young squids (2–14 mm gladius length, GL) occupy near-surface waters during the day (primarily 25–150 m) and have a mode of life different from that of larger individuals. Evidence for the latter feature comes from two sources. First, developmental discontinuities mark the end of the paralarval stage: (a) the number of chromatophores on the dorsal surface of the mantle (and on many other body surfaces) are relatively constant over a considerable growth range (paralarval stage), then abruptly increase in number (Fig. 1); (b) hooks begin to develop on the tentacular clubs as the chromatophoral changes occur. Second, major changes in habitat seem to co-occur with morphological changes. Although little information is available on the distribution of older juveniles, Young (1978) recorded a 19 mm GL specimen of *Onychoteuthis compacta* from about 700 m (caught in an opening-closing net) during the day. In this species the developmental discontinuities listed above occur at 12–16 mm GL.

The three species of *Onychoteuthis* differ considerably in size at the end of the paralarval stage (Fig. 1). Size, therefore, will not be a useful aid for determining the end of the stage and, for other species, chro-

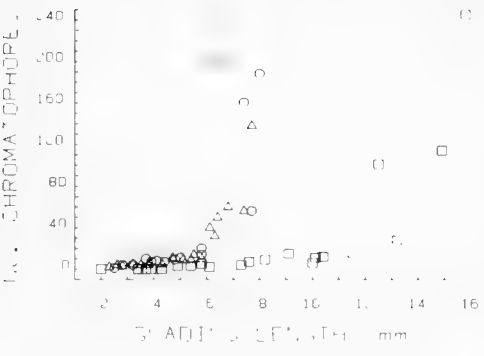

FIG. 1. Number of chromatophores on the dorsal mantle of *Onychoteuthis compacta* (squares; n = 21), *Onychoteuthis* sp. B (circles; n = 14) and *Onychoteuthis* sp. C (triangles; n = 18) at various sizes. Discontinuity in numbers of chromatophores occurs for *O. compacta* at 12–16 mm GL and about 6 and 8 mm GL for the other two species.

matophore development and/or hook development will not necessarily be the best criteria. In many groups, apparently "sudden" morphological changes of other kinds occur in young individuals and should be useful in defining the end of the paralarval stage. For example, in cranchiids the stalked eyes become sessile (e.g. Voss, 1985); in ommastrephids the fused tentacles separate (e.g. Pfeffer, 1912); in *Chiroteuthis* spp. the initial tentacular clubs are lost (Roper & Young, 1967); in *Octopoteuthis* the tentacles are lost (Pfeffer, 1912); in *Histioteuthis* spp. photophores appear and ocular asymmetry develops (personal observations); in *Thysanoteuthis rhombus* mantle and fin shape and muscularity change (personal observations); in *Brachioteuthis* elongate necks become short (Pfeffer, 1912); the planktonic young of benthic octopods lose their Kölliker's tufts (Boletzky, 1974). Numerous other examples exist.

Because the primary criteria for distinguishing the paralarval stage include habitat changes, we have assembled data on the daytime vertical distribution of the early stages for one species in each genus where data permit (Table 1). The table, however, excludes the incirrate (finned) octopods, *Nautilus,* and the neritic sepioids. The first two groups have extremely large eggs and their young are never found in near-surface plankton, while the young of the latter group, with the exception of *Idiosepius* spp. (Boletzky, 1974), occur only occasionally in the plankton (see Boletzky & Hanlon, 1983, for a partial list of species with benthic young). Most incirrate (finless) octopods that spawn small eggs have young in the plankton during the day. However, because of the generic instability in this large group and the difficulties in identifying the planktonic stages, only two genera are represented in the table. Because of similar identification problems, the Loliginidae is represented by a single genus. In many cases the data used to construct the table are few and, in some cases, contradictory data exist for the same species from different areas. Table 1, nevertheless, demonstrates the nearly universal occurrence of young teuthoids in the near-surface plankton during the day and the common absence from these waters of older stages. Paralarvae appear to exist only in the Teuthoidea and Octopoda. Like their neritic relatives, the oceanic, pelagic sepioids (i.e. *Heteroteuthis* spp. and *Spirula spirula*) lack paralarvae.

Identifying paralarvae in species that occupy near-surface waters throughout their life-cycle is difficult. Identity must be determined by careful analyses of morphological changes to determine if developmental discontinuities exist. A similar problem exists in species that exhibit a gradual habitat change (e.g. cranchiids that show gradual ontogenetic descent). In these cases, morphological guidelines must be used. When both morphological and distributional data are lacking, we suggest the following temporary alternative definition for paralarvae: young pelagic cephalopods that can be sampled quantitatively by standard plankton nets in near-surface waters during the day.

SUBADULTS

We define the cephalopod subadult stage as "the life-history stage of a cephalopod beginning with the attainment of all diagnostic morphological features generally used to define the species other than those relating to sex and size, and ending with the attainment of sexual maturity." According to this definition, a subadult, where appropriate, must have: hooks present in the definitive pattern; tentacular clubs and stalks with the definitive shape and sucker pattern; fins of the definitive relative size and shape; photophores in the definitive pattern; chromatophores and iridophores in the definitive pattern; tentacles

TABLE 1. Examples of cephalopods expected or not expected to have paralarva. Mantle lengths in parentheses indicate specimens from open tows; mantle lengths without parentheses indicate specimens from open-closing tows. Y = yes; N = no; ? = unknown; NA = not applicable since young and adult stages occupy same general depth range; * = time of capture not given or captured at night, equal daytime depth assumed.

		Early juvenile <250 m (day)	Late juvenile >300 m (day)	Minimum mantle length (mm) recorded at late-juvenile depth	Paralarva expected
ORDER TEUTHOIDEA					
Loliginidae:	*Loligo opalescens*	Y[1]*	N	NA	Y
Enoploteuthidae:	*Pyroteuthis addolux*	Y[2]*	Y[3]	9[3]	Y
	Pterygioteuthis giardi	Y[2]	Y[3]	9[3]	Y
	Abralia trigonura	Y[4]	Y[3]	18[3]	Y
	Abraliopsis sp. B	Y[4]	Y[3]	9[3]	Y
	Watasenia scintillans	Y[5]*	?	?	Y
	Enoploteuthis higginsi	Y[4]	Y[3]	30[3]	Y
	Ancistrocheirus lesueuri	Y[2]	Y[3]	25[3]	Y
Lycoteuthidae	*Lycoteuthis diadema*	Y[6]*	?	?	Y
	Lampadioteuthis megaleia	Y[7]	?	?	Y
Ommastrephidae:	*Sthenoteuthis oualaniensis*	Y[8]	N[2]	NA	Y
	Hyaloteuthis pelagica	Y[8]	Y[2]	?	Y
	Ommastrephes bartramii	Y[9]*	?	?	Y
	Dosidicus gigas	Y[10]*	?	?	Y
	Nototodarus hawaiiensis	Y[11]	200–400 m[12]	(45)[12]	Y
	Todarodes pacificus	Y[9]*	N?	NA	Y
	Illex illecebrosus	Y[13]*	?	?	Y
Histioteuthidae:	*Histioteuthis dofleini*	Y[2]	Y[3]	13(9)[3]	Y
Neoteuthidae:	*Neoteuthis* sp.	Y[14]	Y[2]	(17)	Y
Gonatidae:	*Gonatus onyx*	Y[5]*	Y[14]	?	Y
	Berryteuthis magister	Y[5]*	?	?	Y
	Gonatopsis borealis	Y[5]*	Y[15]	?	Y
Bathyteuthidae:	*Bathyteuthis abyssicola*	N[11]	Y[11]	2.8[11]	N
Ctenopterygidae:	*Ctenopteryx siculus*	Y[2]	Y[3]	20(11)[3]	Y
Onychoteuthidae:	*Onychoteuthis compacta*	Y[16]	Y[3]	19[3]	Y
	Onykia carribbea	Y[2]	N[2]	NA	?
Octopoteuthidae:	*Octopoteuthis nielseni*	Y[2]	Y[3]	30[3]	Y
	Taningia danae	Y[14]	?	?	Y
Thysanoteuthidae:	*Thysanoteuthis rhombus*	Y[2]	?	?	Y
Cycloteuthidae:	*Cycloteuthis serventyi*	Y[14]	Y[3]	27(17)[3]	Y
	Discoteuthis laciniosa	Y[17]*	Y[3]	56(40)[3]	Y
Lepidoteuthidae:	*Lepidoteuthis grimaldi*	Y[18]	?	?	Y
	Tetronychoteuthis massyae	Y[18]	?	?	Y
Brachioteuthidae:	*Brachioteuthis* sp.	Y[19]	Y[3]	38[3]	Y
Chiroteuthidae:	*Chiroteuthis picteti*	Y[2]	Y[3]	65[3]	Y
	Asperoteuthis sp.	?	Y[3]	73[3]	?
	Planktoteuthis lippula	Y[3]	Y[3]	25(19)[3]	Y
	Grimalditeuthis bonplandii	?	Y[3]	30[3]	?
Mastigoteuthidae:	*Mastigoteuthis schmidti*	N[18]	Y[18]	NA	N
Joubiniteuthidae:	*Joubiniteuthis portieri*	Y[2]*	Y[14]	<22[14]	Y

TABLE 1. (Continued)

		Early juvenile < 250 m (day)	Late juvenile >300 m (day)	Minimum mantle length (mm) recorded at late-juvenile depth	Paralarva expected
ORDER TEUTHOIDEA (Continued)					
Cranchiidae:	*Liocranchia valdiviae*	Y[2]	Y[3]	16(7)[3]	Y
	Cranchia scabra	Y[18]	Y[18]	49[18]	Y
	Leachia pacifica	Y[3]	Y[3]	49[3]	Y
	Megalocranchia fisheri	Y[3]	Y[3]	45[3]	Y
	Sandalops melancholicus	Y[2*]	Y[3]	30[3]	Y
	Teuthowenia maculata	Y[20]	Y[20]	40[20]	Y
	Helicocranchia sp.	Y[2]	Y[3]	17[3]	Y
	Galiteuthis pacifica	Y[2]	Y[3]	15[3]	Y
	Taonius pavo	N[14]	Y[14]	11[14]	N
	Bathothauma lyromma	Y[2*]	Y[3]	20[3]	Y
	Egea inermis	Y[21]	?	NA	Y
ORDER OCTOPODA					
Octopodidae:	*Octopus cyanea*	Y[2*]	benthic	(10)[22]	Y
	Eledone cirrhosa	"planktonic"[23]	benthic	?	Y
Argonautidae:	*Argonauta* sp.	Y[11*]	N	NA	?
Tremoctopodidae:	*Tremoctopus violaceus*	Y[11*]	N	NA	?
Ocythoidae:	*Ocythoe turberculata*	"planktonic"[24*]	N	NA	?
Alloposidae:	*Alloposus mollis*	Y[25*]	?	?	Y
Bolitaenidae:	*Eledonella pygmaea*	Y[3]	Y[3]	7[3]	Y
	Japetella diaphana	Y[3]	Y[3]	8[3]	Y
Vitreledonellidae:	*Vitreledonella richardi*	Y[18]	Y[18]	24[18]	Y
Amphitretidae:	*Amphitretus pelagicus*	Y[26*]	?	?	?
ORDER VAMPYROMORPHA					
Vampyroteuthidae:	*Vampyroteuthis infernalis*	N[15]	Y[15]	NA	N
ORDER SEPIOIDEA					
Sepiolidae:	*Heteroteuthis hawaiiensis*	Y[3]	N[3]	NA	N
Spirulidae:	*Spirula spirula*	N[27]	Y[27]	NA	N

References: (1) Okutani & McGowan, 1969; (2) Young, R. E., personal observation; (3) Young, 1978; (4) Young & Harman, 1985; (5) Kubodera & Okutani, 1981; (6) Voss, 1962; (7) Young, 1964; (8) Harman & Young, 1985; (9) Okutani, 1968; (10) Nesis, 1983; (11) Clarke & Lu, 1975; (12) Burgess, 1972; (13) Roper & Lu, 1979; (14) Clarke & Lu, 1974; (15) Roper & Young, 1975; (16) Young & Harman, 1986; (17) Young & Roper, 1969; (18) Lu & Clarke, 1975; (19) Young *et al.*, 1985; (20) Voss, 1985; (21) Voss, 1974; (22) Wells & Wells, 1970; (23) Boletzky, 1977; (24) Naef, 1923; (25) Lu & Roper, 1979; (26) Thore, 1949; (27) Clarke, 1969.

completely resorbed or lost; eyes of the definitive shape and position; body of the definitive form; arms of the definitive relative sizes; suckers with definitive dentition; beaks and gladii of the definitive shape. Sexually dimorphic features such as hectocotylization and some types of photophores need not be present for a cephalopod to qualify as a subadult. The number of structures (e.g. suckers, photophores, chromatophores) and the absolute size of structures is not critical; the patterns, shapes and relative sizes provide the definitive features.

The period prior to the subadult stage is the juvenile stage (*sensu* Boletzky) and some juveniles, according to the earlier definition, may also be termed paralarvae. In some cases the termination of the juvenile stage

may coincide with the end of the paralarval stage. In *Pterygioteuthis microlampas*, development of arm hooks at 9 to 11 mm GL marks the end of the juvenile stage. This species has been recorded at the adult daytime depth by 12 mm GL (Young, 1978) and probably arrives there at an even smaller size. The depth transition would mark the end of the paralarval stage. In most cases, however, we expect the juvenile stage to terminate considerably after the end of the paralarval stage. In *Onychoteuthis* species C from Hawaiian waters, the paralarval stage terminates at 6 to 8 mm GL while the juvenile stage terminates between 30 and 35 mm GL when the definitive armature is present on the tentacular club. In *Sthenoteuthis oualaniensis*, the paralarval stage terminates with the separation of the tentacles at 9 or 10 mm ML while the juvenile period ends around 100 mm ML with the development of mantle photophores. In a few cases, the attainment of the definitive characters nearly coincides with attainment of sexual maturity (e.g. *Leachia pacifica;* Young, 1975) and a subadult stage may be virtually absent.

See the Abstract for the final synopsis of the definitions.

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SYSTEMATICS AND ZOOGEOGRAPHY OF CEPHALOPODS

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ZOOGEOGRAPHY OF OEGOPSID SQUIDS

Systematics and zoogeography are closely intertwined, the former usually embracing the latter field. Most recent studies in marine zoogeography have centered on open-ocean organisms, chiefly zooplankton and midwater fishes (McGowan, 1974; Backus *et al.*, 1977; Hulley, 1981; Johnson, 1982; Van der Spoel, 1983, and many others). Though cephalopods are a major component of the marine nekton, comparable zoogeographic studies have been lacking and the question often arises whether the zoogeography of pelagic cephalopods differs from that of pelagic fishes, zooplankton and other marine organisms. Because the query is of paramount interest, I will here respond to it before commenting on the subsequent papers from this section of the Symposium. Clearly the answer is to be found in detailed systematic studies where the emphasis is at the specific and subspecific levels. Even though few such investigations have been undertaken in cephalopods, sufficient information is available, I believe, in the literature and unpublished data.

As in most oceanic organisms, higher taxa in oegopsid squids (which represent the vast majority of oceanic species of cephalopods and occupy a broad variety of habitats from surface waters to great depths) can be classified typically as warm-water, cold-water or widespread groups, and tend to be cosmopolitan or nearly so in their distributions. Although the literature contains an unusually large number of so-called cosmopolitan species, growing evidence from the few detailed studies of families, for example the cranchiids (N. Voss, 1985, 1988, unpublished data), the histioteuthids (N. Voss, 1969, unpublished data) and the gonatids (Young, 1972; Kubodera & Okutani, 1981; Kristensen, 1981; Kubodera *et al.*, 1983; Kubodera & Jefferts, 1984a, b; Jefferts, 1985, and others), show that species formerly considered worldwide are composed of multiple species or subspecies, exhibiting a complex of individual distributional patterns.

Epi- and mesopelagic squids

The cranchiids, which range from small to giant in size, form one of the most widely and abundantly represented families of squids in oceanic midwaters, and occupy the water column from the surface to below 2000 m in every ocean except the Arctic. In all species, spawning appears to occur in open ocean, either at the surface or in the midwaters, and over the entire geographic range of the species. Early growth takes place in the upper layers of the water column, followed by ontogenetic descent to deep water where maturation occurs. Cranchiids show varying degrees of diel vertical movement but are not known to migrate horizontally. Of the 13 genera, 10 occur circumglobally in tropical-subtropical waters, with 1 extending its range to temperate waters, 1 to temperate and subpolar waters, and 1 to temperate, subpolar and antarctic waters. One genus is restricted to tropical waters and another to the Antarctic; both are circumglobal. Contiguous geographic distributions of closely related congeners are typical for the family, but considerable overlap of ranges occurs in some genera. One genus (*Teuthowenia*) is atypically allopatric in its distribution.

Though my knowledge of the distributions of the more than 60 species of cranchiids is incomplete, I have been able to discern the following patterns (broadly described in terms of water-mass regions): (1) in the Atlantic—Subarctic-Temperate, North Temperate, Temperate-Subtropical-Semitropical, North Subtropical, Tropical, Tropical-Semisubtropical, Tropical-Subtropical and Eastern Tropical; (2) in the Pacific—Subarctic, North Subtropical, Kuroshio Current, California Current, North Tropical, Eastern Tropical, Western Tropical, Peru-Chile Current and Southeast Australian

Current; (3) in the Southern Ocean—Southern Subtropical Convergence, Subantarctic and Antarctic; (4) in the Indian Ocean, and in multiple oceans—Arabian Sea, Benguela-Agulhas Currents, Tropical Indian-West Pacific and Subtropical Indian-West Pacific. Only 3 species remain, all poorly studied, that appear to show widespread "warm-water" distributional patterns: Pacific-Indian Ocean "Warm-water" (1 species), and Circumglobal "Warm-water" (2 species). Occurrence and within range abundance in the various distributional patterns are related largely to the primary productivity of the waters. Undoubtedly additional patterns will be observed as study of the family continues and new material is acquired, especially to fill noticeable gaps in geographic coverage (e.g. southwestern Atlantic and northwestern Pacific).

The histioteuthids are small to large squids found in large numbers worldwide from the Subarctic to the Subantarctic, from the surface to possibly 2000 m. Spawning occurs in some species on continental and island slopes, and possibly on slopes of submarine ridges and mounts; in others, it occurs in open ocean. Most, if not all species, appear to undergo diel vertical migration. The extent of horizontal movement is not known, but findings support my belief that a species range is composed of a number of localized, breeding populations. The observed differences in occurrence and abundance within ranges is related to productivity and differences in spawning sites. The monotypic family contains 13 species or species groups. New material examined subsequent to my 1969 revision shows that at least 5 of the formerly considered widespread or cosmopolitan species (*Histioteuthis reversa, H. elongata, H. atlantica, H. hoylei* and *H. bonnellii*) actually represent groups of 2 or more closely related taxa. Although specific and subspecific distributions are still poorly known, a number of distributional patterns are strongly suggested: (1) in the Atlantic—Subarctic-Temperate, Tropical-North Subtropical, Tropical-Subtropical and Tropical-South Subtropical; (2) in the Pacific—California Current, Equatorial-Tropical-North Subtropical, Eastern Antitropical Transition and Peru-Chile Current; (3) in the Southern Ocean—Southern Subtropical Convergence, Southern Subtropical Convergence-North Subantarctic, Southern Subtropical Convergence-Subantarctic and Subantarctic; (4) in multiple oceans—South Subtropical Pacific-Indian Ocean and Pacific-Indian Ocean "Warm-water." Atlantic and Pacific-Indian Ocean species whose ranges include southern subtropical waters and Southern-Subtropical-Convergence species co-occur in the Benguela and Agulhas currents. At present only 1 species (*H. meleagroteuthis*) appears to have a circumglobal "warm-water" distribution, and that species has been little studied. As in the case of the cranchiids, future detailed work will reveal additional distributional patterns and better define those listed above.

The gonatids are medium to large squids primarily restricted to the higher latitudes of both hemispheres. They occur in large numbers from the surface to about 1200 m, and exhibit varying degrees of diel vertical movement and horizontal dispersal or migration. The 19 described and estimated 4 undescribed species are grouped into 3 genera, of which only 1 (*Gonatus*) is found in both the Atlantic and Pacific and in both hemispheres. The remaining 2 genera are restricted to the North Pacific. Recent detailed study of *Gonatus* has resulted in the recognition of 12 species: 2 in the North Atlantic, 9 in the North Pacific and 1 in the Antarctic, rather than the 2 circumglobal species (1 each in the northern and southern cold waters) recognized prior to 1972. In the Atlantic, the distributions of the 2 species are parapatric with the southern limit of one and the northern limit of the other, well marked by the Polar fronts. In the Pacific, distributions tend to be sympatric. Gonatid species distribution patterns can be classified broadly: (1) in the Atlantic—Arctic-Subarctic and North Temperate: (2) in the Pacific—Subarctic, Eastern Subarctic, Western Subarctic, California Current and Sea of Okhotsk; and (3) in the Southern Ocean—Antarctic. Differences found in the North Pacific in the breadth of the distributions and in the occurrence of the various growth stages over the geographic ranges suggest probable differences in spawning sites, whether in association with continental slopes or in open ocean, and whether broad or restricted in extent.

Bathypelagic squids

Sufficient information is available on the species of two families of bathypelagic squids, Bathyteuthidae (Roper, 1969; Lu & Clarke, 1975; Toll, 1982, unpublished data; Hess, 1987) and Promachoteuthidae (Roper & Young, 1968; Toll, 1982; Okutani, 1983,

unpublished data; Hess, unpublished data) to reveal the general nature of their distributions. The bathyteuthids are small squids primarily inhabiting depths between 500 m and 4200 m. Reported night captures in the upper 500 m indicate that some diel vertical movement occurs in at least part of the population of *Bathyteuthis abyssicola* in the eastern North Atlantic. Two types of distributional patterns, widespread and restricted, are shown by the 3 currently recognized species belonging to the monotypic genus *Bathyteuthis. B. abyssicola* occurs circumglobally in the Southern Ocean, where it is one of the most abundant cephalopods in the Antarctic, and in scattered areas in the Atlantic and Pacific, and possibly northern Indian Ocean, in deep waters beneath areas of high productivity. Population differences have been found over the wide range of the species. The known distributions of the other 2 species are limited to the California Current (*B. berryi*) and to the Tropical Eastern Pacific (*B. bacidifera*). New evidence from recent comparative morphological studies of the gladius and spermatophores in the teuthoids indicates the presence of a probable fourth species in the Caribbean Sea. In the 3 areas, the possible endemics co-occur with the widespread *B. abyssicola.*

The promachoteuthids are small to medium-sized squids known from only 9 specimens, ranging from small juveniles to a mature male, taken separately in nets fishing between about 1400 m and 3500 m in widely scattered areas in the North and South Atlantic and Pacific oceans. The scant material represents at least 3 well-characterized species, currently recognized as belonging to a single genus: one species known from 2 captures in the western North Pacific; one from a single capture in the eastern North Atlantic; and the third from 4 captures in the Peru-Chile Current and in the Pacific sector of the Subantarctic. Paucity of data precludes description of patterns of distribution in the family, but it is adequate to suggest that they may be relatively restricted.

The distributional patterns in the above families of squids (selected on the basis of sufficient available data) are complex and of varying breadth, sometimes widespread, more often relatively restricted. They reflect the influences of various biological and physical factors largely related to the circulation of the oceans. Biological productivity, major and lesser hydrographic fronts, currents, temperature, vertical physical structure and other environmental features, as well as life history demands, all play roles of varying importance in defining the distributions of the individual species. The evidence, though limited, clearly echoes the findings of studies of other pelagic groups and, I believe, is adequate to show that the zoogeography of pelagic cephalopods does not differ substantially from that of pelagic fishes, zooplankton and other organisms.

SYMPOSIUM PAPERS

The expanding interest in the systematics and zoogeography of cephalopods is shown by the varied subjects of the 7 papers from the Symposium published in the following pages.

The paper by J. Augustyn & W. Grant is particularly noteworthy because it is one of the few cephalopod studies to apply electrophoretic analysis to population and taxonomic investigations. The authors use evidence from morphological and biochemical (protein electrophoresis) analyses to compare the closely related nominal species of coastal squids, *Loligo vulgaris* and *L. reynaudii,* that occur off western Africa. Although the taxa occupy ranges that are not known to overlap, the data sets indicate that morphological and biochemical divergence is minimal. The differences found are estimated to be of subspecific level. It is proposed that the cold-water upwelling off the coast of Namibia, dating back to the late Pliocene, acts as a barrier between the 2 subspecies.

E. Dawe and S. Stephen describe the cephalopod fauna of the upper 100 m of the Gulf Stream and adjacent waters between 37° and 44°N, 53° and 60°W, on the basis of Bongo net and midwater trawl collections taken over a 4-year period. In the collections, which contained at least 51 species belonging to 22 families, ommastrephids occurred in highest abundance followed by enoploteuthids, onychoteuthids and cranchiids, with the enoploteuthids and cranchiids represented by the most species. Analysis showed certain species to be characteristic of Shelf, Slope or northern Sargasso Sea waters. The authors found that cephalopod abundance in the surface 100 m increased from the Slope Water to the northern Sargasso Sea. The reported evidence that tropical-subtropical and subtropical species find their

northern limit at the north edge of the Gulf Stream mirrors the findings of studies on zooplankton, phytoplankton and midwater fishes.

Numerous existing problems in taxonomy of shallow-water cephalopods may find clarification in careful observations of the behavior of the living animals. Such is proposed in R. Hanlon's exploration of behavior and body patterning as valuable sources of innovative characters to complement morphological investigations. Acute body patterns (highly stereotyped, usually locomotory with specific skin patterning) used as responses to predators, prey and conspecifics, daily activity patterns, habitat selection, body-color patterns and their chromatic components, and, in deep-water squids, photophore signal patterns are all aspects of behavior considered to be potentially useful, as is behavior associated with courtship, mating, egg care, feeding, migration and ink use. Hanlon also encourages use of morphological features such as chromatophores, iridescent cells, white leucophore markings, papillae, ocelli, photophores and ink, and gives simple guidelines for data collection and analyses. He concludes with a demonstration of the use of body patterning and behavioral cues in distinguishing the different shallow-water octopods of the Caribbean Sea and between several pairs of morphologically similar species in which current problems in identification exist: *Octopus bimaculoides/O. bimaculatus, Loligo plei/L. pealei,* and *L. vulgaris/L. forbesi.* Systematic, zoogeographic and phylogenetic investigations will benefit from the new comparative studies that this paper will surely stimulate.

The vertical distribution of the Antarctic neoteuthid *Alluroteuthis antarcticus* is described by P. Rodhouse primarily from juveniles collected in opening/closing RMT-8 nets in the Atlantic sector of the Southern Ocean. The data indicate that this meso-bathypelagic squid undergoes ontogenetic descent and some diel vertical movement. A comparison of the vertical distributions of *A. antarcticus* and another Antarctic squid commonly found in the net collections, the cranchild *Galiteuthis glacialis,* suggests to the author that ecological separation occurs between the 2 species.

A revision of the genus *Octopus* in Australian waters is greatly needed both from scientific and commercial points of view. T. Stranks' initial paper of a proposed monographic revision of the genus in southeastern continental waters in which he redescribes *Octopus pallidus* Hoyle, 1889 is an important first step toward that broader goal.

In one of the few studies on cephalopods involving the historical rather than the ecological approach to zoogeography, J. Voight relates the distributions of "geminate" species (pairs of morphologically similar species that share a recent common ancestor) of shallow-water octopods on either side of the Central American Isthmus to the vicariant event of the recent closure of the Central American Seaway. Her close-up study, restricted to the octopods of the shallow American tropical waters, revealed 6, and possibly 7, "geminate" species-pairs, a higher number and higher proportion of the fauna studied than reported in the earlier, less detailed investigation of Nesis (1978). Voight's findings are similar to those of investigations on other marine organisms and did not suggest an exceptional rate of evolution previously proposed for cephalopods.

The systematics are poorly known and the biogeography never analyzed for the deep-sea octopods. In the final symposium paper, G. Voss attempts a first sketch of the distributions of this important faunal group, defined by the author as those benthic and near-benthic species of octopods that normally live below the edge of the continental shelf (except in polar waters where they may range to the surface). Deep-sea octopods occur worldwide in all oceans and have been taken at depths >7000 m. They include all members of the Cirrata, or finned octopods, which are near-benthic in habitat, and members of 11 genera belonging to the benthic Octopodidae of the Incirrata. All are large-egg forms whose hatchlings are supposed to assume the adult habitat directly. Voss's survey and all reported captures show that generic distributions are usually cosmopolitan, or nearly so, as in other marine organisms including meso- and bathypelagic squids (see above). Paucity of captures and frequently uncertain identifications preclude a detailed study, but sufficient published and unpublished data are available to demonstrate that species distributions are often surprisingly limited, and an unexpectedly high degree of sympatry exists among congeners in certain geographic areas where detailed faunal studies have been made. Of the 77 currently recognized species, only 2 found North of the Southern Ocean occur in both the Atlantic and Pacific oceans. Good correlation is found between

areas of species richness and areas of high productivity in overlying waters.

CONCLUDING REMARKS

Cephalopods play a significant role in marine food chains and ecosystems, yet they have been, and continue to be, neglected in multi-organism reviews and investigations of marine resources and various marine systems. This is due largely to the inaccurate picture of cephalopod abundance produced by use of conventional fishing gear and techniques, and the current situation where species identifications in the majority of families and species distributions are poorly known. Studies reported here have increased our knowledge of cephalopods, suggested promising approaches for research, and accentuated the critical need for alpha taxonomic investigations. To know what species there are and where they occur, and to gain an understanding of why they occur as they do, requires additional researchers and increased funding, together with resolution of existing problems in design and use of gear to adequately sample populations and the various growth stages. Since morphological distinctions between closely related species are often observed to be maturity-related and difficult (and in some cases nearly impossible) to detect in the paralarval and juvenile stages, it is essential to know the subadult and adult animals which at present are largely unknown for oceanic species. There is much work to be done. Few other groups of marine animals offer greater challenges and opportunities for innovative studies in systematics, phylogeny and zoogeography than cephalopods.

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BIOCHEMICAL AND MORPHOLOGICAL SYSTEMATICS OF *LOLIGO VULGARIS VULGARIS* LAMARCK AND *LOLIGO VULGARIS REYNAUDII* D'ORBIGNY *NOV. COMB.* (CEPHALOPODA: MYOPSIDA)

C.J. Augustyn[1] & W.S. Grant[2]

ABSTRACT

Two nominal species of squids, *Loligo vulgaris* and *L. reynaudii,* were compared on the basis of morphological measurements, meristic counts of selected characters, and biochemically by means of protein electrophoresis. Results indicated that there were differences in most body dimensions and particularly in club sucker counts and dentition. Canonical and classificatory discriminant analysis were used to summarize these differences. These findings were confirmed by the genetic analysis, which included the calculation of genetic distance between the two nominal species. Differences were found to be of subspecific rather than specific nature, and *L. reynaudii* is demoted to subspecific standing with *L. vulgaris.* The two nominal species therefore become *L. vulgaris reynaudii* and *L. vulgaris vulgaris.* It was also shown that geographical separation between the two taxa is probably caused by an environmental barrier of cold, oxygen-deficient water off the W coast of southern Africa, and that these conditions have existed long enough to have led to divergence between the subspecies.

Key words: systematics; protein electrophoresis; *Loligo vulgaris;* ecology, and distribution

INTRODUCTION

Few squid systematic studies have attempted to compare taxa on the basis of both morphological analyses and biochemical genetic analyses. Smith *et al.* (1981) used morphological and biochemical criteria, but did not carry out detailed morphological comparisons; the male hectocotyli only were compared. We have examined the relationship of two nominal species using both simple statistical comparisons of individual characters and multivariate techniques, namely canonical and classificatory discriminant analyses, and for the genetic comparison, examining banding phenotypes and calculating genetic distance. This multidisciplinary approach has advantages not conferred by the use of either technique individually.

Several species of the squid genus *Loligo* occur in the Atlantic Ocean. Many of these have been well described and can easily be separated by means of morphological and life history differences (Roper *et al.,* 1984). There is, however, some confusion about the status of two nominal species, *L. reynaudii* d'Orbigny, found off the southern coast of Africa, and *L. vulgaris* Lamarck, which inhabits the coastal waters of western Europe and the Mediterranean Sea. The latter species is also caught commercially off NW Africa (Porebski, 1970), but neither species is known along the African coast S of 20° S or N of 28° degrees S and are not among commercial species listed by ICSEAF (International Commission for Southeast Atlantic Fisheries) for this area.

There appear to be few morphological differences between the two forms; *L. reynaudii* was originally described as a distinct species by d'Orbigny (1845) on the basis of shorter arm lengths and a more slender shape. Adam (1952), in his description of *L. vulgaris,* also included measurements of two specimens of *L. reynaudii* from Algoa Bay, South Africa. He alluded to the uncertain taxonomic status of the species, and was of the opinion that differing arm measurements probably resulted from a different method of measurement from those of Hoyle (1886) and Massy (1925). Voss (1962) also examined several specimens of *L. reynaudii,* but could make no conclusions regarding the problem. Roper *et al.* (1984) mentioned that *L. reynaudii* may be synonymous with *L. vulgaris* or a subspecies of *L. vulgaris* and that its systematic position needed clarification.

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TABLE 1. Sample dates and localities for *Loligo reynaudii* and *L. vulgaris* used in morphometric measurements and meristic character counts.

Species	Date	Vessel and/or locality	Position	Composition
L. reynaudii	25/8/80	M/V "St. Briac," near Cape Hangklip	35°13′S 18°41′E	7♂, 15♀
L. reynaudii	21/1/80	R/S "Africana II," Cape Peninsula	34°12′S 18°15′E	9♂
L. reynaudii	22/8/80	M/V "St. Briac," Central Agulhas Bank	35°33′S 20°45′E	16♂, 5♀
L. reynaudii	14/2/84	R/S "Trachurus," Simonstown, False Bay	34°11.5′S 18°26′E	22♂, 11♀
L. reynaudii	1/5/86	P/B "Protector," Kalk Bay, False Bay	34°8′S 18°27′E	7♂, 5♀
L. vulgaris	16/6/54	Belgian coast	51°29′N 2°56′E	20♂, 15♀
L. vulgaris	19/12/51	Calais	—	6♂, 9♀
L. vulgaris	30/3/51	Don du Chalateur, English Channel	—	1♀
L. vulgaris	13/12/83	Alicante, Spain (Mediterranean Sea)	—	4♂, 11♀

The species known in South Africa as *L. reynaudii* has for years been a small but relatively important component of the catches of local white-fish trawlers, and of foreign trawlers operating on the Agulhas Bank off the South Coast. Total catches have fluctuated at the level of about 4000 metric tons per annum. Since 1983, a small-boat jigging industry has begun to develop rapidly in spawning areas, and catches exceeded 3000 m.t. in 1985 (Augustyn, 1986). To aid the management of this valuable industry, it has become imperative to gain as much knowledge as possible about its biology and population dynamics. A prerequisite for such studies is that the systematic status of the species should be clarified, and the present investigation was undertaken with this objective in mind. We shall refer throughout to the subspecific names that we confer, namely *Loligo vulgaris reynaudii* and *Loligo vulgaris vulgaris*.

MATERIALS AND METHODS

Two basic approaches were used in the study: morphological comparisons between the two forms by selected body measurements and counts of certain meristic characters; and proteins were analyzed electrophoretically.

Morphological analysis

Samples of both forms were obtained from several different sources (Table 1). These were originally fixed in 10% formaldehyde and subsequently preserved in 70% ethyl alcohol. After determination of the sex, the following body measurements were made on each specimen:

ML: length of mantle from anterior tip of mid-dorsal point to posterior body tip.

FL: fin length from fin insertion to posterior body tip.

FW: greatest width across both fins.

AL I, II, III and IV: lengths of right dorsal (arm I), dorso-lateral (II), ventro-lateral (III) and ventral (IV) arms, respectively, measured from the most proximal sucker to the arm tip

ASD: diameter of largest sucker on arm III.

CL: length of right tentacular club from the most proximal sucker to the tip.

TSD: diameter of largest sucker on right tentacular club

Several other measurements such as mantle width, head width, and marginal manal club sucker diameters were also made, but were discarded because they were difficult to measure accurately or were deformed during preservation. All measurements were made to the nearest mm with stainless steel vernier

calipers, but in the case of sucker diameters, to the nearest 0.1 mm.

Meristic characters measured were:

AS I, II, III and IV: total number of suckers respectively on arms I, II, III and IV.

BLS I, II, III and IV: number of suckers on buccal lappets, counting counter-clockwise from mid-dorsal (I) to right ventral lappet (IV).

HP: number of papillae on hectocotylus of males.

CSR: number of rows of suckers on right tentacular club.

AD: number of denticles (i.e. knobs and teeth) on the corneous ring of the most proximal sucker of arm III.

AT: number of teeth on the corneous ring of the most proximal sucker of arm III.

DCSD: number of denticles on the largest dactyl sucker corneous ring.

PCSD: number of denticles on the largest carpal sucker corneous ring.

The dentition of the large manal sucker rings was also compared.

Only the 15 Spanish specimens of *L. v. vulgaris,* consisting of 11 females and four males, were available for the meristic character counts, whereas 25 specimens (10 males, 15 females) from the False Bay and Agulhas Bank collections of *L. v. reynaudii* were used for comparison. Linear regression parameters for each character vs. mantle length were obtained, and a procedure (described by Zar, 1974) was carried out to test whether the slopes and intercepts of the regression lines differed significantly. For each meristic character, mean values were compared between species by sex and sexes combined using Student's *t* statistic for the comparison of two means. Canonical discriminant analysis and classificatory discriminant analysis were carried out separately on the morphological and meristic data using procedures of the SAS statistical computer package (SAS Institute Inc., 1982). The canonical procedure derives canonical variables that are linear combinations of the quantitive variables that summarize between-class variation. Pairs of canonical variables were plotted to aid visual interpretation of group differences. The classificatory analysis computes linear or quadratic discriminant functions for classifying observations into groups (in this case the four groups made up by the two sexes in each species). For the meristic data, not all the variables were used in the discriminant analyses, since observations are omitted entirely by the procedure if any single measurement is missing. Those used were: AS III, AS IV, BLS I–IV, CSR and AT.

A number of small specimens of *L. reynaudii* (78–120 mm in length), obtained at a late stage of the study, were used to check on the presence or absence of denticles on the large manal suckers of the tentacular clubs in specimens smaller than those examined earlier.

Genetic analysis

For protein analysis, the 15 Spanish specimens of *L. v. vulgaris* were compared with 44 specimens of *L. v. reynaudii* originating from False Bay. Forty-five specimens of the Californian market squid, *L. opalescens,* were used as an outgroup for comparison to measure the sensitivity of electrophoresis to detect differences between species. After tissue samples of mantle muscle, branchial heart muscle, digestive gland and vitreous fluid of eye were taken for electrophoresis, the specimens were preserved for morphological analysis. Four electrophoretic buffers were used:

(1) gel: Tris 0.03 M, citric acid 0.005 M, 0.0006 M lithium hydroxide, 0.003 M boric acid (pH 8.5); electrode: 0.06 M lithium hydroxide, 0.3 M boric acid (pH 8.1).

(2) gel: 1 : 15 dilution of electrode solution; electrode: 0.15 M Tris, 0.05 M citric acid (pH 6.9).

(3) gel: 1 : 4 dilution of electrode solution; electrode: Tris 0.18 M, boric acid 0.1 M, EDTA 0.004 M (pH 8.7).

(4) gel: 1 : 9 dilution of electrode solution; electrode: 0.1 M phosphate (pH 7.0).

Soluble proteins were extracted from 1 g samples of tissues with equal volumes of 0.1 M phosphate buffer with 0.01 M dithiothreitol (pH 7.0). Horizontal starch-gel electrophoretic methods by May *et al.* (1979) were used, where gels consisted of 12% hydrolysed potato starch (Sigma Chemical Co.). Histochemical stains for specific proteins were applied to the cut surface of a gel using a 1% agar overlay and the recipes of Harris & Hopkinson (1976), except for stains for aspartate aminotransferase (Aat) and esterase (Est), which consisted of a liquid solution. Additionally, octopine dehydrogenase activity was detected using 30 mg octopine, 20 mg NAD, 20 mg methyl thiazolyl tetrazolium (MTT) and 5 mg phenazine methosulphate.

Banding phenotypes on the gels were interpreted to reflect inherited mendelian variation by fit to patterns predicted by the subunit structure of the allozymes and by comparison

TABLE 2. Basic statistics for morphometric measurements made on *Loligo reynaudii* and *L. vulgaris* males.

Species	Variable	N	$\bar{X}$ (mm)	Range	Mean % of ML	S.D.
L. reynaudii	ML	61	162.44	107–313		37.96
	FL	47	109.02	69–209	65.20	29.47
	FW	46	86.13	55–152	51.48	19.74
	AL I	45	45.72	25–71	27.01	10.73
	II	45	53.51	27–87	32.01	13.36
	III	47	57.41	33–90	34.34	14.00
	IV	46	51.51	21–90	30.87	13.71
	ASD	38	1.53	1.0–2.6	1.02	0.48
	CL	43	57.50	32–81	34.24	12.20
	TSD	56	5.77	3.0–8.5	3.5	1.38
L. vulgaris	ML	30	188.40	65–304		72.89
	FL	30	126.23	43–222	67.00	53.39
	FW	30	96.90	41–155	51.43	32.62
	AL I	30	43.70	20–73	23.20	12.61
	II	30	51.93	22–79	27.56	13.93
	III	30	55.52	31–81	29.46	14.81
	IV	30	55.73	27–78	29.58	13.91
	ASD	26	1.78	1.0–2.8	0.93	0.50
	CL	29	49.17	25–78	25.98	14.44
	TSD	29	4.97	2.1–8.5	2.63	1.70

TABLE 3. Basic statistics for morphometric measurements made on *Loligo reynaudii* and *L. vulgaris* females.

Species	Variable	N	$\bar{X}$ (mm)	Range	Mean % of ML	S.D.
L. reynaudii	ML	36	156.81	110–244		32.18
	FL	29	104.34	70–167	65.10	24.20
	FW	28	85.39	49–137	53.35	19.35
	AL I	29	40.36	29–53	25.18	7.43
	II	28	47.13	33–65	29.46	8.75
	III	29	52.66	36–71	32.85	8.86
	IV	29	47.48	31–68	29.63	9.13
	ASD	25	1.47	1.0–2.0	0.98	0.34
	CL	23	51.78	34–67	32.09	8.65
	TSD	30	5.49	4.0–7.0	3.50	0.84
L. vulgaris	ML	36	163.36	77–240		45.41
	FL	36	108.17	46–164	66.21	32.10
	FW	35	92.12	46–140	56.66	26.61
	AL I	35	44.57	23–64	27.37	12.04
	II	35	54.57	28–87	33.51	14.85
	III	36	58.06	29–80	35.54	15.28
	IV	36	57.50	31–80	35.20	14.04
	ASD	25	1.77	1.0–3.3	1.15	0.56
	CL	33	53.18	27–90	32.84	16.86
	TSD	33	5.58	2.8–9.0	3.45	1.73

of gene expression of *L. reynaudii* and *L. vulgaris* with that of *L. opalescens*. Deviation from Hardy-Weinberg (random mating) proportions was tested using the goodness-of-fit statistic *G* (Sokal & Rohlf, 1981) for each polymorphic locus and sample. Locus heterozygosity was defined as $h = 1 - \Sigma x_i^2$, where x_i was allelic frequency. Average heterozygosity (H) is the mean of h, including monomorphic loci. The amount of genetic divergence between taxa was estimated with Nei's (1972) genetic distance (D)

TABLE 4. Basic statistics for morphometric measurements made on both sexes of *Loligo reynaudii* and *L. vulgaris*.

Species	Variable	N	$\bar{X}$ (mm)	Range	Mean % of ML	S.D.
L. reynaudii	ML	97	160.35	107–313		35.85
	FL	76	107.24	69–209	65.16	27.50
	FW	74	85.85	49–152	52.17	19.46
	AL I	74	43.62	25–71	26.32	9.88
	II	73	51.06	27–87	31.06	12.15
	III	76	55.60	33–90	33.79	12.45
	IV	75	49.95	21–90	30.40	12.24
	ASD	63	1.51	1.0–2.6	1.00	0.43
	CL	66	55.51	32–81	33.51	11.36
	TSD	86	5.67	3.0–8.5	3.50	1.22
L. vulgaris	ML	66	174.74	65–304		7.42
	FL	66	116.38	43–222	66.60	5.38
	FW	65	94.32	41–155	54.06	3.65
	AL I	65	44.17	20–73	25.29	1.52
	II	65	53.35	22–87	30.55	1.78
	III	66	56.90	29–81	32.56	1.85
	IV	66	56.70	27–80	32.45	1.71
	ASD	51	1.77	1.0–3.3	1.03	0.07
	CL	62	51.30	25–90	29.36	2.00
	TSD	62	5.29	2.1–9.0	3.03	0.22

TABLE 5. Basic statistics for meristic characters examined in *Loligo reynaudii* and *L. vulgaris* males.

Species	Variable	N	$\bar{X}$ (mm)	Range	S.D.
L. reynaudii	AS I	10	107.1	88–120	10.4
	II	8	111.8	74–129	17.0
	III	9	118.2	111–127	5.7
	IV	9	105.3	90–111	6.5
	BLS I	10	9.9	8–16	2.3
	II	10	9.0	6–12	1.7
	III	10	12.9	10–18	2.2
	IV	10	6.8	5–9	1.3
	HP	9	58.4	44–68	7.4
	CSR	10	43.6	39–48	2.6
	AD	7	26.3	24–30	1.9
	AT	10	17.5	13–30	4.8
	DCSD	10	19.8	17–23	2.1
	PCSD	6	18.3	15–21	2.2
L. vulgaris	AS I	4	97.5	78–109	14.5
	II	4	101.5	66–114	23.7
	III	4	110.3	86–120	16.2
	IV	4	101.3	88–110	10.0
	BLS I	3	4.3	3–5	1.2
	II	4	6.3	5–8	1.3
	III	3	7.7	4–10	3.2
	IV	3	5.0	3–6	1.7
	HP	4	54.5	50–62	5.3
	CSR	3	35.7	35–36	0.6
	AD	1	19.0	19	—
	AT	4	12.3	10–14	1.7
	DCSD	3	17.7	16–19	1.5
	PCSD	3	17.3	15–20	2.5

TABLE 6. Basic statistics for meristic characters examined in *Loligo reynaudii* and *L. vulgaris* females.

Species	Variable	N	$\bar{X}$ (mm)	Range	S.D.
L. reynaudii	AS I	12	103.3	94–112	6.0
	II	11	111.8	101–121	5.6
	III	13	109.2	89–122	9.3
	IV	11	108.9	96–121	8.1
	BLS I	12	7.9	6–10	1.3
	II	12	7.8	5–11	1.7
	III	12	10.5	7–13	1.8
	IV	11	5.9	5–8	0.9
	CSR	10	44.4	42–46	1.2
	AD	11	24.4	21–28	2.2
	AT	15	15.5	12–18	2.0
	DCSD	10	20.4	17–23	2.0
	PCSD	8	14.3	9–18	2.9
L. vulgaris	AS I	10	101.3	86–113	8.4
	II	10	112.1	90–128	12.2
	III	11	111.5	84–126	12.5
	IV	11	114.7	96–127	9.8
	BLS I	11	7.4	4–11	1.9
	II	11	7.4	5–11	2.1
	III	11	9.9	6–12	1.7
	IV	11	6.1	4–8	1.0
	CSR	9	35.9	35–37	0.8
	AD	5	22.8	20–25	2.3
	AT	10	14.4	10–17	2.4
	DCSD	10	19.4	17–21	1.3
	PCSD	9	21.7	16–25	3.0

$$D = -\log_e (J_{xy}/(J_x J_y)^{1/2})$$

where J_{xy}, J_x and J_y represent the probabilities of allelic identity between and within samples x and y, respectively. J_x was calculated as: $J_x = (\Sigma p_{xij}^2)$/loci, where p_{xij} is the allelic frequency of the j-th allele in the i-th locus in population x. J_y is calculated in the same way. J_{xy} is the average over loci of the cross products of allelic frequencies in the two populations: $J_{xy} = (\Sigma p_{xij} \cdot p_{yij})$/loci. Standard errors of *D* were calculated according to Nei & Roychoudhury (1974).

RESULTS

Morphology

The results of morphological measurements of males, females and both sexes combined are given in Tables 2 to 4, respectively, and of the meristic counts in Tables 5 to 7, respectively. Plots of the first two canonical variables from the canonical discriminant analyses for morphological and meristic data are presented in Fig. 1. Results of the *t*-test procedure for significantly differing slopes and regressions are given in Table 8. Tables 9 and 10 present classification summaries obtained from the discriminant function analysis.

For the morphological measurements, a reasonably wide size range of each species was analysed. The results showed significant differences between the two nominal species with regard to most of the characters examined, as indicated by the comparison of the slopes and intercepts of their regressions with mantle length. From Table 8 it is apparent that, except for fin width and fin length regressions with mantle length, all of the comparisons of the slopes, in both sexes, differed significantly between species. The comparison of intercepts in these cases therefore becomes meaningless. In the case of the fin measurements vs. mantle length (FL and FW), where the regression lines are parallel, the intercepts differed significantly in males, but not in females. In general, the characters that did differ significantly were more highly significantly different between the males, in-

TABLE 7. Basic statistics for meristic characters examined in both sexes of *Loligo reynaudii* and *L. vulgaris*.

Species	Variable	N	$\bar{X}$ (mm)	Range	S.D.
L. reynaudii	AS I	22	105.0	88–120	8.3
	II	19	111.8	74–129	11.4
	III	22	112.9	89–127	9.1
	IV	20	107.3	90–121	7.5
	BLS I	22	8.8	6–16	2.1
	II	22	8.3	5–12	1.8
	III	22	11.6	7–18	2.3
	IV	21	6.3	5–9	1.2
	HP	9	58.4	44–68	7.4
	CSR	20	44.0	39–48	2.1
	AD	18	25.1	21–30	2.3
	AT	25	16.3	12–30	3.5
	DCSD	20	20.1	17–23	2.0
	PCSD	14	16.0	9–21	3.3
L. vulgaris	AS I	14	100.2	78–113	10.0
	II	14	109.1	66–128	16.0
	III	15	111.1	84–126	13.0
	IV	15	111.1	88–127	11.3
	BLS I	14	6.7	3–11	2.1
	II	15	7.1	5–11	1.9
	III	14	9.4	4–12	2.2
	IV	14	5.9	3–8	1.2
	HP	4	54.5	50–62	5.3
	CSR	12	35.8	35–37	0.7
	AD	6	22.2	19–25	2.6
	AT	14	13.8	10–17	2.4
	DCSD	13	19.0	16–21	1.5
	PCSD	12	20.6	15–25	3.4

dicating that morphological differentiation is more marked among males of the two nominal species. When the sex-combined data are compared, only AL I, II, III, IV and ASD differ significantly between the two forms with regard to slope of the regression, but in cases where the slopes did not differ, the intercepts all differed significantly.

For the meristic counts, only four *L. v. vulgaris* males were available, a rather low number considering the variation in measurements encountered, but this did not affect our conclusions. The results for the Student's *t* tests indicated significant differences between the species for AD, AT and PCSD at the $P = 0.05$ level. Two major consistent differences were found in the examination of meristic characters. In all *L. v. reynaudii* specimens examined, the large manal sucker corneous rings were smooth, whereas in all 15 *L. v. vulgaris* specimens they bore teeth (Fig. 2). In *L. v. vulgaris*, variation occurred from denticles around most of the circumference in some of the smaller specimens, to only a few on several raised ridges on the circumference of the rings in some larger specimens.

The second major difference between the nominal species (significant at the $P = 0.001$ level in the Student's *t* test analysis) was in the number of rows of suckers on the tentacular clubs. This varied between 39 and 48 with a mean of 44.0 in *L. v. reynaudii*, and between 35 and 37 with a mean of 35.8 in *L. v. vulgaris* (Table 7). No differences were found in the mean number of suckers on the arms (AS I to IV), but there were significant differences in the number of suckers on the buccal lappets in males (BLS I to IV), in the number of denticles on the carpal club suckers (PCSD) in females, and in the number of teeth on arm III proximal corneous ring (AT) in males. The number of papillae in the hectocotylus (HP) of males also differed significantly between the nominal species.

Fig. 1 shows the groupings resulting from the canonical analysis of the morphological measurements and meristic counts of selected variables. It is clear that there is better discrimination between species based on

TABLE 8. Regression equations and results of *t*-test analyses to test whether the slopes and intercepts of regressions of mantle lengths (x) vs. selected morphological characters (y) differed significantly between samples of *Loligo vulgaris reynaudii* and *L. v. vulgaris*.

Sex	Character vs. ML	Equation		*t* Test			
				Slope		Intcpt.	
		L. v. reynaudii	*L. v. vulgaris*	df	Sign.	df	Sign
Males	FL	$y = 0.728x - 12.34$	$y = 0.728x - 10.87$	73	N.S.	74	$P < 0.001$
	FW	$y = 0.444x + 11.81$	$y = 0.438x + 14.42$	72	N.S.	73	$P < 0.001$
	AL I	$y = 0.217x + 8.99$	$y = 0.152x + 15.12$	71	$P < 0.05$	72	N.S.
	II	$y = 0.298x + 3.71$	$y = 0.168x + 20.22$	71	$P < 0.001$	72	$P < 0.001$
	III	$y = 0.309x + 5.76$	$y = 0.186x + 20.41$	73	$P < 0.001$	74	$P < 0.001$
	IV	$y = 0.288x + 3.52$	$y = 0.178x + 22.24$	72	$P < 0.001$	73	$P < 0.001$
	ASD	$y = 0.014x - 0.64$	$y = 0.006x + 0.682$	60	$P < 0.001$	61	$P < 0.001$
	CL	$y = 0.241x + 17.04$	$y = 0.165x + 17.89$	68	$P < 0.05$	69	N.S.
	TSD	$y = 0.028x + 1.15$	$y = 0.020x + 1.16$	81	$P < 0.05$	82	$P < 0.01$
Females	FL	$y = 0.688x - 5.98$	$y = 0.700x - 6.13$	61	N.S.	62	N.S.
	FW	$y = 0.522x + 1.82$	$y = 0.542x + 4.01$	59	N.S.	60	N.S.
	AL I	$y = 0.174x + 12.46$	$y = 0.242x + 5.22$	60	$P < 0.05$	61	$P < 0.001$
	II	$y = 0.189x + 16.83$	$y = 0.296x + 6.33$	59	$P < 0.05$	60	$P < 0.001$
	III	$y = 0.215x + 18.22$	$y = 0.308x + 7.67$	61	$P < 0.05$	62	$P < 0.001$
	IV	$y = 0.205x + 14.67$	$y = 0.285x + 10.87$	61	$P < 0.05$	62	$P < 0.01$
	ASD	$y = 0.006x + 0.54$	$y = 0.011x + 0.07$	46	$P < 0.05$	47	$P < 0.001$
	CL	$y = 0.153x + 27.17$	$y = 0.305x + 3.77$	52	$P < 0.05$	53	$P < 0.001$
	TSD	$y = 0.018x + 2.70$	$y = 0.031x + 0.54$	59	$P < 0.05$	60	$P < 0.001$
Both sexes	FL	$y = 0.714x - 10.29$	$y = 0.719x - 9.24$	138	N.S.	139	$P < 0.001$
	FW	$y = 0.465x + 9.41$	$y = 0.458x + 14.46$	135	N.S.	136	N.S.
	AL I	$y = 0.209x + 9.00$	$y = 0.171x + 14.36$	135	N.S.	136	$P < 0.001$
	II	$y = 0.270x + 6.61$	$y = 0.195x + 19.29$	134	$P < 0.01$	135	$P < 0.001$
	III	$y = 0.284x + 8.88$	$y = 0.211x + 20.01$	138	$P < 0.05$	139	$P < 0.001$
	IV	$y = 0.265x + 6.39$	$y = 0.200x + 21.77$	137	$P < 0.05$	138	$P < 0.001$
	ASD	$y = 0.010x - 0.06$	$y = 0.007x + 0.65$	110	$P < 0.01$	111	$P < 0.001$
	CL	$y = 0.219x + 19.28$	$y = 0.192x + 17.81$	124	N.S.	125	$P < 0.05$
	TSD	$y = 0.025x + 1.60$	$y = 0.021x + 1.57$	144	N.S.	145	$P < 0.05$

TABLE 9. Classification (hit and miss) table for the male and female classes in the two *Loligo* taxa, for morphological measurements. Number of observations and percentages (in boldface type) are shown. Classes are: 1. *L. v. reynaudii* females. 2. *L. v. reynaudii* males. 3. *L. v. vulgaris* females. 4. *L. v. vulgaris* males.

Sample actually in class	Sample allocated to				
	1	2	3	4	Total
1	11	3	0	0	14
	78.57	**21.43**	**0**	**0**	**100**
2	3	15	1	0	19
	15.79	**78.95**	**5.26**	**0**	**100**
3	0	0	21	2	23
	0	**0**	**91.3**	**8.7**	**100**
4	1	0	6	19	26
	3.85	**0**	**23.08**	**73.08**	**100**
Total	15	18	28	21	82
Percent	**18.29**	**21.95**	**34.15**	**25.61**	**100**

TABLE 10. Classification (hit and miss) table for the male and female classes in the two *Loligo* taxa, for meristic counts. Number of observations and percentages (in boldface type) are shown. Classes are: 1. *L. v. reynaudii* females. 2. *L. v. reynaudii* males. 3. *L. v. vulgaris* females. 4. *L. v. vulgaris* males.

Sample actually in class	Sample allocated to				
	1	2	3	4	Total
1	7	0	0	0	7
	100	**0**	**0**	**0**	**100**
2	1	7	0	0	8
	12.5	**87.5**	**0**	**0**	**100**
3	0	0	8	0	8
	0	**0**	**100**	**0**	**100**
4	0	0	0	2	2
	0	**0**	**0**	**100**	**100**
Total	8	7	8	2	25
Percent	**32**	**28**	**32**	**8**	**100**

meristic counts than on the morphological measurements, but that there is some overlapping, indicating that separation between the species based on this criterion is not complete. The coefficients of the first canonical variate showed that DLS and MTSD were the variates largely producing group differences along the first and second canonical axes, respectively, in the morphological analysis, while CSR and BLS I were the corresponding most influential variates in the meristic data.

The classification matrices for the morphological measurements of males and females of the two species (Tables 8, 9) show that only 2 out of 82 specimens were misclassified with respect to species, while 14 were misclassified with respect to sex. For the meristic data (Table 10) no misclassifications with respect to species occurred and there was only one misclassification with respect to sex.

Electrophoretic variation

The enzymes examined, locus abbreviations and buffer/tissue combinations giving the best results are presented in Table 11. An examination of relative banding intensities in mantle, branchial heart muscle, digestive gland and eye fluids did not reveal any differences in the tissue expressions of genes in *L. v. vulgaris, L. v. reynaudii* or *L. opalescens*. We identified the gene products of 30 homologous loci in the three taxa. Twelve proteins showed invariant bands for all three taxa and were interpreted to represent the products of *Adh, Aat-2, Idh-1, Mdh-1, Mdh-2, Mpi, Nsp-1, Nsp-2, Nsp-3, Nsp-4, Pep-2* and *Pep-4*. An additional 10 loci were fixed for different alleles between the Atlantic Ocean squids *L. v. vulgaris* and *L. v. reynaudii*, and the Pacific Ocean squid *L. opalescens*, including *Gpi-1, Gpi-2, Gap-2, Ldh-1, Mdh-3, Me-1, Pep-1, Pgm-3, Sod-1* and *Sod-2* (Table 12). The remaining 8 loci showed allele frequency differences among taxa (Table 12). Heterozygotes for *Est-1* had three-banded phenotypes typical of a dimeric enzyme, whereas heterozygotes for *Est-2* were two-banded, suggesting a monomeric subunit construction for this enzyme. We observed two separate zones of banding for *Idh* on the gels where the most anodal proteins encoded by *Idh-2* had three-banded heterozygotes typical of a dimeric enzyme. Proteins encoded by *Ldh-2* showed five-banded heterozygotes typical of a tetrameric enzyme. Proteins encoded by *Odh* showed two-banded heterozygotes typical of a monomeric enzyme. Proteins encoded by *Pgm-1* showed two-banded heterozygotes in *L. opalescens*, but were monomorphic in the Atlantic Ocean taxa. Proteins encoded by *Pgd* showed three-banded heterozygotes in *L. v. reynaudii* but were monomorphic in the other two taxa. Proteins encoded by *Xo* showed narrow- and broad-banded phenotypes that were interpreted to represent homozygotes and heterozygotes, respectively.

A single significant deviation from Hardy-Weinberg proportions was detected for *Ldh-2* in *L. v. reynaudii* ($G_1 = 5.2$, $0.05 > P > 0.01$)

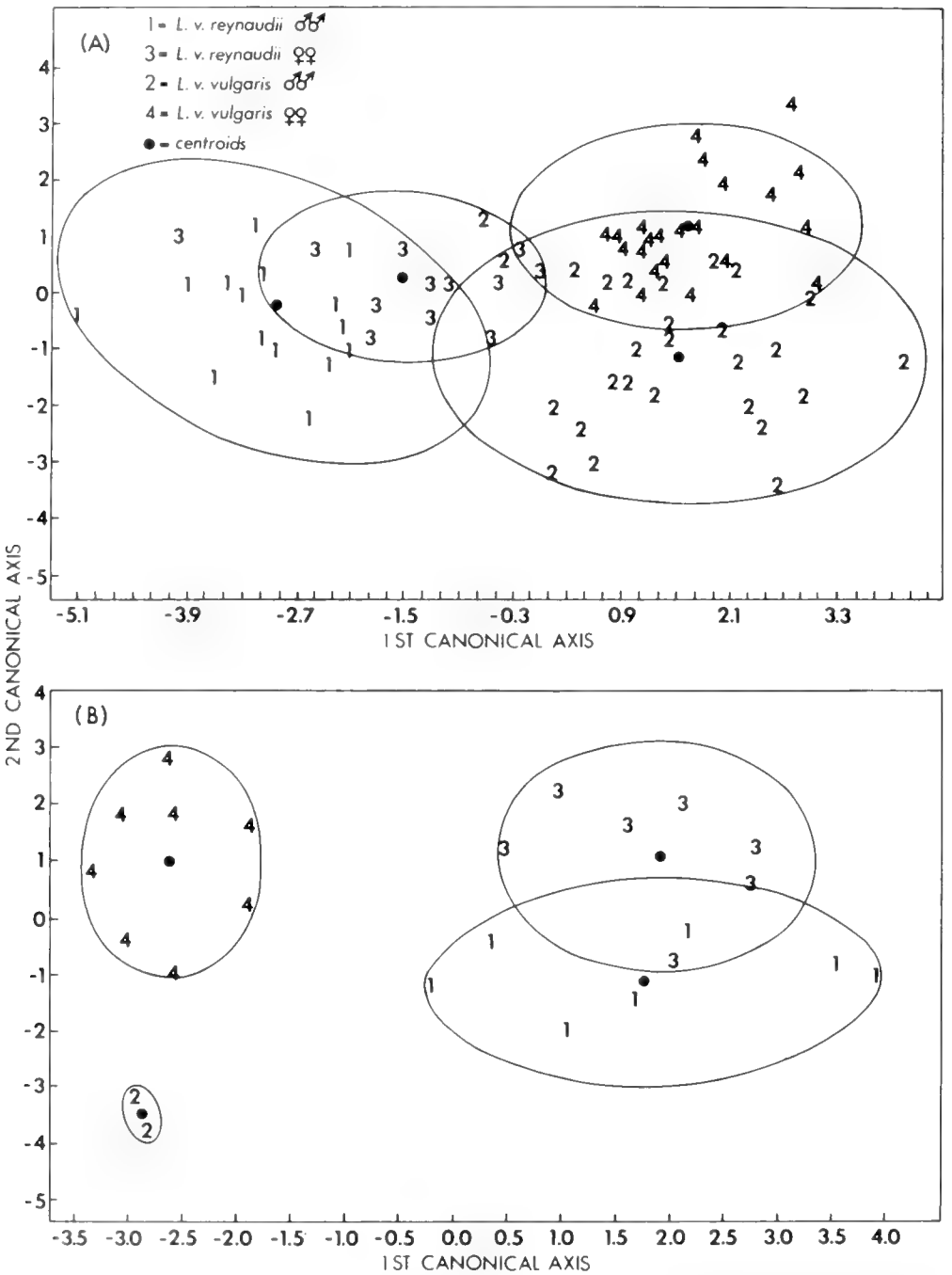

FIG 1. Plots of the first two canonical variables from the canonical discriminant analyses for (A) morphological and (B) meristic data. Ovals were drawn by hand to aid visual interpretation of groupings, the furthest outlier being excluded in some cases.

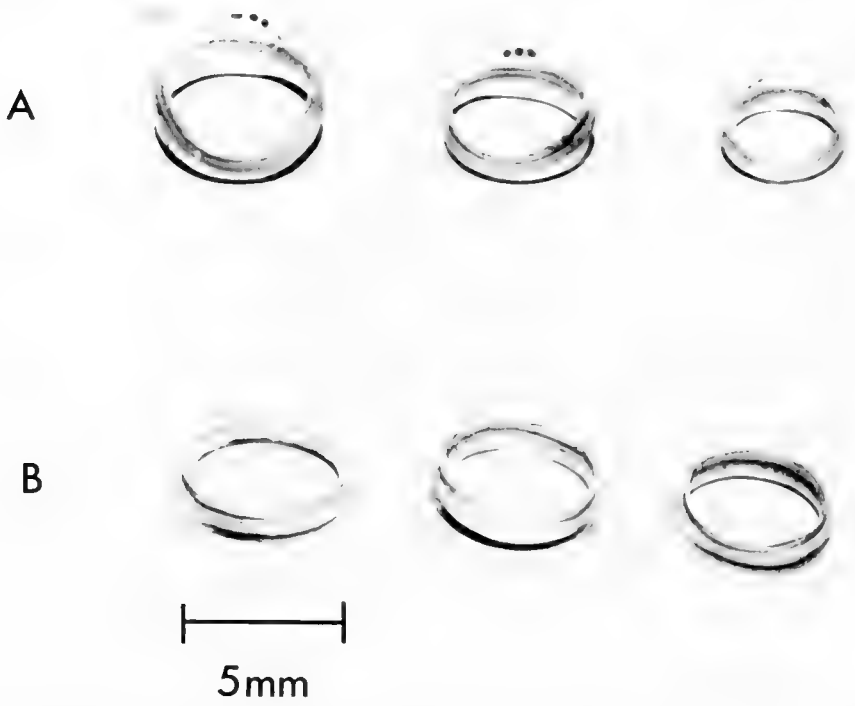

FIG. 2. Large manal club sucker corneous rings from A. *Loligo vulgaris vulgaris* and B. *Loligo vulgaris reynaudii.*

and this was due to a deficit of heterozygotes. A re-examination of genotypes by sex for this locus showed that the frequency of the 180 allele was 0.50 in males ($N = 23$) but only 0.10 in females ($N = 5$).

The proportions of polymorphic loci for *L. v. vulgaris, L. v. reynaudii* and *L. opalescens* using the 0.95 criterion of polymorphism were 0.07, 0.07 and 0.10, respectively; using the 0.99 criterion, the proportions of polymorphic loci were 0.07, 0.23 and 0.17, respectively. Average heterozygosities were 0.011 (S.E. 0.008), 0.030 (S.E. 0.018) and 0.037 (S.E. 0.020), respectively. Nei's (1972) genetic distance, *D*, between *L. v. vulgaris* and *L. v. reynaudii* was 0.030 (S.E. 0.028) and the average *D* between these two subspecies and *L. opalescens* was 0.686 (S.E. 0.184).

DISCUSSION

Morphology

The basis for the historical separation of the taxa into two species has been somewhat doubtful, judging from the comments of several authors, notably Adam (1952), Voss (1962) and Roper *et al.* (1984).

Roper *et al.* (1984) gave, as an identifying feature, shorter arm lengths in *L. reynaudii* than in *L. vulgaris*. This is probably based on d'Orbigny's (1845) original descriptions. Table 4 shows that this does not agree with the present findings, since arms I, II and III were, on average (mean % of ML), slightly longer in *L. v. reynaudii* and, according to Table 8, significantly so.

Voss (1962) mentioned that the most conspicuous feature of all the specimens examined by him was the exceptionally large size of the "median" suckers in comparison with the marginals and more distal ones. Our measurements showed that TSD was, in fact, relatively larger in males of *L. v. reynaudii* than in *L. v. vulgaris*, but not in females (Tables 2 to 4).

Regarding the dentition of the corneous rings, Voss (1962) also mentioned that in some smaller specimens of *L. reynaudii*, 4 or

Table 11. Enzymatic proteins, Enzyme Commission numbers, locus designations and tissues examined in study of squids. M = mantle muscle, H = heart muscle, D = digestive gland, E = eye. See text for buffer formulae 1—4.

Enzyme	Abbreviation	Tissue	Buffer
Alcohol dehydrogenase	*Adh*	M	1
Aspartate aminotransferase	*Aat-1*[1]	H,D	1
	Aat-2	E	1
Esterase	*Est-1*	E	1
	Est-2	D	1
Glucosephosphate isomerase	*Gpi-1*	M	1
	Gpi-2	M,H,D,E	1
Glyceraldehydephosphate dehydrogenase	*Gap-1*[1]	E	2
	Gap-2	E,D,H,M	2
Isocitrate dehydrogenase	*Idh-1*	E	2
	Idh-2	M	2
Lactate dehydrogenase	*Ldh-1*	D	1
	Ldh-2	H,D,M	1
Malate dehydrogenase	*Mdh-1*	M,H,D	2
	Mdh-2	M,H	2
	Mdh-3	M,H,E	2
Malic enzyme	*Me-1*	E	2
	Me-2[1]	H,D	2
Mannosephosphate isomerase	*Mpi*	M,E	3
Nonspecific protein	*Nsp-1*	M,E	1
	Nsp-2	M	1
	Nsp-3	H	1
	Nsp-4	H	1
Octopine dehydrogenase	*Odh*	E,M	
Peptidase	*Pep-1*[2]	M,D,H	3
	Pep-2[2]	M,D,H	3
	Pep-3[1,2]	M,H,D,E	3
	Pep-4[3]	D	3
Phosphoglucomutase	*Pgm-1*	M	1
	Pgm-2[1]	E	1
	Pgm-3	M	1
Phosphogluconate dehydrogenase	*Pgd*	E,M	4
Superoxide dismutase	*Sod-1*	E,D	1
	Sod-2	E,D	1
Xanthine oxidase	*Xo*	D	3

[1]Not sufficiently resolved to be included in data.
[2]Substrate: Leucyl-tyrosine.
[3]Substrate: Phenylalanyl-proline

5 teeth were seen on the corneous rings of the largest suckers. His smallest specimen had a ML of 66.5 mm, which is somewhat smaller than the smallest specimen (78 mm) examined by us. Denticles may occur in very young specimens, but inadequate material precluded further investigation.

The differing number of papillae counted on the male hectocotyli (HP) of the two taxa may have resulted from differing rates of sexual development, since the hectocotylus in many species only develops at an advanced stage of sexual maturity and in some cases is only fully developed in very large mature adult males. According to M. Roeleveld of the South African Museum (personal communication), this is a common phenomenon among male ommastrephids. The number of male *L. v. vulgaris*

TABLE 12. Allele frequencies and average heterozygosities for three taxa of squid (*Loligo*). Sample sizes: *L. reynaudii*, N = 44; *L. vulgaris*, N = 15; *L. opalescens*, N = 45.

Locus	Allele	*Loligo reynaudii*	*Loligo vulgaris*	*Loligo opalescens*
Est-1	75	—	—	0.011
	85	—	—	0.022
	100	0.989	0.933	0.856
	115	0.011	0.067	0.111
Est-2	90	0.011	—	—
	100	0.966	1.000	1.000
	105	0.011	—	—
	110	0.011	—	—
Gap-2	100	1.000	1.000	—
	110	—	—	1.000
Gpi-1	95	—	—	1.000
	100	1.000	1.000	—
Gpi-2	85	—	—	1.000
	100	1.000	1.000	—
Idn-2	50	—	—	0.011
	70	—	—	0.989
	100	0.978	1.000	—
	110	0.011	—	—
	115	0.011	—	—
Ldh-1	50	1.000	1.000	—
	100	—	—	1.000
Ldh-2	70	—	—	1.000
	100	0.569[1]	0.893	—
	180	0.431	0.107	—
Mdh-3	100	1.000	1.000	—
	110	—	—	1.000
Me	100	1.000	1.000	—
	300	—	—	0.622
	350	—	—	0.378
Odh	90	—	—	0.978
	95	0.105	1.000	0.022
	100	0.884	—	—
	105	0.011	—	—
Pep-1	100	1.000	1.000	—
	180	—	—	1.000
Pgd	70	0.011	—	—
	92	0.011	—	—
	100	0.967	1.000	—
	130	0.011	—	1.000
Pgm-1	100	1.000	1.000	—
	110	—	—	0.022
	130	—	—	0.978
Pgm-2	95	—	—	1.000
	100	1.000	1.000	—
Sod-1	60	—	—	1.000
	100	1.000	1.000	—
Sod-2	50	1.000	1.000	—
	100	—	—	1.000
Xo	90	0.011	—	0.174
	100	0.989	1.000	0.826

[1]Departure from Hardy-Weinberg proportions $0.05 < P < 0.01$

was not large enough to draw any definite conclusions regarding differences in the hectocotyli of the two nominal species.

Adam (1952) reported that his Algoa Bay specimens of *L. reynaudii* had up to 28 teeth on the suckers of the sessile arms, compared to about 20 in *L. vulgaris,* but the paucity of specimens prevented him from drawing any further conclusions. In the present study we found that there was a significantly higher number of denticles (i.e. teeth and knobs), at the P = 0.05 level, in *L. v. reynaudii* (AD) than in *L. v. vulgaris.*

The discriminant analyses tend to support the view that while there are definite differences between individual characters, morphological differentiation in general is not strongly developed and that the differences between the taxa are more typical of subspecies than of species.

Electrophoretic variation

Gene expression in *L. v. vulgaris* and *L. v. reynaudii* is similar to that in *L. opalescens.* Ally & Keck (1978) examined *L. opalescens* for *Gpi, Ldh* and *Pgm,* enzymes that were in common with the present study. They reported that the isozymes for *Gpi* and *Ldh* were monomorphic and this agrees with our results for this species. In addition, they presented data for a *Pgm* locus in mantle tissue having two common and three rare alleles. In the present study, we observed two loci (one monomorphic and the other with one rare allele in *L. opalescens*) in mantle tissue and a third locus in eye fluids. We are not able, however, to assign homologies of loci between these studies either on the basis of similarities in allele frequencies or tissue expression. Christofferson *et al.* (1978) reported a polymorphic locus of *Aat* in mantle tissue of *L. opalescens.* Our results, however, showed one protein (*Aat-1*) in heart and digestive gland and a second more anodal protein (*Aat-2*) in eye fluids. Tissue expressions were similar between this study and the present study for *Gap, Mdh, Me* and *Sod.*

The distributions of electrophoretic phenotypes conformed to Hardy-Weinberg expectations for all of the polymorphic loci, except *Ldh-2.* This deviation appears to have been due to an apparent allele frequency difference between males and females. The numbers of individuals in this sample (23 males and 5 females, respectively) was very small and this difference may have been due to sample error. The distribution of phenotypes between sexes for this locus did not fit a pattern expected if this locus were located on a sex chromosome.

The level of genetic variation as estimated by the proportion of polymorphic loci ($P_{0.95}$: 0.07, 0.07 and 0.10 for *L. v. vulgaris, L. v. reynaudii* and *L. opalescens,* respectively) and average heterozygosity (*H:* 0.011, 0.030 and 0.037, respectively) was very low for the three species examined in this study. Nevo (1978) found an average proportion of polymorphic loci ($P_{0.95}$) among 27 invertebrate species (excluding insects) of 0.399 (S.E. 0.275) and an average *H* of 0.100 (S.E. 0.074). It is difficult, with the relatively small sample of loci used in this study (N = 30), to show that the heterozygosity estimates for the squids are significantly less than the average for other invertebrates because of the uncertainty of the underlying statistical distribution of locus heterozygosities (Archie, 1985). For the purpose of this discussion we assume that the observed differences of about 0.07 with other invertebrates is in fact significant.

There have been few studies of squids in which sufficient loci have been examined accurately to estimate heterozygosity, so we are not sure whether squids in general show less variation than other invertebrates or whether species in the genus *Loligo* represent a special case. The amount of genetic variation contained within a species is influenced chiefly by three forces: migration, random genetic drift and natural selection. When the number of migrants between populations is much greater than one, then there is little divergence between populations and the species acts more or less as a single evolutionary unit (Wright, 1940; Kimura, 1955). Several theoretical models show that random genetic drift is insignificant in populations with very large populations because the reproductive sampling error from one generation to the next is small (Wright, 1943; Kimura & Weiss, 1964). Random drift, however, may be important when speciation is initiated by small founding populations followed by slow population growth (Nei *et al.,* 1975; Chakraborty & Nei, 1977).

The results of population studies of *L. opalescens* off California using allozymes showed little differentiation among populations (Ally & Keck, 1978; Christofferson *et al.,* 1978). We interpret these results to indicate that there is a significant amount of gene flow between populations of *L. opalescens,* which

prevents populations from diverging genetically from one another, and assume that *L. v. vulgaris* and *L. v. reynaudii* have similar amounts of migration between populations. Thus, it is unlikely that the low amount of within-species gene diversity results from random genetic drift in small populations. We cannot, however, eliminate the possibility that random drift was important in founding populations.

Numerous models of selection have been proposed to account for the various levels of heterozygosity in natural populations, but it is difficult to distinguish among them with electrophoretic data or else data give conflicting results. The niche-width variation hypothesis, for example, predicts that there is a positive association between heterozygosity and the heterogeneity of the physical and biological habitat. A test of this hypothesis, however, using marine fishes (Somero & Soule, 1974) and insects (Metcalf *et al.*, 1975) has failed to confirm this prediction. Data from mole rats, on the other hand, appear to support this hypothesis (Nevo & Shaw, 1972).

Molecular taxonomy

The analysis of inherited molecular variation can provide a valuable means of estimating evolutionary divergence between taxa because evolutionary change at the molecular level appears to proceed roughly in a clocklike fashion independent of morphological change (Wilson *et al.*, 1977). There is generally good correspondence between biochemical-genetic estimates of divergence between taxa and their taxonomic categories based on morphological analyses (Avise, 1974; Thorpe, 1982). The use of molecular estimates of divergence are particularly useful in situations where morphological changes between diverging taxa have been minimal (e.g. Shaklee & Tamaru, 1981; Smith *et al.*, 1981) or where variable morphological differences among taxa do not reflect genetic isolation (e.g. Kornfield *et al.*, 1982).

Thorpe (1982) compared Nei's genetic distance for 2,664 pairs of taxa with their taxonomic classification and we used these results to gauge the taxonomic status of the squids examined in this study. The average genetic distance (*D*) between conspecific populations was 0.04, whereas the average *D* between congeneric species was 0.62. There was considerable overlap between these two groups and no critical value of genetic distance could be used unambiguously to assign taxa to one taxonomic category or the other. The genetic distance between the two Atlantic Ocean squids and *L. opalescens* ($D = 0.686$) is typical of distance values between related species, but the genetic distance ($D = 0.030$) between *L. v. vulgaris* and *L. v. reynaudii* is more typical of values between conspecific populations. Nevertheless, because of the geographic isolation between the latter two taxa we confer subspecific status to the Southern African squid.

Evolution

Voss (1977) noted that most species of squids consist of a series of geographically distinct subspecies or races and argued that the systematics of squids should reflect these phylogenetic relationships. The amount of morphological and genetic divergence between *L. vulgaris vulgaris* and *L. v. reynaudii* is typical of that between conspecific populations or weakly differentiated subspecies. If one ascribes to the assumption that such differences arise primarily in allopatry (Mayr, 1970), then some kind of barrier to dispersal between the two groups must have been present along the W coast of Africa at some time in the past. The small amount of divergence between the two groups suggests that such a barrier existed relatively recently. Although there have been several episodes of lowered sea level during the Pliocene and Pleistocene, it seems unlikely that there were any effective land barriers to dispersal along the western African coast during these times (Shannon, 1985).

The remaining possibility is that there have been or are now hydrographic barriers to migration. A major environmental barrier of cold, turbulent water, due to wind-driven upwelling, exists between 24° and 28° S off the coast of Namibia (Shannon, 1985) and these conditions have prevailed during several episodes in the recent past. The formation of the characteristic features of the Benguela Upwelling System began about 12 million years ago (late Miocene) but the system probably only became fully established as we know it about 2.5 million years ago in the late Pliocene (Shannon, 1985). Furthermore, Chapman & Shannon (1985) note that oxygen-depleted subsurface water is characteristic of much of the Namibian shelf.

It is well known from their worldwide distributions (Roper *et al.*, 1984) that loliginids

prefer temperate or tropical conditions, especially in coastal waters where most species spawn. Since these squids are highly active predators, they have a relatively high metabolic rate and consequently almost certainly avoid oxygen-deficient waters, particularly when these conditions occur near the bottom where most feeding takes place. During periods of global cooling, populations of *L. vulgaris* may have been displaced in opposite directions into areas of warmer water (CLIMAP, 1976; Thunell & Belyea, 1982). It is probable that the environmental barriers that isolated the southern African form of *L. vulgaris* and that led to the differentiation of a subspecies have been operating since the late Pliocene and may account for the present absence of loliginid squids between 28° and 20° S.

SYSTEMATICS

Loligo vulgaris Lamarck, 1798 subsp. **reynaudii** d'Orbigny, 1845

Loligo reynaudii d'Orbigny, 1845: 315, 346; pl. 11, fig. 3; pl. 19, fig. 3; pl. 24, figs. 1–8. Gray, 1849: 73. Hoyle, 1910: 263; 1912: 280. Thiele, 1920: 440. Massy, 1925: 207; 1927: 155. Voss, 1962: 261–262, fig. 1f–g. Barnard, 1974: 746. *Loligo reynaudi* Roeleveld, 1975: 239. Roper *et al.*, 1984: 100.

Material examined: Table 1.

Diagnosis: Large club suckers without teeth or knobs at mantle lengths as small as 78 mm and larger; clubs with between 39 and 48 rows of suckers (mean 44).

Description: Specimens examined in detail were between 107 and 313 mm mantle length.

The mantle is cylindrical before the fin insertion, after which it begins to taper to a blunt point posteriorly. A groove, widening from the fin insertion posteriorly, runs from just posterior of the anterior dorsal lobe along the dorsal length of the mantle. The mantle width averages 17 to 20% of the mantle length in adults, but this is greater in juveniles than in very large specimens. It is also greater in mature females than in mature males. A prominent lobe of the mantle projects anterodorsally to just behind the iris of the eye. The anterior margin of the mantle is excised ventrally around the funnel.

The fins together are rhomboid in shape and joined posteriorly. Their length averages about 65% of mantle length from the anterior to posterior attachments, and their width, 51%. The anterior edge of the fins is slightly convex, whereas the posterior edge is very slightly concave. The length of the anterior edge is approximately 70% that of the posterior edge. The fins in general are relatively larger in large specimens, but there is no difference between the sexes.

The head is relatively short and about the width of the mantle. The head length relative to mantle length decreases with growth; in adults head length averages 12% of mantle length. The funnel is set in a deep funnel groove. The funnel locking cartilage is long (10 to 14% of ML) and straight. The funnel opening reaches the anterior edge of the iris of the eye, is lens-shaped and equipped with a dorsally attached valve.

Arm length order is usually III > II > IV > I, sometimes III > IV > II > I. Mean arm lengths are 26, 31, 34 and 30% of mantle length for ALI, II, III and IV, respectively. Arm III is the most robust, followed by IV and II, with arm I being the least robust. There is a swimming keel present along the length of each arm, the best-developed one occurring on arm III where it flares prominently about half-way along its length. Protective membranes and trabeculae are present on all the arms, being particularly well-developed on arms I and III.

The arm suckers are arranged biserially. Suckers are of approximately equal size up to proximal sucker pair 6 on arm I and up to pair 10 on arms II, III and IV; more distally, they gradually decrease in size. Proximal sucker sizes are usually in the order: arm III > II > I > IV. The dentition on arm suckers is variable. Teeth often occur only on the distal circumference of the corneous rings, small knobs only often being present on the proximal circumference. The number of teeth on the most proximal sucker ring of arm III, for instance, varies between 12 and 30, while the total number of denticles (teeth and knobs) varies between 21 and 30. In shape, the teeth are usually rounded or square.

The left ventral arm in males is hectocotylized and is very similar to that in *L. vulgaris vulgaris* (Adam, 1952: 51, fig C). The modified part of the arm measures between 25 and 35% of the arm length. The number of papil-

lae, also arranged biserially, is variable (44 to 68) and increases with size and maturity.

Tentacles are robust and fairly short, with clubs expanded with respect to the stalks, in length averaging 34% of mantle length. Tentacular clubs have 39 to 48 (mean 44) rows of four suckers and can be divided into carpal, manal and dactyl areas, in each of which the sucker configuration is different. The carpal suckers are not clearly grouped tetraserially; there are usually 9 or 10 suckers in 3 or 4 rows. The manus usually consists of about 6 rows of suckers, the medial pair large. The ventral one of each pair is the larger. The large suckers are flanked laterally by two much smaller suckers. The dactylus is made up of a large number of tetraserially arranged rows diminishing in size to tiny stalkless suckers distally. The tip of the club ends in a sucker-less flange. The diameter of the largest manal sucker is on average 3.5% of mantle length. There is a swimming keel that runs laterally along the length of the tentacle to the tip of the club, widening slightly on the club itself.

Club sucker dentition is variable, with a combination of teeth and small knobs on the carpal and dactyl sucker corneous rings, but the large manal sucker corneous rings are smooth (Fig. 2). The mean number of denticles on the largest carpal and dactyl suckers are 16 and 20, respectively.

There are 7 buccal lappets with a variable number of suckers on each. The mean number of suckers on buccal lappets I, II, III and IV are 8.8, 8.3, 11.5 and 6.3, respectively. The buccal suckers also have corneous rings armed with about 20 teeth. The lappets are attached as follows: I between left and right arms I; II dorsally to arm II; III and IV ventrally to arms III and IV, respectively.

Colour: Reddish-brown chromatophores occur on the mantle and head, and in nature the colour can change from dark red to almost translucent. The chromatophores in preserved specimens are more concentrated on the dorsal midline of the mantle and on the dorsal surface of the head, particularly above the eyes.

Distribution: Continental shelf and slope of the southern African Coast from southern Namibian waters (28° S) in the W to East London (33° S) on the E coast.

Comparison with L. v. vulgaris: *L. vulgaris reynaudii* differs from *L. vulgaris vulgaris* in the absence of dentition on the corneous rings of the large medial suckers of the tentacular clubs at all sizes above 78 mm ML, the smallest size examined in the present study (Fig. 2), and in a larger number of rows of suckers on the tentacular club. The number of rows averages 44 ± 2.1 (S.D.) in *L. v. reynaudii* and 36 ± 0.7 in *L. v. vulgaris.*

Nomenclature

There is some confusion about the spelling of the subspecific name, *reynaudii.* It was referred to as *L. reynaudi* by e.g. Roeleveld (1975) and Roper *et al.* (1984). The original name given by d'Orbigny (1845) to the southern African form was *Loligo reynaudii,* the terminal *–ii* being correct according to the International Code of Zoological Nomenclature (ed. 3, 1985, Article 31a (ii)) if he used the latinized form of the name Reynaud, i.e. Reynaudius. Considering the date when he described the species, it is likely that d'Orbigny used the latinized form. We therefore recommend that the subspecies name be *Loligo vulgaris reynaudii.* According to the Code (Article 47a: nominotypical subspecies): In the classification of the southern African form as a subspecies of *L. vulgaris,* the nominotypical subspecies automatically becomes *L. vulgaris vulgaris* Lamarck, 1798. Each subspecies retains its own holotype.

Distribution

L. v. reynaudii has been collected between 0 and 384 m in southern African waters, but is seldom taken in water deeper than 250 m, i.e. off the continental shelf and slope. Off the W coast of southern Africa, very small numbers are occasionally taken by trawlers operating in southern Namibian waters, but it is not known to occur north of 28°S. Its distribution is continuous around the South African coast from the Orange River on the W coast to at least as far as East London on the E coast (33°S). According to M. Roeleveld (personal communication), the most common species of *Loligo* N of this is *Loligo duvacelii* d'Orbigny, and there may be some overlapping of distributions in the vicinity of East London. Massy (1925) lists a specimen from Durban (30° S) but this record is suspect. The greatest densities of *L. v. reynaudii* are on the SE and, particularly, on the SW Agulhas Bank (Hatanaka, *et al.,* 1983; Uozumi *et al.,* 1984, 1985), and the major spawning areas are located in the inshore region of the SE coast (Augustyn, 1986).

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THE CEPHALOPOD ASSEMBLAGE OF THE GULF STREAM SYSTEM EAST OF 60°W

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ABSTRACT

The epipelagic cephalopod fauna of the Gulf Stream System E of 60°W was sampled during winter of four years using BONGO nets and large open midwater trawls. Planktonic cephalopods from BONGO nets totaled 528 specimens, representing at least 16 families and 26 species, whereas juveniles and adults from midwater trawls totaled 7,045 specimens, representing at least 22 families and 51 species. Except for *Illex* sp., which was the most abundant species, the cephalopod fauna was dominated by tropical-subtropical forms. The Enoploteuthidae and Cranchiidae were the most speciose families and were also well represented in terms of total numbers of specimens.

Catch rates of total cephalopods from BONGO nets (excluding *Illex* sp.) indicated that diel effects were not great for early life stages. However, for juveniles and adults from midwater trawls, catch rates were much greater during darkness than during daylight. For cephalopods from midwater trawls, overall abundance was much higher during 1984 than 1985. Trawl differences and insufficient sampling precluded comparisons with 1981 and 1982 data. For both the planktonic and midwater trawl cephalopods, catch rates increased dramatically from Shelf Water to the northern Sargasso Sea. The N wall of the Gulf Stream appears to serve as a boundary in restricting the distribution of tropical-subtropical cephalopods to higher latitudes.

Key words: cephalopods; species composition; distribution; water masses; diel effects.

INTRODUCTION

Within the North Atlantic, cephalopod faunal assemblages have been described for a number of more-or-less distinct areas or localities, generally S of 40°N. In the Northwest Atlantic, Voss has described the cephalopods of the Gulf of Mexico (Voss, 1956), Cuba (Voss, 1955) and Bermuda (Voss, 1960) and Gibbs & Roper (1970) described the cephalopod fauna in an area SE of Bermuda. However, very little has been published regarding the cephalopods of the Gulf Stream and associated water masses. Cairns (1976) described the cephalopods of the Straits of Florida and Lu & Roper (1979) described species composition and vertical distribution of cephalopods in an area influenced by the Gulf Stream off Delaware. Most recent studies of vertical distribution and latitudinal variation in species composition have come from the northeastern Atlantic (Clarke & Lu, 1974, 1975; Lu & Clarke, 1975a, 1975b) and the Mediterranean Sea (Roper, 1972, 1974).

The Gulf Stream and associated water masses represent an area of considerable zoogeographical interest in that the northern edge of the Gulf Stream is considered to be a faunal boundary for mesopelagic fishes (Backus *et al.*, 1970; Jahn & Backus, 1976; Backus *et al.*, 1977) as well as for zooplankton (Grice & Hart, 1962) and phytoplankton (Hulbert, 1964). In fact, based largely on distribution of myctophids, Backus & Craddock (1977) have subdivided the water masses of the Gulf Stream System into three faunal regions, with Shelf Water representing part of the Atlantic Subarctic Region. Slope Water is included in the North Atlantic Temperate Region, and the Gulf Stream and Sargasso Sea are included in the North Atlantic Subtropical Region.

The objectives of this paper are to describe the species composition and relative abundance of pelagic cephalopods from the Gulf Stream System E of 60°W and to describe the distribution of total cephalopods among water masses to investigate the existence of faunal boundaries for cephalopod distribution. Vertical distribution of total cephalopods will be

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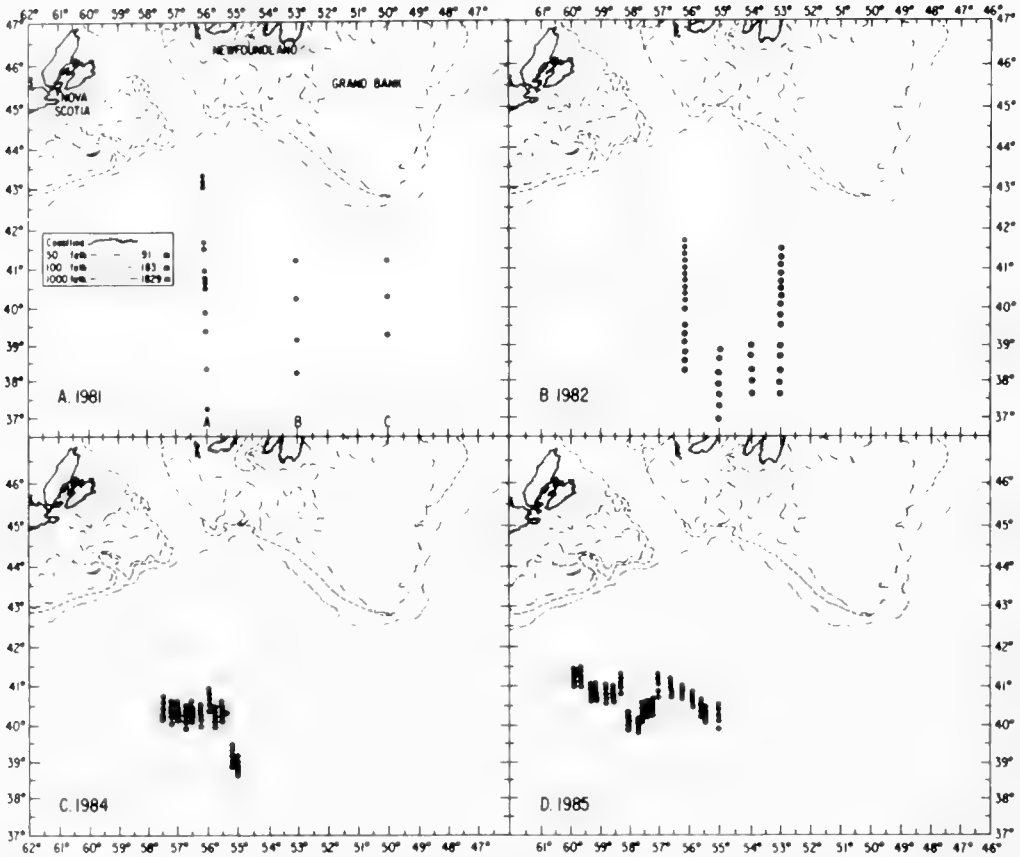

FIG. 1. Location of biological sampling stations (BONGO and midwater trawls) for the four survey years. The 1982 stations were sampled using only BONGO nets.

addressed within the constraints of the sampling gear and methods used.

MATERIALS AND METHODS

Cephalopods were collected during four annual surveys within the Gulf Stream System, each carried out during February-March aboard the R/V GADUS ATLANTICA. Survey design varied among years but the general area of surveys was similar and in all surveys sampling was along transects that extended in the N–S direction (Fig. 1). Other details of sampling methodology are presented in Table 1. At each station, except during 1982, sets were executed using both BONGO nets and midwater trawls. Midwater trawl samples for 1982 are not included here since very few trawl sets were executed.

For all four years plankton was sampled using 61 cm paired net BONGO samplers according to procedures outlined by Smith & Richardson (1977). During 1981 and 1982 multiple sets were executed to various maximum depths at each station (Table 1) but a single tow to 200 m was executed at each station during 1984 and 1985. All plankton samples were initially preserved in 5% buffered formalin and later examined for cephalopods. All cephalopods were identified to the lowest taxonomic level possible.

Midwater trawling was carried out using an Engels-80 midwater trawl (EMT-80) in 1981 and a somewhat similar Diamond IX midwater trawl in 1984 and 1985 (Table 1). During 1981, several sets were generally executed at each station to various maximum depths, whereas sets were consistently to a maximum depth of 100 m during 1984 and 1985.

TABLE 1. Summary of time, area, sampling gear and methodology for surveys carried out during 1981, 1982, 1984 and 1985.

Dates	Latitudinal range	BONGOS[1] Max. depth (m)	BONGOS[1] No. sets	Midwater trawl Type	Midwater trawl Max. depth (m)	Midwater trawl No. sets
1981						
Feb. 22–Mar. 6	50–56°W	50	6	EMT-80[2]	100	15
		100	1		300	14
		200	21		500	7
		300	1		1000	7
1982						
Feb. 21–Mar. 8	53–56°W	50	46	—	—	—
		200	45	—	—	—
1984						
Feb. 24–Mar. 9	55–57°30′W	200	56	Diamond IX[3]	100	56
1985						
Feb. 22–Mar. 10	55–60°W	200	96	Diamond IX	100	94

[1]60-cm diameter paired BONGO sampler with 0.333 mm mesh size
[2]Engels-80 midwater trawl 12-mm mesh codend (stretched mesh).
[3]Diamond IX midwater trawl and 12-mm mesh codend (stretched mesh)

All midwater trawl sets involved towing the trawl for 30 min at the maximum depth before retrieval. *Illex* sp. catches from midwater trawls were sometimes subsampled for length measurement due to the great magnitude of some catches, but all other cephalopods were measured in dorsal mantle length to the nearest whole mm.

The oceanographic sampling regime differed among the four surveys. During all surveys, expendable bathythermographs (XBT's) were used on and between stations to elaborate the temperature distribution along transects. Temperature and salinity were sampled on stations using Knudson reversing bottles in 1981 and 1982, whereas a CTD system, which measures conductivity, temperature and depth, was used during 1984. Only temperature data were collected during the 1985 survey using XBT's since it was found that salinity and density reflected oceanographic features that were also indicated by temperature (Dawe & Beck, 1985). Vertical profiles of temperature, salinity and density along transects were constructed and served as the basis for assigning stations to the various water masses. Satellite-derived oceanographic analysis maps, which are produced twice weekly by the U.S. National Earth Satellite Service (NESS), and sea surface temperature charts, which are produced weekly by the Canadian Meteorological and Oceanographic Centre (METOC), were also used to help identify the Gulf Stream and other circulation features.

To examine distribution of cephalopods among water masses, four water masses are defined:

Shelf Water—temperature and salinity of less than 9°C and 35.00 ppt, respectively.

Slope Water—temperature range 9–15°C and salinity range 35.00–36.00 ppt.

Northern Gulf Stream—stations located N of the Gulf Stream core of maximum surface velocity as indicated by the 15°C isotherm at 200 m. Temperature and salinity exceed 15°C and 36.00 ppt, respectively.

Northern Sargasso Sea—stations located at or S of the Gulf Stream core of maximum surface velocity.

Since BONGO nets and midwater trawls fished during retrieval, stations were assigned to the above water types even if those water types were present only near the surface, which was often the case for Shelf Water and Northern Gulf Stream Water. The somewhat arbitrary distinction between the Northern Gulf Stream and the Northern Sargasso Sea is made here since one well-studied squid *(Illex illecebrosus)* is known to be more abundant overall in the Gulf Stream near its northern edge than throughout the southern Gulf Stream and Sargasso Sea (Dawe & Beck, 1985, 1986; Hatanaka *et al.*, 1985). The

TABLE 2. Cephalopods collected using BONGO nets from surveys carried out during February–March of 1981, 1982, 1984 and 1985, with numbers of specimens, species and sets. (Numbers for higher taxa include specimens that could not be further identified.)

Taxon	No. specimens
Class Cephalopoda	528 (13)
Suborder Oegopsida	518 (177)
Family Brachioteuthidae	34
Brachioteuthis sp.	34
Family Chiroteuthidae	4 (1)
Chiroteuthis sp.	3
Family Cranchiidae	99 (38)
Cranchia scabra	3
Leachia sp.	57
Teuthowenia megalops	1
Family Ctenopterygidae	7
Ctenopteryx sicula	7
Family Joubiniteuthidae	1
Family Enoploteuthidae	39 (19)
Enoploteuthis anapsis	2
Abraliopsis pfefferi	9
Abraliopsis (Micrabralia) sp.	1
Pterygioteuthis gemmata	5
Pterygioteuthis giardi	1
Pterygioteuthis sp.	1
Pyroteuthis margaritifera	1
Family Gonatidae	11
Gonatus sp.	10
Gonatus fabricii	1
Family Histioteuthidae	1
Histioteuthis sp.	1
Family Lycoteuthidae	2 (1)
Lycoteuthis diadema	1
Family Mastigoteuthidae	1
Mastigoteuthis sp.	1
Family Octopoteuthidae	6 (1)
Octopoteuthis sp.	5
Family Ommastrephidae	98 (20)
Illex sp.	78
Family Onychoteuthidae	25 (2)
Onychoteuthis banksi	15
Ancistroteuthis lichtensteini	8
Order Octopoda	10 (1)
Family Octopodidae	6 (3)
Octopus defilippi	2
Octopus vulgaris	1
Family Alloposidae	2
Alloposus mollis	2
Family Argonautidae	1
Argonauta sp.	1
Total cephalopods	528
Number of species	28
Number of sets	272

oceanographic data used to distinguish water masses are not presented here, since they have been described elsewhere (Dawe & Beck, 1985, 1986).

In examining diel differences in catch rates and sizes, twilight periods were defined to ensure the integrity of day-night comparisons. Twilight sets included all tows for which any trawling time occurred within the periods one hour before and after sunrise (0643 hr) or sunset (1745 hr).

All cephalopod specimens described here are presently located at the Northwest Atlantic Fisheries Centre, St. John's, Newfoundland, with the hope of eventual deposition in the National Museum of Natural Sciences, National Museums of Canada. A portion of the material has been reported on previously by Stephen (1982).

RESULTS

Family and species composition

Planktonic cephalopods. The location of stations on transects for the four survey years is shown in Fig. 1. The family and species composition of planktonic cephalopods collected using BONGO nets during the four survey years is presented in Table 2. The number of families and species must be considered as minimal since many of the cephalopods could only be identified to higher taxa. Severe damage to some of the material made further identification impossible. A total of 528 planktonic cephalopods was collected overall, representing at least 16 families and 26 species. This collection included 99 cranchiids (including 57 *Leachia* sp.), 98 ommastrephids (including 78 *Illex* sp.), 39 enoploteuthids, 34 brachioteuthids (all *Brachioteuthis* sp.), 25 onychoteuthids (including 15 *Onychoteuthis banksi*) and 11 gonatids. The other families, including the octopods, were of minor importance, being represented by no more than seven specimens each.

Yearly differences were apparent in family and species composition of cephalopods, which may have been at least in part related to yearly variation in survey design (Fig. 1). In particular, surveys differed with respect to maximum depth of BONGO tows (Table 1) and allocation of sampling effort among water masses. Certain major differences are particularly noteworthy. During 1981 and 1982,

TABLE 3. Cephalopods collected using midwater trawls from surveys carried out during February–March of 1981, 1984 and 1985, with numbers of specimens, species and sets. Numbers for higher taxa include specimens which could not be further identified.

Taxon	No. specimens
Suborder Oegopsida	7028 (38)
Family Brachioteuthidae	3
Brachioteuthis sp.	3
Family Chiroteuthidae	18
Chiroteuthis lacertosa	12
Chiroteuthis joubini	4
Chiroteuthis sp.	2
Family Cranchiidae	217 (8)
Cranchia scabra	46
Leachia sp.	140
Megalocranchia sp.	12
Liocranchia sp.	2
Galiteuthis armata	1
Sandalops sp.	1
Egea sp.	4
Heliocranchia sp.	3
Family Ctenopterygidae	14
Ctenopteryx sicula	14
Family Enoploteuthidae	753 (23)
Enoploteuthis anapsis	24
Enoploteuthis sp.	7
Abralia redfieldi	26
Abralia veranyi	6
Abralia grimpei	14
Abralia sp.	10
Abraliopsis pfefferi	104
Abraliopsis (Micrabralia) sp.	8
Pterygioteuthis gemmata	195
Pterygioteuthis giardi	18
Pterygioteuthis sp.	2
Pyroteuthis margaritifera	313
Ancistrocheirus lesueuri	3
Family Gonatidae	2
Gonatus sp.	2
Family Histioteuthidae	13
Histioteuthis bonnelli	3
Histioteuthis dofleini	7
Histioteuthis corona corona	2
Histioteuthis meleagroteuthis	1
Family Lycoteuthidae	109
Lampadioteuthis megaleia	45
Selenoteuthis scintillans	64
Family Mastigoteuthidae	37 (2)
Mastigoteuthis hjorti	1
Mastigoteuthis sp.	34
Family Octopoteuthidae	27 (1)
Octopoteuthis danae	9
Octopoteuthis sp.	16
Taningia danae	1
Family Ommastrephidae	5266 (14)
Illex sp.	5154
Ommastrephes sp.	12
Ommastrephes bartrami	5
Ornithoteuthis antillarum	81
Family Onychoteuthidae	515 (8)
Onykia carribaea	35
Onychoteuthis banksi	329
Onychoteuthis sp.	1
Ancistroteuthis lichtensteini	142
Family Thysanoteuthidae	1
Thysanoteuthis rhombus	1
Family Cycloteuthidae	1 (1)
Family Lepidoteuthidae	5
Lepidoteuthis grimaldii	1
Tetronychoteuthis dussumieri	4
Family Bathyteuthidae	1
Bathyteuthis sp.	1
Family Spirulidae	1
Spirula spirula	1
Family Sepiolidae	6
Heteroteuthis dispar	5
Heteroteuthis atlantis	1
Family Architeuthidae (?)	1
Architeuthis sp.	1
Order Octopoda	17 (4)
Family Octopodidae	1 (1)
Family Alloposidae	11
Alloposus mollis	11
Family Bolitaenidae	1
Japetella diaphana	1
Total cephalopods	7045
Number of species	52
Number of sets	193

only one of the total of 25 onychoteuthids was collected. Also, during those years, only four of the total of 34 brachioteuthids and four of the total of 39 enoploteuthids were collected. The reverse was true only for the Ommastrephidae, with only four of the overall total of 98 ommastrephids collected during 1984 and 1985.

Cephalopods from midwater trawls. The family and species composition of cephalopods from midwater trawls is shown in Table 3. A total of 7045 juvenile and adult cephalopods was collected representing at least 22 families and 51 species. However, excluding *Illex* sp., only 1891 other cephalopods were collected. The dominant families, in order of abundance, were the Ommastrephidae (mostly *Illex* sp.), Enoploteuthidae (especially *Pyroteuthis margaritifera, Pterygioteuthis gemmata* and *Abraliopsis pfefferi*), Onychoteuthidae (especially *Onychoteuthis banksi* and *Ancistroteuthis lichtensteini*),

Cranchiidae (especially *Leachia* sp. and *Cranchia scabra*) and Lycoteuthidae (*Lampadioteuthis megaleia* and *Selenoteuthis scintillans*). Other families were less common, with seven families being represented by a single specimen. The largest number of species came from two families, the Enoploteuthidae and Cranchiidae.

When the midwater trawl collection (Table 3) is compared to that from BONGO nets (Table 2), several dissimilarities are evident. As planktonic cephalopods, the Brachioteuthidae and Gonatidae were relatively abundant (34 and 11 specimens, respectively) whereas they were quite rare in the midwater trawl collections (three and two specimens, respectively). The Cranchiidae were more common than the Enoploteuthidae from BONGO nets (99 and 39 specimens, respectively), whereas the reverse was true for the midwater trawl collection (217 and 753 specimens, respectively). The Lycoteuthidae were relatively abundant from midwater trawl catches (109 specimens) but were virtually absent from BONGO catches. Several species, most notably *Pyroteuthis margaritifera*, were abundant from midwater trawls but rare or absent from BONGO catches. These also include *Pterygioteuthis gemmata*, *Ornithoteuthis antillarum* and *Selenoteuthis scintillans*. There were no remarkable yearly differences in the midwater trawl cephalopod fauna, except that for virtually all of the dominant families and species (except *Illex* sp.) catches were much lower during 1985 than during the other two years (1981 and 1984).

Distribution of total cephalopods

Planktonic cephalopods. The distribution among water masses of total cephalopods from BONGO nets is presented in Fig. 2 by year, maximum depth of tow and time of day. For this analysis, 1981 data are not included since there were few sets at any of the four depth regimes sampled (Table 1). Also, *Illex* sp. catches were not used in the calculation of catch rates since that species was the most numerous and its early life stages are known to be most abundant in the northern Gulf Stream (Dawe & Beck, 1985).

In most cases, catch rates (number per set) and number of species were highest in the warm-water types, especially the northern Sargasso Sea. However, for 1982, catch rates were highest overall in the northern Sargasso Sea at 200 m but highest in the northern Gulf Stream at 50 m. Yearly effects were also evident, most notably the relative abundance of cephalopods in Shelf Water during 1985, as opposed to their complete absence from Shelf Water in 1982.

There appeared to be little effect of time of day on distribution of cephalopods from BONGO nets (Fig. 2). For 1985 (200 m) the distribution of catch rates among water masses was quite similar during daylight and darkness, but for each water mass catch rates (specimens/set) were somewhat higher during darkness (range 1.5–3.5) than daylight (range 0.9–2.3). However that trend was not consistently evident for other years and depths. In fact, the reverse was true for 1982 sets to 50 m in the warmer water types, with higher catch rates (specimens/set) during daylight (0.8 and 3.0) than darkness (0.6 and 1.3).

Since the effect of time of day was not great and since the distribution of BONGO sets among water masses was reasonably similar during daylight and darkness (Fig. 2), the data were pooled over the 24-hr cycle to further examine the effects of water type, year and depth (Fig. 3). The 1981 data for all depths (combined) are also included here. With the exception of 1982 sets to 50 m, there was consistently a clear increase in catch rates from the coldest (Shelf Water) to the warmest (northern Sargasso Sea) water masses. In the cases of 1981 (all depths) and 1984 (200 m), catch rates in the northern Sargasso Sea (2.6 and 4.4 specimens/set, respectively) were at least twice as high as those from the northern Gulf Stream (1.3 and 1.5 specimens/set, respectively). During 1982, no cephalopods were collected in Shelf Water from eight BONGO sets to 50 m and 200 m, whereas in 1985 the catch rate for Shelf Water (1.4 specimens/set) was comparable to that from Slope Water (1.3 specimens/set). Of the cephalopods that were identified at least to the family level, only the Gonatidae were most common in Shelf Water (during 1985 only) and only the Brachioteuthidae were most common in Slope Water, almost exclusively during 1984 and 1985. Comparison of 50 m and 200 m sets for 1982 indicates that cephalopods were most abundant below the 50 m layer. In the northern Sargasso Sea and Slope Water, catch rates (specimens/set) were 0.1 and 0.6, respectively, for the 0–50 m layer, compared to 0.6 and 1.9, respectively, for the 0–200 m layer. The higher catch rate in the northern Gulf Stream for the 0–50 m layer

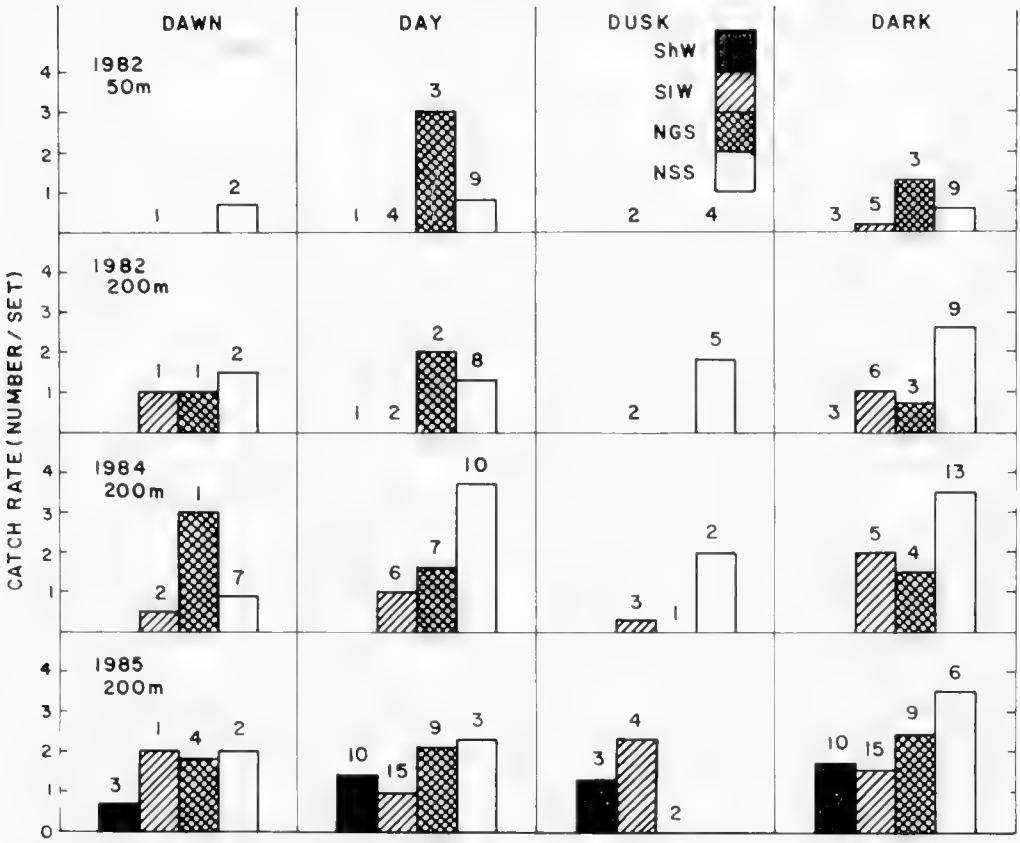

FIG. 2. Catch rates of total cephalopods (excluding *Illex* sp.) from 1982, 1984 and 1985 BONGO sets by depth, period of the day and water mass (ShW—Shelf Water, SlW—Slope Water, NGS—Northern Gulf Stream, NSS—Northern Sargasso Sea). Values above bars are numbers of sets.

is difficult to reconcile since oblique tows to 200 m also sampled throughout the 0–50 m layer.

Cephalopods from midwater Trawls. The distribution of midwater trawl cephalopods among water masses is shown in Fig. 4 by time of day and year. Since *Illex* sp. for all years represented 73% of all the cephalopods, it is excluded from this analysis. The distribution of *Illex* sp. from midwater trawls has been presented in detail elsewhere (Dawe & Beck, 1986). For midwater trawl cephalopods, distribution among water masses was quite similar to the planktonic cephalopods, with catch rates highest in the warm water types, especially the northern Sargasso Sea. That relationship is best exemplified during darkness, when most cephalopods were captured. For 1985, during darkness catch rates were the same in Shelf Water and Slope Water (1.8 specimens/set). For all years, during darkness there was a pronounced increase in catch rates from Slope Water to the northern Sargasso Sea, ranging in specimens/set from 7.8 to 68.0 in 1981, from 20.4 to 29.9 in 1984 and from 1.8 to 5.0 in 1985.

This trend was evident for most families and species, with only a few exceptions. Chiroteuthids were most abundant in Slope Water during 1981 and in Shelf Water during 1985. *Mastigoteuthis* sp. was most abundant in Slope Water during both 1981 and 1984. *Ancistroteuthis lichtensteini* was most abundant in Slope Water during 1984 and Shelf Water during 1985, and that species represented 51% of the total cephalopods (excluding *Illex*) from Slope Water in 1984. For all

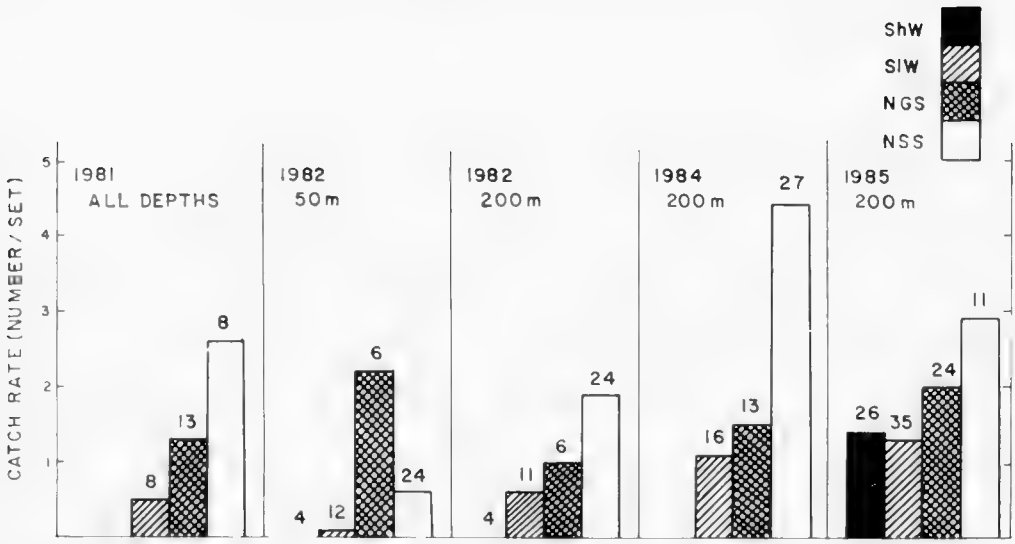

FIG. 3. Catch rates of total cephalopods (excluding *Illex* sp.), from BONGO sets for all years, by year, depth (except for 1981) and water mass (ShW—Shelf Water, SIW—Slope Water, NGS—Northern Gulf Stream, NSS—Northern Sargasso Sea). Values above bars are numbers of sets.

species, mean sizes were compared among water masses but no differences were evident for any species (except *Illex* sp.). Details of distribution of catches and sizes for individual families and species will be presented elsewhere.

Diel effects on catch rates of total cephalopods from midwater trawls (Fig. 4) were far more pronounced than for those from BONGO nets. Catch rates were consistently higher during darkness than daylight for all years and water masses. Catch rates (specimens/set) during daylight and darkness ranged, respectively, from 2.0 to 9.7 and from 7.8 to 68.0 during 1981, from 2.1 to 6.8 and from 20.4 to 29.9 during 1984 and from 0.3 to 1.3 and from 1.8 to 5.0 during 1985. The low catch rate in Slope Water during 1981 was likely in part related to survey design, since in that year many Slope Water stations were far removed from the Gulf Stream (Fig. 1). In some cases, catch rates were also high during twilight periods, probably due to more limited sampling during those periods and the selection of broad time frames for those periods, such that some towing time during darkness was included in twilight sets.

Yearly differences in catch rates of total cephalopods from midwater trawls were also quite pronounced (Fig. 4). Catch rates cited above clearly indicate that cephalopod abundance was much lower in 1985 than during the other two years. High catch rates during darkness of 1981 was probably related to the use of the much larger Engels-80 midwater trawl. However, cephalopods were obviously much more abundant in 1984 than 1985, since sampling gear and depths sampled were the same for those years (Table 1).

DISCUSSION

Family and species composition

The cephalopod collection described here from E of 60°W is quite similar in family and species composition to other collections of pelagic cephalopods from other areas within the Gulf Stream System (Voss, 1960; Gibbs & Roper, 1970; Cairns, 1976). The present collection was decidedly depauperate in myopsids, which are generally neritic (Clarke, 1966) and included many of the circumtropical cephalopods reported by Cairns (1976) from the Straits of Florida. This collection is also similar to one from the Sargasso Sea to the SE of Bermuda (Gibbs & Roper, 1970) in that the Cranchiidae and Enoploteuthidae were the most speciose families from both areas, to-

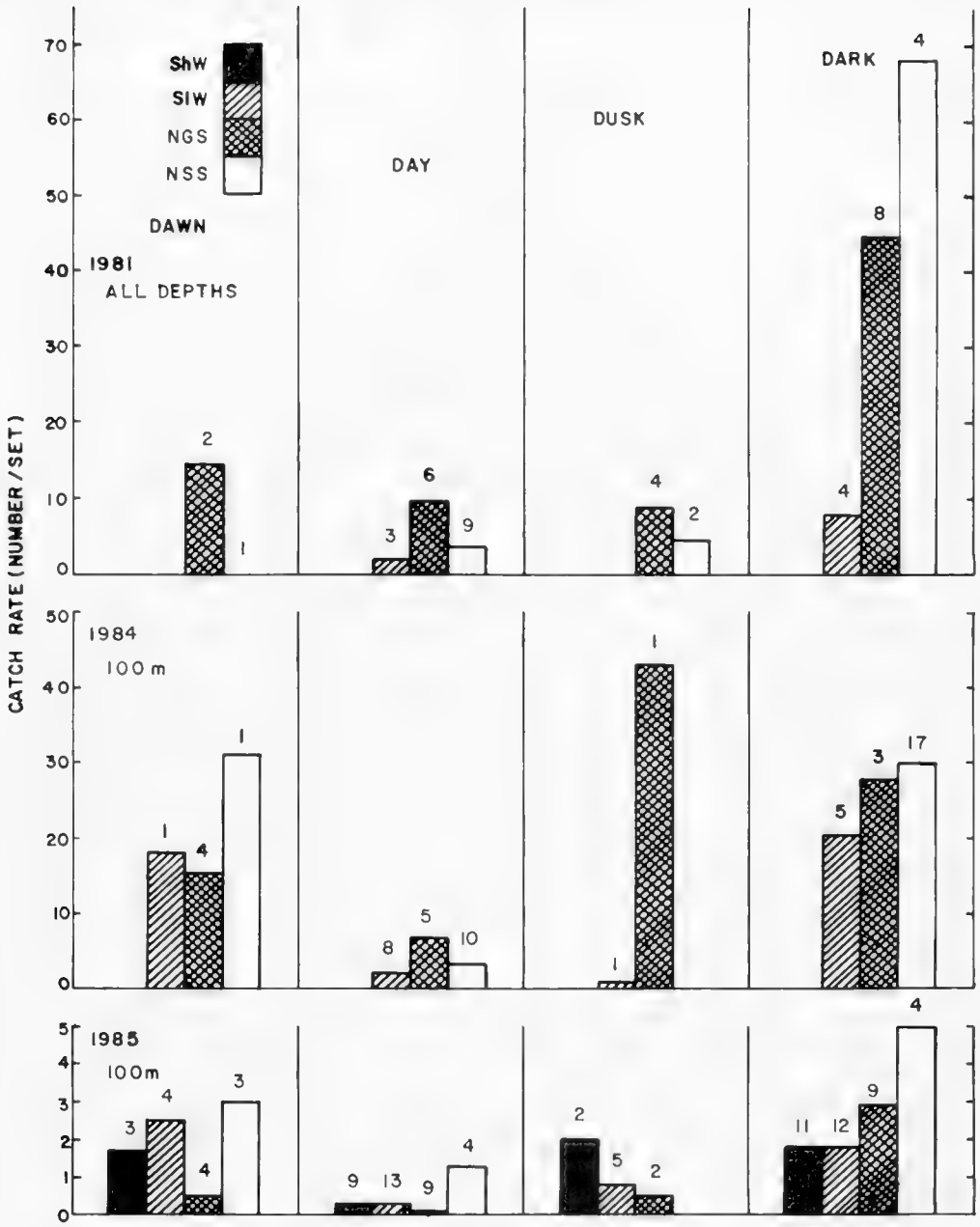

FIG. 4. Catch rates of total cephalopods (excluding *Illex* sp.) from midwater trawls for 1981, 1984 and 1985 by year, period of the day and water mass (ShW—Shelf Water, SIW—Slope Water, NGS—Northern Gulf Stream, NSS— Northern Sargasso Sea). Values above bars are numbers of sets.

gether representing a high proportion of total cephalopods. At deepwater dumpsite 106, Delaware, Lu & Roper (1979) found that during winter the most abundant species were *Pterygioteuthis gemmata*, *Abraliopsis pfefferi* and *Illex illecebrosus*, all of which featured

prominently in the present collection. Overall, however, their winter collection was not strikingly similar to that described here, probably because they did not sample within the Gulf Stream or Sargasso Sea.

Distribution of total cephalopods

Diel effects on catch rates of total cephalopods from BONGO nets were slight, with catch rates somewhat higher during darkness than daylight of 1985. This indicates that for most cephalopods diel vertical migration is not a general characteristic of early planktonic life stages. Diel effects were quite pronounced for juveniles and adults from midwater trawls, however, with catch rates much greater during darkness than daylight. Although there is considerable variation among families and species in patterns of vertical migration exhibited (Roper & Young, 1975), the most common pattern is for cephalopods to be concentrated in the upper 200 m during darkness with a shifting or spreading to greater depths during daylight. That type of vertical migration pattern is particularly characteristic of the Enoploteuthidae (Roper & Young, 1975), which featured prominently in the midwater trawl collections described here. Further details of diel effects for the most common species will be elaborated upon elsewhere.

While there were no pronounced yearly differences in overall abundance of early life stages, catch rates of midwater trawl cephalopods during 1984 were higher than during 1985. That may be related to a strong presence of Shelf Water very close to the Gulf Stream during 1985 (Dawe & Beck, 1986).

In considering distribution of total cephalopods among water masses, it must be recognized that the only truly valid comparison is between Slope Water and the northern Sargasso Sea (which includes the Gulf Stream core). Along the axis of Gulf Stream flow, northern Gulf Stream water overlies Slope Water in a layer that frequently extends to depths of less than 100 m. On the landward side, Shelf Water may also overlie Slope Water with a thin surface layer, such as was particularly evident in the survey area to a maximum depth of 60 m in 1985 (Dawe & Beck, 1986). Thus for some stations that were assigned to Shelf Water or the northern Gulf Stream based on surface characteristics, a large portion of the sampling effort may have been expended within underlying Slope Water. That probably accounts for the similarity of catch rates for 1985 planktonic cephalopods from Shelf Water and Slope Water, since there were no BONGO catches from Shelf Water in 1982 when sampling extended over a broader latitudinal range.

For collections from both BONGO nets and midwater trawls, total cephalopod abundance was much higher in the northern Sargasso Sea than in Slope Water. In fact, except for the 1984 midwater trawl collection, cephalopod catch rates in the northern Sargasso Sea were more than twice as great as those from Slope Water. Lu & Clarke (1975b) found that cephalopod abundance increases from N to S in the eastern North Atlantic, as does number of species, which doubles at 10° intervals of latitude.

Cephalopod distribution among water masses conforms to the scheme of pelagic faunal provinces and regions, as proposed by Backus & Craddock (1977) and Backus *et al.* (1977) based on the distribution of mesopelagic myctophid fishes. Most cephalopods, being more abundant in the northern Sargasso Sea than in Slope Water, could be considered subtropical forms, but many species likely exhibit a tropical-subtropical distribution pattern, having been reported from tropical provinces (not sampled in this study) such as the Straits of Florida (Cairns, 1976).

Not all cephalopods were most abundant in the Sargasso Sea. From BONGO catches the gonatids, known to be subarctic cephalopods, were most abundant in Shelf Water, whereas planktonic *Brachioteuthis* sp. appeared to be a temperate form with greatest catch rates in Slope Water. Some cephalopods from midwater trawls appeared to be temperate forms as well, with centers of abundance located in Slope Water. This was especially true for *Ancistroteuthis lichtensteini,* but other temperate forms appear to include the chiroteuthids, mastigoteuthids and histioteuthids. *A. lichtensteini* and the chiroteuthids also seemed to be relatively abundant in Shelf Water, but at those stations catches may have occurred in subsurface Slope Water.

Myctophid fishes (Backus *et al.,* 1977) and zooplankton (Grice & Hart, 1962) also show considerable variation in relative abundance among water masses, but in both cases numerical abundance overall decreases from neritic waters to the northern Sargasso Sea. In the case of cephalopods, the reverse is true and the N wall of the Gulf Stream appears to serve as a boundary, mainly restrict-

ing the distribution of tropical-subtropical cephalopods to higher latitudes.

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BEHAVIORAL AND BODY PATTERNING CHARACTERS USEFUL IN TAXONOMY AND FIELD IDENTIFICATION OF CEPHALOPODS

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ABSTRACT

A framework is developed for using behavioral and body patterning characters to identify live cephalopods in the natural environment, in aquaria, from photographs and in some cases from preserved specimens. Attention is drawn to Acute body patterns (lasting seconds or minutes) that are stereotyped responses to inter- and intraspecific stimuli. The following characters are suggested as a starting point: *Behavioral characters* such as responses to predators (e.g. Acute body patterns such as Deimatic, Flamboyant, Acute Mottle, Acute Disruptive, Passing Cloud, etc.), motor patterns during foraging and prey attack, intraspecific agonistic behavior, courtship, mating, egg care, chromatic components of patterning, arm postures, burying, habitat, daily activity pattern, diet, behavior at night and migration; *Morphological characters* related to behavior and body patterns: chromatophores, iridescent cells, white leucophore markings, papillae, ocelli, photophores and ink. Simple guidelines are given for data collection and analysis. These criteria are used to construct a behavioral taxonomic guide to the shallow-water octopods of the Caribbean Sea (10 species). Selected examples are given for differentiating sympatric species of *Loligo (L. pealei* and *L. plei; L. vulgaris* and *L. forbesi),* which are difficult to identify alive or preserved. Certain traits are species-specific and thus useful in taxonomy, while others are conservative, widespread characters that have potential use in broader studies of systematics and phylogeny.

Key words: behavior; taxonomy; cephalopods; chromatophore patterns.

INTRODUCTION

I wish to draw attention to ethological aspects of cephalopods to aid field researchers as well as taxonomists. As a group, live cephalopods are known for their dynamic behavior and ability to change color, pattern, texture and shape by neural control. Once preserved, however, cephalopods are difficult to identify because their anatomy includes few hard parts and, in general, standard systematic characters in Mollusca and other phyla often are inadequate to distinguish specimens clearly, especially octopods. Numerous problems exist in cephalopod taxonomy and systematics and these are reviewed by Voss (1977) and Roper (1983). At present, approximately 650 species and 150 genera are recognized in the Cephalopoda. Some peculiarities of the class are that 85% of the genera contain five or fewer species, nearly 50% of the genera are monotypic while two genera (*Octopus* and *Sepia*) contain over 100 species each (or 1/3 of the recognized species), and few subgenera or subspecies have been described *(op. cit.).* Certain genera of squids and octopods are in a constant state of taxonomic confusion and revision, and there is also disagreement regarding higher-order taxa (e.g. some authors split the order Sepioidea into the orders Sepiida and Sepiolida; Donovan, 1977; Fioroni, 1981). Considerations of behavioral taxonomy can, and should, complement standard morphological tools of systematists. Roper & Voss (1983) listed the range of characters suggested for descriptions of species. While they list behavior and chromatophore patterns, no details or guidelines for their use have yet been developed and published. Packard and coworkers (e.g. Packard & Hochberg, 1977) have done an excellent job of laying the groundwork for understanding the relationship of body patterning to behavior. This paper presents a framework for the use of behavioral characters to distinguish cephalopods at the species level.

BEHAVIOR AS A TAXONOMIC CHARACTER

Since the early 1900's the usefulness of behavioral characters in taxonomy has been recognized. Among the most detailed of these studies were: Heinroth (1911), Lorenz (1941) and Johnsgard (1961) on ducks and geese; Whitman (1919) on pigeons; Tinbergen (1959) on gulls; Crane (1949, 1952) on spiders and mantids; Baerends & Baerends-van-Roon (1950) on cichlid fishes, and Jacobs (1953) and Faber (1953) on grasshoppers. For reviews of the assessment of taxonomic affinities using behavioral criteria see Lorenz (1941), Marler (1957), Mayr (1958), Cullen (1959), Atz (1970) and Eibl-Eibesfeldt (1975). Bekoff (1977) has compiled a convenient table of behavioral taxonomic characters of a wide range of organisms. Examples include aspects of general behavior common to most animal groups: drinking, feeding, grooming, playing, sociality patterns, courting, mating calls, parental behavior, nest building, reproductive patterns, aggressive displays, defensive mechanisms, acoustic signalling, and specialized behaviors such as flash characteristics in fireflies and fin movements and color patterns in fishes. Many invertebrate and vertebrate phyla have been examined, including man (e.g. Lomax & Berkowitz, 1972; Eibl-Eibesfeldt, 1975; Cone, 1978). There are cases in which behavioral characters provided the first clue to taxonomic distinctness (e.g. Tinbergen, 1963; Cole, 1984 for some recent examples). It has proved most profitable, of course, to combine behavioral data with morphological and physiological information.

The rationale for using behavior as a valid taxonomic or phylogenetic trait is based upon the concept of evolution. Individual selection is based largely upon behavior (Williams, 1966; Mayr, 1982) and it is recognized widely that all behavioral traits are the product of a dynamic interaction between genetic and environmental influences (e.g. Caspari, 1958; Hinde, 1970; Alcock, 1984). It is beyond the scope of this paper to review or discuss phylogenetic development or value assessment of behavioral traits (e.g. Simpson, 1961; Mayr, 1982).

The task at hand is to identify characters in cephalopods that are species-specific. In other taxa as well as cephalopods, the more rigidly restricted and stereotyped behaviors are usually the easiest to observe and classify. Such "Fixed" or "Modal" Action Patterns are (1) performed in a functional manner the first time an animal encounters the appropriate environmental cue (a sign stimulus or releaser) for the behavior, (2) usually stable with respect to environmental influences, and (3) directly observable, can be recorded on film and described qualitatively and quantitatively. Action patterns are not truly fixed (Schleidt, 1974) and Barlow (1968, 1977) has provided behavioral and neurophysiological arguments why such behaviors might be termed more accurately "Modal Action Patterns," or MAPS. This terminology will be followed here.

The question is: Which Modal Action Patterns are specific to species, and which are shared characters of higher-order groupings? My observations of living cephalopods (in the field and laboratory) indicate that there are plentiful examples of both. More importantly, many homologous Modal Action Patterns among cephalopods (see Packard, 1972; Packard & Hochberg, 1977; Moynihan, 1985) have nuances that are specific. For example, the so-called "Dymantic display," shown by many cephalopods as a response to threat, is characterized by general paling of the body, flaring of the arms, web or fins and expression of two large dark false eyespots. But there are recognizable differences even among octopus species of similar size and habitat, either in the eyespots (size and position), the color and textures of the paled skin, the degree of flattening of the body, etc.

One aspect of cephalopod behavior not yet fully appreciated or used by taxonomists or ethologists is the often strong association of a particular "body pattern" with the behavior. This association was first described in detail for octopuses by Andrew Packard (Packard, 1963; Packard & Sanders, 1969, 1971; Packard & Hochberg, 1977). It has also been described for the squids *Sepioteuthis sepioidea* (Moynihan & Rodaniche, 1982) and *Loligo plei* (Hanlon, 1978, 1982), and has been developed in detail for cuttlefish by Hanlon & Messenger (1988). Color and pattern change in cephalopods are under direct neural control and can occur quickly. Body patterns are physiological entities and fit many of the criteria of a Modal Action Pattern (Barlow, 1977; Hanlon & Messenger, 1988). The *body pattern* is the overall appearance of the animal, and each pattern is composed of chromatic, textural, postural and locomotor *components*. Chronic body patterns (lasting

hours or days) are used mainly for concealment; they are varied and not particularly useful in taxonomy. Acute body patterns (lasting seconds or minutes) are mainly Modal Action Patterns in response to predators, prey or conspecifics; these are valuable characters in taxonomy. Details of the hierarchical organization of body patterning and behavior are found in Packard & Hochberg (1977), Packard (1982) and Hanlon & Messenger (1988).

DATA COLLECTION AND ANALYSIS

Direct observation of the animals under varied conditions—often for only a short period—can reveal sufficient information for species identification. One simple and effective method is to chase or frighten an animal abruptly and to observe, film or photograph (in color) the acute body patterns and behavioral responses. Underwater, this is done by moving quickly towards the animal and perhaps pushing a hand or camera directly toward it. The general response is similar to that of a large predator and will be some form of Modal Action Pattern such as a "Dymantic display" or a Flamboyant pattern (see Packard & Hochberg, 1977). The animals habituate quickly to this method, so the data must be obtained the first few times. Slower approaches by the human observer before or after the quick response will yield other grades of response that are useful as well. In the field, Hanlon & Hixon (1980) have used this method effectively with *O. burryi.* Moynihan & Rodaniche (1982) used it with *Sepioteuthis sepioidea,* Hanlon & Messenger (1988) with *Sepia officinalis,* and Corner & Moore (1980) observed ritualized courtship patterns in *Sepia latimanus.* The same general approach can be taken in laboratory aquaria, where Packard (1963), Van Heukelem (1966), Packard & Sanders (1969, 1971), Packard & Hochberg (1977) and Boyle & Dubas (1981) have, among others, used it effectively. Roper & Hochberg (1988 in this volume) and Hanlon & Wolterding (in press) give examples of both field and laboratory data gathered this way.

The difficulty lies in recognizing those aspects of behavior that are stereotyped or "modal" versus those that are plastic and more influenced by environment. For this reason it seems more reasonable to concentrate on Modal Action Patterns used as acute responses to threat stimuli rather than chronic body patterns used for concealment on diverse substrates (although some of these qualify as MAPS). Courtship behavior (including body patterns) is also stereotyped and species-specific and can often be observed in the laboratory as well as the field. Deciding upon the exact unit of behavior to describe as a Modal Action Pattern depends ultimately upon the observer's perceptual capability. Tinbergen (1959) provided a rationale for choosing and assessing behavioral characters. Lack of standardization of this selection process constitutes a difficult problem in behavioral sciences (Condon & Ogston, 1967), but attention to stereotypy can render the choices fairly simple in many cases. Rearing related species in the same environment is one way to observe species differences that are not determined environmentally; if differences persist, then the differences must be ultimately of genetic origin. Of course this is not always practical; nor is observing the animal extensively in the natural environment. In practice some compromise of the two methods will be used, but extensive observations should be made lest poor behavioral characters be chosen and pursued in future work.

Simple analyses of color photographs or video recordings will enable one to catalog behaviors and body patterns for each species (an ethogram). Having a body pattern associated with most cephalopod behaviors is a distinct advantage to the taxonomist. Most studies in other animal groups have used the "Yes–No" approach in which checklists among species were made with notations of the presence/absence of particular behavioral traits. The work of Lorenz (translated in 1971) on ducks and geese is the most quoted example. Dewsbury (1972) presented another application of the yes–no method by applying it to four major questions concerning male reproductive behavior in mammals; he found that closely related species tended to fall within the same pattern, thus highlighting taxonomic affinities. More rigorous quantitative procedures are suitable for behavioral taxonomy (Sneath & Sokal, 1973) but few studies have used them and their applicability to cephalopods is unknown. Bekoff (1977) reviewed these studies (including pattern similarity coefficients and discriminant function analyses) and outlined cogent reasons concerning how and why they should be used more often in future work.

CHARACTERS OF POTENTIAL USE IN CEPHALOPODS

Cephalopod behavior has not been studied extensively under natural conditions, and few studies have used behavioral cues for taxonomic purposes. Roper & Voss (1983) listed "behavior, non-permanent color patterns observed on live specimens, bottom or habitat preference, abundance, prey, predators, etc." and observations and photographs of live animals as potential identifying features of species. Historically, it has been useful to note features of chromatophore patterns in the skin that persist in preservation (e.g. Jatta, 1896; Naef, 1923; Voss, 1950), but, while helpful, they are limited because they represent only one transient body pattern (which is a physiological entity) shown by the animal at the moment of death. There are a few exceptions in which the morphological arrangement of chromatophores in the skin is different (e.g. Hanlon, 1982). Taki (1941, 1964) was one of the early workers to include coloration, patterning and some habits of live animals in his descriptions of several Japanese octopods. While other ethological data have been published on cephalopods, there is no account of their specific use in taxonomy. Moynihan (summarized in 1985) presented many aspects of behavioral repertoires of some teuthoid and sepioid squids. However, his interest was mainly in communication and phylogeny, not species identification.

The following list is not meant to be comprehensive, but to provide a framework for ideas, definitions and future work. Additions and deletions will be necessary as the usefulness of these characters is assessed. My major purpose is to stimulate interest in certain behavioral traits and to accumulate, define and organize those characters that (1) will aid in identification of species and (2) form the basis of behavioral data that can be used eventually in the broader areas of systematics, ontogeny and phylogeny.

Behavioral characters

Emphasis is placed on Acute body patterns and other Modal Action Patterns. Examples of how these can be used are given in the next section.

1. Responses to predators. For cephalopods, the primary defense is concealment by a variety of methods (Hanlon & Messenger, 1988), most of which involve Chronic body patterns to match the bottom, produce disruptive coloration, countershading in the water column, etc. These patterns are diverse and not especially useful in species taxonomy.

For secondary defense (after detection by the predator), cephalopods use several categories of Acute body patterns helpful in taxonomy.

Deimatic pattern (or "Dymantic display") exaggerates the size, contrast and image of octopuses, squids and cuttlefishes, and has been described above. It is shown near or on the bottom, even in squids and cuttlefish. Packard & Sanders (1971: fig. 14) illustrate one example of a variation in *Octopus vulgaris* that is probably species-specific.

Flamboyant pattern of octopuses, squids and cuttlefishes is shown in the water column (even by octopuses) and consists of contortion of the arms, strong papillation, mottled skin and other specific variations. Packard & Sanders (1971) and Hanlon & Messenger (1988) showed that this pattern is replaced by the Deimatic as *Octopus vulgaris* and *Sepia officinalis* grow larger, so there may be ontogenetic specificity in some species.

Acute Mottle is a high-contrast pattern of dark mottles on a light background; the arrangement of the mottles in the skin shows specificity in many species.

Acute Disruptive seems unique to *Sepia* spp. (Hanlon & Messenger, 1988; Roper & Hochberg, 1988 this volume) and many of the chromatic components of the pattern are species-specific.

Passing Cloud is a pattern in which waves of expanded chromatophores pass over the bodies of octopuses and cuttlefishes; the origin and destination of the waves differ among octopuses and among cuttlefish, and some related species within those groups do not show it at all.

This list is not all-inclusive. There are other stereotyped responses to predators with potential use in species taxonomy, such as the sequence and nuances of the typical cephalopod escape response that generally involves: Darkening (of the whole body)—Blanching—Inking—Jetting, followed by a specific behavior and body pattern (e.g. Flamboyant in young cuttlefish or some concealment pattern in octopuses).

2. Motor patterns during foraging and prey attack. *Octopus cyanea* forages by making "speculative pounces" on likely spots

around a reef (Yarnall, 1969) and this is a distinctive feature not observed in most octopuses. Hochberg & Couch (1971) and Hanlon & Wolterding (in press) have described a similar, but distinctive, foraging method in *O. briareus* termed Parachute Attack Maneuver; different size classes and year classes of *O. briareus* perform it too. Packard (1963) described yet another method for *O. vulgaris.* Warren, Scheier & Riley (1974) described specific sequences for *O. rubescens.* In the cuttlefishes *Sepia officinalis* and *S. latimanus* a Passing Cloud pattern along with waving arms is used to attract (distract?) prey just before seizure, but the species differences are noticeable. Flores (1983) described attack sequences for the oceanic squid *Todarodes pacificus* that may be used to compare similar oceanic species.

3. Intraspecific agonistic behavior. Social, schooling male squids use various forms of ritualized behavior to achieve dominance over rival males, presumably for increased access to females. For example, *Loligo plei* uses a distinctive "Lateral display" in such behavior and this character is very different from other loliginid species with which it overlaps (Hanlon, 1978, 1982). Zebra-type patterns are used by many cephalopod species. Moynihan (1985) tabulated many examples of such ritualized patterns. While many are homologous, the details of the patterns are often species-specific.

4. Courtship. There are many specifics in this category, but rather few reported. Males (and sometimes females) of *Octopus horridus* and *O. cyanea* often show a bold pattern of dark vertical stripes, while *O. vulgaris* does not (Young, 1962; Van Heukelem, 1966; Wells & Wells, 1972). *Octopus vulgaris* males often show a "Sucker display" to females during courtship and copulation (Packard, 1963). Pickford & McConnaughey (1949) described courtship and mating in the sibling species *O. bimaculatus* and *O. bimaculoides;* no interbreeding occurred, but nor did any obvious behavioral or patterning differences arise in these few observations. This is an important problem that deserves more investigation, with particular emphasis placed on the sequence of events.

The dazzling courtship display of male *Sepia officinalis* involves Zebra patterning, lateral positioning, extended fourth arm and circling, all in a stereotyped and species-characteristic manner (Tinbergen, 1939), and Corner & Moore (1980) described comparable, yet specifically different, courtship behavior and patterning for *Sepia latimanus.* The squid *Sepioteuthis sepioidea* also shows elaborate and specific courtship behavior and body patterns (Arnold, 1962; Moynihan & Rodaniche, 1982). The use of light organs in courtship of bathypelagic species is probable but not yet studied.

5. Mating. Some octopuses mate after mounting the female (e.g. *Octopus briareus*) while others mate by remaining apart while the male extends the hectocotylized arm to the female and passes a spermatophore with minimal contact (e.g. *Octopus vulgaris*). See the synopsis in Mangold (1987: 167). The duration of mating varies substantially among species, from 5 min to 3 hr (Boyle, 1983). The sepiolid squid *Euprymna scolopes* is reported to show particular body patterns during mating (Moynihan, 1983); most cephalopods do not. Teuthoid squids mate very quickly (seconds) and by two methods, but there do not seem to be species differences (Mangold, 1987).

6. Spawning and egg care. Many species have specific spawning periods (Mangold, 1987: 192) and some closely related species spawn different numbers and sizes of eggs (e.g. *Octopus bimaculatus* vs. *O. bimaculoides;* Pickford & McConnaughey, 1949). Some loliginid squids spawn eggs in large communal beds *(Loligo opalescens)* or in one general area (*L. pealei* near Woods Hole, Mass.), while others *(L. plei)* spawn smaller clumps of eggs in widely separated areas. Some octopuses brood their eggs by carrying them around in a pouch formed by the arms and web (e.g. *Octopus defilippi* and *Octopus burryi* in the Caribbean, *Hapalochlaena maculosa* in Australia, and the bathypelagic octopus *Eledonella pygmaea*), while most attach their eggs to a substrate within a lair.

7. Chromatic components of body patterns. These are in the form of distinctly placed rings, stripes, bars, spots, etc. of expanded chromatophores on particular parts of the body. They occur repeatedly in the same area of skin, are species-specific and have a specific neural substrate that produces and controls expression of the component (Dubas *et al.,* 1986; Messenger & Miyan, 1986). Some chromatic components result from concentrations of iridescent cells or white leucophore cells. These chromatic components are the building blocks of body patterns and may be the best criteria for shallow-water cephalopods in diverse habitats and with

TABLE 1. Behavioral, body patterning and ecological characteristics of the shallow-water octopuses of the

	Octopus vulgaris Cuvier, 1797	*Octopus hummelincki* Adam, 1936	*Octopus defilippi* Verany, 1851	*Octopus burryi* Voss, 1950	*Octopus macropus* Risso, 1826
Adult size	large 5000 g	70 mm ML	moderate 90 mm ML	small 60 mm ML	moderate 1000 g
Small eggs and planktonic young	yes	yes	yes	yes	yes
Large eggs and benthic young	no	no	no	no	no
Eggs carried around by female	no	no	yes	yes	no
Mating style	male covers female and distance	?	?	?	?
Arm length	moderate 3–5 × ML	moderate 3–4 × ML	very long 6 × ML	short 2–3 × ML	long 4–6 × ML
Activity period	diurnal-nocturnal	?	nocturnal	nocturnal	nocturnal
Primary habitat	coral reef and sand-seagrass	coral reef	sand-mud	sand flats near reef	sand flats near reef
Can bury in sand	yes	no	yes	yes	no
Type of lair	Hole in hard substrate—littered	?	?	?	hole in sand
Ocelli in skin	no	yes	no	no	no
Most distinguishing body pattern feature	Highly variable patterns; classic Deimatic (Packard & Sanders, 1971)	Ocelli between eye and 2nd/3rd arms; blue ring conspicuous.	Slim transverse arm bars; heart-shaped white leucophore pattern on mantle; long, narrow mantle and very long arms.	Only octopus with dark longitudinal stripe on dorsal arms (Hanlon & Hixon, 1980).	Only octopus with red base color and bright white oval leucophore spots all over.
Distinguishing skin features	Patch and groove trellis arrangement; distinct frontal white spots; Figs. 1, 2.	Ocelli; patch and groove trellis arrangement; diffuse frontal white spots; 2 large mantle white spots; Figs. 3, 4.	Widespread dark flecks of mottle; inconspicuous frontal white spots; distinctive round white spots placed along arms and one on mantle near eyes; no reddish coloration; pink iridescence under eye; Figs. 11, 12.	Patch and groove trellis arrangement; small frontal white spots; distinctive mantle papillae and transverse white streak; Fig. 9.	Mainly red chromatophores; large white oval leucophore patches; no frontal white spots; distinctive large papillae on mantle; Figs. 13–15.
Distinguishing behavior	Active during day; bivalves and crab shells around lair; response to threat is Deimatic pattern—very startling; often found on top of reef crest; very wide range of body patterns.	Little known; very common in Haiti; common in very shallow water on reefs. Similar patterns to *O. vulgaris* but ocelli separate them as well as all other octopods since *O. maya* is restricted in distribution. Response to threat unknown.	Crawls across open substrate with long arms spread radially, presumably hunting prey; can easily bury directly into substrate from this position. Response to threat unknown.	Found mainly in sandy and muddy bottoms that provide no cover; ability to dive straight into sand and disappear; extremely fast forward attack on crabs; response to threat is not Deimatic but Flamboyant pattern (Hanlon & Hixon, 1980).	Forages on coral reef edge in sand; uses long arms to pull crabs and shrimp from holes; response to threat is to turn bright red with bold white spots.

Caribbean Sea.

Octopus briareus Robson, 1929	*Octopus joubini* Robson, 1929	*Octopus maya* Voss & Solis, 1966	*Octopus zonatus* Voss, 1968	*Euaxoctopus pillsburyae* Voss, 1975
moderate 1000 g	very small 30 g	large 2000 g	small 30 mm ML	small 24 mm ML
no	no	no	no	?
yes	yes	yes	yes	?
no	no	no	no	no
male covers female	close contact and distance	distance	?	?
long 4–6 × ML	short 2–3 × ML	moderate 3–4 × ML	short 2 × ML	very long 6–9 × ML
nocturnal	nocturnal	diurnal-nocturnal	?	?
coral reef	seagrass or coral reef	sand-mud or seagrass	?	?
no	no	no	no	?
hole in reef or sponge	empty bivalve shell	hole in hard substrate—littered	?	?
no	no	yes	no	yes
Only octopus with blue-green appearance when chromatophores retracted (Hanlon & Wolterding, in press).	Very drab—uniform light, dark and weak mottle only (Mather, 1972).	Ocelli between eye and 2nd/3rd arms, but blue ring absent in adults.	Only octopus with broad dark transverse bands on body (Voss, 1968).	Large semicircular ocelli on mantle; extremely long arms (Voss, 1975).
Uniform distribution of chromatophores; no frontal white spots; uniform blue-green iridescence; Figs. 7, 8.	Uniform distribution of yellow and brown chromatophores—no reds; no frontal white spots; very few components of patterning; Fig. 16.	Ocelli; patch and groove trellis arrangement; distinctive frontal white spots; Figs. 5, 6.	Dark gray-brown bands interspersed with yellow-white areas; Fig. 10.	Only species with ocelli on mantle. No figure available.
Parachute Attack Maneuver is unique; broad web distinctive; most common octopus on coral reefs at night; response to threat is modified Diematic with pale body, dark eye ring but no dark edged suckers as in *O. vulgaris*.	Very small; lives in empty shells or bottles in sand-seagrass area; very limited repertoire of body patterns; strongly nocturnal. Response to threat is weak. Mottle pattern.	Active during day; bivalve and crab shells around lair; response to threat is Deimatic as in *O. vulgaris;* very wide range of body patterns; very restricted distribution—S. Gulf of Mexico only.	Response to threat is intense light-dark banding; otherwise bands almost indistinguishable.	?

good patterning repertoires. For example, the Dark hood of *Octopus vulgaris* has different forms in other octopods; Ring components on the mantle of squids are differently placed; dark transverse lines and bars on *Sepia* spp. are different.

8. Arm postures and papillae. Some of these postural and textural components of body patterns are species-specific. Raised first arms during courtship by male *Loligo pealei* distinguishes this species from the nearly identical *Loligo plei.* Other postures during courtship, defense from predators and attack of prey may be specific. The degree of papillation on octopuses and *Sepia* is *not* a good character because they can all show the range from smooth to highly papillate. However, the location of specifically shaped papillae is sometimes specific and a good character. For example, see Packard & Hochberg (1977) and Hanlon & Hixon (1980) on octopuses, and Hanlon & Messenger (1988) and Roper & Hochberg (1988 this volume) on *Sepia* spp.

9. Burying. Most sepioid squids and a few octopuses can bury directly into the substrate as a response to predators (e.g. *Octopus burryi;* Hanlon & Hixon, 1980).

10. Habitat preference. Species of the same genus may prefer different habitats, especially during specific periods of development. For example, some neritic species of *Loligo* sit on the substrate in a concealment pattern if the substrate and behavioral circumstances are appropriate. Some *Octopus* species have specific den and substrate preferences (i.e. sand, mud, coral reef; Table 1) or are planktonic for the first portion of the life cycle. Mesopelagic and bathypelagic cephalopods sometimes prefer specific depths (Voss, 1967; Young, 1978).

11. Daily activity pattern. Some species are strictly diurnal, crepuscular or nocturnal (e.g. see Table 1). Some squids school continually *(Loligo opalescens)* while others school in the daytime and forage separately or in small groups at night (*Sepioteuthis sepioidea* and adult *Loligo pealei* in the Caribbean Sea). Many squids are attracted to lights at night, but behave differently. For example, *Loligo opalescens* swarms very near the lights, adult *L. pealei* and *Sepioteuthis sepioidea* approach singly or in pairs and *L. plei* schools just on the periphery of light or under the boat.

12. Diet. In general, cephalopods are opportunistic carnivores. However, some species have strong preferences for particular prey species. See Boletzky & Hanlon (1983) and Nixon (1987). Some octopuses feed on particular prey and litter their lair with specific remains (see *O. vulgaris* in Table 1).

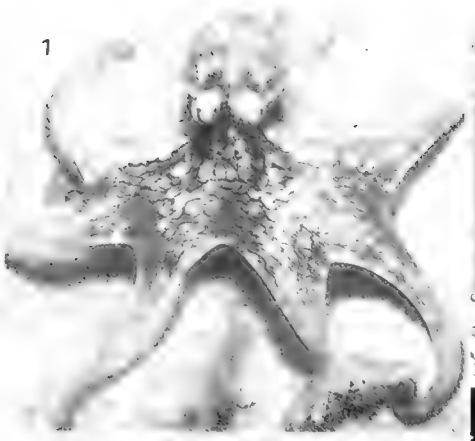
13. Migration. Vertical migrations can be species-specific (see no. 10 above). Horizontal migrations are common in cephalopods, are usually species-specific and are related to overwintering, following prey or spawning (see Mangold, 1987: 192).

Morphological characters related to behavior and body patterns

These selected traits can be assessed in the living animal, in photographs or in preserved specimens.

1. Chromatophores. Species have some characteristic combinations of either yellow, red, brown or black chromatophores. In general, yellows are smallest, reds larger, etc. These influence the overall color of the animal and can be a good diagnostic character in the living animal or in photographs. Size, density

FIG. 1. Adult *Octopus vulgaris* at Bimini, Bahamas. Note Dark hood component, mottle, and patch and groove trellis arrangement of skin.
FIG. 2. Juvenile *O. vulgaris* from northern Gulf of Mexico. Note Frontal white spots, Arm bars, reddish color. John W. Forsythe photograph.
FIG. 3. Adult *O. hummelincki* at Bimini, Bahamas. Note two large Mantle white spots, Frontal white spots, ocellus (below eye) and patch and groove trellis arrangement of skin.
FIG. 4. Same animal as Fig. 3. Note Mantle white spots still visible, ocellus enhanced and prominent cirrus at posterior.
FIG. 5. Adult *O. maya* off Campeche, Mexico. Note ocellus (no iridescent blue) and patch and groove skin.
FIG. 6. Laboratory-reared juvenile *O. maya* in Deimatic pattern. John W. Forsythe photograph.
FIG. 7. Adult *O. briareus* at night off Roatan Island, Honduras. Note overall green iridescence and extensive web spread during this speculative Parachute Attack Manuever over a small coral.
FIG. 8. Adult *O. briareus* at night off Grand Cayman Island, West Indies. Note overall blue iridescence, mottle pattern and web spread. Raymond F. Hixon photograph.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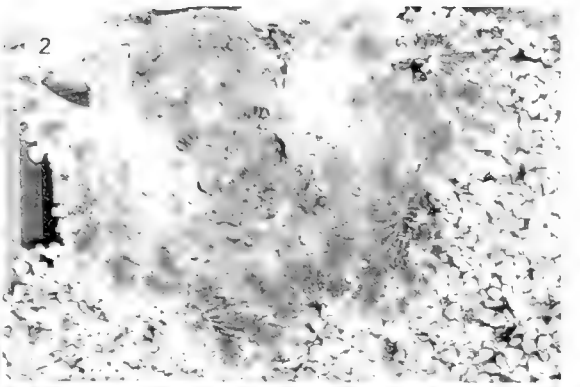
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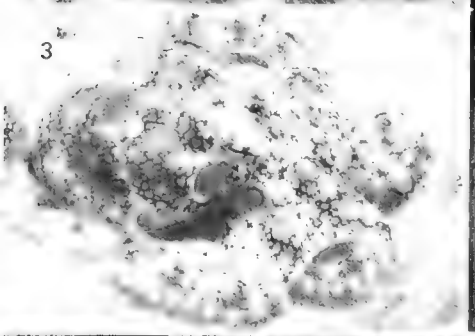
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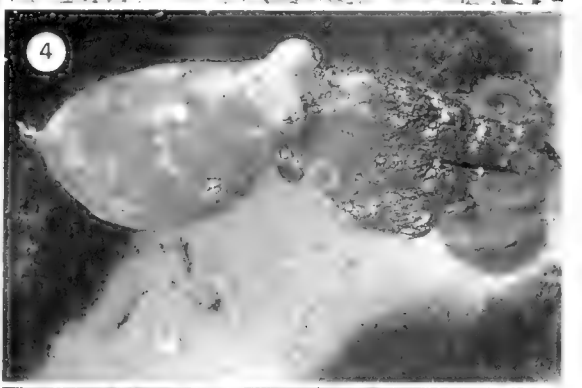
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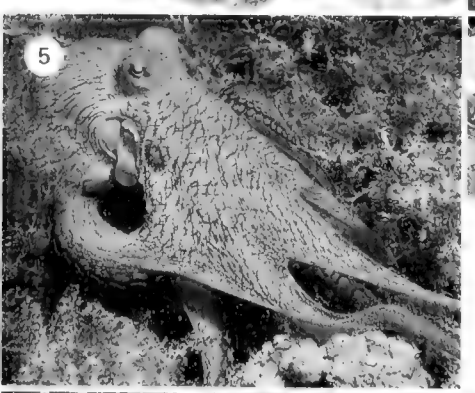
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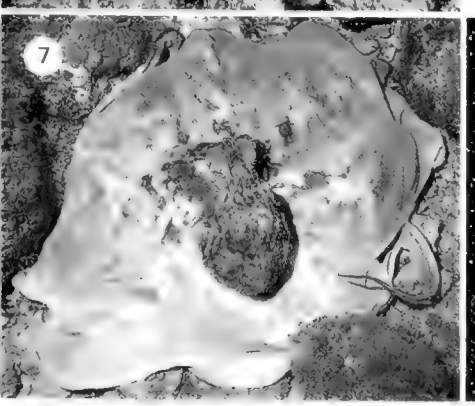
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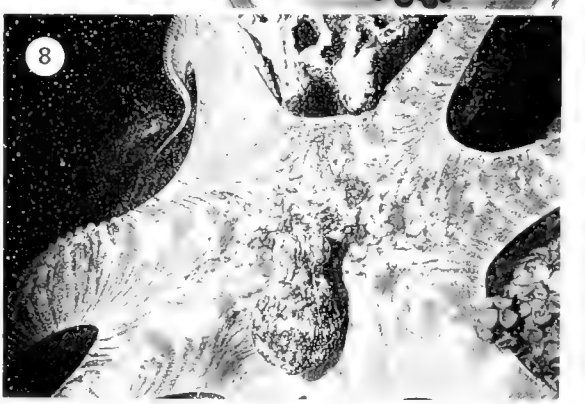
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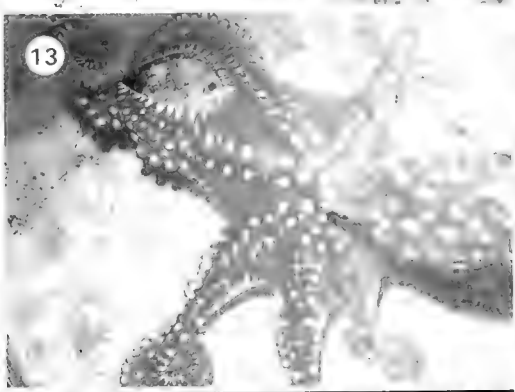
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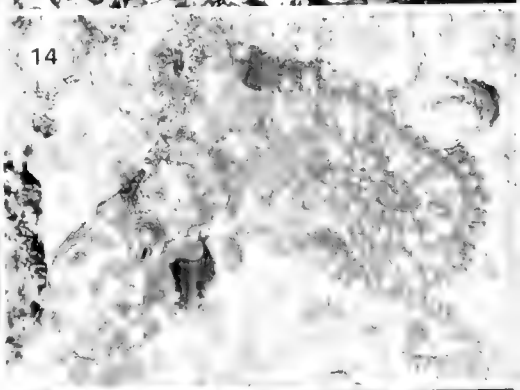
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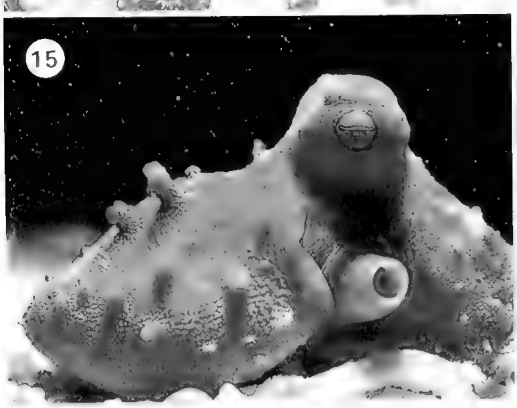
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and morphological arrangement of chromatophores are often species-specific; for example, the "patch and groove" system in the skin of *Octopus vulgaris* and certain other octopuses (details in Packard & Hochberg, 1977; Packard, 1982). Packard (1985) provided other ways to view species-specific characteristics of chromatophores during ontogeny.

2. Iridescent cells. The distribution and color (silver, pink, yellow, green or blue) of these dermal cells contribute strongly to species-specific patterning of the live animal. Color photographs are especially useful. Recent evidence (Hanlon, 1982; Cooper & Hanlon, 1986) indicates that some iridophores are controlled actively by squids and are shown during intraspecific signalling.

3. Leucophores and white spots. Leucophores and reflective cells reflect white (Brocco & Cloney, 1980) and are common and good identifying features in octopuses and *Sepia* spp., including the youngest stages (Packard & Hochberg, 1977; Hanlon & Messenger, 1988). These are observed easily in many photographs (especially Frontal white spots of octopuses) and sometimes are evident in preserved material.

4. Ocelli. Placement and form of ocelli are species-specific and this has been used in taxonomy. There is probably specificity in the way ocelli are expressed behaviorally, but this has not yet been reported.

5. Photophores. There are several types of photophores and their morphological arrangement is an important taxonomic features in deep-water cephalopods (Berry, 1920a,b). Observations *in situ* or on shipboard should reveal signalling patterns (in addition to a countershading function; Herring, 1977; Young & Mencher, 1980) similar to that reported for flash patterns in fireflies (e.g. Barber, 1953).

6. Ink. The ink is sometimes differently colored between species (even in preservation) and may have taxonomic significance, although it has not been analyzed carefully. Behaviorally, species use the ink differently—some shoot small distinct blobs (pseudomorphs) while others produce huge clouds to disorient and confuse predators (i.e. flash behavior).

BEHAVIORAL TAXONOMY OF THE SHALLOW-WATER OCTOPODS OF THE CARIBBEAN SEA

Voss (1968, 1975) provided keys to the shallow-water species of octopods in the tropical Western Atlantic. These keys are primarily morphometric, but do include some notes on color and body patterning. It is still difficult and time-consuming to key out animals once they are preserved. Behavior and coloration of live animals render identification easier. Hochberg & Couch (1971) provided some good behavioral and patterning distinctions for the identification of *Octopus vulgaris, Octopus macropus* and *Octopus briareus.*

Table 1 lists characters with which the shallow-water octopods may be distinguished by divers, from laboratory observations or photographs. Figures 1 to 16 complement Table 1 and illustrate some of the key points.

Octopus vulgaris (Figs. 1, 2) and *O. hummelincki* (Figs. 3, 4) of the same size are particularly similar in appearance for a wide range of body patterns, but the distinctive ocelli of *O. hummelincki* separate the two. *Octopus maya* (Figs. 5, 6) is similar in appearance and body patterning to these two species as well, but its restricted distribution and large eggs help with identification, and *Octopus vulgaris* is the only species likely to be

FIG. 9. Adult *O. burryi* off St. Croix, U.S. Virgin Islands. Note dark Longitudinal arm stripe, patch and groove skin and white Transverse streak on mantle.
FIG. 10. Adult *O. zonatus* from off Venezuela. Freddie Arocha Pietri photograph.
FIG. 11. Adult *O. defilippi* reared in the laboratory. Note Transverse arm bars, heart-shaped pattern on mantle and single white spot on extreme anterior mantle.
FIG. 12. Juvenile laboratory-reared *O. defilippi.* Note pink iridescence under eye, small Frontal and Arm white spots and spread arms during foraging. John W. Forsythe photograph.
FIG. 13. Adult *O. macropus* from off Miami, Florida.
FIG. 14. Adult *O. macropus* at night off St. Croix, U.S. Virgin Islands. Note reddish mottle and white papillae.
FIG. 15. Adult *O. macropus* from off Miami, Florida. Note coloration and prominent, flat mantle papillae. Martin R. Wolterding photograph.
FIG. 16. Adult *O. joubini* from off Miami, Florida. Note drab coloration and uniform distribution of chromatophores in skin.

TABLE 2. Comparisons of chromatic components of body patterning in two pairs of sympatric *Loligo* spp. See Hanlon (1982) for general descriptions of these types of components.

A. W Atlantic:

Loligo (Doryteuthis) plei Blainville, 1823	*Loligo pealei* LeSueur, 1821
Ring	Ring
Arm spots	Arm spots
*(No Dark arms and head)	*Dark arms and head
*(No Dark eye ring)	*Dark eye ring
*Stitchwork fins	*Fin edge spots
*Accentuated testis	*(No Accentuated testis)
*Shaded testis	*(No Shaded testis)
*Mid-ventral ridge	*Mid-ventral stripe
*Lateral flame	*Weak Lateral flame
*Lateral blush	*(No Lateral blush)
*Dorsal arm iridophores	*Dorsal arm iridophores

Data derived from Hanlon (1978, 1982), Hanlon, Hixon & Hulet (1983) and unpublished data.

B. E Atlantic:

Loligo vulgaris Lamarck, 1798	*Loligo forbesi* Steenstrup, 1856
Fin and mantle spots	Fin and mantle spots
Lateral mantle stripe	Lateral mantle stripe
Dark fin edge	Dark fin edge
Mid-ventral stripe	Mid-ventral stripe
Red spot under eye	?
Lateral blush	?
*Lateral mantle streaks	*Lateral mantle streaks
*Iridescent posterior mantle	*(No Iridescent posterior mantle)
*?	*Gold iridescent sheen

Data derived from Jatta (1896), Naef (1923), Tardent (1962) and Neill (1977). Also from (1) 21 adult *L. vulgaris* (10–16 cm ML) maintained in tanks for three weeks at the Stazione Zoologica in Naples, Italy and (2) from observations on adult *L. forbesi* in the tanks of the Marine Biological Laboratory in Plymouth, England as well as four weeks of observing live *L. forbesi* in the Azores Islands in tanks and in nature; further observations were obtained from animals reared to adult size in Galveston (Hanlon *et al.*, 1985).

C. Shared chromatic components among all four species.

Clear	Shaded eye
All dark	Dorsal mantle collar iridophores
Dorsal stripe	Dorsal iridophore splotches

*Components that apparently differ between species.
? Not yet observed.

found in the same areas as *O. maya;* the ocelli of *O. maya* distinguishes it. Small *O. vulgaris* and *O. joubini* of the same size can be distinguished early on by noticing their response to a startling stimulus; *O. joubini* shows only a pale mottle pattern or general darkening (Fig. 16), whereas *O. vulgaris* shows a Deimatic pattern (Packard & Sanders, 1971: fig. 14) that is dramatically different. *Octopus vulgaris, O. macropus, O. briareus* and occasionally *O. burryi* all may be seen together around many reef areas. Usually it is easy to distinguish them because *O. vulgaris* is active mainly during the day and lives on the top of the reef crest with lairs that are littered by bivalve, gastropod or crab shells. *Octopus briareus* (Figs. 7, 8) is extremely nocturnal and lives on the forereef and backreef areas and has a unique blue-green iridescence in many of its body patterns. This coloration is particularly striking and easy to see when a light is flashed on it at night. *Octopus macropus* (Figs. 13–15) and *O. burryi* (Fig. 9) are both restricted most of the time to sandy areas on the periphery of the reef. *Octopus macropus* is so distinctive as never to be confused; even in its pale body patterns, the large flat papillae (Fig. 15) and

general reddish tone identify it. The distinctive, dark, longitudinal arm stripes of *O. burryi* (Fig. 9 and color plate in Hanlon & Hixon, 1980) easily separate this species from all others, even in the smallest post-settling individuals (Forsythe & Hanlon, 1985). Observations or photographs of the skin also help to identify the species: only *O. vulgaris, O. hummelincki, O. maya* and *O. burryi* have the patch-and-groove arrangement of the skin (see Figs. 1, 3, 5, 9). Egg-carrying by female *Octopus defilippi* and *O. burryi* possibly reflect their preference for open sandy or muddy habitats. In such areas, naturally occurring lairs are scarce and one can imagine that carrying eggs and having the ability to bury into the sand and disappear when a predator approaches would be a useful adaptation. However this has not been observed in nature.

CHARACTERS OF POTENTIAL USE IN SEPARATING SYMPATRIC SPECIES OF *LOLIGO*

The Loliginidae represent a family with considerable systematic problems because body morphometrics are very similar, especially in the younger stages. Continuing disagreement exists regarding the generic or subgeneric status of *Loligo, Heterololigo, Photololigo, Nipponloligo* and *Doryteuthis* as well as the placement of the 25 or so species within this group (e.g. Cohen, 1976; Natsukari, 1984; Roper, Sweeney & Nauen, 1984; Brakoniecki, 1986). There is particular interest in these species because of their importance to fisheries and the biomedical research community. I provide here some examples of behavioral and body patterning features that may aid in distinguishing sympatric species that are extremely similar in appearance and morphometry.

Loligo plei— Loligo pealei in the W. Atlantic

Cohen (1976) and Hixon (1980) described the extensive geographic and ecological overlap of these two species. Hatchlings (McConathy, Hanlon & Hixon, 1980) and juveniles smaller than approximately 70 mm ML of these two species are nearly indistinguishable, even with detailed, internal morphometric information.

The chromatic components of patterning of the two species are listed in Table 2. Male *Loligo plei* greater then 70 mm ML can show

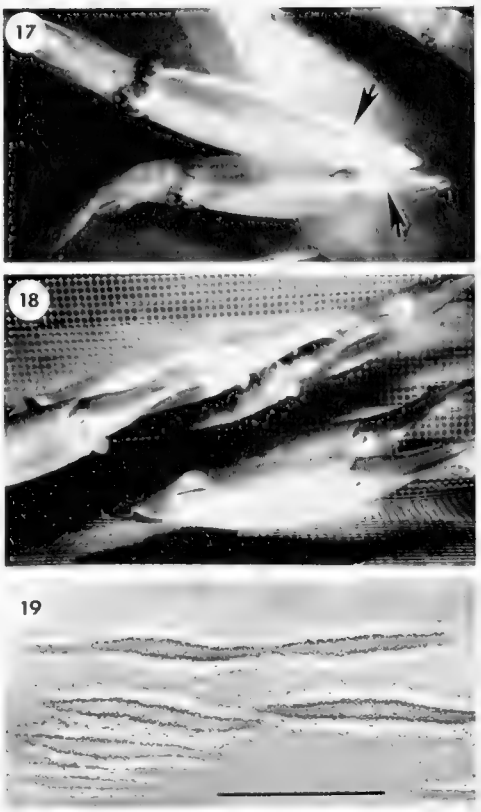

FIG. 17. *Loligo vulgaris* (15 cm ML) with arrow indicating the bright green Iridescent posterior mantle.
FIG. 18. Female *Loligo forbesi* (24–29 cm ML) in a submerged tank on the Azores Islands. Note Lateral mantle streaks. John W. Forsythe photograph.
FIG. 19. Lateral mantle streaks of a male *L. forbesi* (56 cm ML). Scale 50 mm.

Accentuated or Shaded testis (regulated by the chromatophores in the skin over the testis) whereas *Loligo pealei* cannot. The Mid-ventral ridge in *L. plei* is a protrusable flap of darkened skin in males, but in *L. pealei* there is only a dark Mid-ventral stripe (of expanded chromatophores) and no flap of skin that extends downward. Juvenile male *L. plei* and females can show a Lateral blush that has not been observed in *L. pealei.* Conversely, Dark arms and head and Dark eye ring have been seen only on *L. pealei.*

Loligo pealei and *L. plei* swimming in a tank together are nearly identical (Hanlon, Hixon & Hulet, 1983: fig. 5). One distinguishing behavior is that *L. pealei* will sit naturally on the

TABLE 3. *Loligo forbesi* from the Azores Islands, 1985. Number and length of Lateral mantle streaks (see Figs. 18 & 19) on males and females. Mean size of mature males 57 cm ML, maximal 95 cm ML. Mean size of adult females 34 cm ML, maximal 41 cm ML (Martins, 1982).

		Mantle length (cm)	No. streaks per side	Mean streak length and (range)
Males:	1.	9	8L, 7R	4 (3–5)
	2.	10	13L, 17R	5 (3–11)
	3.	12	9L, 8R	6 (3–8)
	4.	12	15L, 14R	6 (3–10)
	5.	14	18L, 16R	10 (4–17)
	6.	27	16L, 15R	15 (5–37)
	7.	29	14L, 16R	19 (5–35)
	8.	30	14L, 18R	20 (5–37)
	9.	33	10L, 11R	20 (10–33)
	10.	34	12L, 15R	17 (6–32)
	11.	36	18L, 18R	21 (11–35)
	12.	37	15L, 13R	21 (10–40)
	13.	38	16L, 15R	30 (7–72)
	14.	40	19L, 19R	23 (8–45)
	15.	40	16L, 17R	19 (6–40)
	16.	40	15L, 18R	23 (5–85)
	17.	48	16L, 16R	30 (12–55)
	18.	66	15L, 18R	51 (22–115)
	19.	77	16L, 17R	82 (28–213)
Females:	1.	28	10L, 11R	14 (10–17)
	2.	29	12L, 12R	11 (5–24)
	3.	31	8L, 8R	14 (8–21)
	4.	31	10L, 8R	13 (8–21)
	5.	31	8L, 9R	13 (5–26)
	6.	32	8L, 8R	13 (4–20)
	7.	32	10L, 9R	12 (4–18)

bottom with a ring pattern on the mantle *(op. cit.)* while *L. plei* never will. During agonistic encounters, *L. plei* males will show a dramatic Modal Action Pattern termed "Lateral display" characterized by the adult Lateral flame component (Hanlon, 1982: fig. 1) while *L. pealei* can show only a very weak Lateral flame component in fairly large animals. During the Lateral display, *L. plei* arches the first pair of arms and shows the brilliant solid iridescent color of the Dorsal arm iridophores. In *L. pealei,* the iridescence is less concentrated and these arms are never arched in that fashion. Courtship behavior of *L. pealei* is described in some detail by Arnold (1962) and differs substantialy from that of *L. plei* (Waller & Wicklund, 1968; Hanlon, 1978, 1982).

In nature, *L. pealei* comes to lights at night singly or in pairs near the surface; in general, they are not attracted strongly to lights. *Loligo plei* usually moves to the lighted area in large schools, although generally the schools stay on the periphery of light.

Loligo vulgaris—Loligo forbesi in the E Atlantic

These species overlap in distribution over an extensive area (Roper, Sweeney & Nauen, 1984). Even large adults (ca. 25–90 cm ML) are similar but are usually distinguishable by fin proportions and the arrangement of suckers on the tentacular club. However, all other sizes appear nearly identical, including hatchlings. The chromatic components of patterning are listed in Table 2. The most noteworthy finding is how very similar their chromatic expression is. Live adults of *L. vulgaris* may be distinguished by the bright green or blue iridescence (depending upon lighting and viewing direction) on the posterior 1/4 of the dorsal mantle (Fig. 17). This has never been seen in *L. forbesi,* which does not have the cellular arrangement of iridophores to produce this chromatic component of patterning. Other distinguishing features are that only *L. forbesi* will sit naturally on the bottom

as reported for *L. pealei*. *L. forbesi* has a "golden sheen" of iridescence over its dorsal surfaces that has not been noted in *L. vulgaris*.

There are some differences in the Lateral mantle streaks: only in *L. forbesi* do the streaks appear to get very long (Figs. 17, 18, 19). As indicated in Table 3: (1) only mature males and females greater than 9 cm ML have the streaks, (2) length of streaks is roughly proportional to ML, (3) the streaks reach a length of 213 mm in the largest males (i.e. 60–90 cm ML), (4) the number of streaks per animal varies and the streaks are not symmetrical on each side of individual animals. Long streaks on *L. vulgaris* have not been seen or reported; the longest streaks on the squids I observed in Naples were 8 mm for a male of 15 cm ML. Photographs of *L. vulgaris* from the Sahara Bank indicate streaks of only 10–20 mm for squids of 50 cm ML. This character may separate the species, but it is necessary to check more large *L. vulgaris* to verify the difference in streak length. Jatta (1896) and Naef (1923) noted these streaks in preserved specimens of both sexes of *L. forbesi*, but Naef (1923) stated wrongly that in *L. vulgaris* only males had them (my observations in Naples confirm that females do). Holme (1974) stated erroneously that *L. vulgaris* did not have streaks. Naef (1923) also stated that fin spots are present only on males of *L. vulgaris*, but fin and mantle spots are present on both sexes of both species; only observations of live animals can verify chromatic components because they are physiological entities controlled by the nervous system.

CONCLUDING THOUGHTS

Behavioral taxonomy has been beneficial in other animal groups and there is reason to believe that it will augment studies in cephalopod taxonomy, systematics and phylogeny. The real advantage in the cephalopod ethogram is that body patterns of chromatophores (neural control) are associated closely with specific behaviors and provide the opportunity to obtain highly visible and specific patterns as well as more "typical" behaviors used in behavioral taxonomy. These may be used for identification purposes if one is careful to choose the most stereotyped and specific characters. I would argue that the chromatic components of patterning may prove more useful in species identification than whole body patterns because components are more discrete, recognizable and repeatable within the species, and they represent a more discrete and smaller unit of behavior to analyze.

Analyses of body patterns and their components will reveal many examples of shared characters across species, genera, families and even orders. Moynihan (1975, 1985; Moynihan & Rodaniche, 1977, 1982) has begun to outline relationships of conservative body patterns among various groups of cephalopods, with *Sepioteuthis sepioidea* providing his baseline information. Since his primary focus is on communication in cephalopods, his analyses are based upon body patterns and not the more detailed components of patterning. If future baseline information on species is described according to a framework similar to that provided here, with particular emphasis on components of patterns and the hierarchical classification of patterning described by Packard and his collaborators (e.g. Packard & Hochberg, 1977) then this information will be more comparable when sufficient representatives of different cephalopod groups are analyzed. Such information, used initially for species identification, may then provide the building blocks for studies of systematics and phylogeny. It would seem particularly worthwhile to pursue aspects of behavior and patterning that are related to courtship and reproduction because these characters may yield more species-specific information. Indeed, it is likely that specific body patterns may be the key to prevention of interbreeding between sympatric sibling species.

Behavioral taxonomy as I outline it will be more useful for some groups of cephalopods than others. My examples include only shallow-water, nearshore cephalopods, which may be easier to distinguish because of their fairly rich patterning repertoires (a reflection of their behavioral diversity). However, I have deliberately chosen some species groups within *Octopus* and *Loligo* that are difficult to separate. In the future, ethologists and others working with live animals must help taxonomists by collecting behavioral and patterning information and providing it along with voucher specimens. Conversely, taxonomists must recognize the value of such information and synthesize it with classical morphological data.

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DISTRIBUTION OF THE NEOTEUTHID SQUID *ALLUROTEUTHIS ANTARCTICUS* ODHNER IN THE ATLANTIC SECTOR OF THE SOUTHERN OCEAN

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ABSTRACT

The "Discovery" collections contain 51 specimens of *Alluroteuthis antarcticus* from the Southern Ocean 0°–30°E, S of the Antarctic Polar Front. Of these, 45 specimens were caught during "Discovery" Cruise 100 with the opening-closing "RMT 8" net. Capture rate of the species was highest in the 800–900 m depth layer. At night, *Alluroteuthis* appears to spread vertically and there is evidence of ontogenetic descent. When vertical distribution of *A. antarcticus* is plotted as a function of mantle length and compared with the distribution of the cranchiid *Galiteuthis glacialis* apparently there is spacial separation between the two species in the water column. The degree of ecological separation cannot be determined in the absence of information on the trophic ecology of these squids.

Key words: *Alluroteuthis;* squid; Antarctic; distribution.

INTRODUCTION

The oegopsid squid *Alluroteuthis antarcticus* Odhner, 1923 is a member of the familiy Neoteuthidae. Its taxonomic status within the family is discussed by Roper, Young & Voss (1969). The distribution of *A. antarcticus* is restricted to the Southern Ocean (Roper, Sweeney and Nauen, 1984) and beaks identified to this species have been found in the stomach contents and regurgitations of Antarctic vertebrate predators (Clarke, 1980; Clarke, Croxall & Prince, 1981; Clarke & McLeod, 1982a, b). Clarke (op. cit.) and Clarke, Croxall & Prince (op. cit.) originally attributed *A. antarcticus* beaks to *? Crystalloteuthis* (see Clarke & McLeod, op. cit. a, b).

The aim of this study was to describe the distribution of the catch of *Alluroteuthis antarcticus* from the sector of the Southern Ocean 0°–30°E. The work is based largely on 45 specimens from "Discovery" Cruise 100 that, in 1979, made four transects across the Southern Ocean between the Cape of Good Hope and the ice edge of Antarctica, within the sector 15°–30°E. Six additional specimens of *A. antarcticus* in the "Discovery" collections from earlier cruises in the sector 0°–30°E have also been included. Together these specimens constitute the second largest collection of the species available for study, exceeded only by the "Eltanin" collection of 69 specimens (Roper, 1969). The specimens forming the present collection were caught largely by opening-closing nets that sampled discrete depth layers so that vertical distribution of the catch is particularly well defined.

MATERIALS AND METHODS

The track of the ship in the Southern Ocean and sampling stations of RRS "Discovery" during cruise 100 (30 January–4 April 1979) are shown in Fig. 1a. All specimens of *A. antarcticus* captured during this cruise were caught in the opening-closing Rectangular Midwater Trawl ("RMT 8") Multi-net (Roe & Shale, 1979). Samples were fixed in 5% formalin and preserved in Steedman's Solution (Steedman, 1976). Dorsal mantle length (ML) of each squid was measured to the nearest 0.1 mm. Capture rate (CR) was calculated from the number of specimens caught in each 100 m depth layer, divided by the number of hauls that sampled each layer. Where specimens were caught in a haul that spanned a layer >100 m, it was assumed that the capture rate was constant throughout the vertical range of the haul and the catch was divided by the number of 100 m layers sampled. Six additional specimens of *A. antarcticus* were obtained from samples collected during former "Discovery" cruises.

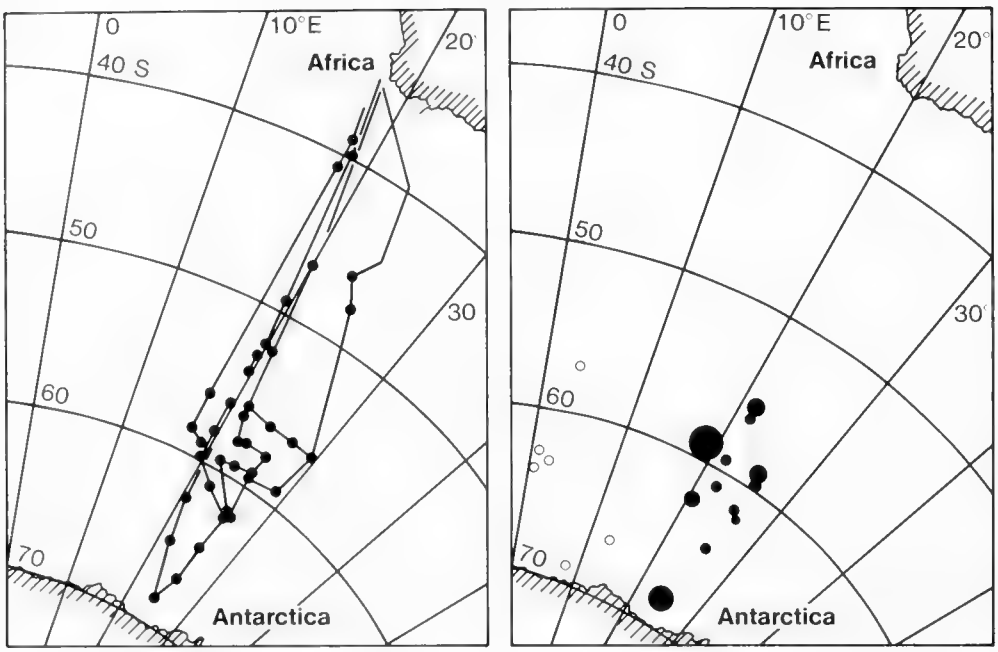

FIG. 1. *Alluroteuthis antarcticus*. Left, RRS "Discovery" Cruise 100 track and sampling stations. Right, distribution of the catch. Size of shaded circles indicates relative size of catch at each station (open circles represent specimens from earlier cruises).

TABLE 1. Details of specimens of *Alluroteuthis antarcticus* in the "Discovery" collections, from the sector of the Southern Ocean 0 –30° E. N70—70 cm ring-frame net; N100—100 cm ring-frame net; O—deployed obliquely; TYF—Peterson young-fish trawl; V—deployed vertically.

Station	Date	Time	Depth (m)	Net	ML (mm)
1154	12 Mar 1933	1949–2039	240–0	TYFB	110.0
1784	4 June 1936	1845–1925	800–400	TYF70B	20.4
2012	22 Mar 1937	–1810	1500–1000*	N70V	44.6
2322	17 Apr 1938	–2245	1000–750*	N70V	17.4
2543	20 Jan 1939	2247–2330	760–540	N100B	15.3
2556	25 Jan 1939	2226–2256	360–200	TYFB	13.6

*Estimated depth layer

RESULTS

Horizontal distribution of the catch

The positions where *Alluroteuthis antarcticus* were caught during the "Discovery" 100 cruise and during previous expeditions are shown in Fig. 1b. Relative numbers captured at each station are indicated also. The stations differed in overall fishing intensity and in the intensity of fishing at different depths, so the data should not be taken to indicate differences in population density. Details of the material from the "Discovery" expeditions prior to cruise 100 are given in Table 1. Kemp & Hardy (1929) described the nets used.

The northernmost haul to capture *A. antarcticus* was at a station 55° 36′ S, well to the

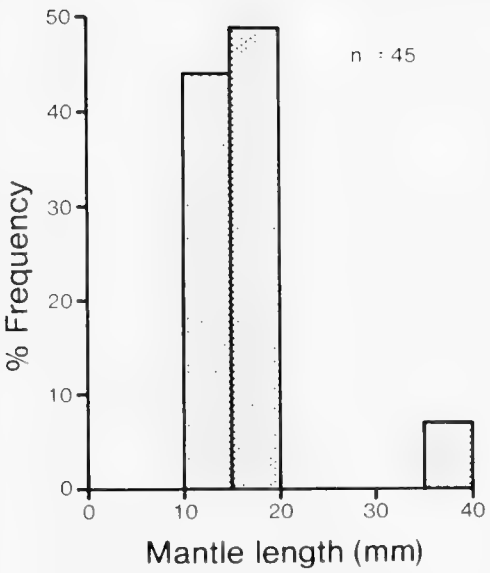

FIG. 2. *Alluroteuthis antarcticus.* Frequency distribution of the catch from "Discovery" Cruise 100.

S of the Antarctic Polar Front (APF); the most southerly catch of the species was at 69° 21′ S.

Size structure of population sample

The "Discovery" 100 cruise sample was dominated by small individuals <20 mm ML (Fig. 2). The sample population was skewed strongly positively and the largest size caught by the net was 36 mm ML. The largest individual from the earlier expeditions was 110 mm ML (Table 1).

Vertical distribution of the catch

Capture rate in each 100 m layer for the "Discovery" 100 sample is shown in Fig. 3, together with the fishing effort in each layer. The catch was concentrated in the 500–1000 m zone, with a pronounced peak of numbers in the 800–900 m layer. Small numbers appeared in hauls to a depth of 2000 m but were not caught at the deepest depths sampled to 2800 m.

The "Discovery" 100 sample was divided into three size classes: 11–15 mm, 16–20 mm and 36–40 mm ML. The capture rate, as a fraction of the total for each size class, was calculated for each 100 m layer (Fig. 4). The 11–15 mm size class was concentrated in the 700–800 m depth layer, with a median depth of 650 m. The 16–20 mm size class was concentrated in the 800–900 m depth layer, with a median depth of 750 m. The 36–40 mm size class was concentrated in the 1000–1200 m depth layer, with a median depth of 1250 m.

The specimens from the earlier expeditions (Table 1) showed a general increase in size with depth, with the exception of the largest specimen (110 mm ML), which was caught in a haul relatively close to the surface (0–240 m).

The "Discovery" 100 sample was divided into individuals caught during daylight (including dusk) and those caught during darkness (including dawn). The capture rate, calculated for each 100 m layer as a fraction of the total (Fig. 5), showed that there was a tendency for the population to spread vertically during the hours of darkness.

When the vertical distributions of *A. antarcticus* and the other common species of squid in the "Discovery" 100 collection, *Galiteuthis glacialis* (Rodhouse & Clarke, 1986), was plotted as a function of ML, there appeared to be spacial separation in the water column between the two species (Fig. 6).

DISCUSSION

All specimens of *Alluroteuthis antarcticus* were caught to the S of the APF. The data suggest that juveniles of about 10–20 mm ML were concentrated in the 800–900 m depth layer and that growth was accompanied by ontogenetic descent. That is to say, smaller individuals tend to occur relatively close to the surface and they migrate downwards as they grow. It is notable, however, that the largest specimen in the collection was caught in a net that sampled a layer from 0–240 m. This specimen was a female in an early stage of maturity. It is known that larger specimens of the species are sometimes present near the surface because squids, identified from beaks to this species, with an estimated mean weight of 278 g occur in the diet of surface-feeding birds (Clarke, Croxall & Prince, 1981). However, there are insufficient data to allow the distribution of large specimens to be characterized. It appears from the catch that the juveniles live in the upper zone of the "Warm Deep Water" (Deacon, 1937) and descend

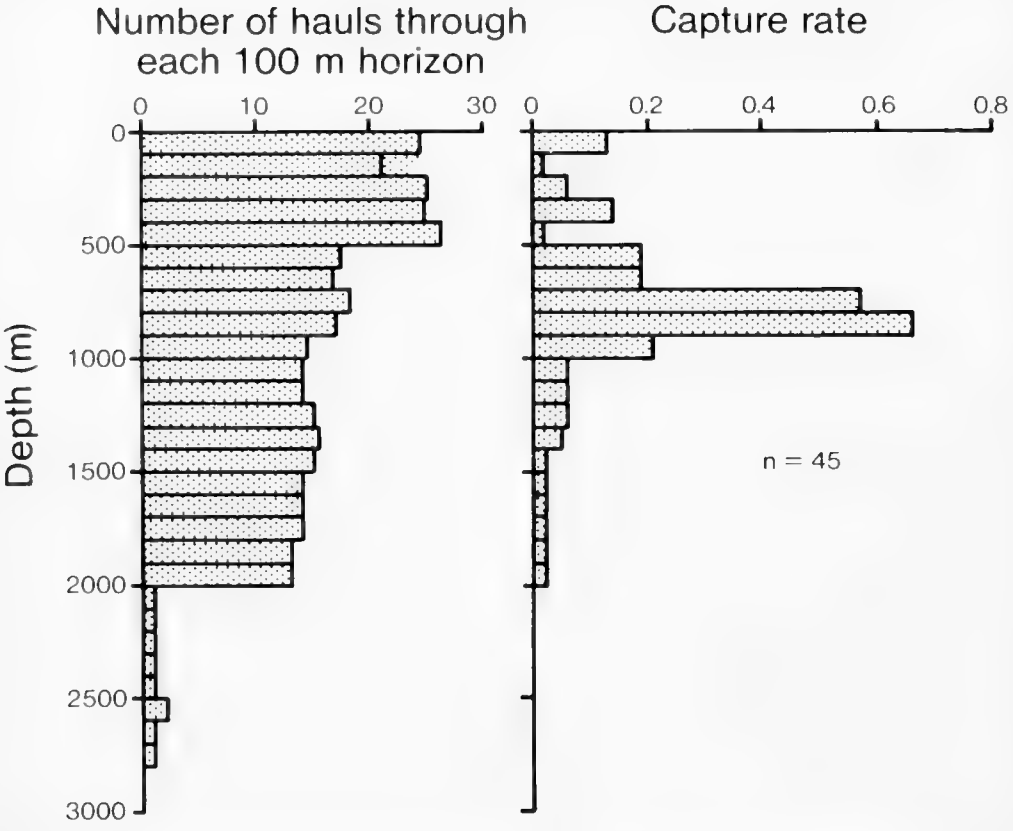

FIG. 3. *Alluroteuthis antarcticus*. Capture rate at each 100 m depth layer and fishing effort at each layer.

further towards the "Antarctic Bottom Water" during growth. Adults may ascend towards the surface to feed, mate or spawn. Alternatively, they may be moribund individuals that float up or are carried in localized upwellings (Imber & Berruti, 1981) or are regurgitated near the surface by deep-diving predators (Clarke, Croxall & Prince, 1981). The distribution of the catch of juveniles during daylight-dusk and darkness-dawn suggests that the species does undergo some diel vertical movement.

The distribution in the water column of several species of oceanic squids from other latitudes has been described by Clarke & Lu (1974, 1975), Lu & Clarke (1975a, b) and Roper & Young (1975). In many instances, vertical distribution is characterized by ontogenetic spreading or descent. Two other species were sufficiently well represented in the "Discovery" 100 collection to allow a meaningful description of the vertical distribution of the catch. These were the cranchiids *Galiteuthis glacialis* (Chun) (Rodhouse & Clarke, 1986) and *Mesonychoteuthis hamiltoni* Robson (Rodhouse & Clarke, 1985). Neither species was caught to the N of the Antarctic Polar Front (APF). The size range and number of *G. glacialis* caught was much greater than *M. hamiltoni.* The catch of the smaller individuals was concentrated in the 300 to 400 m depth layer and larger individuals were caught deeper in the "Warm Deep Water;" this suggests that there is ontogenetic descent. There is also some evidence of diel vertical migration by *G. glacialis:* the modal peak of the population in the nighttime-dawn samples was 100 m higher in the water column than in the daylight-dusk samples. The size range of *M. hamiltoni* was small and the catch was concentrated in the 300 to 400 m depth layer, beneath the discontinuity marked by the interface of the Antarctic Surface Water and the "Warm Deep Water"

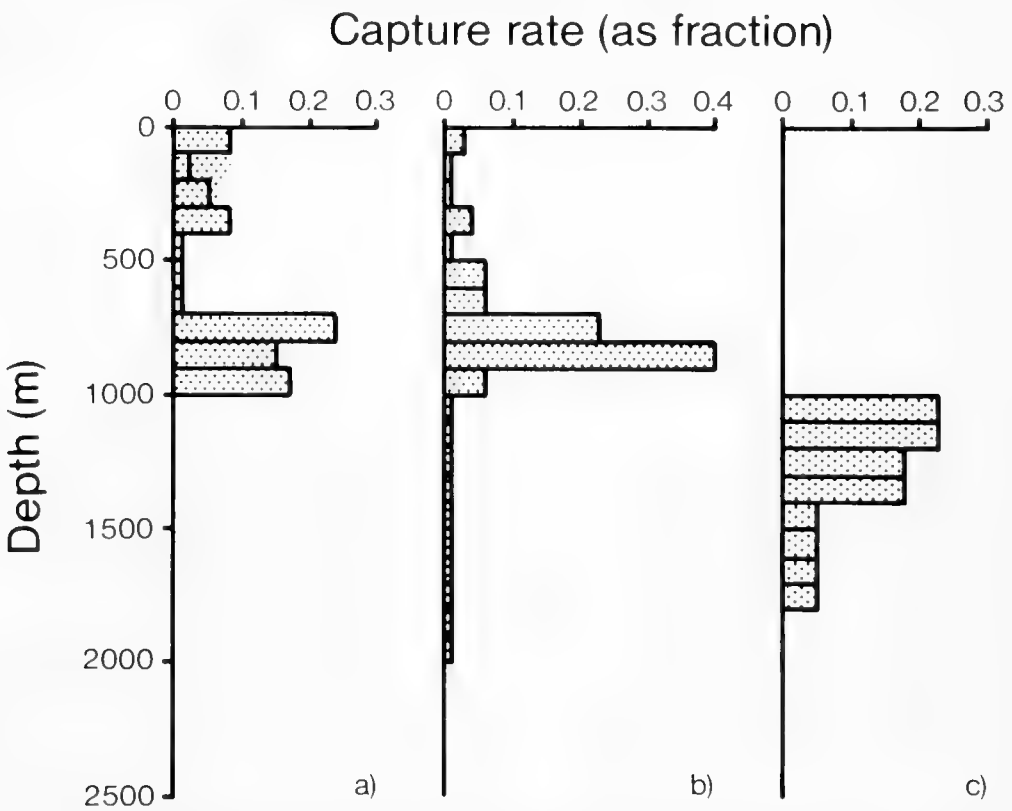

FIG. 4. *Alluroteuthis antarcticus*. Capture rate at each 100 m depth layer as a fraction of the total for each size class. a) 11–15 mm ML; b) 16–20 mm ML; c) 35–40 mm ML.

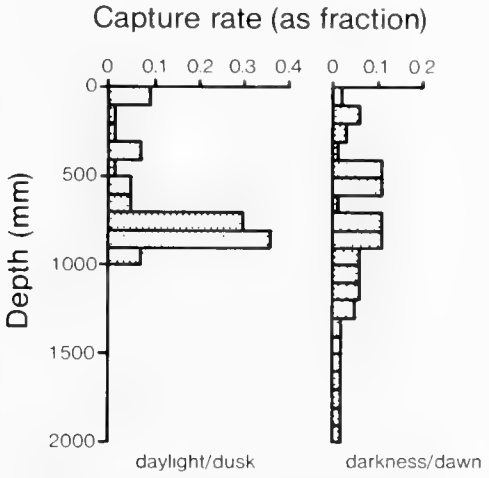

FIG. 5. *Alluroteuthis antarcticus*. Capture rate at each 100 m depth layer, as a fraction of the total, for daylight-dusk hauls and darkness-dawn hauls.

(Deacon, 1937). On the basis of open net data, McSweeny (1971) concluded that the vertical range of *G. glacialis*, in a different sector of the Southern Ocean, is 200 m to 1500 m, that ontogenetic descent exists and vertical migrations occur.

The data presented in Fig. 6 suggest spacial segregation in the water column between *Alluroteuthis antarcticus* and *Galiteuthis glacialis*, but in the absence of information on the trophic ecology of Antarctic squids it is not possible to assess the degree of ecological separation between the two species. The allometric relations between arm length and body length are quite different in *A. antarcticus* and *G. glacialis* (Fig. 7). The arm lengths of *A. antarcticus* are much greater in relation to ML than in *G. glacialis*, so that if their vertical distributions are plotted as a function of arm span, a measure of the functional size of these predators, the differences

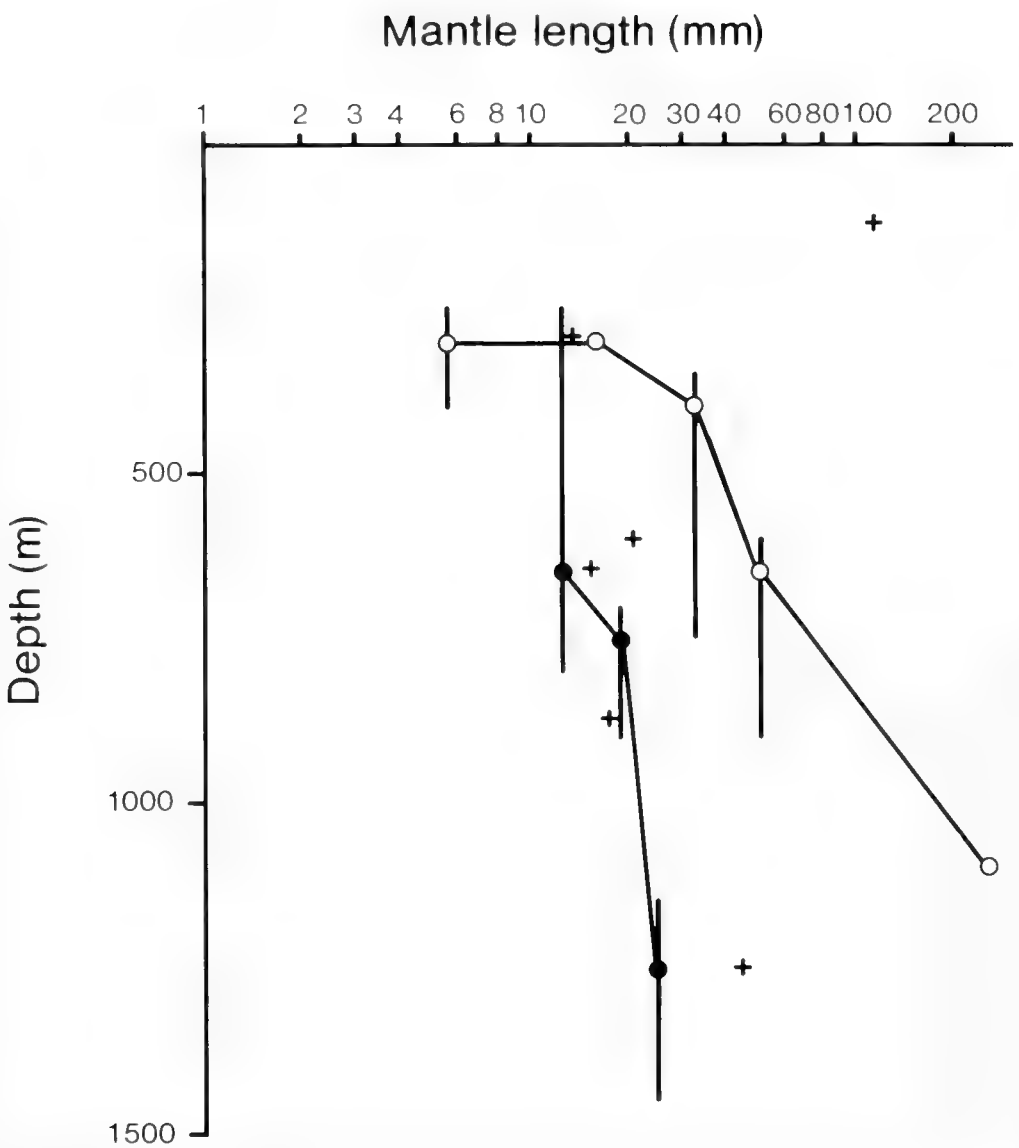

FIG. 6. Vertical distribution of *Alluroteuthis antarcticus* (●) and *Galiteuthis glacialis* (○) as a function of mantle length (ML). Vertical lines: interquartile range. Individual specimens of *A. antarcticus* from earlier "Discovery" expeditions are indicated by crosses.

between their distributions in the water column narrows (Rodhouse, unpublished data). Analysis of the relationship between niche width and feeding structures and the influence of water depth on the spectral composition of the prey community may provide a functional explanation for vertical distribution patterns and ontogenetic descent of oceanic cephalopods.

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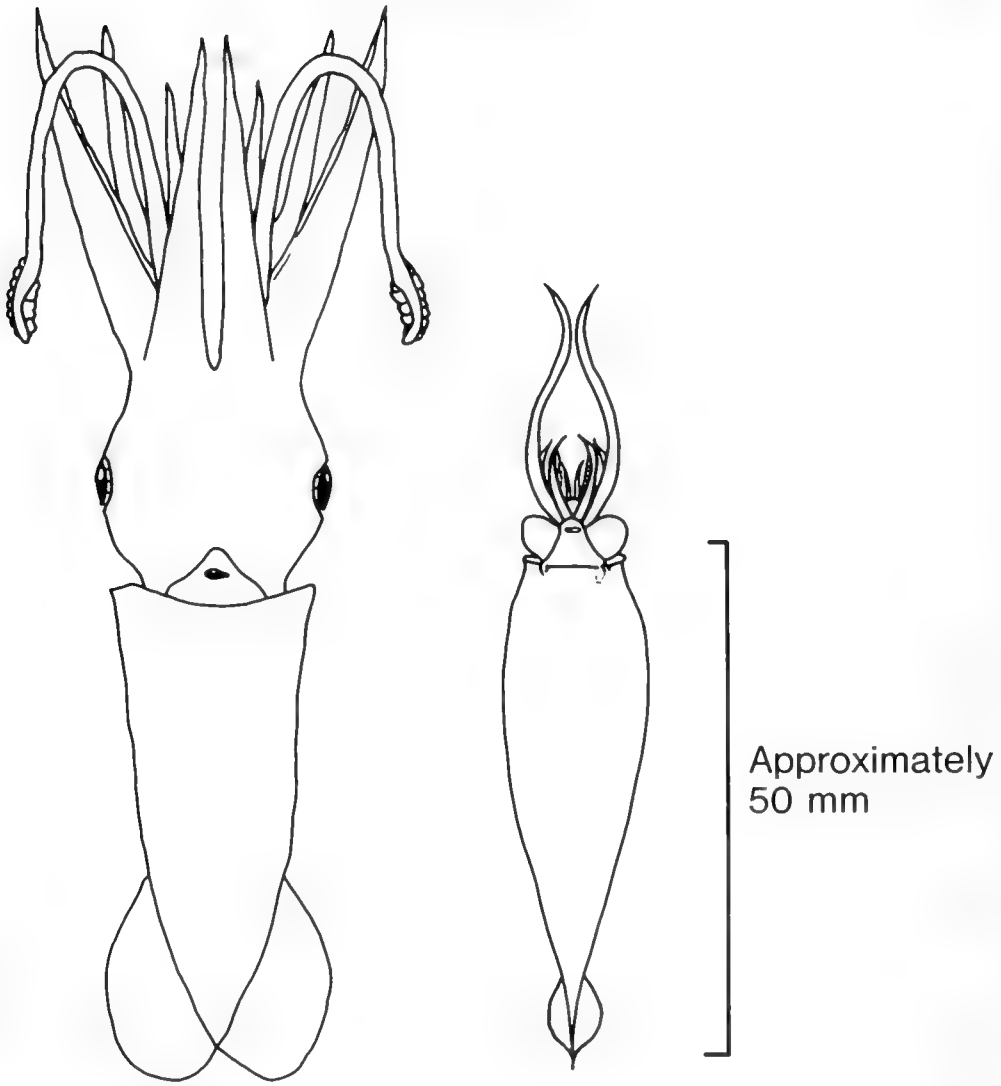

FIG. 7. *Alluroteuthis antarcticus* (left) and *Galiteuthis glacialis* (right) showing relative arm lengths.

working on detached duty at the Plymouth Laboratory.

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REDESCRIPTION OF *OCTOPUS PALLIDUS* (CEPHALOPODA: OCTOPODIDAE) FROM SOUTH-EASTERN AUSTRALIA

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ABSTRACT

Octopus pallidus Hoyle, 1885 from south-eastern Australia is redescribed and illustrated. The species was originally described from specimens collected during the cruise of the H.M.S. "Challenger" (1873–1876) at East Moncoeur Island, off Victoria, and Twofold Bay, New South Wales. *O. pallidus* is now known to be distributed from southern New South Wales to the Great Australian Bight.

O. pallidus is readily distinguished from other species of *Octopus* by a number of characters that include: short arms, a broadly ovoid mantle, a distinctive pattern of closely set tubercles and prominent papillae on the dorsum, enlarged suckers on all arms of mature males, a medium-sized ligula (8–16% of third right arm length), large eggs (11–13 mm long), and 7–9 gill lamellae.

O. pallidus is an inshore species, living on sand and among sponges and ascidians at depths from 7 to 275 m. The animal is medium-sized; males are mature at approximately 50 mm mantle length, and females attain ovarian maturity at a mantle length of about 60 mm.

Key words: cephalopod; Octopodidae; *Octopus;* systematics; morphology.

INTRODUCTION

The status of *Octopus pallidus* has been confused systematically since Hoyle's (1885a) original diagnosis. This south-eastern Australian taxon has been described under various names, including *O. boscii* Lesueur, 1821 (Brazier, 1892; Pritchard & Gatliff, 1898), *O. variolatus* Blainville, 1826 (Berry, 1918) and *O. boscii* var. *pallida* Hoyle, 1885. To revise and supplement the work of Hoyle (1886), Berry (1918) and Robson (1929), a comprehensive re-evaluation of *O. pallidus* was undertaken, based upon the examination of new material from south-eastern Australia. It should be noted that the validity of records based upon descriptions of specimens from outside Australia (see Joubin, 1897; Hoyle, 1904; and Massy, 1916a,b) is not determined in this study. The counts, measurements and indices listed in Tables 2–5 are as defined by Roper & Voss (1983). Other abbreviations used are: BMNH—British Museum (Natural History), and MV— Museum of Victoria. This paper is the first in a monographic revision of the genus *Octopus* in south-eastern Australian waters.

HISTORICAL RESUMÉ OF *OCTOPUS PALLIDUS*

Hoyle (1885a) described *Octopus boscii* var. *pallida* based upon specimens collected in south-eastern Australia during the cruise of the H.M.S. "Challenger" (1873–1876). Additional details appeared in a subsequent description (Hoyle, 1885b), which was later expanded with the inclusion of measurements and figures (Hoyle, 1886). The sexes of the specimens and locality details differ in all three papers. Hoyle (1886) listed the correct information. The described material included one male from off East Moncoeur Island, Victoria, and one female and a juvenile from off Twofold Bay, New South Wales. Hoyle (1886) included measurements and figures of the large female specimen (total length of 325 mm), and gave the hectocotylised arm measurements from the male specimen (total length of 160 mm). There were no details of the juvenile. Hoyle (1886) maintained that, rather than erect a new species, he would refer the "Challenger" specimens to *O. boscii* var. *pallida,* comparing it with a specimen of *O. boscii* in the British Museum that had been identified by J. E. Gray. Hoyle was uncertain whether Gray had based his identification

TABLE 1. Material examined: *Octopus pallidus*.

Sex	ML (mm)	MV reg.	Location	Date	Depth (m)
1♀	14.3	F52095	Western Port Bay, Vic. [39°S, 145°E]	—	—
1♀	25.3	F52094	38°00′S, 148°05′E	14.II .1971	44
1♀	26.4	F31559	38°54′S, 147°07′E	18.XI .1981	58
1♀	31.2	F52093	Port Lincoln, S.A. [35°S, 136°E]	—	—
1♂	43.0	F30855	40°56′S, 146°06′E	4.II .1981	64–68
1♂	54.1	F52108	43°39′S, 147°49′E	16.II .1976	160
1♂,1♀	54.5–60.8	F52503	38°02′S, 145°05′E	10.X .1984	—
1♂	54.7	F30872	40°27′S, 147°25′E	6.II .1981	55
1♂	56.9	F24453	38°05′S, 145°05′E	14.XII.1958	15
1♂	60.8	F24499	Port Melbourne, Vic. [38°S, 145°E]	29.V .1925	—
1♂	64.8	F52497	Bruny Is., Tas. [43°S, 148°E]	II .1972	12
1♀	66.2	F52090	32°24′S, 133°24′E	26.X .1973	40
2♂,2♀	69.4–89.7	F52087	39°46′S, 145°34′E	3.II .1981	79
2♀	69.5–70.7	F30871	40°33′S, 145°45′E	4.II .1981	68
1♀	72.5	F24451	Mentone, Vic. [38°S, 145°E]	—	—
1♀	73.5	F52502	38°02′S, 145°05′E	29.VII.1985	—
1♀	80.8	F52505	Wilson's Promontory, Vic. [39°S, 146°E]	5.II .1982	—
2♂,1♀	89.4–99.8	F52506	Wilson's Promontory, Vic. [39°S, 146°E]	IX .1984	–
1♀	98.0	F52504	Bass Strait [40°S, 146°E]	—	—
1♂	98.1	F52499	39°08′S, 143°25′E	31.I .1981	55–84
1♂	105.0	F52500	38°02′S, 145°05′E	12.VII.1984	—
1♂	117.4	F52507	38°02′S, 149°12′E	16.IX .1984	118
1♂	147.3	F52501	Stanley, Tas. [40°S, 145°E]	V .1980	37

upon the description of *boscii* by Lesueur (1821) or Férussac & d'Orbigny (1835–1848), but decided that "it appeared better to accept Gray's opinion and to give a new description of the old species."

Both Brazier (1892) and Pritchard & Gatliff (1898) listed *O. boscii* var. *pallida* Hoyle in synonymy with *O. boscii* Lesueur. Brazier's (1892) listing was based upon Gray (1849), and used existing locality records; Pritchard & Gatliff (1898) added a new record (Port Phillip Heads, Victoria), but no further information was included.

Berry (1918) revised the species' nomenclature and described specimens collected in the waters off Victoria, Tasmania and the south-east of Western Australia by the F.I.S. "Endeavour" (1909–1914). He pointed out that *O. boscii* Lesueur as an indeterminate species could not be accepted, and concluded that if any of the older names were valid, *variolatus* had priority. He included *O. boscii* var. *pallida* in that synonymy. Berry (1918) provided descriptions, measurements and figures of the "Endeavour" specimens, noting particularly the animals' integumental sculpture.

Robson (1929) raised *pallida* to specific rank. He concurred with Berry's (1918) dismissal of *boscii* Lesueur. Robson, however, disagreed with Berry's use of *variolatus* Blainville, explaining that the type specimen of *variolatus* was lost, and that Blainville's type description was ambiguous. Robson (1929)

TABLE 2. Measurements (mm) and indices of 10 female *Octopus pallidus*.

	F52095	F52094	F31559	F52093	F52090	F30871	F30871	F24451	F52087	F52087
ML	14.3	25.3	26.4	31.2	66.2	69.5	70.7	72.5	73.7	86.4
TL	37.0	60.1	76.3	103.2	203.4	207.0	219.9	222.3	235.5	308.3
MWI	81.8	73.1	86.4	85.9	74.9	74.0	72.8	81.4	75.3	85.2
HWI	88.8	71.1	72.0	72.4	55.4	52.5	51.5	48.0	54.3	51.2
MAI	61.1	63.2	49.8	43.3	47.0	50.0	46.8	45.3	45.5	38.1
ALI	L R	L R	L R	L R	L R	L R	L R	L R	L R	L R
I	153.1 143.4	134.4 138.3	166.7 166.7	208.3 198.7	185.8 187.3	174.1 178.4	200.8 196.6	190.3 180.7	203.5 202.2	204.8 180.6
II	155.2 153.8	142.3 154.2	189.4 170.5	214.7 214.7	196.4 202.4	200.0 194.2	213.6 202.3	201.4 200.0	213 0 214.4	246.5 237.3
III	163.6 155.2	154.2 156.1	200.8 162.9	221.2 230.8	197.9 213.0	198.6 179.9	210.7 203.7	209 7 197 2	218.5 217.1	262.7 238.4
IV	162.2 156.6	158.1 158.1	193.2 193.2	222.8 227.6	203.9 208.5	192.8 200.0	186.7 200.8	220.7 213.8	219.8 218.5	247.7 258.1
AWI	11.9	8.7	9.5	7.7	8.5	8.9	9.2	9.1	7.6	10.0
ASIn	5.6–7.0	6.3–7.1	7.2–7.6	6.7–7.4	5.7–6.2	5.8–6.9	5.8–6.5	6.9–7.9	6.0–6.9	6.0–7.6
WDI	35.0	41.3	32.6	29.9	27.0	33.1	29.8	33.1	27.8	24.2
WF	DCBEA	DCBEA	DCEBA	DCBEA	D CEBA	DCBEA	CBDAE	DCBEA	DCBEA	DCBEA
GiLC	7 7	8 8	7 7	8 8	8 8	8 8	8 8	8 8	8 8	8 9
EgLI	—	—	—	—	4.2–5.4*	—	3.4–3.5*	16.4	4.3–5.8*	12.7
EgWI	—	—	—	—	0.9–1.1*	—	0.6*	3.4–3.9	0.7–0.9*	2.9–3.2
FuLI	41.3	38.7	36.4	40.1	39.4	33.1	36.5	31.4	36.6	38.2
FFuI	22.4	22.5	25.4	19.6	24.6	19.0	20.4	14.3	24.4	24.5
PAI	83.9	79.1	94.7	89.7	80.1	79.1	87.7	80.0	76.0	92.6

*Immature eggs.

TABLE 3. Measurements (mm) and indices of 10 male *Octopus pallidus*.

	F30855	F52108	F30872	F24453	F24499	F52497	F52087	F52087	F52499	F52500
ML	43.0	54.1	54.7	56.9	60.8	64.8	69.4	89.7	98.1	105.0
TL	132.2	182.3	184.9	158.8	222.1	225.9	204.9	302.2	364.1	353.6
MWI	73.7	81.7	78.1	67.0	91.0	76.4	79.8	78.0	70.8	63.2
HWI	59.3	58.0	59.4	50.4	56.3	52.9	57.6	48.7	47.2	44.2
MAI	47.8	43.3	46.1	49.5	38.2	41.3	46.0	42.9	38.7	44.9
ALI	L R	L R	L R	L R	L R	L R	L R	L R	L R	L R
I	186.0 181.4	199.6 199.6	205.4 200.7	165.2 170.5	241.8 236.8	214.5 220.7	194.5 190.2	204.0 212.9	235.0 234.6	200.0 197.1
II	202.3 193.0	203.3 201.5	207.5 216.1	184.5 181.0	248.4 258.2	228.4 231.5	203.2 191.6	211.8 216.3	245.6 258.1	205.7 211.4
III	207.0 172.1	221.8 184.8	216.6 193.2	202.1 159.9	258.2 218.8	223.0 194.4	197.4 155.6	229.7 190.6	256.1 201.6	222.9 173.3
IV	209.3 197.7	229.2 231.1	217.7 221.6	196.8 189.8	261.5 241.8	242.3 236.1	208.9 217.6	233.0 230.8	242.3 248.4	214.3 212.4
AWI	9.3	8.8	11.7	9.7	10.9	8.6	11.7	10.0	8.5	10.1
ASIn	5.6–6.0	6.7–6.8	7.3–9.0	5.8–7.2	8.9–11.0	9.1–10.0	7.5–9.1	8.8–10.1	10.2–11.7	11.5–13.5
WDI	34.4	28.0	36.3	33.0	23.9	27.7	27.2	25.4	24.1	29.9
WF	DCB = EA	DCBEA	DCEBA	DCEBA	DCBEA	DCBEA	DCBEA	DCBEA	DCBEA	DCEBA
GiLC	8 8	8 8	8 8	7 7	8 8	8 8	8 8	9 9	8 8	8 8
HcAI	172.1	184.8	193.2	159.9	218.8	194.4	155.6	190.6	201.6	173.3
OAI	83.1	83.3	89.2	79.1	84.7	83.4	78.8	83.0	78.7	77.8
LLI	4.6	6.8	4.9	7.5	15.6	10.3	8.6	9.6	8.7	12.4
CaLI	41.2	52.9	59.6	55.9	34.3	42.2	54.8	35.1	39.0	32.3
PLI	12.6	16.3	22.9	14.1	22.7	26.5	14.8	22.5	21.9	18.2
SpLI	—	—	—	—	91.3–98.4	98.0–118.2	42.8*	82.2–87.1	87.0–91.2	62.6–71.0
SpWI	—	—	—	—	2.7–2.8	2.7–2.8	3.3	2.6–2.8	2.1–2.2	2.8–3.0
SpRI	—	—	—	—	44.0–47.7	34.5–41.9	51.2	38.5–45.7	38.8–52.3	39.6–40.9
FuLI	36.0	41.0	36.6	33.4	51.5	44.4	35.0	39.0	41.3	40.9
FFuI	27.9	23.1	10.8	19.3	30.1	21.1	19.0	26.4	30.3	23.2
PAI	90.7	101.7	91.4	77.3	102.0	71.0	79.3	98.1	77.7	73.3

*Only one ill-formed spermatophore in Needham's sac; animal apparently just maturing

TABLE 4 Combined ranges, means and standard deviations of indices of 10 male and 10 female *Octopus pallidus*.

Index	Range and mean	S.D.(n−1)
MWI	67.0– 77.5– 91.0	6.8
HWI	44.2– 57.6– 88.8	10.9
MAI	38.1– 46.4– 63.2	6.5
ALI I	134.4–192.1–241.8	25.3
II	142.3–205.4–258.2	27.1
III	154.2–201.8–262.7	28.6
IV	156.6–213.1–261.5	26.3
AWI	7.6– 9.5– 11.9	1.3
ASIn	5.6– 7.7– 13.5	1.9
WDI	23.9– 30.2– 41.3	4.6
HcAI	155.6–184.4–218.8	19.4
OAI	78.7– 82.1– 89.2	3.5
LLI	8.6– 10.9– 15.6	2.7
CaLI	32.3– 39.6– 54.8	8.2
PLI	12.6– 19.3– 26.5	4.7
SpLI	42.8– 84.5–118.2	20.1
SpWI	2.1– 2.7– 3.3	0.2
SpRI	34.5– 43.2– 52.3	5.6
EgLI	12.7– 14.5– 16.4	2.6
EgWI	2.9– 3.4– 3.9	0.4
FuLI	31.4– 38.5– 51.5	4.4
FFuI	10.8– 22.4– 30.3	4.8
PAI	71.0– 85.3–102.0	9.4

TABLE 5. Ranges, means and standard deviations of selected characters showing sexual dimorphism in female and male (60–90 mm ML) *Octopus pallidus*.

	Females (n = 6)		Males (n = 4)	
Character	Range and mean	S.D. (n−1)	Range and mean	S.D. (n−1)
ML	66.2– 73.2– 86.4		60.8– 71.2– 89.7	
ALI III L	197.9–216.4–262.7	24.0	197.4–227.1–258.2	25.0
III R	179.9–208.2–238.4	19.8	155.6–189.9–218.8	26.0
ASIn	5.7– 6.5– 7.9	0.7	7.5– 9.3– 11.0	1.1

based his description of *O. pallida* upon a re-examination of Hoyle's type material, and on information provided by Berry (1918).

Cotton (1932), Cotton & Godfrey (1940), Macpherson & Gabriel (1962) and Macpherson (1966) have since briefly described *O. pallidus* from specimens collected in south-eastern Australian waters. Planktonic specimens, attributed to *O. pallidus* by Allan (1945), were collected from south-eastern and eastern Australian waters as far north as Bundaberg, Queensland. As that account apparently indicates a tropical as well as temperate distribution for the species, the identification should be considered with caution. On the basis of large eggs attributed to this species, the identification by Allan (1945) cannot be accepted since the juveniles are demersal not planktonic.

Octopus pallidus Hoyle, 1885

Octopus boscii var. *pallida* Hoyle, 1885a: 223; 1885b: 97; 1886: 81, pls. 1, 3, fig. 2.

Octopus boscii, Brazier (not Lesueur, 1821),

TABLE 6. Comparison of selected characters of *Octopus pallidus* and *O. tetricus*.

	O. pallidus Hoyle	*O. tetricus* Gould
Size	medium (up to 150 mm ML, 350 mm TL, and to 800 g in weight)	large (up to 160 mm ML, 800 mm TL, and to 3000 g in weight)[1]
Arm length	60–70% of TL	80–90% of TL[1]
Arm formula	IV.III.II.I	III.II.IV.I[2]
Web depth	30% of arm length	20–25% of arm length[1]
Funnel organ	V V shaped	W shaped[2]
Gill count	7–9 lamellae on outer demibranch	9–10 lamellae on outer demibranch[1]
Ligula	medium sized, well developed (LLI 8–16%)	very small, poorly developed (LLI 1.5%)[1]
Eggs	large (11–13 mm long), attached singly to substrate	small (2.4 mm long), attached in egg strings to substrate[3]

[1]Roper *et al.* (1984).
[2]Robson (1929).
[3]Joll (1983)

1892: 3 *(partim);* Pritchard & Gatliff, 1898: 241

Polypus variolatus, Berry (not Blainville, 1826), 1918: 278, pls. 79–81, figs. 2, 3, pl. 82, figs. 1–4.

Octopus pallida, Robson, 1929: 126, text fig. 38 *(partim);* Cotton, 1932: 545; Cotton & Godfrey, 1940: 449, text figs. 432–435 *(partim);* Macpherson & Gabriel, 1962: 415, text fig. 484 *(partim);* Macpherson, 1966: 241.

MATERIAL EXAMINED

See Table 1.

DESCRIPTION

Medium-sized animals with firm consistency (Fig. 1a). Mantle saccular, broadly ovoid (MWI 67.0–*77.5*–91.0); mantle wall thick, muscular. Head wide, but narrower than mantle (HWI 44.2–*57.6*–88.8); demarked from mantle by moderate constriction. Eyes large, but not projecting far above surface of head. Funnel large, stout, bluntly tapered (FuLI 31.4–*38.5*–51.5); free for about half its length (Fig. 1b; FFuI 10.8–*22.4*–30.3). Funnel organ consisting of two closely opposed V-shaped units (Fig. 1c); limbs thick. Mantle aperture wide (PAI 71.0–*85.3*–102.0).

Brachial crown very strong, well developed. Arms short (MAI 38.1–*46.4*–63.2), stout (AWI 7.6–*9.5*–11.9), tapering to fine tips. Arm lengths subequal; arm order usually IV.III.II.I. Suckers biserial, with obvious radial grooves; moderately sized (ASIn females 5.7–*6.5*–7.9, males 7.5–*9.3*–11.0); 10th to 13th suckers usually largest, enlarged on all arms of mature males only.

Web formula usually DCBEA; dorsal and ventral sectors always shallower. Webs shallow (WDI 23.9–*30.2*–41.3); web remnants extend up ventral side of arms for approximately 3/4 of their length. Third right arm of males hectocotylised (Figs. 1d, e); shorter than its opposite number (OAI 78.7–*82.1*–89.2; HcAI 155.6–*184.4*–218.8). Spermatophoral groove well developed, with conspicuous thickening of web membrane. Ligula 8–16% of third right arm length in mature animals; usually recurved orally (LLI 8.6–*10.9*–15.6). Ligula groove long, well marked and deep, with incomplete transverse ridges. Calamus short, acutely pointed (CaLI 32.3–*39.6*–54.8).

Gills possess 7–9 lamellae on outer demibranch, plus the terminal lamella.

Digestive tract typical of the genus (Fig. 2a). Upper beak has short, blunt, curved rostrum; curved crest; large wings; large lateral walls, with posterior margin deeply indented (Fig. 2b). Lower beak has short, blunt rostrum, and short hood; wings have tear shaped darkened areas, lightening towards margins (Fig. 2c). Rostrum, hood, crest and lateral walls, of both upper and lower beaks

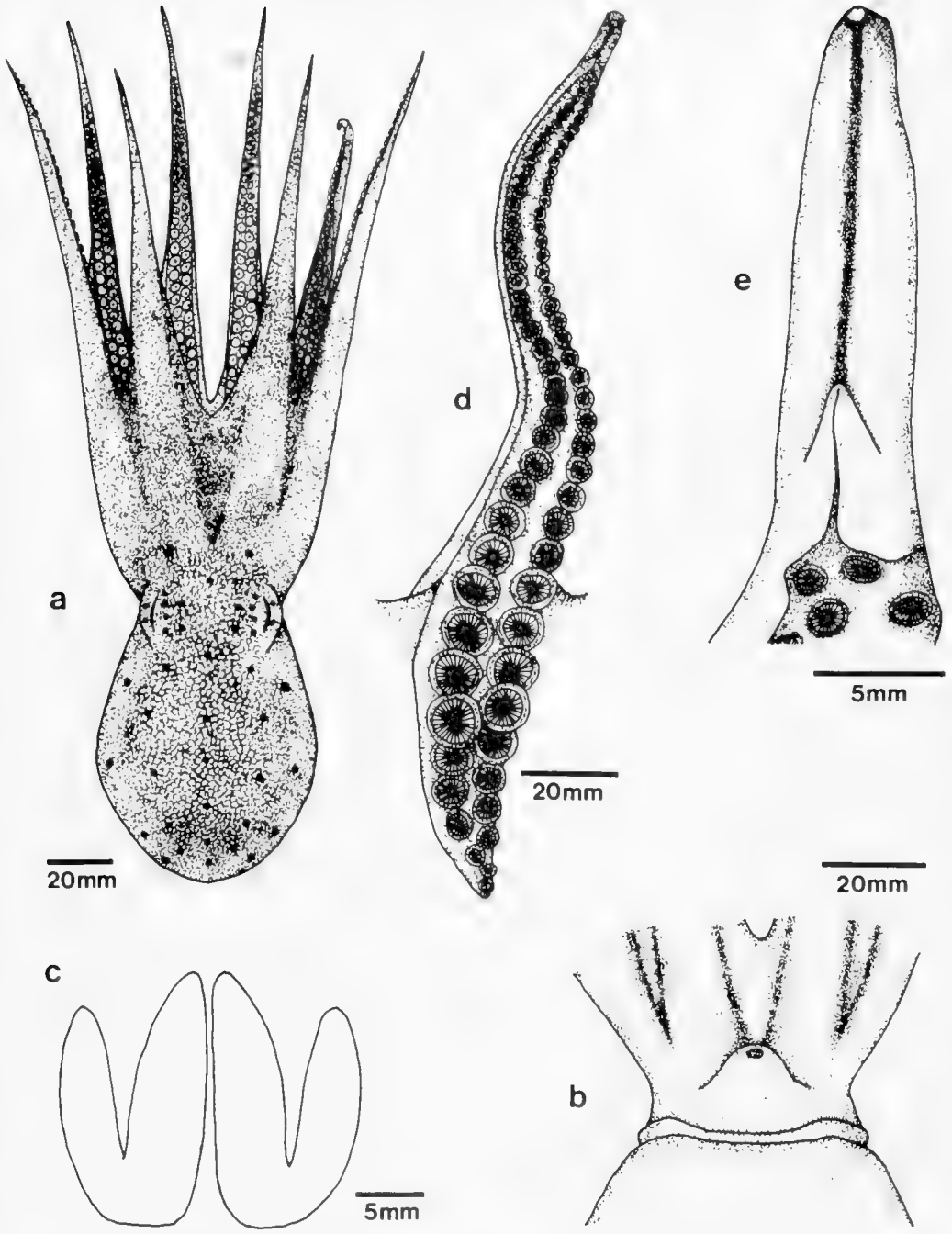

FIG. 1. *Octopus pallidus* Hoyle: **a,** dorsal view of MV F52087, ♂, 89.7 mm ML; **b,** ventral view of mantle opening and funnel, and **c,** funnel organ, of MVF 52505, ♀, 80.8 mm ML; **d,** hectocotylised arm of MVF 52499, 98.1 mm ML; **e,** detail of hectocotylus of MV F52500, 105.0 mm ML.

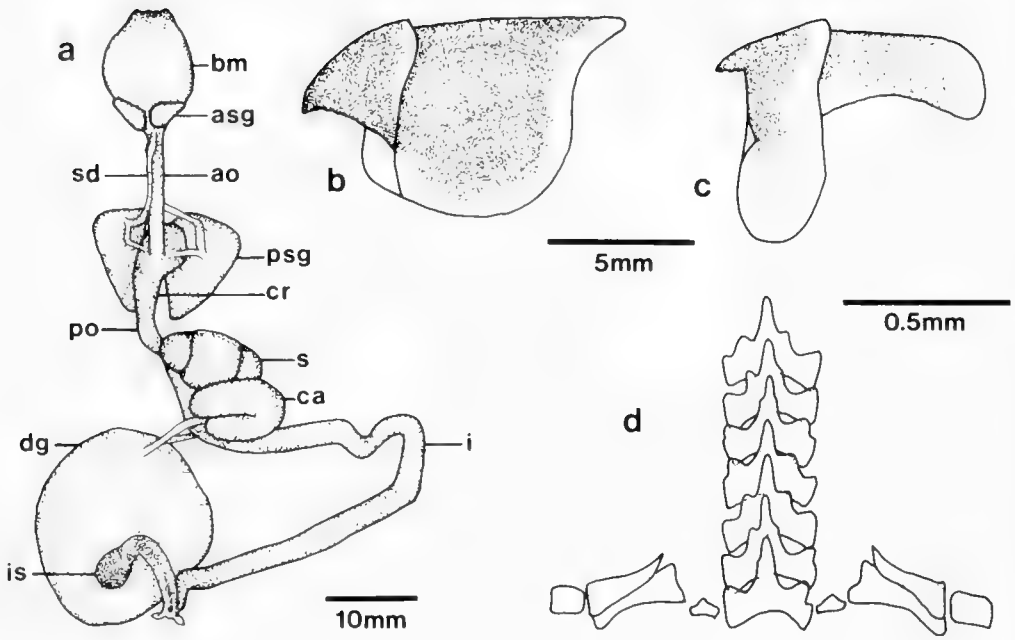

FIG. 2. *Octopus pallidus* Hoyle: **a,** digestive tract of MV F52503, ♂, 54.5 mm ML (*ao*—anterior oesophagus, *asg*—anterior salivary gland, *bm*—buccal mass, *ca*—caecum, *cr*—crop, *dg*—digestive gland, *i*—intestine, *is*—ink sac, *po*—posterior oesophagus, *psg*—posterior salivary gland, *s*—stomach, *sd*—salivary duct); **b,** upper beak, and **c,** lower beak, of MV F52503, ♀, 60.8 mm ML; **d,** radula of MV F52504, ♀, 98 .0 mm ML.

heavily pigmented, dark brown to black; margins of wing, hood, crest and lateral walls of both beaks transparent. Radula typically octopodan (Fig. 2d), with seven transverse rows of teeth. Rhachidian tooth has an asymmetrical seriation of B_{4-5} type, and is slender, with 1–2 small lateral cusps on either side. First lateral teeth small and unicuspidate; second lateral teeth large with long curved base; third lateral teeth long and slightly curved; marginal plates oblong and plain.

Anterior salivary glands small, bordering posterior buccal mass. Posterior salivary glands stout anteriorly, tapering posteriorly, with one salivary duct from each gland running forward independently, then uniting (at a point halfway along the anterior oesophagus) to form single duct running alongside the oesophagus. Duct enters buccal mass dorsal to oesophagus. A second shorter duct runs from each posterior salivary gland to crop. Crop has anterior caecum of about 20% of its length. Posterior oesophagus short. Stomach typically bipartite. Caecum has single loose coil. Two separate ducts connect digestive gland (near the midline) with stomach and caecum. Intestine undifferentiated, although two coils occur midway, but these are not enlarged to form pouches. Ink sac large, lying superficially in groove on ventral face of digestive gland. A short, stout duct connects ink sac with dorsal side of intestine near anus. Anus bears a pair of anal flaps.

Testis posterior in position. Vas deferens long, delicate, tightly coiled, entering spermatophoral gland at proximal end. Spermatophoral gland swollen proximally, with muscular walls, but becoming thin walled towards its junction with the long accessory gland. A short tube connects accessory gland and Needham's sac. Needham's sac long, conical, pointed at apex. There is some variation in the shape of the penis, but generally the organ is long (PLI 12.6–*19.3*–26.5), with a single coiled diverticulum. Genital aperture subterminal, on right side of penis (Figs. 3a, b).

Spermatophores relatively long (SpLI 42.8–*84.5*–118.2) and slender (SpWI 2.1–*2.7*–3.3) (Figs. 3c–f). Oral cap simple, not markedly expanded, with a long cap thread. Ejaculatory apparatus is a tightly coiled tube, which narrows orally, with one coil close to the oral end. Thick, bulbous cement body connects with both oral and aboral ends by narrow necks. Sperm reservoir spirally wound

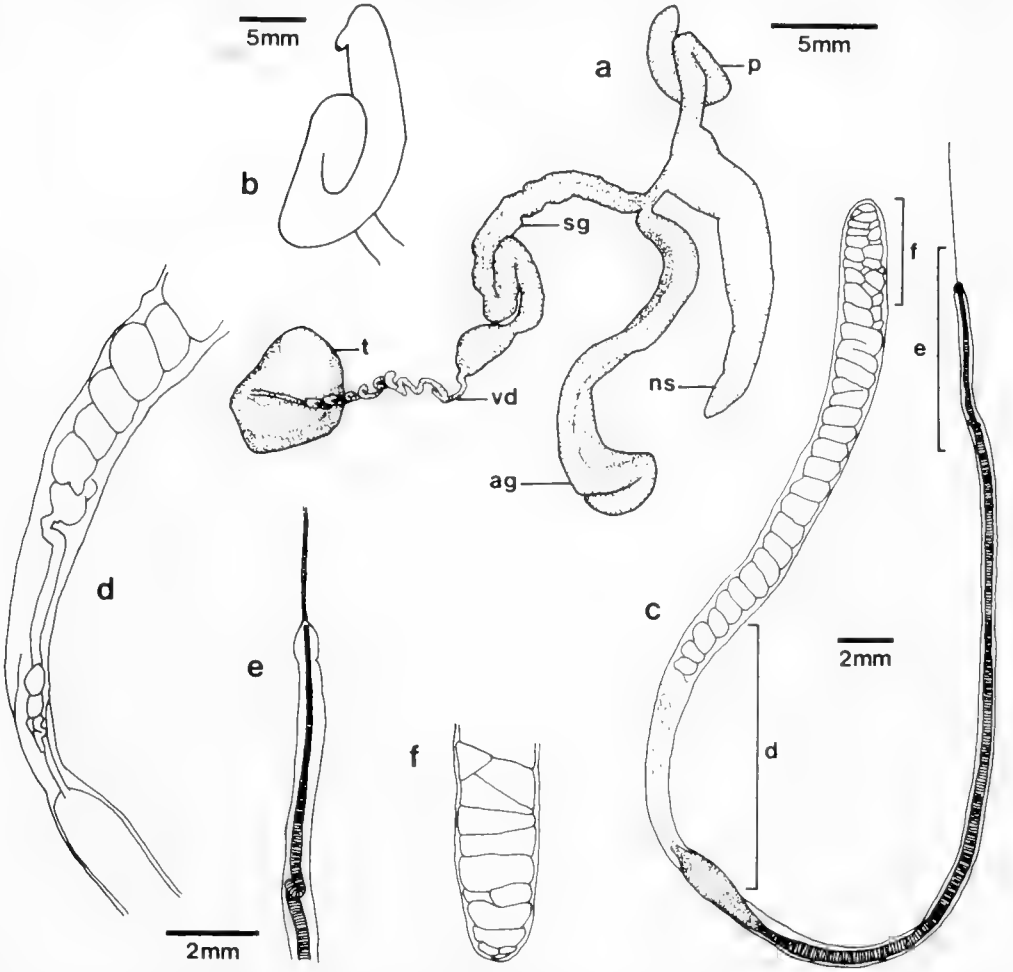

FIG. 3. *Octopus pallidus* Hoyle: **a,** male reproductive organs of MV F52506, 89.4 mm ML (*ag*—accessory gland, *ns*—Needham's sac, *p*—penis, *sg*—spermatophoral gland, *t*—testis, *vd*—vas deferens); **b,** penis of MV F52506, 94.7 mm ML; **c–f,** spermatophore from MV F52507, 117.4 mm ML; **c,** whole spermatophore; **d,** spermatophore midsection, cement body to sperm reservoir; **e,** oral cap and cap thread; **f,** aboral end of sperm reservoir.

with a rounded aboral end; comprises approximately half of the spermatophore length (SpRI 34.5–*43.2*–52.3); forms widest region of spermatophore.

Ovary large, roundly triangular, displacing adjacent organs when mature (Fig. 4a). Proximal oviducts short, straight, attaching to spherical oviductal glands, which are darker in color. Distal oviducts sharply curved, tapering gradually. One female (MV F52502) was observed brooding eggs. Mature eggs large (11–13 mm long, 3–4 mm wide), white, translucent (Fig. 4b; EgLI 12.7–*14.5*–16.4; EgWI 2.9–*3.4*–3.9). Eggs attached singly to substrate by long, thin stalks (6–7 mm long). Egg striation absent.

Integumental sculpture consists of a pattern of coarse, uniformly shaped and closely set epidermal tubercles. These "rosette" shaped tubercles cover both dorsal and ventral surfaces (Fig. 4c). Tubercles reach the largest size on dorsum near base of arms; those on ventral surface are smaller and less prominent. Branched and unbranched papillae present on dorsum. Pattern of papillae on mantle dorsum includes approximately five

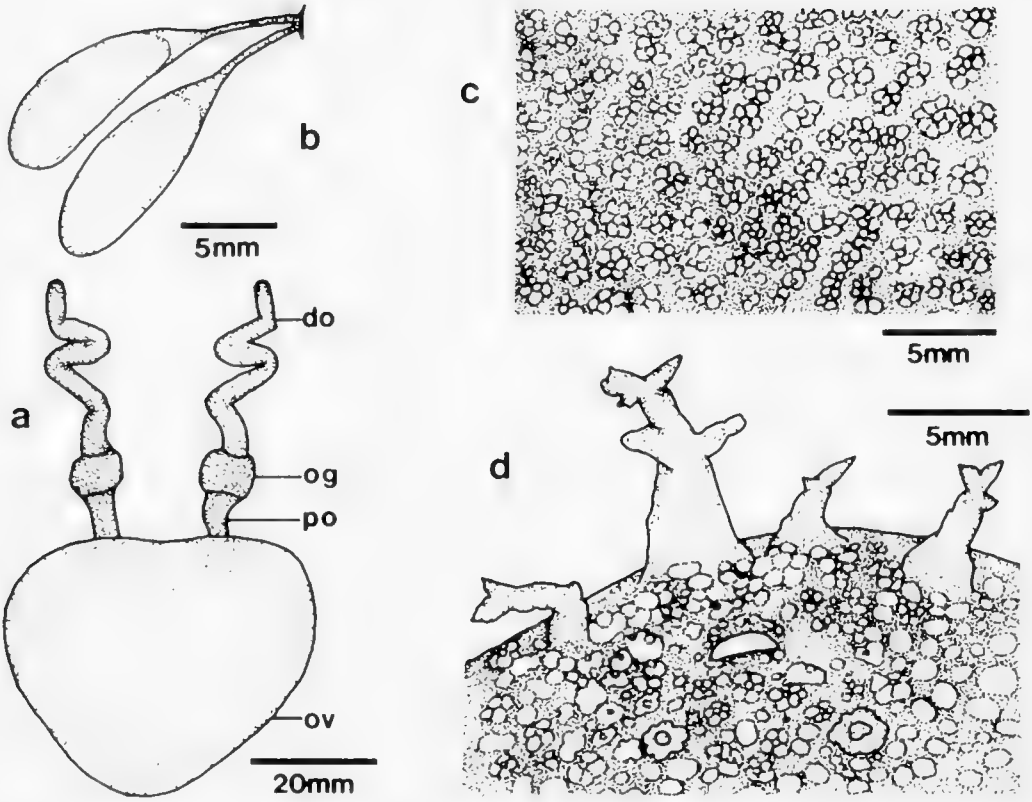

FIG. 4. *Octopus pallidus* Hoyle: **a,** female reproductive organs of MV F52506, 98.8 mm ML (*do*—distal oviduct, *og*— oviductal gland, *ov*—ovary, *po*—proximal oviduct); **b,** mature, laid eggs of MV F52502, 73.5 mm ML; **c,** rosette shaped tubercles on mantle dorsum, and **d,** lateral view of arborescent ocular papillae, of MV F52500, ♂, 105.0 mm ML.

sub-parallel rows of simple, usually unbranched papillae along the mantle length. Each row has 4–6 papillae. Larger arborescent papillae obvious in ocular region (Fig. 4d), with four supraocular and two subocular papillae. Three rows, of two papillae each, lie on the dorsal surface of the web and dorsal pair of arms. Lateral integumentary ridge or fold around mantle circumference absent.

In life, color of resting animals is brown and cream mottled dorsally, paler ventrally; when stimulated, animals become uniformly dark brown to purple. Preserved specimens in isopropyl alcohol reddish brown to orange dorsally, slightly paler ventrally. In both live and preserved specimens, a faint orange stripe is often present along length of dorsal arms. Surface of the raised tubercles usually darker than the background, giving a reticulate pattern. Ocelli absent.

Sexual dimorphism was observed in third right arm length, which is shorter in males, and in sucker diameter, showing enlargement in mature males (see Table 5). Males mature at approximately 50 mm mantle length. Females attain ovarian maturity at about 60 mm mantle length. The largest specimen studied was a male of 147 mm mantle length from off Stanley, Tasmania (MV F52501).

TYPES

Three syntypes extant, British Museum (Natural History):

i) BMNH 1889.4.24.19, 1 ♂ (39°10′30″S, 146°37′E, off East Moncoeur Island, Bass Strait, 70 m, sand and shell bottom);

ii) and iii) BMNH 1889.4.24.20–21, 1♀ and 1 juvenile (36°59′S, 150°20′E, off Twofold Bay, New South Wales, 275m, green mud bottom).

DISTRIBUTION

Octopus pallidus is distributed in the temperate waters of south-eastern Australia, from southern New South Wales to the Great Australian Bight, including Bass Strait and Tasmania (Fig. 5). It is an inshore species, living on sand bottoms, and among sponges and ascidians, at depths from 7 to 275 m.

DISCUSSION

The taxonomic confusion concerning *O. pallidus* has resulted from the variety of names used for the species. The source of the problem was Hoyle's (1885a) designation of the species as *O. boscii* var. *pallida*.

O. boscii Lesueur, 1821, as well as *O. variolatus* Blainville, 1826, were described from Péron's manuscript notes on a specimen from Dorre Island, Shark Bay, Western Australia (Robson, 1929). The descriptions were brief and lacked figures. The type specimen is apparently no longer extant (Robson, 1929). Hoyle (1886) recognised the uncertainty surrounding the name *boscii*. Consequently, he used a specimen, identified by Gray and attributed to *boscii*, when comparing the "Challenger" material. Hoyle (1886) thought the "Challenger" material sufficiently similar to Gray's *boscii* that he named them *O. boscii* var. *pallida*.

Robson (1929) subsequently reidentified Gray's specimen of *boscii* as *O. tetricus* Gould, 1852. So Hoyle (1886) had been attempting to compare a specimen of *O. tetricus* with the *O. pallidus* material from "Challenger." It appears that specimens previously identified as *O. boscii* are now referable to either *O. pallidus* or *O. tetricus*.

Hoyle (1886) and Robson (1929) have remarked upon the similar integumental sculpture of *O. pallidus* and *O. tetricus*, but Robson (1929) mentioned factors that obviously distinguish the two species. Robson (1929), Joll (1983) and Roper, Sweeney & Nauen (1984) have provided details of *O. tetricus* from subtropical south-western Australian waters. Apart from the superficial resemblance of the integumental sculpture, there appear to be few features in common between the two species (see Table 6).

Octopus pallidus is a distinctive species endemic to temperate waters of south-eastern Australia. It can be distinguished easily from other sympatric species of *Octopus* and other known species of the genus on the basis of a combination of characters: a broadly ovoid mantle, and stout arms (1-1/2–2 times mantle length), giving the animal a robust appearance; a characteristic pattern of epidermal tubercles, and enlarged papillae over each eye; enlarged suckers on all arms of mature males; a medium-sized ligula (8–16% of third right arm length); large eggs (11–13 mm long), attached singly to the substrate; and 7–9 gill lamellae.

Little is known of the biology of the species. Macpherson & Gabriel (1962) reported that "this species lives in deep water and has been taken in depths of up to 200 fathoms [366 m]. It is often trapped in crayfish pots which it is fond of raiding." Current information, though, indicates that this account was the result of incorrect identification (Winstanley, Potter & Caton, 1983).

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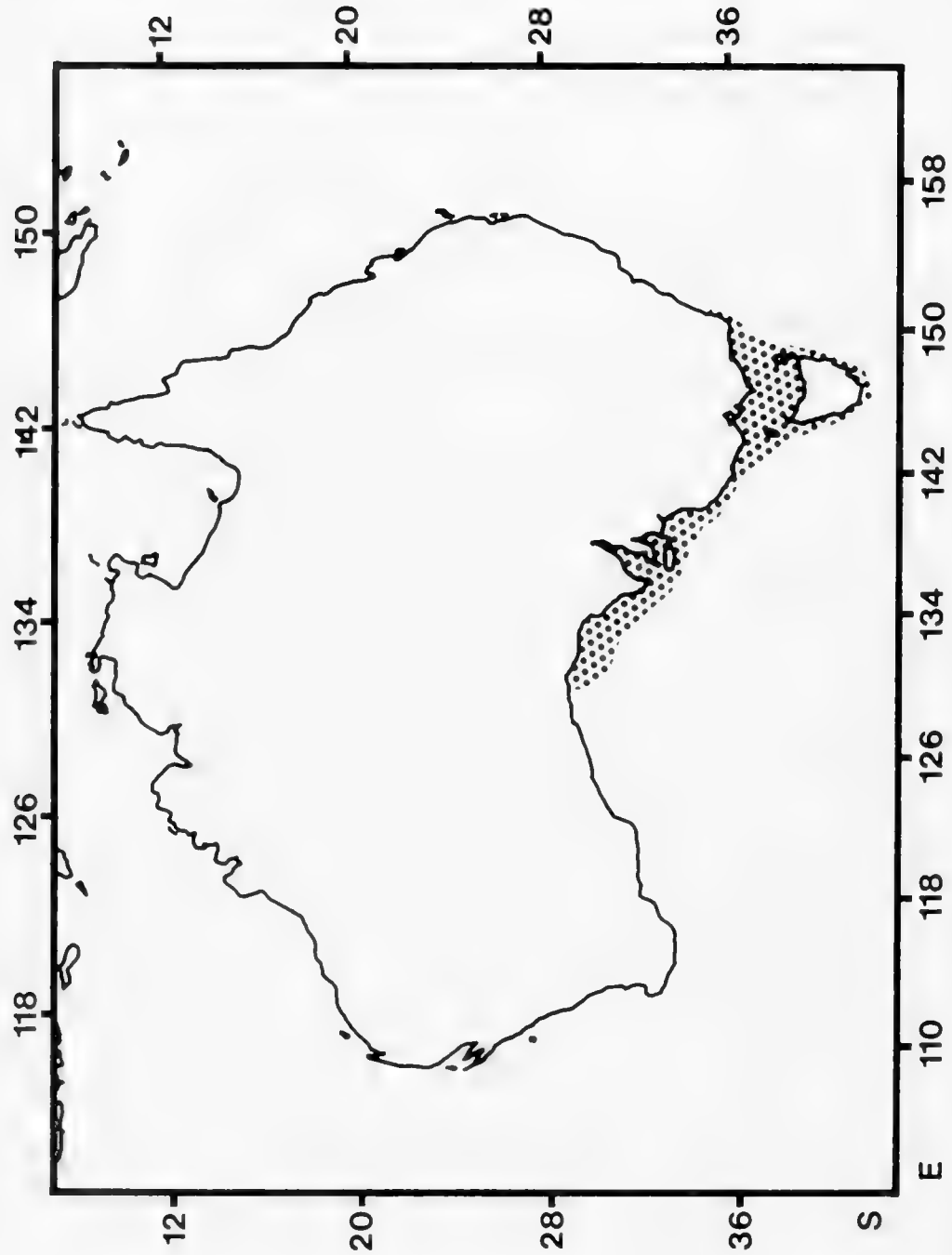

FIG. 5. Geographical distribution of *Octopus pallidus* around the south-eastern coast of Australia.

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TRANS-PANAMANIAN GEMINATE OCTOPODS (MOLLUSCA: OCTOPODA)

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ABSTRACT

The many similar marine species (geminate congeneric pairs) on either side of the Panamanian isthmus demonstrate the vicariant effect of the recent closure of the central American seaway. An earlier study of the American tropical cephalopods (Nesis, 1978) concluded that cephalopods fail to show evidence of this vicariant event. However, reinvestigation of the tropical American shallow-water octopods revealed a higher number of geminate species pairs than known at the time of the earlier analysis. The trans-Panamanian geminate species include *Octopus bimaculatus – O. maya, O. oculifer – O. hummelincki, O. digueti – O.* sp. A, *O. chierchiae – O. zonatus, O. alecto – O. briareus* and *Euaxoctopus panamensis – E. pillsburyae.* A seventh species pair, *O. fitchi – O. joubini,* may also be geminate. These additional data, based upon consideration of littoral octopods, do not support Nesis' view that the evolutionary rate of cephalopods is so rapid that phylogenies are obscured in a few million years.

Key words: geminate species; *Octopus;* biogeography; Gulf of California, eastern tropical Pacific, western tropical Atlantic.

INTRODUCTION

The previous connection between the Pacific and the Atlantic Oceans through what is now Central America was first postulated on the basis of faunal affinities between the two regions (Ekman, 1953). These faunal affinities consist mainly of geminate species, i. e. morphologically similar species with distributions on either side of the isthmus. Close relationships between the species are postulated due to their similarity and the geologic history of the area, which indicates that the areas have been isolated for only 3.0 – 3.5 million years (Jones & Hasson, 1985).

Few geminate species are currently recognized in the class Cephalopoda. Nesis (1978) compared Pacific cephalopods (ranging from the southern coast of California, U.S.A. to mid-Chile) to those of the tropical western Atlantic (from Cape Canaveral, Florida to the Orinoco River, Venezuela). Of the 210 species considered, he felt that the similarity between only four of the pairs was sufficient to indicate their origin as linked closely to the origin of the central American isthmus. These four pairs are *Octopus zonatus – O. chierchiae, O. digueti – O. joubini, O. bimaculatus – O. maya* and *O. oculifer – O. hummelincki.* Nesis concluded that the evolutionary rate of cephalopods had been so rapid that evidence of previous faunal contacts had been obliterated.

The present study re-examines whether evidence of trans-isthmian faunal affinities remains among the octopods. I show that there are more geminate species of octopods than Nesis indicated. This conclusion is based on a more detailed comparison of octopods from the eastern tropical Pacific (including the Gulf of California) to those of the western tropical Atlantic.

METHODS

My comparison was based on overall phenetic similarity. Widely distributed species, ranging beyond the study area, were excluded from the study because they do not contribute substantially to the zoogeographic analysis. The data base consisted of taxonomic treatments containing proposed relationships, morphology and natural history, supplemented by additional personal collections from the Gulf of California. Aspects of internal anatomy were not included due to uncertainties regarding intraspecific variation.

Data for the western Atlantic species were obtained from Hanlon (1983a), Pickford (1945) and Voss (1956, 1968). For the eastern Pacific species, data were obtained from

TABLE 1. A list of hypothesized geminate species pairs of shallow-water octopuses between the eastern Pacific and the western Atlantic.

Eastern Pacific	Western Atlantic	Source
O. oculifer	*O. hummelincki*	Voss, 1971
O. bimaculatus	*O. maya*	Nesis, 1978
O. digueti	*O. joubini*	Pickford, 1945
O. chierchiae	*O. zonatus*	Voss, 1968
Euaxoctopus panamensis	*E. pillsburyae*	Voss, 1975
O. alecto	*O. briareus*	This study

Berry (1954) and Voss (1968, 1971). My own collections, housed in the University of Arizona Invertebrate Collection, and species diagnoses of Berry (1953) and Perrier & de Rochebrune (1894), contributed data for the octopuses of the Gulf of California.

Because the species comparisons were based upon overall similarity, the species pairs presented in this paper represent hypotheses that remain to be tested by phylogenetic analysis.

RESULTS

The species of *Octopus* and *Euaxoctopus* known from each region are listed in the Appendix. Table 1 lists the species hypothesized to represent geminate species pairs. A brief review of the evidence supporting these hypotheses is presented below.

Ocellated species

A group of octopuses with blue ocelli occur throughout the American seas. Similarities in gill lamellae counts, enlarged sucker distributions, hectocotylus morphology and the placement, color and overall morphology of the ocellus unify the group (see Burgess, 1966; Pickford & McConnaughey, 1949; Voss, 1971; Voss & Solis, 1966). They include *O. bimaculatus* Verrill, 1883 and *O. bimaculoides* Pickford & McConnaughey, 1949 from the southern Californian coast, and *O. oculifer* Hoyle, 1904 and an unnamed species (Pickford, 1945) from the eastern tropical Pacific, a region *O. bimaculatus* also enters, and *O. maya* Voss & Solis, 1966 and *O. hummelincki* Adam, 1936 from the western Atlantic.

Unfortunately, the two tropical Pacific species are poorly known. *Octopus oculifer* Hoyle, 1904 was described from a single female collected in the Galapagos Islands. Voss (1971), based on two additional specimens, suggested that the specimens might be *O. oculifer,* although he was unable to confirm the identities. Pickford (1945) removed Central American specimens of *O. bimaculatus* from synonymy on the basis of ligula length and small body size.

Voss (1971) stated that *O. oculifer* is very closely related to *O. hummelincki,* and that both may be related to *O. bimaculatus* and *O. bimaculoides* of the temperate Pacific. Pickford (1945) suggested that *O. hummelincki* was closely related to the small undescribed species she had separated from *O. bimaculatus.* Nesis (1978) cited *O. oculifer* and *O. hummelincki,* and *O. bimaculatus* and *O. maya* as geminate pairs separated by the Central American isthmus.

Pygmy octopuses

A second group of possibly related octopuses occurs in the American seas. The characters delineating this group include small adult size (<40 g), 5 to 8 gill lamellae per demibranch and similar overall morphologies. Additionally, their eggs are attached to the substrate individually rather than in the festoon arrangement that is typical of many species of *Octopus.* This group consists of *O. fitchi* Berry, 1953 from the northern Gulf of California, *O. micropyrsus* Berry, 1953 from southern California, *O. digueti* Perrier & Rocheburne, 1894 from the Gulf of California and *O. joubini* Robson, 1929 from the Western Atlantic.

R. Toll (personal communication, 1986; see also Hanlon, 1983b) has suggested that there are two distinct species included in our current concept of *O. joubini. Octopus joubini sensu stricto* is apparently a small octopus that has small eggs and planktonic young. The specimens that have been misidentified

as *O. joubini* are very similar, but have large eggs with direct development of embryos into demersal young. I will refer to this species as *O.* sp. A since it has yet to be described.

Relationships within this group have been postulated previously. Berry (1953) noted that *O. fitchi* clearly pertains to *O. digueti. Octopus* sp. A and *O. digueti* are geminate species (Nesis, 1975, 1978; Pickford, 1945).

A strong similarity is also seen between *O. fitchi* and *O. micropyrsus* (F. G. Hochberg, personal communication, Voight, personal observations). Both have 5 to 6 gill lamellae per demibranch, one enlarged sucker on each arm of both sexes and solid red coloration. They differ in both calamus size and egg type. *Octopus fitchi* has eggs 5 mm long and planktonic young (Voight, personal observations). *Octopus micropyrsus* has eggs 10 to 12 mm long and demersal young (Hochberg & Fields, 1980). The disjunct distribution of these very similar and probably closely related species, between the northern Gulf of California and the Californian coast, is seen in many other taxa and may result from recent climatic events (Brusca, 1983; Wallerstein & Brusca, 1982).

Egg length in *O. joubini s. s.* and *O. fitchi* is similar and both species have planktonic young (J. W. Forsythe, personal communication). This is especially notable because small octopuses rarely have planktonic young (Boletzky, 1984; Green, 1973 in Strathmann & Strathmann, 1982). However, until *O.* sp. A is known more fully, its relationship with *O. fitchi* will remain unresolved.

Octopus sp. A is morphologically and ecologically similar to *O. digueti,* as noted by Pickford (1945). Both live in sandflat habitats and use the refuge provided by empty mollusc shells. Eggs 8 mm long and demersal young typify the species. They differ only in that *O. digueti* exhibits a more mottled coloration in life (J. W. Forsythe, personal communication) and grows faster in laboratory studies (Hanlon & Forsythe, 1985).

Barred and spotted octopuses

A group of small octopuses, characterized by distinct patterns of spots, stripes and bars on the mantle and arms, seems unique to the American tropics (Voss, 1968). This group consists of *O. zonatus* Voss, 1968 from the western Atlantic and four species from the eastern Pacific: *O. chierchiae* Jatta, 1889, *O. penicillifer* Berry, 1954, *O. stictochrus* Voss, 1971 and an as-yet-undescribed species, given the vernacular name "the larger Pacific striped octopus" by Rodaniche (1984).

Voss (1968) considered *O. zonatus* from the western Atlantic to be very similar to the larger Pacific striped octopus that he had erroneously identified as *O. chierchiae. Octopus zonatus* is very similar to *O. chierchiae* and to the larger Pacific striped octopus.

Hochberg (1980) suggested that *O. penicillifer,* from near the tip of Baja California, and *O. stictochrus,* from the Gulf of Panama, are synonymous. The extensive similarities in morphology and the unusual coloration shared by these species strongly support Hochberg's suggestion.

Euaxoctopus

Among other octopods, *Euaxoctopus panamensis* Voss, 1971 and *E. pillsburyae* Voss, 1975 are Trans-Panamanian twin species, as demonstrated by Voss (1975).

Octopus alecto – O. briareus

I propose an additional geminate pair that has apparently escaped the attention of previous workers. *Octopus alecto* Berry, 1953, from the Gulf of California, shares several diagnostic characters with *O. briareus* Robson, 1929 from the western Atlantic. Table 2 presents a summary comparison of these species. In both, the second and third pairs of arms are the longest and stoutest, an extensive interbrachial web is used as a net to capture prey and a membrane fringes the ligula. The species flush solid maroon when disturbed, but typically have creamy white skin with lime-green iridocytes, associated with granulations scattered over the skin.

The species differ in egg size. *Octopus briareus* has eggs 10–14 mm long and demersal young (Hanlon, 1983a); *O. alecto* has eggs 2 mm long and planktonic young (Voight, personal observation).

DISCUSSION

The strong similarity between six species pairs of octopods straddling the central American isthmus is evidence that each of the pairs shares a recent common ancestor. Because the ranges of the putative ancestral species were split by the uplift of Central America only 3.0 – 3.5 million years ago (Jones & Hasson,

TABLE 2. Comparison of *O. alecto* Berry, 1953 and *O. briareus* Robson, 1929.

Shared characters between *O. alecto* and *O. briareus.*
Mantle round or ovoid elongately
Constricted neck
Robust, well-developed funnel
Second and third arm pairs both longest and stoutest.
Arm lengths over 80% of total length
Extensive interbrachial web
Ligula with fringing membrane
Coloration tan or maroon with green iridescence
Characters separating *O. alecto* and *O. briareus:*
Eggs: 2 mm long in *O. alecto;* young planktonic
15 mm long in *O. briareus;* young demersal

1985; Keigwin, 1978; Saito, 1976), recent common ancestry is a more parsimonious explanation for the observed similarity than is convergence between distinct evolutionary lineages. However, that the observed similarity accurately reflects recent common ancestry remains to be demonstrated. I believe that a cladistic analysis of the group will support the hypotheses proposed here.

Nesis (1975, 1978) found a low proportion of geminate species between the cephalopods of the Pacific and Atlantic. However, Nesis' species list was inflated in two ways. First, he included all cephalopods, shallow-water to deep-water. As the deep-sea separation of the eastern tropical Pacific and the western tropical Atlantic is now thought to have occurred 30 million years ago (Jones & Hasson, 1985), divergence between deep-water species over that time is not expected to be comparable with that seen between shallow-water species that were isolated for only 3 million years. Second, Nesis included species from non-tropical areas of the Pacific in his comparison with tropical species from the western Atlantic. My study is restricted in scope to shallow-water octopods from tropical and subtropical areas. These restrictions are in keeping with other studies of trans-Panamanian geminate taxa.

Nesis' inflated species list and the resultant low proportion of geminate species (4 of 110 Pacific species) led him to the conclusion that the evolutionary rate of cephalopods is such that phylogenies are obscured within a few million years. My study, because of its restricted scope and access to data unavailable to Nesis (personal communication), finds a higher proportion of geminate species (6 of 16 species). This is in keeping with other marine taxa and fails to demonstrate an unusual evolutionary rate.

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APPENDIX 1. Species of each of the areas considered; species names, authors and distributions. Sources: Burgess, 1966; Hanlon, 1983a, 1983b; Hochberg, 1980; Hochberg & Fields, 1980; Voight, unpublished data; Voss, 1968, 1971.

Species (author)	Distribution
EASTERN PACIFIC	
O. oculifer (Hoyle, 1904)	Galapagos, Costa Rica, Panama?
O. n. sp. (Pickford, 1945)	Panama, San Salvador
O. bimaculatus (Verrill, 1883)	Southern California, Gulf of California
O. fitchi (Berry, 1953)	Northern Gulf of California
O. digueti (Perrier & Rochebrune, 1894)	Gulf of California
O. penicillifer (Berry, 1954)	Tip of Baja California
O. chierchiae (Jatta, 1889)	Gulf of Panama to Central Gulf of California
O. stictochrus (Voss, 1971)	Gulf of Panama
Larger Pacific striped octopus	Gulf of Panama to Oaxaca, Mexico
O. hubbsorum (Berry, 1953)	Gulf of California
O. alecto (Berry, 1953)	Gulf of California
O. veligero (Berry, 1953)	Gulf of California to Sinaloa, Mex.
O. balboai (Voss, 1971)	Gulf of Panama
O. selene (Voss, 1971)	Gulf of Panama
O. ?vulgaris (Cuvier, 1797)	Worldwide, except near poles
E. panamensis (Voss, 1971)	Gulf of Panama
WESTERN ATLANTIC	
O. hummelincki (Adam, 1936)	Florida Keys to Brazil
O. maya (Voss & Solis, 1966)	Yucatan Peninsula
O. sp. A	Georgia, Gulf of Mexico, NW Caribbean
O. joubini s. s. (Robson, 1929)	Virgin Islands, Gulf of Mexico
O. zonatus (Voss, 1968)	Southwest Caribbean
O. briareus (Robson, 1929)	Caribbean, Gulf of Mexico, Florida, northern South America, Nicaragua, Yucatan, Honduras
O. burryi (Voss, 1950)	Trans-Atlantic
O. defilippi (Verany, 1851)	Trans-Atlantic
O. macropus (Risso, 1826)	Circum-tropical?
O. vulgaris (Cuvier, 1797)	Worldwide, except polar regions
E. pillsburyae (Voss, 1975)	Gulf of Uraba to Surinam

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THE BIOGEOGRAPHY OF THE DEEP-SEA OCTOPODA

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ABSTRACT

The deep-sea octopods are in both octopod suborders, the Cirrata with three families, eight genera and 29 species, and the Incirrata with three octopodid subfamilies, 11 genera and 48 species. In addition, some families in the Incirrata contain deep-sea pelagic species. Only benthic or "near benthic" species are dealt with in this paper.

Among the Cirrata, only the Cirroteuthidae contain species with a multi-ocean distribution. *Cirrothauma murrayi* is possibly cosmopolitan, occurring from polar seas to the depths of the tropics. *Cirroteuthis muelleri* has an amphiboreal distribution in the North Atlantic and North Pacific. In the monotypic Stauroteuthidae, *Stauroteuthis syrtensis* is known only from the temperate western North Atlantic. The Opisthoteuthidae are represented by two genera. *Opisthoteuthis* with 10 species is the shallowest-dwelling of the cirrates, with all but three species living between 100 and 1000 m. The genus is distributed throughout the oceans except in high latitudes. *Grimpoteuthis*, with 13 species, occurs in all oceans but no species is recorded from more than one ocean. A specimen has been trawled from 7279 m.

The deep-sea Incirrata are found among three subfamilies of the Octopodidae. In the Bathypolypodinae, *Bathypolypus* has five species in the Atlantic, one off Japan, one in the Indian Ocean; *Teretoctopus* has two species in the Indian Ocean, and *Benthoctopus* has approximately 15 species distributed worldwide from the Antarctic to the Arctic, with individual species restricted to single oceans. Among the Pareledoninae, *Pareledone, Vosseledone* and *Velodona* are found only in the southern hemisphere, and *Tetracheledone* only in the western North Atlantic. *Pareledone* is the most speciose and has a circum-Antarctic distribution; the monotypic *Vosseledone* is known only from Brazil; the two species of *Velodona* occur off East Africa. Among the Graneledoninae, *Graneledone* has two species in the Southern Ocean, one in the North Atlantic, one in the North Pacific and one in the Panamic region. *Thaumeledone* and *Bentheledone* are confined to the Southern Ocean.

Key words: biogeography; deep sea; Octopoda; Cirrata; Incirrata.

INTRODUCTION

The deep-sea octopods (defined as those octopods that live below the edge of the continental shelf) are in both suborders, the Cirrata and the Incirrata. The cirrates, or the finned octopods, represent the oldest evolutionary lineage of the octopods (Voss, 1988). The cirrates are soft-bodied, semigelatinous animals with a pair of small to large fins, a deep and sometimes complicated web and a relatively large, single internal shell. They swim by medusoid action of the web, by jet propulsion, by beating the fins or by a combination of all three (Pereyra, 1965; Roper & Brundage, 1972). Although swimmers, they live just above the bottom and are rarely captured in the mid-depths.

The deep-sea benthic incirrates resemble, in general appearance, the typical shallow-water octopods. They are soft to firm bodied, have a moderate to deep web and vestigial paired shell-remnants or stylets. They crawl about the bottom or swim by jet propulsion for short distances. While there are several families that have deep-sea pelagic octopods (see Thore, 1949), only the benthic species are considered here.

In both orders, the benthic and "near benthic" females produce large eggs resulting in young that, we suppose, take up the adult habitat and mode of life immediately after hatching from the egg.

One might expect that the genera and species of the deep-sea cirrates would have a much broader distribution than would the slower moving, bottom crawling deep-sea incirrates. Such, however, is not the case although our studies are limited by a paucity of specimens and much confusion concerning identifications.

TABLE 1. List of cirrate octopods arranged according to Voss (1988) with depth of capture and nomenclatural status.

Cirroteuthidae
Cirroteuthis muelleri Eschricht, 1836. 0–2342 m
Cirrothauma murrayi Chun, 1911. 1500–4500 m.
Stauroteuthidae
Stauroteuthis syrtensis Verrill, 1879. 457–2463 m.
Opisthoteuthidae
Grimpoteuthis megaptera (Verrill, 1885). 1929–4710 m.
Grimpoteuthis umbellata (Fischer, 1883). 1140–5274 m.
Grimpoteuthis pacifica (Hoyle, 1885). 4465 m
Grimpoteuthis meangensis (Hoyle, 1885). 915–1098 m.
Grimpoteuthis caudani (Joubin, 1896). 650 m.
Nomen dubium.
Grimpoteuthis plena (Verrill, 1885). 1964 m.
Grimpoteuthis hippocrepium (Hoyle, 1904). 3336 m.
Nomen dubium.
Grimpoteuthis mawsoni (Berry, 1917). 527–549 m.
Nomen dubium.
Grimpoteuthis grimaldi (Joubin, 1903). 1804–1901 m.
(= *Opisthoteuthis* ?)
Grimpoteuthis wuelkeri (Grimpe, 1920). 2057 m.
Nomen dubium.
Grimpoteuthis albatrossi (Sasaki, 1920). 487–1680 m.
Grimpoteuthis glacialis (Robson, 1930). 500 m.
Grimpoteuthis bruuni Voss, 1982. 250–360 m.
Opisthoteuthis agassizi Verrill, 1883. 922–2250 m.
Opisthoteuthis depressa Iijima & Ikeda, 1895
128–1074 m.
Opisthoteuthis pluto Berry, 1918. 833 m
Opisthoteuthis persephone Berry, 1918. 549 m.
Opisthoteuthis medusoides Thiele, 1915. 399 m.
Opisthoteuthis extensa Thiele, 1915. 769 m.
Opisthoteuthis californiana Berry, 1949. 125–1100 m.
Opisthoteuthis japonica Taki, 1962. 150 m
Opisthoteuthis phillippi Oomen, 1976. 275–365 m
Uncertain status
Chunioteuthis ebersbachi (Grimpe, 1916). 1100 m
Chunioteuthis gilchristi (Robson, 1924). 2562 m.
Froekenia clara Hoyle, 1904. 1016 m.
Cirroteuthopsis massyae Grimpe, 1920. 1409 m.

CIRRATA

The Cirrata contains 29 species in 8 genera (Table 1). These are divided into three families based upon web and shell types: Cirroteuthidae, Stauroteuthidae and Opisthoteuthidae.

Cirroteuthidae

The cirroteuthids contain two monotypic genera represented by *Cirroteuthis muelleri* and *Cirrothauma murrayi.* All of the cirroteuthids are fragile, soft-bodied animals, especially *Cirroteuthis.* Meaningful measurements are exceedingly difficult to obtain from the specimens, and internal organs are relatively unstudied. Perhaps these genera will be found to be more speciose when sufficient specimens are available.

Cirroteuthis muelleri is known from the northwestern Atlantic (Voss, unpublished), Baffin Bay to Iceland (Eschricht, 1836; Voss, unpublished), off Jan Mayen (Appellöf, 1893) and from off Oregon (Voss & Pearcy, in preparation). These data suggest an amphiboreal distribution (Fig. 1). Although a number of species of fish show this amphiboreal distribution, most are separated into Atlantic and Pacific subspecies (Ekman, 1953). The apparent absence of a median pallial adductor in Atlantic specimens (Robson, 1932) is erroneous since examination of well-preserved WALTHER HERWIG specimens from the North Atlantic proved that this structure was present. No other characters have been found by which to separate the two populations. Because of the high northern occurrence in the Atlantic, the distribution could be continuous through the Arctic Ocean even though no high Arctic records are known. The species has been taken from the surface (off Greenland) to 2342 m.

Cirrothauma murrayi has been monographed by Aldred *et al.* (1983) who gave all known records. Additional records in Fig. 1 have been added from specimens in the University of Miami collections. This species is known from the coasts of Europe and northwestern Africa to off northeastern South America and along the eastern coast of North America from about New York to the Bahamas and questionably from the Indian Ocean. Its normal depth range is 1500 to 3000 m. One specimen was taken from an ice hole in the Arctic Ocean. If all specimens prove to belong to a single species, this species will be the only widely distributed, nearly cosmopolitan, species among the deep-water octopods.

Stauroteuthidae

Like the cirroteuthids, stauroteuthids are fragile, soft-bodied animals. This family is represented only by *Stauroteuthis syrtensis.* It occurs from Nova Scotia (Verrill, 1879) along the Atlantic coast of North America to north of the Bahamas (Voss, unpublished) (Fig. 1). Except for one questionable record, this species occurs only in the western North Atlantic. Its depth range is 457 to 2463 m.

Opisthoteuthidae

As now understood, this family includes the majority of the cirrates: 22 species belong to

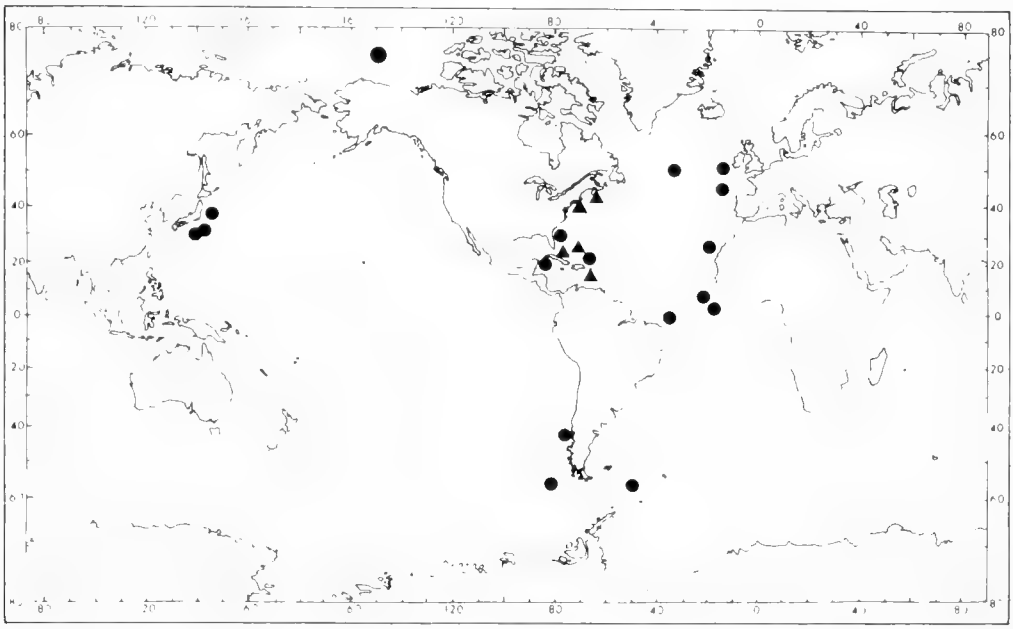

FIG. 1. Cirroteuthidae. ◦ *Cirroteuthis muelleri*, ● *Cirrothauma murrayi*. Stauroteuthidae. ▲ *Stauroteuthis syrtensis*.

the two genera *Grimpoteuthis* and *Opisthoteuthis*.

The genus *Grimpoteuthis* occurs throughout the Atlantic and Pacific oceans and the Antarctic (Fig. 2), living in depths from about 200 m to below 7000 m (Robson, 1932). The identity of the majority of species is uncertain and many species are represented by single specimens. As a result, the geographical range of many species is not yet established. At present no species is known to be widespread.

Nine species have been described and three are in manuscript (Voss & Pearcy, in preparation; Voss, in preparation). Brief inspection of the GALATHEA collections suggests that this number may be doubled when these specimens are reported upon.

Grimpoteuthis glacialis is known from south of the Antarctic Convergence from South Georgia, the Palmer Peninsula and the Ross Sea (Robson, 1930; unpublished ELTANIN records, University of Miami). It probably occurs throughout the Southern Ocean south of the convergence. A second undescribed Southern Ocean species occurs in the Scotia Sea (WALTHER HERWIG specimens, Voss, in preparation). *Grimpoteuthis bruuni* occurs off northern Chile and Peru (Voss, 1982). *G. umbellata*, the type species, is still relatively unknown but occurs off Mauritania and the Azores (Fischer, 1883; Robson, 1932). Several specimens identified as this species (Robson, 1932; personal observations) are questionable.

Verrill (1885) described *G. megaptera* and *G. plena* from off New England. The latter is a *nomen dubium*, but *G. megaptera*, with many misidentified specimens, may be widespread in the North Atlantic.

In the same year Hoyle (1885) described two species taken by the CHALLENGER: *G. pacifica* from near the Coral Sea and *G. meangensis* from north of the Celebes. No subsequent specimens identifiable to either of these two species have been reported. In 1904 Hoyle also described *G. hippocrepium* from off the Gulf of Panama. The validity of all three species is questionable because of the brief descriptions and poor illustrations.

Grimpoteuthis albatrossi was described by Sasaki (1920) from south of the Aleutians from specimens taken by the United States Fish Commission steamer ALBATROSS. It also occurs off Japan (Sasaki, 1929).

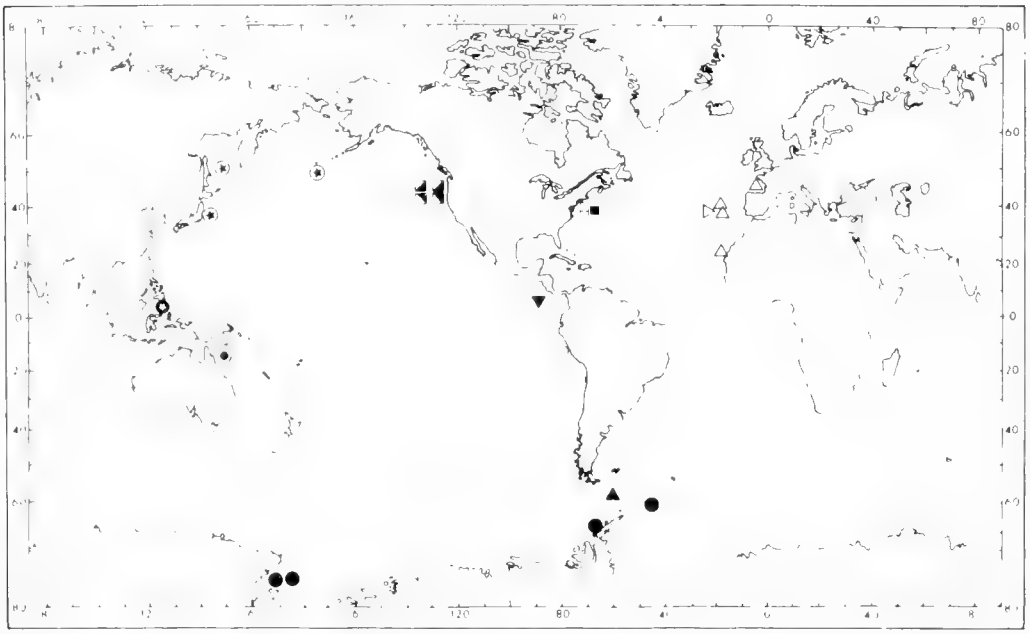

FIG. 2. Opisthoteuthidae. ○ *Grimpoteuthis bruuni*, ● *G. glacialis*, ▲ *G.* sp. A, ◪ *G. sp. B*, ◹ *G. sp. C*, ✪ *G. meangensis*, ● *G. pacifica*, △ *G. umbellata*, ⊔ *G. megaptera*, ■ *G. plena*, ▼ *G. hippocrepium*, ▷ *G. grimaldi*, ⊛ *G. albatrossi*.

The beautifully illustrated and well described *G. grimaldi* Joubin, 1903, is a problem. Since its original description from off the Azores, additional specimens have been assigned to this species in the literature (Joubin, 1920; Bruun, 1945). My study of the optic lobe of cirrates (unpublished) indicates, however, that *G. grimaldi* is probably an *Opisthoteuthis* and may even be *O. agassizi*.

The genus *Opisthoteuthis* occurs in moderate depths, mostly between 100 and 1000 m in tropical and temperate seas (Fig. 3). It ranges from about 50°N to 45°S. Only three species have been taken in depths greater than 1000 m; the deepest record is 2250 m (Verrill, 1885).

If identifications are correct, *Opisthoteuthis agassizi* occurs in the eastern Atlantic from southwest of Ireland (Chun, 1913) to off Spain, and the western Mediterranean (Morales, 1959), the Gulf of Guinea (Voss, in preparation) and south to off southwest Africa (Adam, 1962). Its distribution in the W Atlantic appears to be more limited, ranging from northeast of the Bahamas (Univ. of Miami collections) and Gulf of Mexico (Voss, 1956) to northeastern Brazil (Univ. of Miami collections).

Opisthoteuthis californiana occurs in northern California (Berry, 1949) and Washington (Pereyra, 1965) waters and has since been reported by Taki (1963) from off Honshu. Two other species, *O. depressa* and *O. japonica*, also occur in Japanese waters.

Thiele (1915a) described *O. extensa* from a VALDIVIA station southwest of Sumatra and *0. medusoides* from off Zanzibar. In 1918 Berry described *O. persephone* and *pluto* from captures by the ENDEAVOUR in the Great Australian Bight. Many more specimens of the latter two species have since been taken. Both apparently are limited to Australian waters.

The last species described is *Opisthoteuthis philippi* from off southwest India (Oomen, 1976).

The paucity of records prevents generalizations on the biogeography of the individual species.

INCIRRATA

The incirrate deep-sea octopods contain 48 species distributed unevenly in 11 genera (Ta-

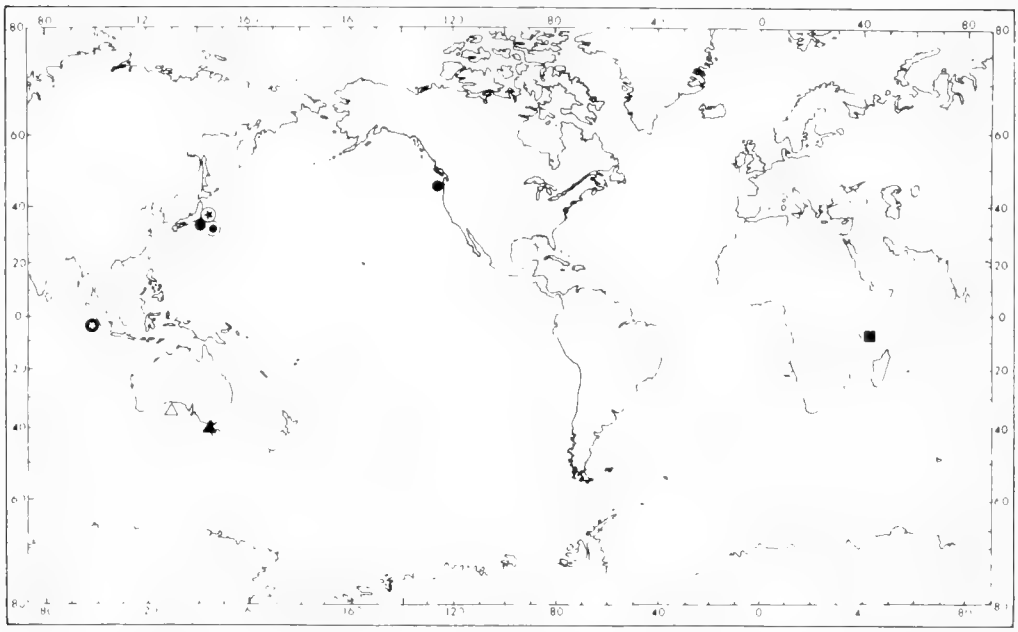

FIG. 3. Opisthoteuthidae. ◯ *Opisthoteuthis agassizi*, ⦿ *O. depressa*, ✪ *O. extensa*, ■ *O. medusoides*, ⦿ *O. japonica*, ● *O. californiana*, □ *O. philippi*, △ *O. pluto*, ▲ *O. persephone*.

ble 2). I have recently (Voss, 1988) grouped these in three subfamilies: Bathypolypodinae, Pareledoninae and Graneledoninae. In general, the deep-sea incirrates do not occupy as great depths as do the cirrates, ranging from about 200 m to nearly 4000 m but with many living to about 500 m. All, however, show modifications to reflect a deep-sea habitat.

Robson's (1932) classification of the incirrates, in my opinion, cannot be maintained. Some major shifts are made here, based partly on the number of longitudinal sucker rows and the presence or absence of an ink sac and crop.

Bathypolypodinae

This subfamily contains three genera, *Bathypolypus, Teretoctopus* and *Benthoctopus*. All have biserial arm suckers and no ink sac.

Bathypolypus contains five species of which three are found in the North Atlantic (Mangold-Wirz, 1963; Toll, 1985), one off Japan (Sasaki, 1929) and one in the Indian Ocean (Thiele, 1915b) (Fig. 4). *B. arcticus* is found in the North Atlantic from the Straits of Florida (in deep water) north to Baffin Bay, Denmark Straits, Iceland, northern Great Britain and the North Sea to Spitzbergen and the Arctic Ocean north of European Russia (Nesis, 1982; Kumpf, 1958). It appears to be an Atlantic Boreal and Arctic species. *B. arcticus* is also confused with *B. faeroensis*, recently reinstated by Toll (1985), and *B. proschi* Muus, 1962. Further work may show that true *B. arcticus* may have a more limited distribution.

B. sponsalis occurs along the Atlantic Spanish coast southward to Senegal (Adam, 1960) and the Cape Verde Islands (Fischer & Fischer, 1892) and the western Mediterranean Basin (Wirz, 1955; Morales, 1958) in depths from 400 to 930 m. Its distribution reflects the typical Mauritanian—Lusitanian—Mediterranean pattern (Ekman, 1953: 80).

B. salebrosus is found off northern Honshu, the Kurile Islands and in the Okhotsk Sea (Sasaki, 1929). It lives between about 450 and 800 m.

B. valdiviae is distributed from near Durban, South Africa, to off Walfisch Bay, Namibia, in depths from 400 to 800 m (Thiele, 1915b; Sanchez & Moli, 1984). Its range encompasses the Cape Province and the Namaquan Province, both dominated by the

TABLE 2. List of incirrate octopods arranged according to Voss (1988) with depths of capture and nomenclatural status.

Bathypolypodinae
Bathypolypus arcticus (Prosch, 1849). To 1543 m
Bathypolypus sponsalis (P. & H. Fischer, 1892). 930–1250 m
Bathypolypus faeroensis (Russell, 1909). 480–1030 m
Bathypolypus valdiviae (Thiele, 1915). 403–860 m
Bathypolypus salebrosus (Sasaki, 1920). 487–805 m
Bathypolypus proschi Muus, 1962. *Nomen dubium*
Teretoctopus indicus Robson, 1932. 996 m
Teretoctopus alcocki Robson, 1932. 353–1281 m
Benthoctopus piscatorum Verrill, 1879. 220–2492 m
Benthoctopus levis (Hoyle, 1885). 13–137 m
Benthoctopus januari (Hoyle, 1885). 350–732 m
Benthoctopus ergasticus (P. & H. Fischer, 1892). 470–915 m
Benthoctopus lothei (Chun, 1914). 1365 m
Benthoctopus hokkaidensis (Berry, 1921). 487–920 m
Benthoctopus abruptus (Sasaki, 1920). 1074 m
Benthoctopus pseudonymus (Grimpe, 1922). 1599 m
Benthoctopus berryi (Robson, 1924). 2196 m
Benthoctopus eureka (Robson, 1929). Depth unknown
Benthoctopus sibiricus Loning, 1930. Stomach of walrus
Nomen dubium
Benthoctopus thielei Robson, 1932. On shore
Benthoctopus profundorum Robson, 1932. 741–3431 m
Benthoctopus magellanicus Robson, 1932. 145 m
Benthoctopus oregonae Toll, 1981. 640–1080 m
Pareledoninae
Pareledone charcoti (Joubin, 1905). 18–595 m
Pareledone turqueti (Joubin, 1905). 25–1116 m
Pareledone harrisoni (Berry, 1917). 494–655 m
Nomen dubium
Pareledone adeliana (Berry, 1917). 527–549 m
Nomen dubium
Pareledone polymorpha (Robson, 1930). 15–1116 m
Velodona togata togata Chun, 1915. 748 m
Velodona togata capensis Robson, 1929. 402–457 m
Vosseledone charrua Palacio, 1978. 10–200 m
Tetracheledone spinicirrus Voss, 1955. 183–544 m
Eledone moschata (Lamarck, 1798). Above 300 m
Eledone cirrhosa (Lamarck, 1798). 10–800 m
Eledone caparti Adam, 1950. 60–170 m
Eledone thysanophora Voss, 1962. Tide pool
Eledone massyae Voss, 1964. 60–170 m
Graneledoninae
Graneledone verrucosa (Verrill, 1881). 852–2297 m
Graneledone challengeri (Berry, 1961). 1153–1867 m
Graneledone verrucosa media (Joubin, 1918). 1458 m
Nomen dubium
Graneledone antarctica Voss, 1976. 2341 m
Graneledone macrotyla Voss, 1976. 1647–2044 m
Graneledone boreopacifica Nesis, 1982. 1165–1500 m
Thaumeledone brevis (Hoyle, 1885). 800–3931 m
Thaumeledone gunteri Robson, 1930. 410 m
Bentheledone rotunda (Hoyle, 1885). 3596 m
Bentheledone albida (Berry, 1917). 3111 m.

Agulhas Current, which probably is responsible for this distribution.

The genus *Teretoctopus* contains only two species, *T. indicus* and *T. alcocki*. *T. indicus* has been reported from the pearl grounds off Ceylon (Robson, 1932) and the upper Arabian Sea in 1000 m (Massy, 1916b). *T. alcocki* is known from the Bay of Bengal and the Andaman Sea to the upper Arabian Sea (Robson, 1932) between 500 and 1281 m. Little is known of these two Indian Ocean species other than Robson's (1932) comments.

The genus *Benthoctopus* occurs from the Arctic Ocean to the Southern Ocean (Fig. 5). Specific identifications are difficult; there are numerous synonyms and the literature is filled with misidentifications. All of the 15 or so species have somewhat limited ranges. They live from about 200 m to in excess of 3000 m.

Benthoctopus piscatorum is an Atlantic Boreal species found from New England to the British Isles, North Sea and off Norway (Robson, 1932). It may also be found in the High Arctic and around Spitsbergen (Robson, 1932) but its northern and eastern ranges are not known. *B. ergasticus* is in the Eastern Atlantic southwest of Ireland (Massy, 1909) and the Mauritanian upwelling area (Ficsher & Fischer, 1892). *B. januari* is strictly Atlantic, distributed from the Gulf of Mexico (Voss, 1956) to middle Brazil (Hoyle, 1886). Toll (1981) described *B. oregonae* from the middle of the former's range.

The only other species with sufficient information to permit speculation on its range is *B. magellanicus* from the Valdés Peninsula, Argentina (Ré, personal communication) to Tierra del Fuego (Robson, 1932) and apparently N to Chiloe Island (Voss, unpublished).

Greater deep-trawling efforts will certainly reveal more species and wider distributions. There are four species off Japan and Voss & Pearcy (in preparation) have four new species from off Oregon. But records are few and scattered widely. Surprisingly, few are known from the Southern Ocean.

Pareledoninae

This subfamily contains the deep-sea genera *Pareledone, Velodona, Tetracheledone* and the closely related shallow-water genus *Vosseledone* and the more distantly related genus *Eledone*. *Eledone* is retained only provisionally in this subfamily as it shares the common characters of uniserial arm suckers, an ink sac and a crop. However, Robson (1932) and others have held that it is not truly related to the other members of the subfamily.

The species of *Pareledone* are all from the Southern Ocean (Fig. 6), living primarily south of the Antarctic Convergence (Robson, 1932; Dell, 1959; Taki, 1961; Voss, in preparation) although *P. turqueti* was recorded off Pará,

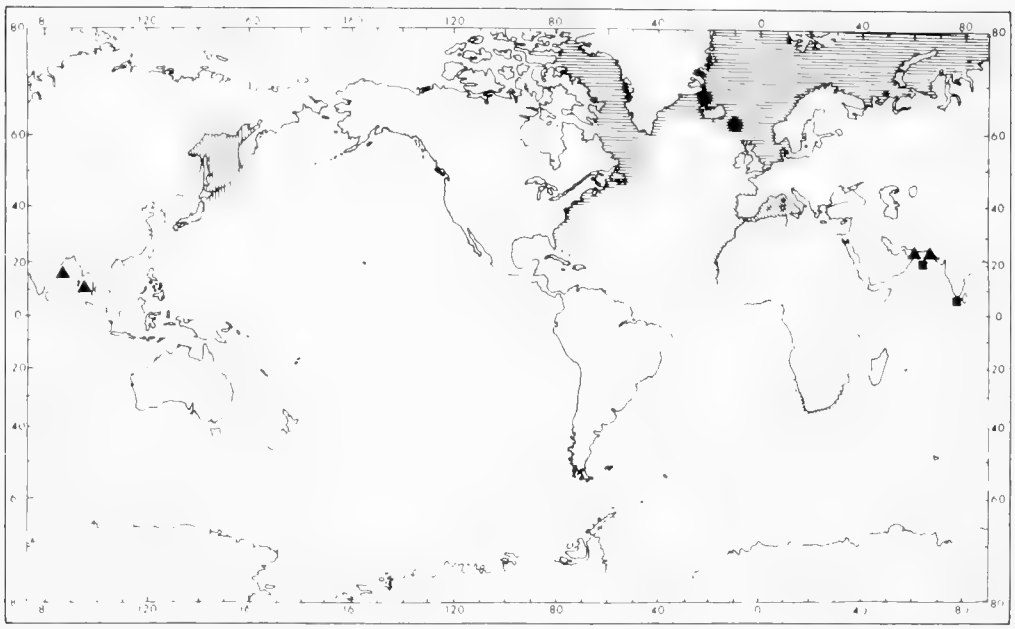

FIG. 4. Bathypolypodinae. ≣ *Bathypolypus arcticus,* ▨ *B. sponsalis,* ▥ *B. salebrosus,* ▤ *B. valdiviae,* ● *B. faeroensis,* ■ *Teretoctopus indicus,* ▲ *T. alcocki.*

Brazil, by Massy (1916a) from deep trawling of the TERRA NOVA expedition. It was also taken in Antarctic Deep Water. All of the species are circumpolar, living from tide pools in the Ross Sea to below 2000 m, with temperature apparently the controlling factor (Voss, in preparation).

Velodona togata has been taken twice from off Zanzibar (Chun, 1915; Voss, unpublished). A subspecies, *capensis,* occurs in the Cape Province (Robson, 1932). This species is probably widely distributed along the East African coast in depths between 400 m and 800 m (British Museum collections). *Vosseledone charrua* has a similar distribution along the eastern coast of South America in shallow water, only 10 m to 200 m (Palacio, 1978), but it is closely related to the deep-sea *Pareledone* in many of its features. *Tetracheledone spinicirrus* is known only from north Florida to the Gulf of Mexico and the northern Caribbean in 183 to 544 m (Voss, 1956). *Vosseledone, Velodona* and *Tetracheledone,* while occurring in shallow to moderate depths, in all other respects are deep-water animals closely resembling the genus *Graneledone* to which, on superficial examination, many of them would seem to belong.

Eledone was originally thought to be Mediterranean with only two species, *cirrosa* and *moschata.* Later a West African species, *E. caparti,* was described (Adam, 1950) from the Guinean Province. More recently another species, *E. massyae,* was added from off Brazil (Voss, 1964) where an additional, undescribed species occurs (Haimovici, in preparation), and finally one from southwest Africa, *E. thysanophora,* was described (Voss, 1962). A new species from Australia has recently been found (Lu, personal communication). Apparently the genus is circumtropical and warm temperate in distribution (Fig. 7). It is found in moderate depths, mostly above 300 m with a single record to 800 m. Its true position and relationship with both the deep-water and shallow-water genera has yet to be solved.

Graneledoninae

This subfamily contains the genera *Graneledone, Thaumeledone* and *Bentheledone* (Fig. 8). They are all deep dwellers, living between 1000 m to in excess of 3500 m. The genus *Graneledone* shows a somewhat

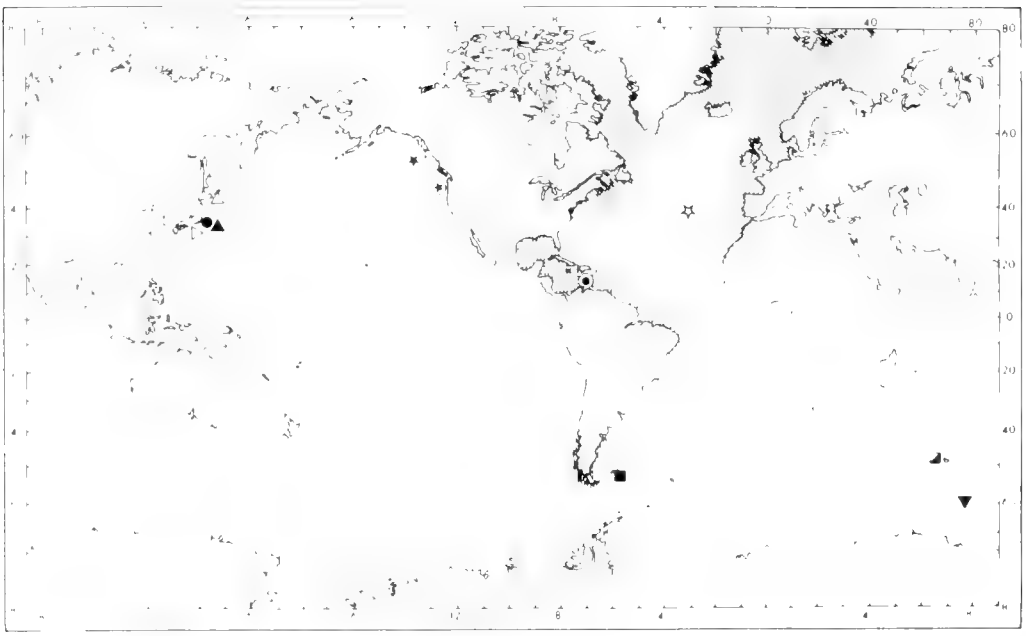

FIG. 5. Bathypolypodinae. ⁂ *Benthoctopus piscatorum*, ⁄⁄ *B. ergasticus*, ⁂ *B. magellanicus*, ⁜ *B. januari*, ⦿ *B. oregonae*, ■ *B. eureka*, □ *B. berryi*, ⁘ *B. pseudonymus*, ▲ *B. fuscus*, △ *B. hokkaidensis*, ▷ *B. abruptus*, ● *B. profundorum*, ▼ *B. levis*, ◪ *B. thielei*, ★ *B.* sp. A, ⍟ *B.* spp. A, B, C, D.

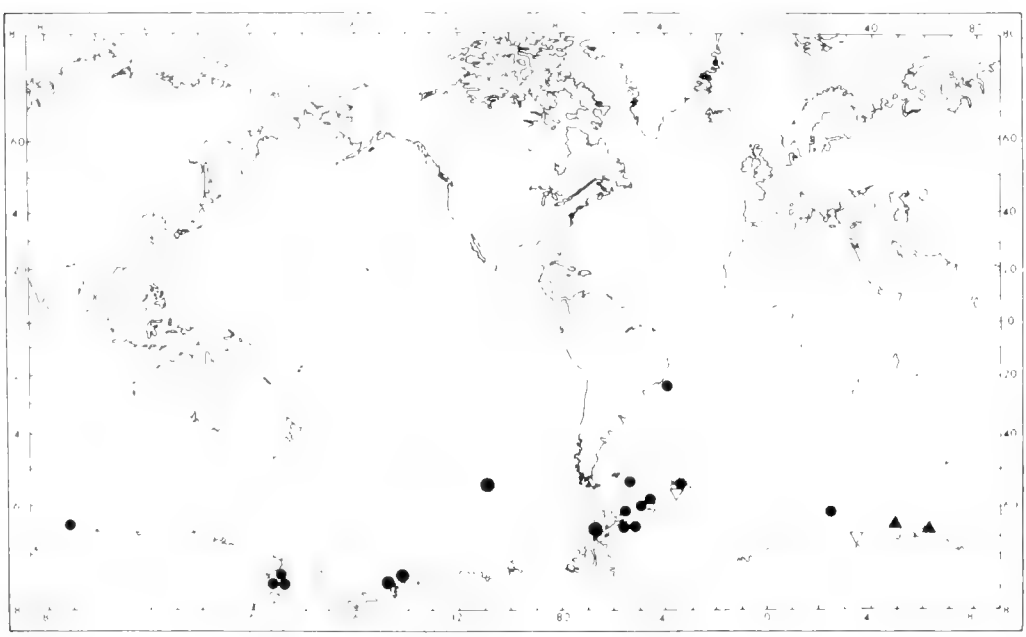

FIG. 6. Pareledoninae. ◯ *Pareledone polymorpha*, ● *P. turqueti*, ▽ *P. senoi*, ▲ *P. harrisoni*, □ *P. charcoti*.

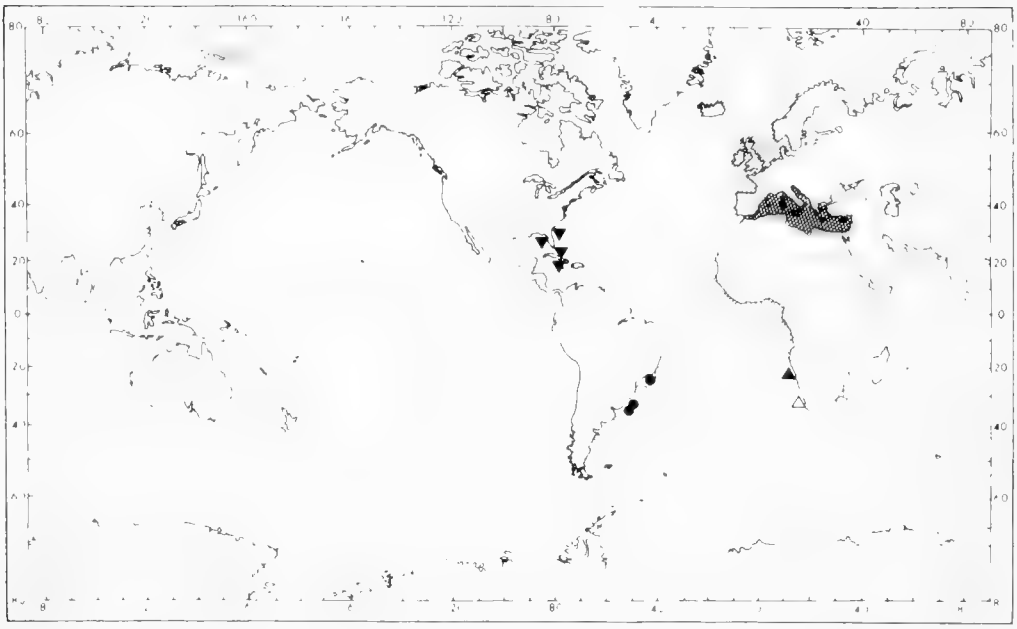

FIG. 7. Pareledoninae. ◯ *Velodona togata*, ● *Vosseledone charrua*, ▼ *Tetracheledone spinicirrus*, □ *Eledone massyae*, ▲ *E. nigra*, △ *E. thysanophora*, *E. cirrosa*, ▒ *E. moschata*, ▦ *E. caparti*.

bipolar distribution with *G. verrucosa*, *G. boreopacifica* and *G.* sp. A (the latter two perhaps identical), in N waters (Voss & Pearcy, in preparation), and *G. antarctica*, *G. macrotyla* and *G. challengeri* (Voss, unpublished) in the Southern Ocean and north of New Zealand. An unidentified specimen, examined by me from off the Gulf of Panama, may represent an additional species (sp. B).

The genus *Thaumeledone* contains two nominal species, *T. brevis* and *T. gunteri*. Unpublished records of *T. brevis* show that it is distributed from the Palmer Peninsula to the Ross Sea and is probably circumpolar south of the Antarctic Convergence. *T. gunteri* is probably a synonym and was reported from off South Georgia. It was taken in 410 m (Robson, 1930) while *T. brevis* lives between 800 m and 3931 m (Robson, 1932; Voss, unpublished).

Bentheledone consists of two described species, *B. rotunda*, and *B. albida*, with two undescribed species (Voss, in preparation). All occur south of the Antarctic Convergence in depths from 3000 m to 3596 m. They are probably circumpolar. Despite the extensive trawling effort in the Southern Ocean, no specimens of *Bentheledone* have been taken from moderate depths.

DISCUSSION

Unfortunately the systematics of the deep-sea octopods, and in particular the cirrates, have been sadly neglected. Robson's (1932) monographic study of the cirrates was the last attempt to make order out of the group. It did not succeed, partly because of Robson's mental decline and partly because of the poor quality and quantity of available collections. The situation has not altered appreciably since then because, while considerable additional material has accumulated, the existing types have deteriorated to such an extent that reliance must be given to the original, often inadequate, descriptions. The next few years, however, should show progress in cirrate systematics and biogeography as a result of study of the collections made by the USNS ELTANIN in the Antarctic and the Danish GALATHEA on her round-the-world, deep-trawling expedition, all now at Miami.

Among the cirrates, several distributional patterns are evident, even at this time. *Cirrothauma* is found in both the Atlantic and Pacific Oceans. *Stauroteuthis* is known definitely only from the western North Atlantic, including the Caribbean Sea.

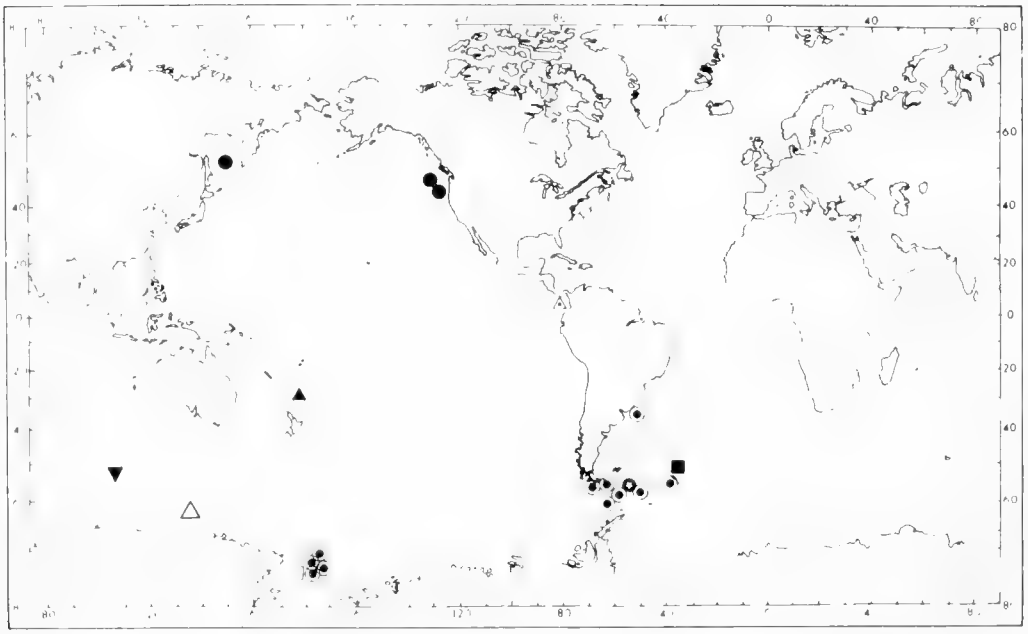

FIG. 8. Graneledoninae. ◯ *Graneledone verrucosa,* ● *G.* sp. A, ▲ *G. challengeri,* ◬ *G.* sp. B, □ *G. antarctica,* ◘ *G. macrotyla,* ◉ *Thaumeledone brevis,* ■ *T. gunteri,* ▼ *Bentheledone rotunda,* △ *B. albida.*

Grimpoteuthis is widely distributed but does not occur in the Arctic, living no further north than about 50°N. It is the only genus of cirrates that has representatives in the Southern Ocean south of the Antarctic Convergence.

Opisthoteuthis is known only from 45°N to 45°S. Living in shallower depths, its distribution reflects the shallow-water hydrographic regimes.

The Incirrata have fared little better. Again Robson (1932) brought together all of the known information on the group but it is a hodge-podge of misidentification by previous authors and reflects a lack of comparative morphological data. Many of the species described both before and after Robson were done without referring to the types, and since 1932 are based upon Robson's confused decisions. Again, many of the types are in poor condition or are immature specimens lacking important specific characters (Voss, in preparation).

The Bathypolypodinae contains primarily northern hemisphere genera and species. Five of the six species of *Bathypolypus* occur from about 20°N to the Atlantic and Pacific Subarctic with one extending to Spitsbergen. The sole southern exception is *B. valdiviae,* which is found off South Africa in the Agulhas Current or the southern end of the cold Benguela Current. *Teretoctopus* is limited to the deep underlying cold water of the tropical Indian Ocean.

Benthoctopus is the most widely distributed of the Bathypolypodinae but only four of the nineteen species are found in the southern hemisphere with two south of the Antarctic Convergence. Of the remaining 15 species, one is widely distributed in the North Atlantic Boreal to the Subarctic and perhaps to the High Arctic. Two are tropical and nine are northern temperate.

The Pareledoninae comprises two groups showing distinctly different distributional patterns. *Pareledone* is strictly Southern Ocean; all of the known species have their main concentrations south of the Antarctic Convergence and are circumpolar. The few catches north of the convergence, one off Montevideo and the others near the Falkland Islands, were in cold Antarctic Deep Water.

The remaining genera of the Pareledoninae are all Atlantic and Indian Ocean in occurrence. Their distribution reflects the general surface hydrographic regimes and all have

restricted distributions, reflecting their moderate-depth, continental slope habitats.

The Graneledoninae are in the western North and South Atlantic, Pacific and Southern Ocean. *Graneledone* has a somewhat bipolar distribution with two species in the colder North Atlantic and North Pacific Subarctic Water and three in Southern Ocean. However, one species occurs north of New Zealand and one off the Gulf of Panama, both in deep water.

The genera *Thaumeledone* and *Benthoctopus* contain the deepest dwelling of all the Incirrata and are found in the Southern Ocean south of the Antarctic Convergence with only one record in Antarctic Deep Water off Argentina.

Of the 77 species of deep-water octopods, 25 percent are confined to the Southern Ocean in Antarctic Water; 22 percent are limited to Atlantic and Pacific Subarctic Water. Ten percent may be considered shallow to moderate depth inhabitants of the warm water regions of the world. The remaining 43 percent occupy the great depths below the tropical and warm temperate regions of the world, living in cold deep water derived from either the Antarctic or the Arctic Deep or Intermediate Water.

The high percentages of species occurring in the Southern Ocean is probably due to the high productivity of the waters between the Antarctic Convergence and the Antarctic continent, with somewhat lower levels northward to the Subtropical Convergence. The same situation is found with regard to the species in the North Atlantic and North Pacific in Subarctic Water. The dearth of species in the Arctic Ocean may well correlate with the lower productivity of the Arctic Ocean in general. If one compares the distributions of the deep-water octopods in the world oceans with the map of ocean productivity as depicted by Ebeling (1962), one finds a high correlation with species richness and high productivity (above 100-200 gC/m^2/year) with few genera and species in less productive waters.

ACKNOWLEDGEMENTS

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GENETIC DIFFERENTIATION AMONG WEST INDIAN POPULATIONS OF THE SCHISTOSOME-TRANSMITTING SNAIL *BIOMPHALARIA GLABRATA*

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ABSTRACT

Genetic variation and population differentiation are described for *Biomphalaria glabrata* from four islands in the West Indies. Estimates of enzyme polymorphism and individual heterozygosity were low relative to many other molluscs and consistent with the geographic isolation of these populations. Total genetic variance for the populations was partitioned as follows: 78% between islands, 2% between samples on islands and 20% between individuals in a sample.

Key words: *Biomphalaria glabrata*, *Schistosoma mansoni*, West Indies, enzyme polymorphism, genetic differentiation.

INTRODUCTION

A study of genetic variation in 28 loci in laboratory stocks of *Biomphalaria glabrata* revealed greater differentiation between some West Indian samples than expected (Mulvey & Vrijenhoek, 1984). In particular, a stock derived from the Dominican Republic (DR) had Nei (1978) genetic distance values of up to 0.43 when compared with stocks derived from Puerto Rico, St. Lucia and Brazil. Most conspecific mollusc populations have genetic distance values of less than 0.10 (Selander & Ochman, 1983). Furthermore, Mulvey & Vrijenhoek (1984) observed partial reproductive incompatibilities between the DR stock and other laboratory stocks of *B. glabrata*. Goldman *et al.* (1984) described chromosomal differences between this DR stock and other *B. glabrata*. To establish the significance of findings based on laboratory stocks, we have examined genetic variation in field-collected populations from four West Indian islands, including the Dominican Republic.

Biomphalaria glabrata is widely distributed throughout northern and eastern South America and the West Indies where it is the most important intermediate host for the human blood fluke, *Schistosoma mansoni* (PAHO, 1968). These snails exist as a series of locally or regionally differentiated races with respect to susceptibility to infection with strains of schistosomes (Kagan & Geiger, 1965; Michelson & DuBois, 1978). Compatibility of host and parasite is often limited to sympatric forms (Basch, 1975, 1976; Woodruff, 1985). Loci differentiation may be promoted by genetic drift associated with ephemeral habitats and the life history characteristics of these snails. The natural history of *B. glabrata* is well known as a result of field studies in Puerto Rico (Jobin, 1979; Pimental & White, 1957) and St. Lucia (Jordan *et al.*, 1978; McKellop & Harrison, 1980, 1982; McKillop *et al.*, 1981). These snails inhabit freshwater marshes, drainage or irrigation ditches, small ponds and slow-flowing streams (McKillop & Harrison, 1980). Suitable aquatic habitat has an inherent patchiness, and dispersal of snails between patches may be restricted. Populations are likely to experience occasional demographic "bottlenecks" due to drought and flooding. As they are potentially self-fertilizing hermaphrodites, a single individual can repopulate a site following a population crash. With a minimum generation time of 8 weeks under optimal conditions and a reproductive potential of 30 eggs/snail/day (Perlowagora-Szumlewicz, 1958), populations are capable of rapid recovery following disturbance (McKellop *et al.*, 1981).

Population structure and genetic variability in *B. glabrata* are important features, as they might affect transmission of the major human parasite, *Schistosoma mansoni*. Michelson & DuBois (1978) suggested that random genetic drift would be important in local differen-

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tiation of snail populations and might be associated with local differences in snail susceptibility to strains of schistosomes. Mulvey & Vrijenhoek (1982) tested this hypothesis by examining seven populations in Puerto Rico. These populations were highly structured; snails exhibited relative genetic homogeneity within populations and differentiation among local populations. Moreover, the snails from the island of Puerto Rico were genetically less variable than those described from Brazil (Narang *et al.*, 1981). An association between reduced genetic variability and genetic differentiation in island populations is not unexpected. The present study was undertaken to ascertain the degree of genetic differentiation occurring among island populations and to clarify the systematic relationships of snails from the various islands, especially the Dominican Republic.

MATERIALS AND METHODS

Specimens

Biomphalaria glabrata were collected with dip net or forceps from 6 locations in the West Indies between 20 August and 4 September 1983 as follows (arranged in geographic sequence from northwest to southeast):

A. Quisquisya, Dominican Republic	roadside ditch
B. Piedra Blanca, Dominican Republic	roadside ditch by cane field
C. Malpica, Puerto Rico	rural stream
D. Gosier, Guadeloupe	small farm pond
E. Bexon, St. Lucia	plantation irrigation ditch
F. Americ, St. Lucia	small hillside stream

Snails were abundant ($> 200/m^2$) at all locations except B and F. In addition, *Biomphalaria havenesis* were collected from a roadside ditch near Las Piedra in the Dominican Republic to serve as an outgroup for analytical purposes. Samples were transported alive to San Diego or stored on dry ice following collection. Voucher specimens are deposited at the University of Georgia's Savannah River Ecology Laboratory.

Electrophoresis

Snails were crushed individually; tissues were removed from the shell and homogenized in 0.2 ml of grinding solution (0.01 M Tris, 0.001 M EDTA, 0.05 mM NADP; pH 7.0). Homogenate fluid was absorbed onto filter paper wicks and inserted into 12.5% horizontal starch gels. Electrophoretic methods, including combinations of buffers and stains, are given elsewhere (Mulvey & Vrijenhoek, 1981a) and, in the case of five previously unstudied proteins, in Table 1. The M stock of *B. glabrata* (NIH albino) was used as a laboratory reference material and mobilities of electromorphs are reported relative to the common allozyme of the M stock which is arbitrarily assigned a value of 100. Typically, individuals from several populations as well as M stock standards were run on each gel to facilitate comparison of alleles across populations.

Statistical Analyses

Data consisting of multilocus genotypes for individual snails were analyzed using the BIOSYS-1 computer program (Swofford & Selander, 1981). A locus was considered polymorphic (*P*) if more than one allele was detected. Mean heterozygosity per individual (*H*) was estimated by direct count. A χ^2 statistic was used to test the fit of the observed data to expectations under a model of panmixia. For loci with three alleles, χ^2 statistics were calculated by pooling the least common alleles. Expected frequencies were calculated using Levene's (1949) correction for small sample sizes. Population structure was examined using F-statistics (Wright, 1978). The total genetic variance was partitioned into the following components: between islands, between subpopulations on islands, and heterozygosity within island subpopulations. Estimates of genetic distances were obtained by the method of Nei (1978) which is unbiased by sample size. Genetic distance values were clustered using the unweighted pair group averaging method.

RESULTS

Eighteen proteins were examined and provided information on 21 loci. Genetic interpretation of electromorph patterns is based on breeding experiments in *B. glabrata* (Mulvey

TABLE 1. Methods used to resolve additional enzyme systems in *Biomphalaria glabrata*.

Enzyme	Abbreviation	Buffer[a]		Stain[b]
Alkaline phosphatase	ALP	TC6.8	50	ml 0.2 M Tris/HCl, pH 8.0
			50	mg β-naphthyl acid phosphate
			50	mg Fast Blue BB
Haemoglobin	HB	TC6.8		Stain: 1.25 g Coomassie blue in fixative (250 ml methanol, 250 ml water, 46 ml acetic acid)
				Destain: several changes of fixative
Isocitrate dehydrogenase	IDH	TC6.8	50	ml 0.2 M Tris/HCl, pH 8.0
			120	mg isocitric acid
			140	mg $MgCl_2$
			10	mg NADP
			10	mg MTT
			5	mg PMS
Nucleoside phosphorylase	NP	TBE	50	ml 0.1 M K-phosphate, pH 6.5
			30	mg inosine
			10	units xanthine oxidase
			10	mg MTT
			5	mg PMS
Xanthine dehydrogenase	XDH	TBE	50	ml 0.2 M Tris/HCl, pH 8.0
			25	mg hypoxanthine
			10	mg NAD
			10	mg MTT
			5	mg PMS

[a]TC6.8 = 0.188 M tris, 0.065 M citrate, pH 6.8; dilute 1:20 for gels and 1:10 for electrode chambers. TBE = 0.5 M tris, 0.65 M borate, 0.2 M EDTA; pH 8.0; dilute 1:10 for gels and use undiluted for electrode chambers

[b]NAD = nicotinamide adenine dinucleotide, NADP = nicotinamide adenine dinucleotide phosphate, MTT = methyl thiazolyl blue, PMS = phenazine methosulphate

& Vrijenhoek, 1984; Mulvey & Woodruff, 1985; Mulvey *et al.*, in press) and descriptions given by Harris & Hopkinson (1976) and Richardson *et al.* (1986). Each of the proteins not previously described for *B. glabrata* (ALP, IDH, HB, NP and XDH) appeared as a single region of activity. Patterns observed for heterozygous individuals were consistent with known subunit structures. Electromorph patterns were identical in tissues frozen in the field or never frozen.

Allozyme frequencies for the seven samples are presented in Appendix A, and summary statistics describing genetic variation are presented in Table 2. Eight loci were monomorphic for all *B. glabrata* examined. Thirteen loci were variable; *Gap, Got-1, Ldh, Mdh-1, Me, Np, Pgd* and *Xdh* had two alleles, and *Est-2, Idh, Pgm-1* and *Pgm-2* had three alleles among populations of *B. glabrata*. The Dominican Republic population of *B. havenensis* had 16 monomorphic loci and was diallelic at five loci (*Gpd, Got-1, Pgd, Pgm-1* and *Pgm-2*). Average individual heterozygosity (*H*) ranged from 0.000 to 0.037 for populations of *B. glabrata* and was 0.006 for the *B. havenensis* population.

Genotype frequencies in each sample were generally in agreement with expectations for random mating. Twenty-eight χ^2 tests for fit to Hardy-Weinberg expectations were performed and no statistically significant deviations were observed. Values for F-statistics for the six populations of *B. glabrata* are presented in Table 3. The mean F_{it} value of 0.838 reflects a larger contribution by F_{st} (between population differentiation) and little contribution by F_{is} (within population differentiation) to the overall fixation index. A hierarchical G-statistic analysis indicates that 78% of the total genetic variance can be accounted for between islands, 2% between samples on islands, and 20% between individuals in samples.

Genetic distance values are presented in Table 4. Values for populations of *B. glabrata* ranged from 0.00 to 0.18. The interspecific genetic distances for *B. glabrata* and *B. havenensis* were all greater than 0.47.

The two St. Lucia samples exhibited no detectable enzyme activity for the *Idh* locus. When tissue from St. Lucia snails was electrophoresed and stained for IDH activity, the gel remained unstained; M stock controls or snails from other islands run alongside showed good activity. An attempt to demon-

TABLE 2. Summary statistics of genetic variation among West Indian populations of *Biomphalaria glabrata*. See text for locations. *P* = % polymorphic loci; > one allele detected. *H* = mean heterozygosity by direct count

Population	Sample Size	Mean No. alleles/locus (± 0.1)	*P*	*H*
Biomphalaria glabrata				
A. Dominican Republic	33.4 ± 1.0	1.2	19.0	0.027
B. Dominican Republic	38.8 ± 1.2	1.3	23.8	0.022
C. Puerto Rico	29.4 ± 1.5	1.3	23.8	0.011
D. Guadeloupe	32.5 ± 2.8	1.1	9.5	0.003
E. St. Lucia	36.9 ± 3.0	1.1	9.5	0.003
F. St. Lucia	37.5 ± 3.2	1.2	23.8	0.037
Biomphalaria havenesis				
G. Dominican Republic	33.0 ± 2.2	1.3	23.8	0.006

strate IDH activity in St. Lucia snails was made by varying conditions of electrophoresis and modifying the staining solution but no activity could be detected. As the St. Lucia samples showed strong activity when stained for all other enzyme systems, an allelic designation of "null" has tentatively been given to these snails for the *Idh* locus.

DISCUSSION

The levels of genetic polymorphism among populations of *B. glabrata* from four islands in the West Indies are in the lower range of values reported for 28 terrestrial and freshwater pulmonate snails (Selander & Ochman, 1983). Mean individual heterozygosity (*H*) in populations of *B. alexandrina* in Egypt (Graven, 1984) and *B. straminea* in Hong Kong (Woodruff *et al.*, 1985) were 0.04–0.09 and 0.06–0.10, respectively. Narang *et al.* (1981) reported that populations of *B. glabrata* in Brazil had levels of allozyme polymorphisms (*P*) that ranged from 0.18 to 0.48 and levels of individual heterozygosity of 0.076 to 0.211. Although there is only partial overlap for the enzymes used in these studies, the lower levels of genetic variation in the West Indian populations are consistent with their island distribution as geographic isolation reduces the probability of dispersal among populations. Estimates of average individual heterozygosity ranged from 0.00 to 0.04. These values are also at the lower end of the range reported for molluscs (Selander & Ochman, 1983). As discussed by Simon & Archie (1985), estimates of *P* and *H* are strongly dependent on the loci chosen as well as sample size. Thus differences in proteins studied in the studies of *Biomphalaria* may account for some of the differences in *P* and *H* estimates.

Selfing is probably not a significant contributor to population structure among these island populations. Although a functional hermaphrodite, *Biomphalaria* is a preferential outcrosser, capable of multiple matings and sperm storage (Mulvey & Vrijenhoek, 1981b). These attributes tend to balance the pressures of small population size which would otherwise reduce genetic diversity. Population structure, as determined by F-statistics, followed a predictable pattern: differentiation among islands > among local populations > within populations.

As expected, Nei's genetic distance values were highest for the between species comparison, D = 0.54. Values for conspecific populations of *B. glabrata* ranged from 0.00 to 0.18. Estimates of genetic distances involving the Dominican Republic and St. Lucia populations have bearing on questions raised by previous work about the relationships of these populations to other *B. glabrata*. Mulvey & Vrijenhoek (1984) studied ten laboratory stocks of *B. glabrata* and found average genetic distance values for stocks originating from the Dominican Republic (DR) and St. Lucia (L-311) to be 0.29 and 0.21, respectively from other stocks of *B. glabrata*. These values are quite high for conspecific comparisons and are more often reported for comparisons of congeneric species. The laboratory stocks previously examined had, however, been isolated many generations and may have undergone genetic divergence reflecting one or more founder events and/or genetic drift or selection associated with maintenance under artificial laboratory conditions. Snails collected in the

TABLE 3. F-statistics for six West Indian populations of *Biomphalaria glabrata* based on 13 polymorphic loci.

Locus	F_{is}	F_{it}	F_{st}
Est-2	0.056	0.888	0.881
Gap	0.200	0.234	0.043
Got-1	−0.014	−0.002	0.011
Idh	–	1.00	1.00
Ldh	−0.231	−0.032	0.161
Lap	−0.036	−0.006	0.029
Mdh-1	−0.014	−0.002	0.011
Me	0.114	0.154	0.045
Np	−0.032	−0.005	0.026
Pgd	0.361	0.976	0.962
Pgm-1	0.344	0.724	0.579
Pgm-2	0.646	0.651	0.013
Xdh	−0.014	−0.004	0.009
Mean	0.171	0.838	0.805

TABLE 4. Genetic distance values (Nei, 1978) for six populations of *B. glabrata* and one population of *B. havenensis*.

	B	C	D	E	F	G
B. glabrata						
A. Dominican Republic	0.002	0.003	0.164	0.143	0.142	0.532
B. Dominican Republic	–	0.000	0.174	0.134	0.132	0.547
C. Puerto Rico		–	0.180	0.139	0.138	0.554
D. Guadeloupe			–	0.091	0.093	0.469
E. St. Lucia				–	0.002	0.560
F. St. Lucia					–	0.555
B. havenesis						
G. Dominican Republic						–

field from the Dominican Republic and St. Lucia and compared with M stock snails had genetic distance values of 0.17 and 0.16, respectively. Field samples thus showed lower levels of genetic differentiation. The DR stock apparently derived from collections made from ponds at the Botanical Gardens in Santo Domingo. The Quisquisya and Piedra Blanca sites are approximately 135 and 150 km respectively, from Santo Domingo, therefore, these samples may not be directly comparable to the DR stock. Among the collections of *B. glabrata* from four islands the genetic distance values were all less than or equal to 0.20; such values are similar to those obtained for conspecific populations of mammals, rodents and molluscs (Nevo, 1978).

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Appendix A. Allele frequencies in West Indian populations of *B. glabrata* and *B. havenensis*. – designates cathodal migration.

Locus/Allele	Population[a]						
	A	B	C	D	E	F	G
Aconitase-2							
100	1.00	1.00	1.00	1.00	1.00	1.00	
130							1.00
Alkaline Phosphatase							
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Aspartate amino transferase-1							
100	1.00	1.00	1.00	1.00	1.00	1.00	0.95
167							0.05
Aspartate amino transferase-2							
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Esterase-2							
50	1.00	1.00	1.00	0.03			
80							1.00
100				0.95	0.83	1.00	
160				0.02	0.17		
Glyceraldehyde-3-phosphate dehydrogenase							
118		0.07	0.04				
110							1.00
100	1.00	0.93	0.96	1.00	1.00	1.00	
α-Glycerophosphate dehydrogenase							
140							0.05
100	1.00	1.00	1.00	1.00	1.00	1.00	0.95
Hemoglobin							
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Isocitrate dehydrogenase							
100						1.00	1.00
114	1.00	1.00	1.00				
Null				1.00	1.00		
Lactate dehydrogenase							
100	1.00	1.00	1.00	1.00	0.81	1.00	1.00
86					0.19		
Leucine amino peptidase							
100	0.97	1.00	1.00	1.00	1.00	1.00	1.00
96	0.03						
Malate dehydrogenase-1							
235							1.00
100	0.99	1.00	1.00	1.00	1.00	1.00	
22	0.01						
Malate dehydrogenase-2							
–100	1.00	1.00	1.00	1.00	1.00	1.00	
–115							1.00
Malic enzyme							
58	0.02	0.09	0.06				
100	0.98	0.91	0.94	1.00	1.00	1.00	
122							1.00
Mannose-6-phosphate isomerase							
100	1.00	1.00	1.00	1.00	1.00	1.00	
132							1.00
Nucleoside phosphorylase							
100	1.00	1.00	1.00	1.00	0.97	1.00	1.00
117					0.03		
6-Phosphogluconate dehydrogenase							
92	1.00	1.00	0.94				
100			0.06	1.00	1.00	1.00	
283							0.97
317							0.03

Appendix A continued

Locus/Allele	Population[a]						
	A	B	C	D	E	F	G
Phosphoglucomutase-1							
92	0.07	0.32	0.08	0.02	0.02	0.98	0.99
100	0.89	0.68	0.88	0.98	0.98	0.02	0.01
106	0.04		0.04				
Phosphoglucomutase-2							
100	1.00	1.00	1.00	1.00	0.98	0.99	
229						0.01	0.04
200							0.94
187							0.02
133					0.02		
Phosphoglucose isomerase							
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Xanthine dehydrogenase							
100		0.02	0.01				
82	1.00	0.98	0.99	1.00	1.00	1.00	1.00

[a]*Biomphalaria glabrata* (A–F): A = Quisquisya, DR; B = Piedra Blanca, DR; C = Malpica, PR; D = Gosier, Guadeloupe; E = Bexon, St. Lucia; F = Americ, St. Lucia; *Biomphalaria havenensis*, G = La Cambia, DR

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THE KARYOLOGY OF *LITTORINA NERITOIDES* (LINNAEUS, 1758) (MOLLUSCA, PROSOBRANCHIA).

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ABSTRACT

From analysis of spermatocyte and oocyte bivalents the haploid number n = 17 has been determined for *Littorina neritoides* (L.) from the Sicilian coast. The diploid number 2n = 33 and the karyotype of male specimens were obtained from mitotic metaphases of gonad tissue with the cellular suspension technique. A male XO sex-determining mechanism is proposed for this species.

Key words: *Littorina neritoides;* karyology; Italy; sex determination

INTRODUCTION

The chromosome information on Littorinidae (Mollusca: Gastropoda: Prosobranchia: Mesogastropoda), briefly summarized in Table 1, indicate that this family has been only partly studied. In particular, within the genus *Littorina,* both the haploid and diploid chromosome numbers have been reported only for one out of the five species examined.

There still is no agreement as to the number of chromosomes characterizing *Littorina neritoides.* The haploid number n = 16 was proposed for male specimens from the Gulf of Palermo, Italy (Vitturi & Catalano, 1984), and n = 17 and 2n = 34 for male specimens collected along the coast near Villefranche-sur-Mer, France (Thiriot-Quievreux & Ayraud, 1982).

According to Thiriot-Quievreux & Ayraud (1982), the specimens of the latter population possessed 8 pairs of metacentric, 2 submetacentric and 6 acrocentric chromosomes. Due to the small size, the morphology of the 17th pair was not identified.

With the aim of analyzing in more detail the cytological condition of this species, the karyology of *L. neritoides* from two different localities of the Sicilian coast has been investigated.

MATERIAL AND METHODS

One hundred sexually mature specimens of *Littorina neritoides* (54 males and 46 females), collected in December 1986 on the rocks along the coast of Sferracavallo (Palermo) and of Castellammare del Golfo (Trapani), were employed. Taxonomic identification of the specimens was made according to the guidelines of Parenzan (1970), and voucher shells of 20 specimens were deposited at the Museum of the Institute of Zoology of the University of Palermo.

Meiotic chromosomes were obtained by treating testes and ovaries according to the squashing technique described for other molluscan species (Vitturi *et al.,* 1983; Vitturi & Catalano, in press). Spermatogonial metaphase chromosomes were prepared following the air-drying technique adopted by Thiriot-Quievreux & Ayraud (1982) and were interpreted on the basis of arm ratio as suggested by Levan *et al.* (1964).

Observation and photomicrography were carried out with the aid of a Wild phase-contrast microscope.

OBSERVATIONS

Mitotic chromosomes

Spermatogonial metaphase chromosomes were observed and their count gave 33 as the diploid number (Table 2). All the elements appeared well separated and randomly distributed on the squashing plane (Figs. 1b, 2b). In order to obtain the average karyotype (Fig. 4) (Table 3), the chromosomes of five photomicrographs were cut and arranged on the basis of their decreasing size and centromere position (Figs. 1a,b; 2a,b; two plates are repre-

TABLE 1. Chromosome numbers of eight species of the family Littorinidae.

Species	n	2n	Source	Reference
Order Mesogastropoda				
Superfamily Littorinoidea				
Family Littorinidae				
Nodilittorina picta	15		Japan	Nishikawa, 1962
Nodilittorina granularis	18		Japan	Nishikawa, 1962
Littoraria strigata	17		Japan	Nishikawa, 1962
Littorina brevicula	17		Japan	Nishikawa, 1962
Littorina neritoides	17	34	France	Thiriot-Quievreux & Ayraud, 1982
Littorina neritoides	16		Italy	Vitturi & Catalano, 1984
Littorina saxatilis		34	Sweden	Janson, 1983
Littorina obtusata		34	Sweden	Janson, 1983
Littorina punctata	16		Italy	Vitturi *et al.*, 1986a

TABLE 2. Number of chromosomes found in 135 metaphase plates observed for *Littorina neritoides*.

	n	≤15	16	17	18
Spermatocyte bivalents	frequence	5	25	38	1
Oocyte bivalents	frequence	1	2	25	
	2n	≤31	32	33	34
Spermatogonial chromosomes	frequence	3	6	27	2

sented). The analysis revealed that the male karyotype of *L. neritoides* consisted of 10 metacentric pairs, 2 sub-metacentric, 3 subtelocentric, 1 acrocentric and one small submetacentric unpaired element, NF = 60. Furthermore, the 13th submetacentric pair often appeared to be polymorphic owing to the presence of a probable satellite element that was slightly longer than its presumed homologue (Figs. 2a, 3, see arrow).

Mitotic polyploid plates were observed (Fig. 5). Sometimes also in these spreads it was possible to note a sub-metacentric element that probably had long-arm satellites (Fig. 5, the element indicated by the arrow belongs to another polyploid plate). As we counted about 66 chromosomes, we considered these spreads as tetraploid.

At mitotic prophase the chromosomes showed a regular outline (Fig. 6).

Meiotic chromosomes

Pachytene was the first spermatogenetic stage we observed (Fig. 7). In addition to the tight pairing of homologous chromosomes, the entire outlines of these bivalents were irregular. We were unable to determine the haploid value due to overlap of some elements.

The count of spermatocyte bivalents at early diakinesis gave the haploid number of n = 17 (Figs. 8,9) (Table 2). In all these plates one element differed from the others in its small size of about 0.6 μm, and there was evidence that an end-to-end connection between this chromosome and another bivalent of the same plate often occurred (Figs. 8, 9, see arrows). Two large, ring-shaped bivalents with probably two terminal chiasmata were observed (Fig. 9). The cross-like morphology of numerous elements was perhaps due to the phenomenon of pre-metaphase stretch. Rod-shaped elements with round extremities were present.

Since in numerous spreads the small chromosome showed the same morphology observed for the unpaired spermatogonial element, following the suggestion of Baker & Callen (1950), we thought it to be a "univalent."

At late diakinesis several plates seemed to possess the haploid number n = 16 (Fig. 10) (Table 2).

The dimensions of the bivalents ranged from 1.8 μm to 3.7 μm.

Oocyte bivalents at metaphase-I (Fig. 11) appeared well separated from one another and their number was n = 17 (Table 2). No elements clearly differed from the others in

TABLE 3. Mean length and arm ratio of the chromosomes of five metaphase plates of *Littorina neritoides*.

Chromosome pairs	Mean length in microns ± SD	Arm ratio mean	Centromere position
1	4.88± 1.30	1.17	M
2	4.19± 1.14	1.62	M
3	3.44± 0.83	1.37	M
4	3.13± 0.85	3.17	ST
5	2.81± 0.77	3.32	ST
6	2.69± 0.75	00	A
7	2.69± 0.75	1.33	M
8	2.56± 0.72	3.65	ST
9	2.38± 0.63	1.26	M
10	2.25± 0.61	1.14	M
11	2.13± 0.52	1.13	M
12	2.06± 0.43	1.36	M
13	2.04± 0.43	1.81	SM
14	1.93 ± 0.41	1.20	M
15	1.83 ± 0.35	2.17	SM
16	1.73± 0.38	1.09	M
17	1± 0.37	2.45	SM

dimensions as we observed at least two or three small elements quite similar in size. All these chromosomes showed the bivalent appearance: ring-, cross-, and rod-shaped. In the latter case, a thin area (Fig. 11, see arrows) might indicate the junction point between the two homologues.

The bivalent indicated by two arrows (Fig. 11) probably possessed one medial chiasma. The dimensions of these chromosomes varies from 2.2 μm to 3.7 μm.

DISCUSSION

In the present paper the same results (2n = 33 in males, n = 17 in both sexes) for *Littorina neritoides* from the two sites at Sferracavallo and Castellammare del Golfo have been obtained.

Our analysis suggests the following cytological features: (1) pachytene chromosomes resembling "lampbrush chromosomes" indicate that *L. neritoides* should be listed among the molluscan species with this peculiarity (Vitturi, 1982; Vitturi *et al.*, 1982; Vitturi *et al.*, 1985); (2) some bivalents of both sexes appear to be chiasmatic; (3) distant somatic pairing between homologous chromosomes at the mitotic metaphase stage, a common phenomenon in the animal kingdom (Kitani, 1964; Colombera, 1973), has not been observed. The same result was previously reported for *Teredo utriculus* (Mollusca, Bivalvia) (Vitturi *et al.*, 1983), *Bursatella leachi leachi* and *Bursatella leachi savignana* (Mollusca: Opisthobranchia) (Vitturi *et al.*, 1985), and for some neogastropod molluscan species (Vitturi *et al.*, 1987), and (4) since only in colchicinized chromosome preparations have tetraploid metaphase plates been seen, the possibility of colchicine have a polyploid action cannot be excluded. In a previous paper (Thiriot-Quievreux & Ayraud, 1982), diakinetic polyploid spreads were described for *L. neritoides*, but in that case also colchicine was used.

From the counts of male and female bivalents the n = 17 haploid number of chromosomes has been determined for the species studied here; this result confirms the datum proposed for the population from Villefranche-sur-Mer, France (Thiriot-Quievreux & Ayraud, 1982).

The n = 16 spermatocyte haploid value given in the paper by Vitturi & Catalano (1984) for *L. neritoides* from the Gulf of Palermo might be explained as a result of the difficult identification of the small-sized element at diakinesis.

The diploid value 2n = 33 consistently observed by us at the spermatogonial metaphase stage is not in agreement with 2n = 34 suggested by Thiriot-Quievreux & Ayraud (1982) for this species at the same stage. This divergence would seem to indicate that *L. neritoides* is a polymorphic species. Due to the presence of the diploid numbers 2n = 33 and 2n = 34, one would hypothesized that a Robertsonian traslocation has occurred. Moreover, autosomal polymorphism in chromosome structure in *Mytilus edulis* and *Mytilus californianus* (Ahmed & Sparks, 1970), and different chromosome numbers for *Isognomon alatus* (n = 26, 28) (Bivalvia: Isognomonidae) (Duran-Gonzales *et al.*, 1984) and for *Purpura lapillus* (Prosobranchia: Neogastropoda) (n = 13,18) (Staiger, 1950; 1954) have been reported. Still, a Robertsonian chromosomal polymorphism has been widely described in fishes (Chen, 1971; Black & Howell, 1978; Ojima & Kashiwagi, 1981; Hartley & Horne, 1984, and authors quoted by them; Vitturi *et al.*, 1984; Vitturi *et al.*, 1986b). The hypothesis of a Robertsonian fusion in *L. neritoides* can, however, be discounted because of the different fundamental numbers in the populations examined, more precisely NF = 54–56 and NF = 60 in *L. neri-*

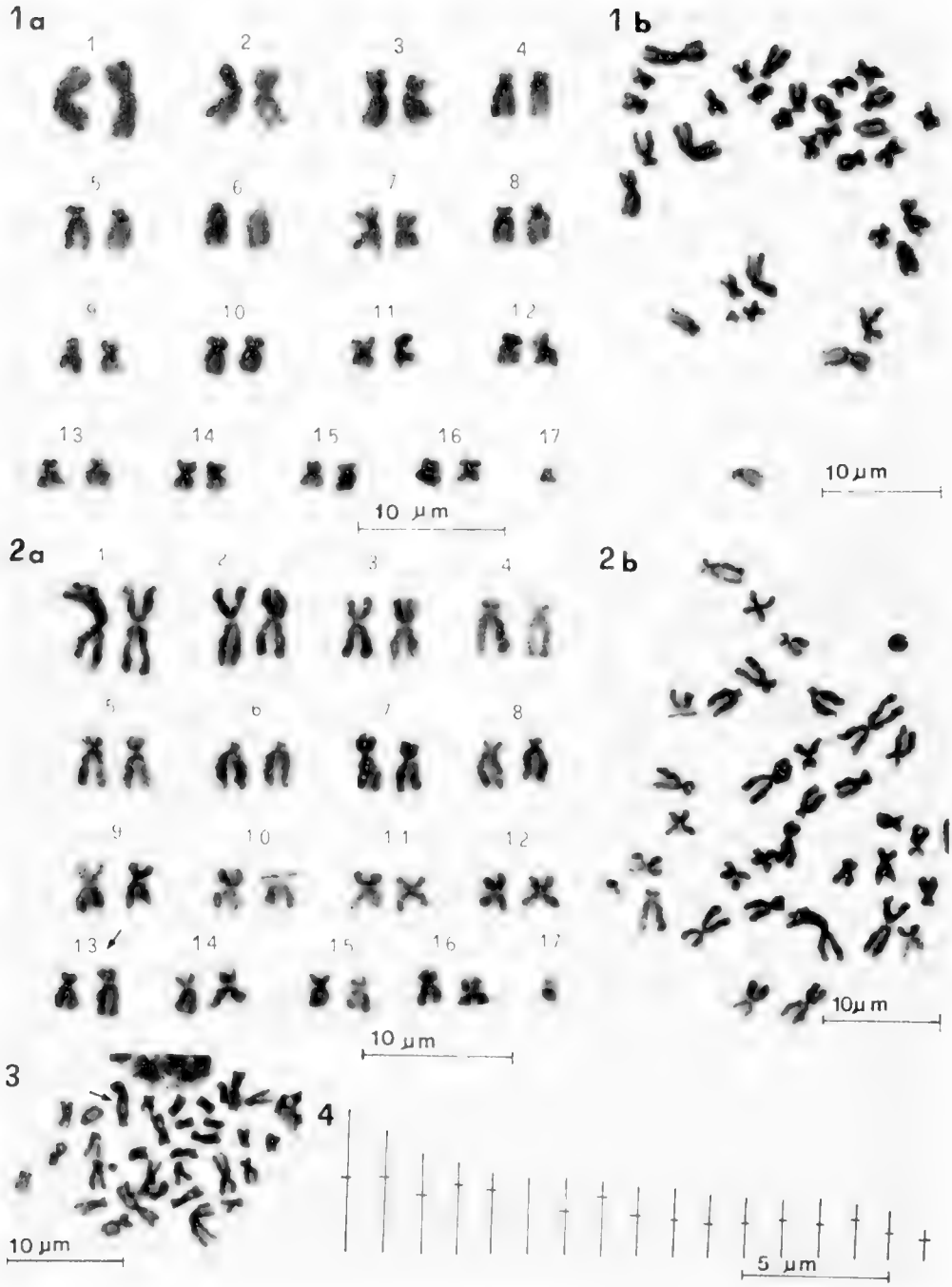

FIG. 1a, 2a—Male representative karyotypes of *L. neritoides*.
FIG. 1b, 2b—Spermatogonial metaphase plates of *L. neritoides*.
FIG. 3—Spermatogonial metaphase plate of *L. neritoides* (arrow indicates a long-arm satellited chromosome).
FIG. 4—Average karyotype obtained from five spermatogonial metaphase plates of *L. neritoides*.

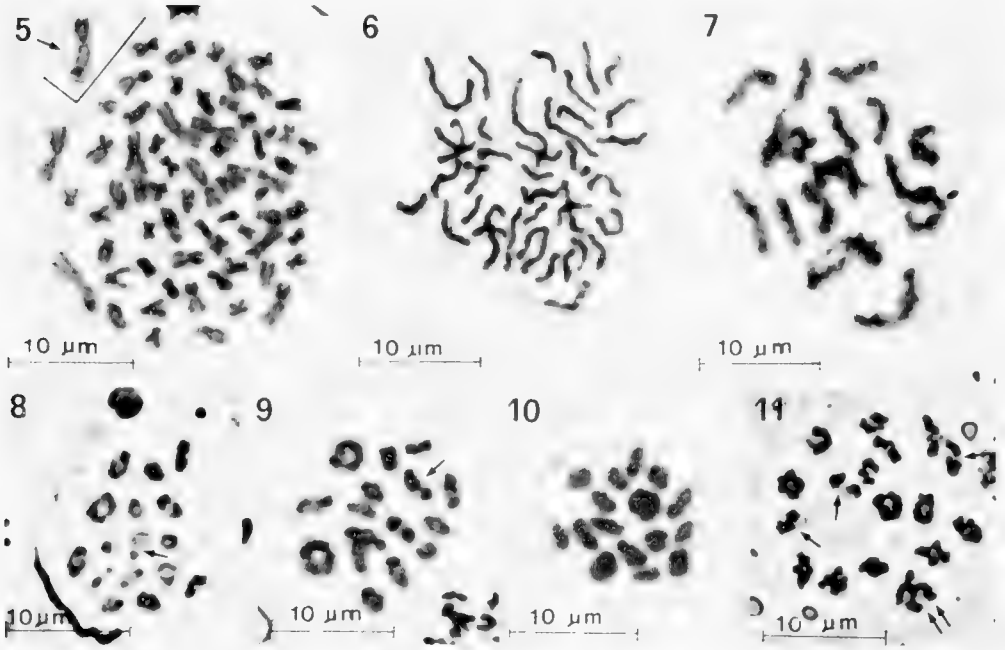

FIG. 5—Tetraploid mitotic metaphase plate in male gonads of *L. neritoides*.
FIG. 6—Mitotic prophase chromosomes in male gonads of *L. neritoides*.
FIG. 7—Pachytene chromosomes in male gonads of *L. neritoides*.
FIG. 8, 9—Early diakinesis in male gonads of *L. neritoides* (arrow indicates an end-to-end connection between two bivalents).
FIG. 10—Late diakinesis in male gonads of *L. neritoides*.
FIG. 11—Oocyte bivalents of *L. neritoides*.

toides from Villefranche-sur-Mer and from the Sicilian coast respectively.

Really, one must interpret the results of Thiriot-Quievreux & Ayraud (1982) with caution as in the karyotype figure by these authors the homologous chromosomes of the 17th pair seem to differ from one another in dimension and in morphology as well.

In order to explain our results, we suggest a male XO sex-determining mechanism in *L. neritoides*. This assumption appears to be upheld not only by the occurrence of a small unpaired chromosome at the spermatogonial metaphase stage, but also by the morphology of oocyte bivalents which allows the supposition of a diploid number of 34 chromosomes in female specimens. Actually, a sex-determining mechanism of this type does not represent an isolated case within the Prosobranchia. In fact, a male XO sex-system is regularly observed in the family Neritidae (Prosobranchia: Archaeogastropoda) (Vitturi & Catalano, in press, and authors quoted by them).

It is of some interest to point out that the finding of the same sex-mechanism in Littorinidae (Mesogastropoda) and Neritidae (Archaeogastropoda) makes still more debatable the uncertain taxonomic position of the latter family (Morton & Yonge, 1964; Fretter, 1965; Franc, 1968; Vitturi & Catalano, in press).

Finally, on the basis of the data now at hand on *L. neritoides*, we think it useful for further cytological analyses to be made of those *Littorina* species that to date have been only partly studied (Janson, 1983; Vitturi *et al.*, 1986a).

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CHARACTER VARIATION IN A COMPLEX OF RISSOID GASTROPODS FROM THE UPPER CONTINENTAL SLOPE OF THE WESTERN NORTH ATLANTIC

Michael A. Rex[1], M. Campbell Watts[1], Ron J. Etter[2] & Susan O'Neill[1]

ABSTRACT

Four deposit-feeding rissoid gastropod species coexist on the upper continental slope (478–1102 m) south of New England. A biometrical analysis revealed pronounced geographic variation in larval and adult shell size and adult sculpture. Differentiation correlates more with depth differences of hundreds of meters than with horizontal separation on scales of tens of kilometers. *Frigidoalvania brychia*, the largest and most abundant species, shows a striking increase in sculptural variation with increased depth. Sculptural expression expands in the direction of shell forms more characteristic of the other three species as these species decrease in relative abundance. A consideration of character variation, dispersion of size ratios, relative abundance and density suggests that interspecific competition plays a role in structuring the rissoid complex. The upper slope is a potentially important site of population differentiation for the deep-sea gastropod fauna.

Key words: Gastropoda; Rissoidae; deep sea; geographic variation; evolution; competition.

INTRODUCTION

" . . . the foundation of most evolutionary theory rests upon inferences drawn from geographic variation or upon the verification of predictions made about it."

S. J. Gould & R. F. Johnston, 1972

Studies of geographic variation have played a fundamental role in elucidating evolutionary mechanisms for species inhabiting terrestrial and coastal-marine environments. However, little is known about even the most basic patterns of geographic variation in the deep-sea benthos. In this paper we measure character variation in shell form among four sympatric rissoid gastropods collected from the upper continental slope south of New England. Our aims are to document the geographic scales of depth and horizontal distance over which significant phenotypic divergence occurs, and to explore morphological evidence that variation is mediated through selection imposed by competition among the species.

The four species, *Frigidoalvania brychia* (Verrill, 1884), *Onoba pelagica* (Stimpson, 1851), *Pusillina harpa* (Verrill, 1880) and *Pusillina pseudoareolata* (Warén, 1974), present a unique opportunity to study morphological variation in deep-sea snails inhabiting the upper slope. In extensive collections dredged south of New England by vessels of the Woods Hole Oceanographic Institution (Sanders, 1977), this is the only case in which four confamilial snail species were retrieved from the same samples at abundances high enough for statistical analysis. All four species are deposit feeders (Rex, 1976) and have lecithotrophic development (Warén, 1974; Rex & Warén, 1982). All have simple ventricose shells (*sensu* Fretter & Graham, 1962) of 2–3 whorls and are similar in size. These shared features of life style and morphology suggest that the assemblage is a guild of competing species much like sympatric members of *Hydrobia*, another deposit-feeding rissoacean taxon (Fenchel, 1975; Fenchel & Kofoed, 1976).

Our attention was drawn to this group by a striking pattern of geographic variation in *Frigidoalvania brychia*. It is the most abundant prosobranch living on the upper continental slope south of New England (Rex & Warén, 1982). It shows little variation in sculpture at the upper limit of its bathymetric range (500 m), where it coexists with the other three species. With increasing depth, the relative abun-

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dance of the three other species decreases, and the sculptural variation of *F. brychia* increases dramatically to include forms more typical of its relatives. At 1100 m, *F. brychia* becomes the most variable deep-sea prosobranch known. The geographic changes in abundance and shell morphology within the complex suggested that *F. brychia* may experience ecological release manifested as increased morphological variation (*sensu* Van Valen, 1965; Grant, 1972) in the deeper parts of its range. Interspecific competition has been proposed as an important determinant of community structure on the upper continental slope of the western North Atlantic (Rex 1976, 1977, 1983; Huston, 1979). Our biometrical analysis quantifies geographic variation in this group and suggests that observed patterns are shaped, in part, by interspecific competition.

BIOGEOGRAPHIC SETTING

Sampling Localities

The snails were collected with an epibenthic sled (Hessler & Sanders, 1967) at five stations on the upper continental slope south of New England. Station data and abundances of the species are presented in Table 1, and the station localities are plotted in Fig. 1. Among all five stations the average horizontal separation is 22.26 km (range 4.09—38.06 km), and the average depth separation is 311.60 m.

The continental slope south of New England is a topographically complex and dynamic environment (MacIlvaine & Ross, 1979; Knebel, 1984) extending from the shelf-slope transition (200 m) to about 2000 m where the more gradually descending continental rise begins (Fig. 1). The gentle gradient (1–2°) of the upper slope steepens to 7.6° at about 1000 m. The slope is traversed by numerous submarine canyons, one of which is near station 87. The surface of the upper slope is smooth on horizontal scales of kilometers, but irregular features related to erosional gullies, and sediment slump scars measuring tens of meters are common (MacIlvaine & Ross, 1979). At mid-slope depths, massive slumping caused by the steepening grade has produced uneven relief on scales of hundreds of meters and exposed underlying rock formations in places.

We do not know the specific sedimentary regimes at our stations because the epibenthic sled filters out fine sediments from samples. However, general characteristics of sediments in this region are well known (Sanders *et al.*, 1965; MacIlvaine & Ross, 1979; Maciolek *et al.*, 1986). Erosion by active bottom currents and sediment spillover from the continental shelf have resulted in coarse-grained sediments (predominantly sands) at the shelf-slope transition. From 500 m to mid-slope depths there is a decrease in mean grain size and the percentage of sand, from sandy silts and silty sands to clayey silts, as erosional processes give way to the influences of hemipelagic sedimentation. Currents become less pronounced and sediments are highly resistant to erosion.

Finer sediments found toward mid-slope depths have a higher organic content (Maciolek *et al.*, 1986), suggesting that more food may become available to deposit feeders with increased depth. However, the opposite appears to be true: density of the macrofauna as a whole declines exponentially with depth across the upper slope (Rex, 1983; Maciolek, *et al.*, 1986), and the density of the rissoid complex drops by an order of magnitude (see below). The exact causes of this marked decrease in density with depth are unclear. Rates of community-wide respiration (Smith & Hinga, 1983) and bacterial degradation (Jannasch & Wirsen, 1983) decrease with depth in the deep sea. Particulate organic carbon flux to the bottom and the percentage of this utilized by the benthos both tend to decrease with depth as well, but the rates show considerable spatio-temporal variation and are poorly known for the narrow region of the upper slope (Pace *et al.*, 1987; Smith & Hinga, 1983). Whatever the cause, it seems clear from the pattern of density that the food resource base available to the complex becomes more limited with increasing depth.

Distributional Patterns

In general, all four species are distributed in deep water off northeastern North America (Warén, 1974; Ponder, 1985). *Frigidoalvania brychia,* the most abundant species at all five sites in this study (Table 1), makes up approximately half the individuals in the assemblage at the shallowest stations (88, 96 and 105). *Onoba pelagica, Pusillina harpa* and *P. pseudoareolata* make up varying proportions of the remaining half. At the intermediate depth (sta. 207), *F. brychia* increases in relative

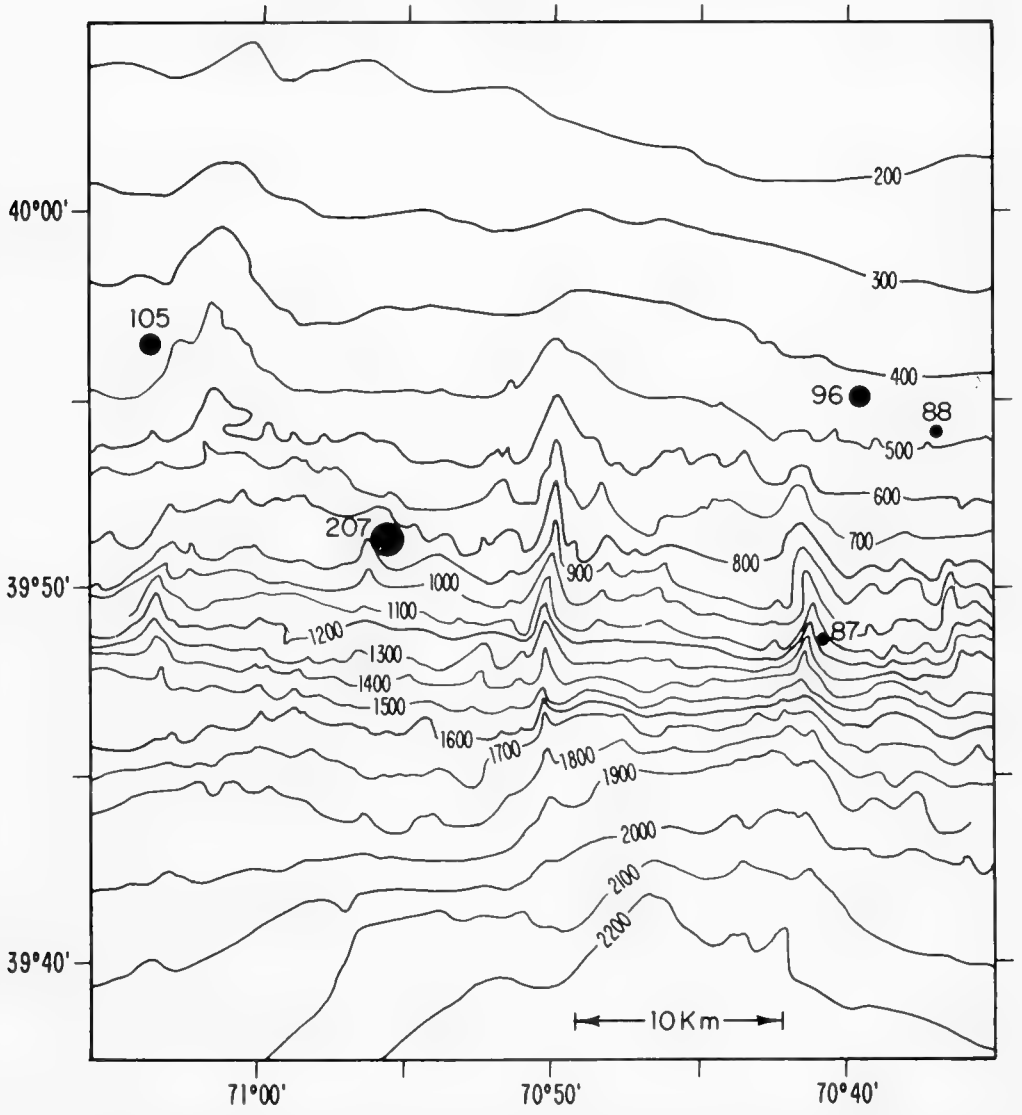

FIG 1. Sample localities for upper-slope rissoids. Contours (in meters) are adapted from MacIlvaine & Ross (1979) and are reproduced with permission of the authors and the Society of Economic Paleontologists and Mineralogists. Diameters of the station localities (circles) correspond to the length of the epibenthic sled tow estimated from time on the bottom. Slight discrepancies between the depths cited in Table 1 and the contour lines result from navigational and sounding errors and small-scale topographic variation.

abundance to 57%. The two species of *Pusillina* remain abundant, but *O. pelagica* becomes quite rare. At 1102 m, *F. brychia* is the only member of the complex present.

The epibenthic sled is a non-quantitative sampling device that is towed for a kilometer or more on the bottom. Hence, the occurrence together of the rissoid species in sled samples does not necessarily imply local coexistence; for example, the sled could have traversed several different habitat patches each with its own distinctive rissoid fauna. A recent extensive sampling program in the western North Atlantic using quantitative 0.25 m^2 box

TABLE 1. Station data and relative abundances for rissoids from the upper continental slope south of New England. Station coordinates are for the beginning of the trawl. Station localities are plotted in Fig. 1.

				Frigidoalvania brychia			*Pusillina harpa*		
Station no.	Latitude (N)	Longitude (W)	Depth (m)	Number of individuals	% of total	Rank*	Number of individuals	% of total	Rank
88	39°54.1′	70°37.0′	478	17	46	4	11	30	5
96	39°55.2′	70°39.5′	498	146	46	1	101	32	3
105	39°56.6′	71°03.6′	530	171	52	1	93	28	3
207	39°51.3′	70°54.3′	808	5318	57	1	2659	28	2
87	39°48.7′	70°40.8′	1102	636	100	1	—	0	—

				Pusillina pseudoareolata			*Onoba pelagica*		
Station no.	Latitude (N)	Longitude (W)	Depth (m)	Number of individuals	%of total	Rank	Number of individuals	%of total	Rank
88	39°54.1′	70°37.0′	478	6	16	10	3	8	13
96	39°55.2′	70°39.5′	498	27	9	9	42	13	7
105	39°56.6′	71°03.6′	530	—	0	—	65	20	4
207	39°51.3′	70°54.3′	808	1410	15	4	11	<1	20
87	39°48.7′	70°40.8′	1102	—	0	—	—	0	—

*Rank in total sample (includes all gastropod species collected)

corers (Maciolek *et al.*, 1986) has shown that the species, in fact, do coexist on very small spatial scales. Eighty-three box core samples were taken on the upper to mid-slope south of New England centered on depths of 255 m, 550–560 m and 1220–1250 m. The fauna was sampled from nine 10×10 cm subcores of each box core (a surface area of 0.09 m^2). Members of the rissoid complex were found in 34 (41%) of the 83 samples. The overall pattern of occurrence was identical to that from sled samples: *Onoba pelagica* was the only species found at 255 m (Rex, 1979), all four were at 550–560 m, and only *Frigidoalvania brychia* at 1220–1250 m. At 550 m, pairs of species were found in five box cores (*F. brychia* and *Pusillina pseudoareolata*, or *F. brychia* and *O. pelagica*), and a trio was recovered from one sample (*F. brychia, P. harpa* and *O. pelagica*). Coexistence clearly occurs on centimeter scales. The box core samples also showed that the density of the assemblage drops from 33.3 m^{-2} at 255 m, to 16.7 m^{-2} at 550–560 m, and 2.5 m^{-2} at 1220–1250 m.

A NOTE ON SYSTEMATICS

We have used the taxonomic names presented in Ponder's (1985) recent review of rissoid genera. His revisions of *Frigidoalvania, Onoba* and *Pusillina* are based on the internal anatomy, radula, operculum and shell form of representative species, including those analyzed in the present paper. Ponder relied on material from station 207 (Table 1) for the overall morphology of *P. harpa* and *P. pseudoareolata*, and aspects of the internal anatomy of *F. brychia;* and he kindly confirmed our species identifications.

Warén (1974) illustrated the wide range of shell variation in *Frigidoalvania brychia,* but referred more boldly sculptured forms to *F. americana* (Friele, 1886) and smoother forms to the "very closely related" (p. 127) *F. brychia* (Verrill, 1884). Examination of additional material led Warén (*in lit.* to Ponder, 1985, p. 50) to conclude that *F. brychia* and *F. americana* were the same species. Ponder (1985) accordingly synonymized them. We concur with this since shell variation in our extensive material of *F. brychia* appears to be continuous.

MEASUREMENT OF SHELL FORM

To study patterns of character variation in the four species, we measured five standardized variables of adult and larval shell form. Samples of 50 individuals, selected at random,

were used whenever possible, but smaller samples (3–26) were used in seven cases because of the low abundance of well-preserved material (Tables 1, 2). Specimens were measured with an ocular micrometer on a Wild binocular microscope. We oriented shells for measuring on a small clay disk so that the axis of coiling was parallel to the base of the microscope and the terminus of the protoconch (transition between the larval and adult shells) was centered facing upwards toward the observer. In this position, protoconch height and the height of the first whorl on the adult shell located precisely one revolution beneath the terminus of the protoconch were measured. We measured the latter dimension to standardize the variables to a common stage of growth (Gould, 1969; Rex, 1979). Then the shell was rotated upward until the axis of coiling was perpendicular to the base of the microscope and the shell apex pointed vertically toward the observer. In this position the maximum protoconch width was measured. We combined this variable with protoconch height to calculate total protoconch size (Gould, 1969). In addition, we counted and recorded three characteristics of shell sculpture: the number of axial ribs of the first adult whorl, the number of axial ribs on the second adult whorl and the number of knobs on the shell, as appropriate for each species. The mean and standard deviation for each variable are reported in millimeters (Table 2).

PATTERNS OF GEOGRAPHIC VARIATION

We performed a one-way ANOVA to detect heterogeneity of means among samples for each variable, and Dunn-Šidák multiple comparison tests (Sokal & Rohlf, 1981) to show which samples differed significantly (Table 2). Inequality signs in Table 2 show the position and direction of significant ($P < 0.05$) differences between stations. The data revealed no significant heteroscedasticity. We tested for differences in the degree of variability of the variables by comparing coefficients of variation and their five-percent confidence limits (Sokal & Rohlf, 1981) for all sample pairs. Again, significant differences (no overlap of confidence limits) are shown by inequality signs in Table 2.

Frigidoalvania brychia

All of the variables measured on *F. brychia* show highly significant ($P < 0.001$) heterogeneity of means among the five samples. The three populations from 500 m are statistically indistinguishable except for a difference in protoconch size between stations 105 and 96. As we move to 808 m, however, there are significant increases in the means of axial ribs I, and in variability of number of knobs on whorl II. Between 808 and 1102 m, there are marked increases in protoconch size, whorl I size and the number of knobs on whorl II.

The striking depth-correlated variation in the expression of sculpture mentioned earlier is more difficult to quantify than simple meristic or continuously varying characteristics. Fig. 2 shows nine specimens picked at random from stations 88, 96 and 105. These are typical of all individuals from 500 m in having axial ribs on whorl I, and both shoulder knobs and strong spiral costae on the body whorl. Fig. 3 shows the range of variation found in the population from station 87 at 1102 m. Axial ribs on whorl I are still present, but sculpture on the body whorl ranges from forms like those found at 500 m (Fig. 3, A) to forms with nearly smooth body whorls (Fig. 3, I). The variation is continuous and tends to increase in the direction of assuming the less massive sculpture more typical of the other three species shown in Fig. 4. To assess the increase in variation of sculptural expression, we scored 500 randomly selected individuals each from station 207 and 87 as either "rugose" (Fig. 3, A-D) or "smooth" (Fig. 3, E-I). The percentage of rugose forms decreases from 100% at 500 m to 78% at 808 m and to 47% at 1102 m. A chi-square analysis of raw data showed a highly significant difference in the representation of smooth and rugose forms between stations 207 and 87 (chi-square = 49.31, $P < 0.001$).

In summary, the three samples from 500 m are very similar. With increasing depth, there are statistically significant shifts to larger larval and adult shell sizes, more axial ribs on whorl I, more shoulder knobs and more variation in the expression of sculpture.

Pusillina species

P. harpa, the second most abundant species (Table 1), shows clear increases in protoconch size and number of axial ribs II between the 500 m stations and 808 m. Except for a difference in number of axial ribs II between stations 96 and 105, the populations from 500 m are similar. Axial ribs on the first whorl were too faint to measure accurately at stations 96, 105 and 207, therefore this qual-

TABLE 2 A comparison of means and coefficients of variation among stations from the western North Atlantic for four deep-sea rissoids. The mean ($\bar{X}$) and standard deviation (SD) of four morphological characters are shown for each sample. Means were compared among stations with an ANOVA and a Dunn-Šidák multiple comparison test (MC). The degrees of freedom (df) and F-value refer to the ANOVA. The inequality signs in the MC rows separate those stations that differed significantly ($P < 0.05$) and indicate the direction of the difference. Stations separated by a comma were statistically indistinguishable, and any station not listed was not different from any of the other stations. For example, the multiple comparisons for whorl I height in *Frigidoalvania brychia* indicate that the means for stations 96, 105 and 207 are not significantly different, but all are smaller than for station 87. In addition, since station 88 does not appear, its mean was not different from the mean of any other station. The results for the coefficient of variation (CV) are presented in a similar manner. The coefficients of variation were compared by using the 95% confidence limits. If the confidence limits did not overlap, coefficients of variation were considered different.

		Frigidoalvania brychia					*Pusillina harpa*				*Pusillina pseudoareolata*			*Onoba pelagica*			
	Sta	88	96	105	207	87	88	96	105	207	88	96	207	88	96	105	207
	N	16	50	50	50	50	10	50	50	50	6	24	50	3	26	50	8
Protoconch	$\bar{X}$	1.14	1.14	1.11	1.15	1.25	0.89	0.88	0.87	0.94	0.86	0.89	0.89	0.87	0.85	0.89	0.94
Size	SD	0.03	0.05	0.04	0.05	0.06	0.04	0.05	0.04	0.04	0.04	0.08	0.04	0.01	0.04	0.03	0.06
	df			4,211					3,156			2,77				3,83	
	F			61.039***					22.445***			0.981				15.334***	
	MC			105 < 96,207 < 87					88,96,105 < 207			—				96 < 105 < 207	
				88<87					—			—				—	
	CV			NS					NS			96 > 207				88 < 96	
Whorl I	$\bar{X}$	0.54	0.52	0.50	0.53	0.58	0.37	0.39	0.39	0.40	0.35	0.35	0.35	0.39	0.39	0.41	0.46
Height	SD	0.06	0.05	0.05	0.07	0.08	0.02	0.03	0.02	0.02	0.01	0.04	0.02	0.01	0.03	0.03	0.04
	df			4,211					3,156			2,77				3,83	
	F			10.309***					2.508			0.074				10.154***	
	MC			96,105,207 < 87					—			—				96 < 105 < 207	
	CV			NS					NS			88 < 96				NS	
Axial	$\bar{X}$	12.44	12.60	12.78	13.70	14.02	25.10	—	—	—	15.33[+]	14.80[++]	—	15.67	16.42	14.74	15.13
Rib I	SD	0.73	0.86	1.00	0.95	1.08	2.38	—	—	—	2.08	2.17	—	0.58	1.72	1.16	1.13
	df			4,211					—			1,6				3,83	
	F			22.353***					—			0.116				9.016***	
	MC			88,96,105 < 207,87					—			—				96 > 105	
	CV			NS					NS			NS				NS	
Axial	$\bar{X}$	11.50	12.02	11.74	12.44	14.08	25.50	28.04	30.12	33.06	16.33	17.0	—	17.33	17.58	16.42	16.63
Rib II[§]	SD	1.03	0.91	0.83	1.96	1.51	2.17	3.24	4.27	3.80	1.97	2.32	—	1.53	1.75	1.62	1.77
	df			4,211					3,156			1,28				3,83	
	F			24.495***					20.102***			0.417				2.872*	
	MC			88,96,105,207 < 87					88,96 < 105 < 207			—				96 > 105	
	CV			88,96,105 < 207					NS			NS				NS	
				105 < 87													

NS = Not Significant
*$P < 0.05$
***$P < 0.001$
[§]Variable = # knobs on whorl II for *F. brychia* only
[+]n = 3
[++]n = 5

itative interpopulation difference is introduced among the 500 m stations. *P. pseudoareolata* showed only two significant differences for measurable traits among stations where it occurred: protoconch variation between stations 96 and 207, and variation of whorl I height between stations 88 and 96. Axial ribs on whorls I and II were too faint to measure accurately at station 207.

Onoba pelagica

O. pelagica shows increases in protoconch size and whorl I height between the 500 m stations and 808 m. There are also differences between stations 96 and 105 for means of all variables, and between 88 and 96 for variation in protoconch size. *O. pelagica* permits us to extend the analysis of one species in the complex onto the continental shelf. Rex (1979) compared geographic variation among five continental shelf (69-196 m) and four continental slope (478–808 m) populations of *O. pelagica* using five variables of larval and adult size. Slope populations differed significantly from shelf populations for all five variables. *O. pelagica* shows steep clines across the shelf-slope transition and some continued clinal effects on the upper slope.

Phenotypic differences among populations of the four species are more related to differences in depth than to horizontal separation. Correlations of the difference in protoconch size and whorl I size between populations with depth difference between sites are highly significant ($r = 0.740$, $P < 0.001$, d.f. = 23; and $r = 0.529$, $P < 0.01$, d.f. = 23, respectively). The same larval and adult size differences are uncorrelated with horizontal separation ($r = 0.004$, n.s., d.f. = 23; and $r = 0.128$, n.s., d.f. = 23). Etter & Rex (in prep.) compare populations of deep-sea snails using Mahalanobis' generalized distance (D^2) as a standardized multivariate measure of phenotypic divergence. Our analysis includes all four rissoid species for large sample sizes ($N \geq 24$, Table 2). Results support the above analysis of individual variables. D^2 values between populations were significantly correlated with depth difference (Spearman's rank correlation $r_s = 0.809$, $N = 10$, $P < 0.01$), but not horizontal separation ($r_s = -0.324$, $N = 10$, n.s.).

COMPETITION AND SIZE RATIOS

Competitive resource partitioning has been related to body size in *Hydrobia*, another small deposit-feeding rissoacean snail. Fenchel (1975) and Fenchel & Kofoed (1976) showed that character displacement of body size in *Hydrobia* appeared to be based on particle-size selection of sediment ingested, with larger snails tending to consume larger particles and smaller snails consuming smaller particles. More recently, Levinton (1982, 1987) has shown experimentally that the feeding modes underlying resource partitioning in hydrobiids are more complex. The snails not only ingest particles, but also scrape food off sediment grains that they are unable to swallow. Also, snails adjust the upper size limit of particles ingested depending on the nutritional quality of particles (Levinton, 1987). Levinton's results suggest that larger snails consume a broader range of particle sizes than smaller snails, and may, therefore, be at a competitive advantage.

Since Hutchinson (1959) proposed that the coexistence of ecologically similar species required a minimum size difference, numerous studies have attempted to explain size ratios of species by competition. While the notion of a universal minimum size ratio has been rejected on both empirical and theoretical grounds (Roth, 1981; Simberloff & Boecklen, 1981; Eadie *et al.*, 1987), the critical interpretation of relative size ratios as a possible function of competition has proven useful in understanding the assembly of species (*e.g.* Grant & Abbott, 1980; Case *et al.*, 1983; Schoener, 1984). Much work on this problem has centered on the framing of null hypotheses. However, choices of a biologically realistic null hypothesis and the appropriate statistical test have proven to be difficult, if not intractable, problems (Harvey *et al.*, 1983; Colwell & Winkler, 1984; Schoener, 1984; Tonkyn & Cole, 1986). We have taken two approaches to testing whether size ratios in the rissoid complex might reflect competition—one based on the constancy of ratios (Simberloff & Boecklen, 1981) and another on the contention that ratios should be greater between larger species of a guild than between smaller species (Schoener, 1970). We were unable to use what, in our view, have been the most successful and convincing null hypotheses, those involving a comparison of local assemblages to regional or global species pools (*e.g.* Case *et al.*, 1983; Schoener, 1984), because not enough is known about the distribution, degree of sympatry and geographic variation of deep-sea rissoids. Our analyses are restricted to the available sam-

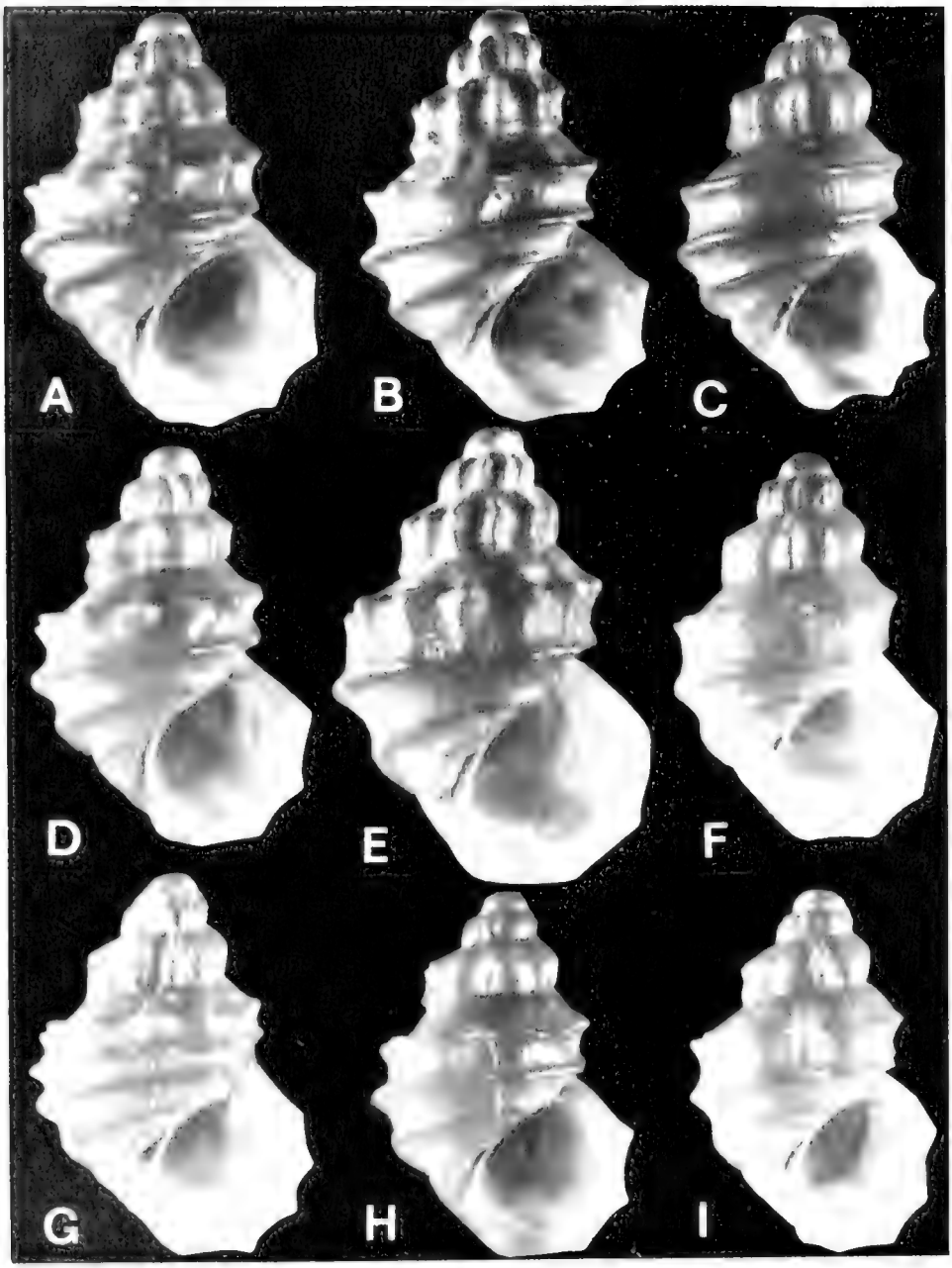

FIG 2 Examples of *Frigidoalvania brychia* (Verrill, 1884) collected from about 500 m on the upper continental slope south of New England. All specimens from this depth have heavy sculpture with axial ribs on whorl I, and both shoulder knobs and spiral costae on the body whorl. A–C (station 88), D–F (station 96), G–I (station 105). The shells measure about 3.4 mm in height.

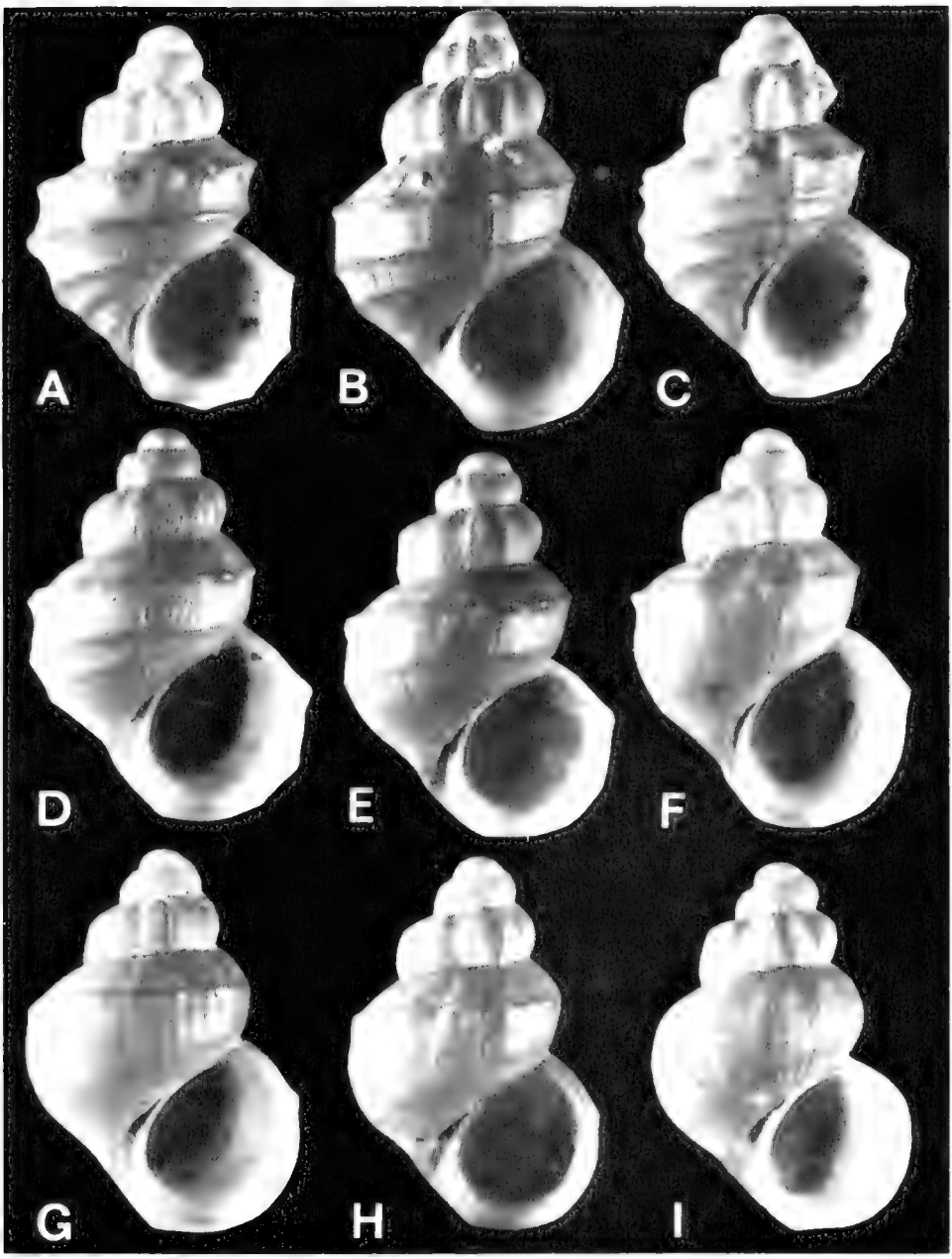

FIG 3. The range of variation in sculpture shown by *Frigidoalvania brychia* (Verrill, 1884) at 1102 m (station 87) on the continental slope south of New England. Sculptural expression varies continuously from heavy features (A) typical of that found at shallower stations (see Fig. 2) to forms with nearly smooth body whorls (I). The shells measure about 3.5 mm in height.

FIG 4. Specimens of *Onoba pelagica* (Stimpson, 1851), left; *Pusillina harpa* (Verrill, 1880), middle; and *Pusillina pseudoareolata* (Warén, 1974), right, collected from station 96 on the upper continental slope south of New England. The shells measure about 2.4 mm, 2.0 mm and 2.2 mm in height respectively.

ples. We stress that our results do not provide direct evidence on competition. Body size may be an adaptation to other biotic or physical factors, or to historical events (Colwell & Winkler, 1984; Tonkyn & Cole, 1986).

Many studies have claimed that natural selection exerted through interspecific competition will result in constant size ratios between adjacent size-ranked species in a guild (reviewed in Simberloff, 1983; Simberloff & Boecklen, 1981). Simberloff & Boecklen (1981) applied a statistical test of Barton & David (1956) to determine whether size ratios of ecologically similar species are either random and independent, or constant. Species' sizes are viewed as points on a logarithmically-scaled line, the endpoints of which are defined by the largest and smallest species. Constant size ratios result from regular spacing of sizes on the log-scaled line. The null hypothesis being tested is that size ratios are random. The alternative hypothesis is that they are constant. The test has been criticized by Harvey *et al.* (1983), Case *et al.* (1983), Schoener (1984) and Colwell & Winkler (1984) on the grounds that the assumed uniform-size distribution is biologically unrealistic and biases the test toward a Type II error. They propose that a log-normal distribution would be more appropriate. More recently, however, in response to this criticism, Boecklen & Ne Smith (1985) have shown that the test is insensitive to the underlying distribution.

We performed the test using mean values of whorl I size. We used this standardized measure of size rather than total size because of uncertainty about whether the snails continue to grow at large sizes. Warén (1974) and Rex (1979) felt that some rissoid species (*e.g. Frigidoalvania brychia* and *Onoba pelagica*) had determinate growth with final adult size indicated by the development of a thickened lip on the aperture. However, the two *Pusillina* species show less evidence of this, and the appearance of a thickened lip on the aperture in another prosobranch, *Nucella lapillus*, does not mark the cessation of growth (Cowell & Crothers, 1970; Crothers, 1971). If growth in the rissoids were indeterminate, then means of total sizes of randomly selected individuals could simply reflect variation in age structure of the population. Coevolved size differences are more apt to be manifested in a standardized measure of size; this seems to be a largely overlooked, but obviously important problem in relating size to competition.

Our analysis is presented in Table 3. Notice that the ranking of species' sizes is consistent among the samples with the exception of station 105 where *Pusillina pseudoareolata* is missing. Mean sizes were converted to logs, and size intervals (g) between size adjacent

TABLE 3. Results of the Barton-David test (Simberloff & Boecklen, 1981) for dispersion of size ratios in deep-sea rissoids. Subscripts 1, 2 and 3 for the intervals (g) refer to the smallest, middle and largest respectively. Subscripts for the ratios (G) refer to the interval combinations compared. The probability of obtaining a ratio greater (more constant) than the ones observed is given as 1 − Pr.

Station	Species	Mean whorl I height (mm)	Logarithm of size	Interval	Ratio	1-Pr
88	*Pusillina pseudoareolata*	0.350	−1.049	0.066 = g_2	G_{12} = 0.470	0.429
	Pusillina harpa	0.374	−0.983	0.031 = g_1	G_{13} = 0.093	0.663
	Onoba pelagica	0.386	−0.952	0.332 = g_3	G_{23} = 0.199	0.858
	Frigidoalvania brychia	0.538	−0.620			
96	*Pusillina pseudoareolata*	0.352	−1.044	0.100 = g_2	G_{12} = 0.020	0.970
	Pusillina harpa	0.389	−0.944	0.002 = g_1	G_{13} = 0.007	0.969
	Onoba pelagica	0.390	−0.942	0.276 = g_3	G_{23} = 0.362	0.665
	Frigidoalvania brychia	0.514	−0.666			
105	*Pusillina harpa*	0.388	−0.947	0.063 = g_1	G_{12} = 0.337	0.496
	Onoba pelagica	0.413	−0.884	0.187 = g_2		
	Frigidoalvania brychia	0.498	−0.697			
207	*Pusillina pseudoareolata*	0.354	−1.038	0.114 = g_1	G_{12} = 0.820	0.129
	Pusillina harpa	0.397	−0.924	0.148 = g_3	G_{13} = 0.770	0.016
	Onoba pelagica	0.460	−0.776	0.139 = g_2	G_{23} = 0.939	0.052
	Frigidoalvania brychia	0.529	−0.637			

species determined. We then calculated size ratios (G) for all interval combinations. The probability of obtaining a ratio greater (more constant) than the one observed is given as 1-Pr.

The null hypothesis of random ratios is rejected ($P = 0.016$, $P = 0.052$) for two of the three possible ratios in station 207 at 808 m. All of the ratios at station 207 are considerably larger than at other stations. The size structures of assemblages from the three 500 m stations appear to be largely random, or in two cases non-random because of significantly small ratios (uneven rather than constant size differences).

Schoener (1965, 1970, 1974) proposed that competition can result in interspecific differences in size, but with a systematic departure from constant size ratios. If larger species exploit a larger range of food sizes, such as they do in *Hydrobia,* then large species will tend to be more different than smaller species. We tested Schoener's hypothesis by comparing the size ratios of the largest pair of species (*Frigidoalvania brychia* and *Onoba pelagica*) to the smallest pair (the *Pusillina* species in stas. 88, 96 and 207, or *P. harpa* and *Onoba pelagica* in sta. 105). Size ratios of the larger species are always higher. The probability that a difference in the order of size ratios between largest and smallest pairs this extreme occurs is $P = 0.06$ (binomial test). The size ratio of the largest pair is larger than ratios among size-ranked smaller species in all cases except for station 207 where ratios for the largest and middle pairs are 1.15 and 1.16 respectively. There is not a perfect rank order relationship between size ratios and increasing size of size-ranked pairs.

DISCUSSION

This is the first biometrical analysis of geographic variation in upper-slope benthic invertebrate species. The most important finding is that pronounced character shifts occur in this narrow region of the deep sea. Bathymetric differences of hundreds of meters produce greater morphological change than tens of kilometers of horizontal separation. The degree of change is less than observed in *Onoba pelagica* on similar spatial and bathymetric scales across the shelf-slope transition (Rex, 1979), but considerably more than found over depth differences of thousands of meters and horizontal separation of hundreds of kilometers at lower bathyal and abyssal depths (Rex & Etter, in prep.). High rates of species replacement with depth on the upper slope suggest that it is a steep environmental gradient (Rex, 1977; Grassle *et al.,* 1979). The marked clinal effects within the bathymetric ranges of individual rissoid species support this view. The upper slope is a potentially important site for population differentiation leading to the di-

versification of the rich and indigenous deep-sea gastropod fauna.

Competition and Size Ratios

The interpretation of evolutionary patterns in this remote environment is necessarily speculative. The selective gradients that cause differentiation in the rissoids are likely to be complex, involving both physical and biotic factors that affect the species independently and mediate biological interactions among them and with other elements in the community. Shell form in marine prosobranchs has been related to a variety of selective agents including predation and physical disturbance (Vermeij, 1978), effects of habitat type on mobility (Palmer, 1980), the need to conserve shell material (Graus, 1974) and factors favoring differences in life-history tactics (Rex, 1979). These are all plausible explanations for the observed patterns. Unfortunately, in this case, the lack of specific information about these potential causes makes them difficult to evaluate in a useful way. However, the shifts in size and variation that accompany changes in relative abundance and density immediately suggest that interspecific competition may play a role in the assembly and morphological structure of the rissoid group (*e.g.* Van Valen, 1965; Simberloff & Boecklen, 1981; Schoener, 1984). Comparative studies on species diversity, normalized species/genus ratios, trophic composition and patterns of nutrient input have suggested that competition is an important determinant of community structure on the upper slope (Rex, 1981, 1983; Rex & Warén, 1981).

The Barton-David test revealed constant size ratios at station 207. If the degree of overdispersion of size ratios is a measure of the intensity of competition, then the assemblage from 808 m appears to be the most structured. One interpretation of these findings is that resources at 500 m are adequate to support all four species at relatively high densities. *Frigidoalvania brychia* is the most abundant species, and its larger size may permit it to exploit a broader range of particle-size food resources. At 800 m the decreased relative abundance of the three smaller species and significant overdispersion of size ratios may represent a combined numerical and functional response to competition for a more limited resource base.

The result of testing Schoener's hypothesis, while based only on the ranks of size ratios of largest and smallest pairs, is consistent with the explanation that competition plays a role in the size structure of the complex where the four species coexist. It does not tell us where, at 500 or 800 m, competition is likely to be more important.

The Simberloff-Boecklen and Schoener tests are based on somewhat different premises and might seem like incompatible approaches for detecting the effects of competition. This is not necessarily the case since competition could result in both significant overdispersion of sizes and a subtle nonlinear size gradient among species. In the limited way that we have been able to apply them, the Simberloff-Boecklen test is the more conservative and stringent of the two.

Competition and Variation

Frigidoalvania brychia, the most abundant and largest snail in the complex, shows significant increased variation in the expression of sculpture with increasing depth. The variation appears at 808 m where the other three species become less abundant, and expands significantly at 1100 m where the other species are absent. The variation increases in the direction of smoother body whorls more characteristic of the three other species. This pattern suggests that *F. brychia* might experience ecological release manifested as increased phenotypic variation (*sensu* Van Valen, 1965) as its potential competitors become less abundant.

The adaptive significance of smooth vs. heavy sculpture for deposit feeding is uncertain. All of the species probably forage at or near the soupy water-sediment interface. Smooth surfaces are common in snails that burrow through sediment and may be an adaptation to reduce drag (Palmer, 1980). It could enable the three smaller less ornamented species to forage slightly deeper in the sediment than *Frigidoalvania brychia.* The heavy knobs of *F. brychia* are identical to those used as anti-predator adaptations in other snails (Palmer, 1979). Heavy armor may confer a protective advantage on the more exposed sediment surface. It is possible that the increased range of sculptural expression in *F. brychia* at 800–1100 m reflects foraging over a broader depth range in the sediment. Also, if larger snails can exploit a broader range of resources, then *F. brychia's*

significant increase in larval and adult sizes at 1100 m may also be a form of ecological release (Grant, 1972). Scarcer resources at 1100 m should select for broader feeding habits (Schoener, 1971).

We cannot provide direct evidence for the influence of competition—that species exploit food resources which limit the populations of other species and that competition has altered fundamental and realized niches in some specific way. Also, the adaptive significance of character variation is uncertain. Taken together, however, the significant character differences and dispersion of size ratios do suggest that competition contributes to shaping patterns of morphology and abundance in the rissoid complex.

SUMMARY

This paper presents an analysis of character variation in a complex of ecologically similar rissoid gastropods collected from the upper continental slope south of New England. Pronounced geographic variation has developed across the depth gradient of the upper slope suggesting that this region is an important site of population differentiation in deep-sea gastropods. Patterns of character variation, size ratios, relative abundance and density are consistent with the hypothesis that interspecific competition contributes to structuring the complex. Biometrical analyses of geographic variation have considerable potential for providing insights into the evolution and ecological assembly of the deep-sea benthos.

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EFFECTS OF FOOD AND SHELL MORPH ON TEMPERATURE PREFERENCES OF *CEPAEA NEMORALIS*

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ABSTRACT

Temperature preferences of the polymorphic land snail *Cepaea nemoralis* were tested with a temperature gradient in the laboratory. We expected starved snails to choose colder locations to conserve energy, and we expected darker morphs to choose cooler temperatures, based on the climatic selection hypothesis. Starved snails moved to a significantly warmer location when food was added. When no food was present, starved snails chose significantly colder temperatures than previously fed snails. Previously fed snails moved to a significantly colder area when food was added, for unknown reasons. When food was present, previous feeding had no consistent effect on temperatures chosen. Morph differences in temperature were less pronounced than those associated with food; only five of the 20 pairwise morph comparisons made were significant. Four of the five significant comparisons were consistent with the climatic selection hypothesis, and all were found in starved snails. Yellow unbanded snails had higher mortality (probably due to starvation) than snails with other shell morphs.

Key words: *Cepaea;* land snails; polymorphism; ecology; behavior; temperature preferences; habitat choice.

INTRODUCTION

The land snail *Cepaea nemoralis* is among the most polymorphic species known. Both shell color and banding patterns vary, and there are often associations of morph frequencies with habitat. Often there are more snails with light shells in hot, sunny areas, and more snails with dark shells in cool, shady areas (Jones *et al.,* 1977). One explanation for this is based on microclimate and body temperature. Dark shells become warmer than light shells when the two types are placed next to each other in the sun (Heath, 1975). Presumably snails with dark shells would be at an advantage in cool habitats, since they would warm more rapidly in sunlight to temperatures required for activity; and snails with light shells would be less likely to reach lethal temperatures in warm, sunny habitats. This explanation is known as the climatic selection hypothesis (Jones *et al.,* 1977). An alternative explanation for the association, visual selection, assumes that each morph is more cryptic to predators in the habitat in which it is more common (Jones *et al.,* 1977).

We examined the temperature preferences of *Cepaea nemoralis* to try to determine the cause of this morph-habitat association, which occurs in our study area. Temperature could be used in habitat choice in *Cepaea* for two reasons, which are not exclusive. One way assumes that climatic selection produced the observed distribution of morphs. Individual snails might use environmental temperature as a cue for finding a suitable habitat, and different morphs would choose different temperatures. This assumes that the distribution of morphs results from active choice and not passively from differential mortality, and that other cues (such as light intensity) are less important. It also assumes that individual snails have a choice of habitats within their dispersal range, which is low (5–10 m/yr, Jones *et al.,* 1977). Movement among habitats has been documented in *Theba,* however (Johnson, 1981). Another reason to choose habitats based on temperature assumes that the observed association of morphs with habitats already exists, without assuming its cause. Morphs living in different microclimates might evolve different physiological adaptations, and different temperature prefer-

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TABLE 1. Mean temperatures (°C) selected by snails in each group in each treatment. Source is the vegetation from which the snails came, days starved is the time between collection and start of the experiment, and no. pinks is how many pink snails were in each group. Numbers are mean temperature (SD, N).

Gp. no.	Source	Days starved	No. pinks	Treatment 1	Treatment 2	Treatment 3	Treatment 4
1	hedge	60	2	17.8 (2.6, 15)	19.7 (4.3, 15)	—	—
2	ivy	13	0	19.2 (2.9, 19)	20.7 (4.4, 19)	19.7 (5.1, 14)	16.3 (4.0, 14)
3	ivy	24	3	15.1 (5.3, 20)	16.2 (4.3, 19)	23.8 (4.5, 16)	22.0 (5.3, 16)
4	ivy	28	10	14.7 (2.7, 19)	19.9 (3.8, 19)	19.6 (3.8, 15)	16.1 (4.1, 14)
5	grass	0	3	—	—	19.7 (5.7, 19)	17.8 (5.1, 19)
6	honeysuckle	8	7	22.3 (5.1, 14)	23.3 (4.1, 14)	23.5 (4.5, 14)	22.8 (3.7, 12)
mean				17.6 (4.7, 87)	19.8 (4.7, 86)	21.2 (5.0, 78)	18.9 (5.2, 75)

ences. There is some evidence for physiological differences among morphs in *Cepaea* (Jones *et al.*, 1977), but this explanation predicts larger differences in temperature preference among habitats than among morphs. If each morph prefers its optimum habitat, that preference would also promote stable polymorphism (Maynard Smith, 1970).

We predicted that darker (banded and/or pink) snails would prefer cooler temperatures, for either of the two reasons outlined above. This was found by Sedlmair (1956) for pink banded compared to yellow banded snails of this species. We also predicted that starved snails would choose colder temperatures than fed snails. Metabolic rate in this species is reduced at low temperatures (Richardson, 1975), so choosing a cold area would conserve energy.

METHODS

Samples

Snails were collected in Lexington, Virginia, from May to July 1986, and on 9 June 1987. This species was introduced to Lexington from Europe in 1883, and originally included individuals with brown, pink, and yellow shells, with or without dark bands (Howe, 1898). However, the brown morph has disappeared here since 1898 (Johnson *et al.*, 1984). Each group of 20 adult snails came from a single plot with homogeneous vegetation 3 m × 3 m in size. The sources of the groups are listed in Table 1. Snails were kept indoors at approx. 21–24°C in paper bags before being tested. Five groups were tested between 24 June and 14 September 1986, and one group between 18 and 30 June 1987. This period is after the May-June peak of activity to which Cameron (1970) limited his experiments, but snail activity indoors did not appear to decrease until after 14 September. Banded (12345) and unbanded (00000) snails with yellow or pink shells were used. Snails were individually marked with holes drilled in the lip and letters painted on their shells.

Apparatus

A temperature gradient was established along an aluminum bar. One end of the bar was in a cold water bath, and the other in a hot water bath. A thin metal tray painted black inside, 112 cm long × 17 cm wide × 3 cm high, was placed on top of the aluminum bar. The snails were kept in the tray with a cover of hardware cloth with 0.6 cm mesh. The cover kept them on the floor of the tray with only a few exceptions. For the few snails on the wire, body temperature was used instead of tray temperature. The tray was enclosed in a chamber covered with a plexiglass sheet, which allowed natural daylight to enter from windows in the room and kept the humidity high in the tray (95–100%).

Procedure

A control stage and four treatments were used to manipulate two food variables: feeding before and during the experiment. At the start of each experiment, 20 starved, marked adults were placed in a double line at the center of the tray with a thin layer of distilled water and no gradient; this was the control. In Treatment 1, the temperature gradient was established (and left on for all successive treatments), without moving the snails or adding

TABLE 2. Effect of shell morph on temperatures chosen in each treatment. Trt. = treatment, numbers are mean temperatures (SD, N), and significant differences are underlined and connected.

Trt.	Yellow unbanded	Yellow banded	Pink unbanded	Pink banded
1	18.8 (5.1, 33)	17.2 (3.8, 36)	19.7 (5.7, 13)	13.6 (1.9, 9)
2	19.0 (4.4, 30)	20.0 (4.4, 35)	23.1 (4.2, 12)	17.1 (5.3, 9)
3	22.1 (5.2, 26)	20.3 (4.7, 31)	22.1 (6.1, 12)	20.2 (4.0, 9)
4	19.0 (5.3, 23)	18.4 (5.1, 31)	19.6 6.2, 11)	19.2 5.3, 9)

any food. In Treatment 2, a single layer of paper towel, 200 g of dry oatmeal, and 3–5 g of dolomitic powdered limestone were added to the tray, with enough distilled water to keep the paper towel moist, without moving the snails. In Treatment 3, the same snails (which had just been fed) were started at the center of the tray with only distilled water and the temperature gradient present. In Treatment 4, food and limestone were added to the tray, without moving the snails.

All groups were not tested in all treatments. Group 1 was not tested in Treatments 3 and 4 because it was used for electrophoresis after Treatment 2. Group 5 was tested first in Treatments 3 and 4, and when we were ready to test it further, almost half of the snails were dead.

When used, paper towels and food were changed every other day. The temperatures along the gradient in the tray varied linearly ($r = 0.99$) and the mean temperatures at the ends of the tray were 11.9°C (SD 2.7) and 32.3°C (SD 1.4). Temperatures on the surface of the tray (or snail body temperatures) were measured with a YSI Model 46 Telethermometer with a #423 probe. Sedlmair (1956) only noted positions after the snails had been in the tray for 0.5 and 1 hr. However, we considered that this was too short a time for the snails to choose their preferred temperature, since they are slow-moving. In three groups in Treatment 3, we noted positions after 1 hr in the tray for comparison to Sedlmair's (1956) results (she also used fed snails with no food present). In all treatments, we waited 24-36 hr for the snails to equilibrate before recording positions of the snails twice a day (at about 0900 and 2100) to the nearest 0.5 cm along the gradient.

Data analysis

Snail positions were recorded until there were at least three consistent ($P > 0.05$) consecutive positions, as tested with repeated measures two-way ANOVA (with time as the repeated factor; Sokal & Rohlf, 1973). The mean of these positions was then used in further analysis. The distribution of the snails in the tray was tested by sorting the positions into 22 bins, each 5 cm long × 17 cm wide, and testing the fit of the resulting frequencies to the Poisson distribution with a G goodness-of-fit test; the coefficient of dispersion (CD = variance/mean) was also calculated (Sokal & Rohlf, 1973). Positions were converted to temperatures using linear regressions, and mean temperatures for each treatment were compared using paired t-tests with two-tailed probabilities. The temperatures were normally distributed (tested with the NSCORES procedure in Minitab). Five pairwise comparisons of morph temperatures were planned to examine banding and color effects: yellow unbanded vs. yellow banded, pink unbanded vs. pink banded, yellow banded vs. pink banded, yellow unbanded vs. pink unbanded, and yellow unbanded vs. pink banded. Comparisons between temperatures of the shell morphs were made with the ONEWAY analysis of variance procedure in SPSS-X (SPSS, 1983), using the CONTRAST subcommand with pooled variance estimates. A log transformation was used on the data from Treatment 1 to equalize the variances (see Table 2).

RESULTS

Spatial distribution

The distribution of the snails in the tray tended to be clumped, rather than random (Poisson) or overdispersed (repulsed). Examining the 16 tests plus the four control stages in 1986, only a few occasions (six out of 20) showed significant clumping, but this was probably due to the small sample size (20

snails at once) which required lumping of categories. The coefficient of dispersion was greater than 1.0 in 19 of 20 occasions, ranging from 0.94 to 4.6 (mean 2.07, SD 0.99), which indicates clumping (Sokal & Rohlf, 1973). Often several snails were touching each other, although none were seen mating. There were no consistent differences among the treatments in the degree of clumping. In spite of the tendency toward clumping, the repeated measures two-way ANOVA always showed significant differences ($P < 0.05$) in the positions of individuals (independent factor), although the repeated factor (time) showed no significant differences.

The snails took 24—36 h to reach consistent positions, much longer than the time (1 h) allowed by Sedlmair (1956). One-hour observations were much less clumped than later observations in 2 of the 3 cases in Treatment 3, in spite of the fact that snails had been started in the middle of the tray. The final positions in Treatment 3 were significantly colder (by 3.5 and 4.3°C) than those after 1 hour in two of the groups.

Response to the temperature gradient

For the starved snails, the control stage was compared to Treatment 1 to ensure that the snails were moving in response to the gradient. The response to the gradient (in position change) was significant ($P < 0.01$ in all four groups, paired t-test, mean 22.2 cm moved in either direction). The temperatures chosen after the gradient was established were significantly colder (by 5.1–10.9°C) than room temperature.

Effects of feeding

The mean temperatures chosen by each group in each treatment are shown in Table 1. Three of the four possible effects of feeding were significant over all six groups; one of these differences was not expected. When food was given to starved snails, they moved a mean of 2.3° warmer (paired $t = 5.44$, $df = 85$, $P = 0.0001$). When no food was present, starved snails (Treatment 1) were a mean of 4.2° colder than previously fed snails (Treatment 3; paired $t = 4.3$, $df = 58$, $P = 0.0001$). However, when food was added to previously fed snails they moved a mean of 2.1° colder (paired $t = 5.29$, $df = 74$, $P = 0.0001$), which was not expected. When there was food in the tray (Treatments 2 and 4), previous feeding had no effect on temperatures chosen (paired $t = 0.41$, $df = 55$, $P = 0.68$). Similar results were found by testing each group separately, except that the comparisons were not significant in five of the 18 tests. The origin, number of days starved, and number of pink snails in each group (Table 1) had no consistent effects on these results, except that groups with more pink snails tended to have lower means, because pink snails prefer cooler temperatures (see below). The group tested in 1987 (Group 6) chose warmer temperatures than the 1986 groups, but it showed similar responses to food.

Morph differences

Each morph chose a broad range of temperatures in each treatment (Fig. 1). Mean temperatures chosen by each morph under the same conditions are shown in Table 2. Because of the significant effects of feeding, morph comparisons were made only within each treatment. One-way ANOVA comparing the temperatures of the four morphs was highly significant for Treatments 1 and 2 ($P = 0.014$ and 0.017 respectively), but not for Treatments 3 and 4.

Five of the planned pairwise contrasts were significant, with four in the predicted direction; these are underlined and connected in Table 2. In Treatment 1, yellow bandeds were significantly warmer than pink bandeds ($t = 2.2$, $df = 87$, $P = 0.03$); pink unbandeds were significantly warmer than pink bandeds ($t = 3.0$, $df = 87$, $P = 0.004$); and yellow unbandeds were significantly warmer than pink bandeds ($t = 3.02$, $df = 87$, $P = 0.003$). In Treatment 2, pink unbandeds were significantly warmer than pink bandeds ($t = 3.03$, $df = 82$, $P = 0.003$); and pink unbandeds were significantly warmer than yellow unbandeds ($t = 2.7$, $df = 82$, $P = 0.009$). The last comparison is contrary to the climatic selection hypothesis. In Treatments 3 and 4, there were no significant contrasts, and the frequency distributions (Fig. 1) show no marked differences between morphs.

Morphs differed significantly in mortality when tested the first time in 1986, but not when tested the second time. In their first test, nine of 100 snails died, and seven of those that died were yellow unbanded ($G = 6.58$, $df = 2$, $P < 0.05$; pinks considered one class due to small N). Mortality was higher in the second test (12 of 56 died), but it was random with respect to shell morph ($G = 1.04$, $df = 2$,

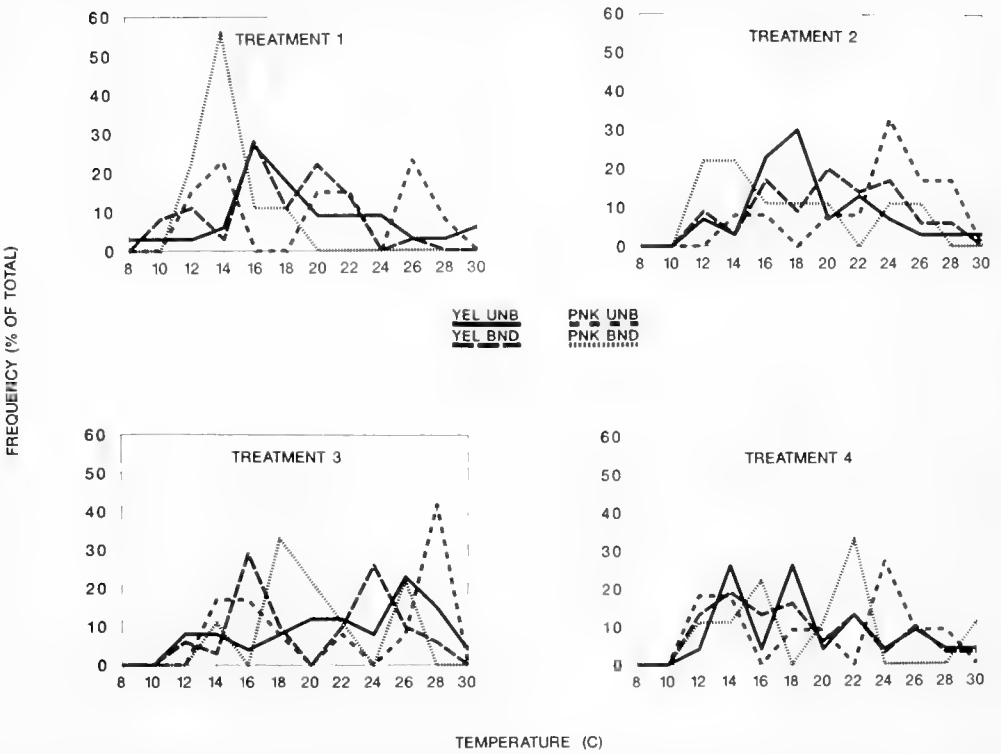

FIG 1. Frequency distributions of temperatures selected by snails of different morphs in each treatment. PNK = pink, YEL = yellow, BND = banded, UNB = unbanded. Treatment 1 = starved, no food; Treatment 2 = food added, Treatment 3 = fed, no food in tray, Treatment 4 = food added. Sample sizes, mean values for each morph, and significant differences are given in Table 2.

NS). The main cause of mortality appeared to be starvation: the two groups with highest mortality (five each) had the longest periods of starvation preceding the tests (60 and 55 days).

DISCUSSION

The clumped distribution of the snails suggests that the tray was large enough to test 20 snails at a time without bias caused by the size of the tray. If they had been overdispersed, their positions might have been affected more by the size of the tray. Why the snails choose to be clumped is not clear from this experiment. The clumping was not caused by temperature, since each group of 20 snails always differed significantly in preferred temperature, and the snails were clumped (significantly in two of the four cases) when the gradient was not established.

Starved snails without food consistently chose colder temperatures than the same snails when fed, whether the feeding came before or during the testing. Snails in Treatment 1 might have selected a colder-than-usual temperature when the gradient was started for two reasons, but the second is more likely: either (1) colder places are more likely to contain food in the wild, or (2) colder temperatures reduce the metabolic rate of the snail, enabling it to conserve energy. Adding food either before or during the experiment made higher metabolic rates possible, and these can be achieved by choosing higher temperatures. Richardson (1975) for *C. nemoralis* and Steigen (1979) for *C. hortensis* both found that metabolic rate was greatly reduced at low temperatures (below 15°C).

Adding food to the tray (Treatments 2 and

4) appeared to make previous feeding unimportant, as it should be according to the metabolic explanation given above. However, previous feeding did not prevent adding food from having a consistent effect on temperature choice. All previously fed groups moved to a colder location when food was added (Table 1). This movement is inconsistent with a metabolic explanation.

Morph differences in temperature preference were more pronounced in starved snails than in previously fed snails, since the only significant ANOVAs and all the significant contrasts were in starved snails (Treatments 1 and 2). Thus, feeding affects the morph differences in temperature. However, morph seems to have little effect on the differences among feeding treatments (Table 1), since the number of pink snails had little effect on the response to temperature. Thus, feeding appeared to have larger effects on temperature preference than shell morph, but four of the five significant morph comparisons were consistent with the climatic selection hypothesis.

Our finding that yellow unbanded morphs had higher mortality when first tested disagrees with the results of Steigen (1979), who predicted for *C. hortensis* that the unbanded form would be more resistant to starvation than the banded form. Sedlmair (1956) found that pink banded *C. nemoralis* had lower mortality after a hot, dry period than yellow banded snails, and that banded *C. hortensis* had lower mortality than unbanded snails under the same conditions, both of which agree with our result. However, Jones *et al.* (1977) and Clarke *et al.* (1978) criticized Sedlmair's analysis, and our sample is too small to enable us to draw any conclusions from it about mortality.

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GENETIC VARIATION IN *ONCOMELANIA HUPENSIS: SCHISTOSOMA JAPONICUM* TRANSMITTING SNAILS IN CHINA AND THE PHILIPPINES ARE DISTINCT SPECIES

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ABSTRACT

The hypothesis that Chinese, *Oncomelania h. hupensis*, and Philippine, *O. h. quadrasi*, are subspecies of a widely distributed polytypic species was tested by studying variation at 21 electrophoretically detected allozyme loci. Field-collected samples from Guichi, Anhui Province, Peoples Republic of China, were compared with samples from the Philippine islands of Mindoro, Luzon, Leyte and Mindanao. Results show these snails are amphimictic and that the Chinese populations are 2-3 times more variable than those in the Philippines. Comparing the continental populations with those of the islands, the mean number of alleles per locus is 2.0 vs. 1.2, the proportion of loci polymorphic is 0.57 vs. 0.20, and the mean individual heterozygosity is 0.19 vs. 0.04.

Nei's multilocus genetic distances between seven Philippine samples were typical values for conspecific comparisons: $\bar{D} = 0.036$ (0.001–0.134). In contrast, the Chinese and Philippine samples have evolved genetic differences at over 20% of their structural gene loci: $\bar{D} = 0.62 \pm 0.20$. Fixed differences characterize snails from each area at six loci, and 14 area-specific alleles were identified. Despite their morphological similarity and a lack of strong postmating reproductive isolation, the Chinese and Philippine taxa deserve recognition as full species: *O. hupensis* and *O. quadrasi*. This recommendation is discussed in the light of studies of genetic differentiation in other molluscs and the changing nature of the evolutionary (cohesion) species concept.

We discuss the evolution of *O. quadrasi* and speculate as to the role of migratory birds in carrying the ancestors of this species to the Philippines perhaps 3 million years ago. We point to the need to establish the degree of genetic differentiation between *O. hupensis* and the other six subspecies found in China. Finally, we note the close parallel between the genetic differentiation of *O. hupensis* and *O. quadrasi* and the Chinese and Philippine populations of the blood fluke *Schistosoma japonicum* ($\bar{D} = 0.57$); the latter taxon may also represent a group of presently unrecognized sibling species.

Key words: snails, evolution, allozyme variation, species, geographic variation, schistosomiasis.

INTRODUCTION

Studies of the evolution of living molluscs are bedeviled by two types of problems. First, molluscan systematists working with traditional conchological characters are plagued by the multifarious effects of phenotypic plasticity, stasis and convergence. Second, and this is a problem shared with many other groups, morphology is rarely useful in determining the taxonomic status of related but allopatric populations. Biochemical genetic techniques can, in many cases, help circumvent these problems and, coupled with traditional approaches, elucidate patterns of evolution and phylogeny. Recent examples of the power of the multifaceted approach in resolving problems of the first type involve such notoriously difficult groups as terrestrial pulmonates of the genus *Cerion* and freshwater unionid clams (Davis, 1983, 1984; Davis & Fuller, 1981; Gould & Woodruff, 1986; Woodruff & Gould, 1980). The present paper has as its focus the more general problem of the second type: the detection of evolutionary differences in related but geographically isolated populations and the interpretation of their significance. We here report the first study of

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genetically interpreted variation in the geographically widespread snail *Oncomelania hupensis*.

Oncomelania hupensis is the intermediate host snail of the Asian blood fluke *Schistosoma japonicum*. The snails are small (adult shell length 4–10 mm) amphibious prosobranchs placed in the family Pomatiopsidae and subfamily Pomatiopsinae. Present understanding, based primarily on the work of George Davis (1979, 1980; and references therein), is that *O. hupensis* is a classic polytypic species with six geographically defined subspecies:

Oncomelania hupensis hupensis Gredler, 1881	Chinese Mainland
O. h. quadrasi (Mollendorff, 1895)	Philippines
O. h. formosana (Pilsbry & Hirase, 1905)	Taiwan Province
O. h. nosophora (Robson, 1915)	Japan
O. h. chiui (Habe & Miyazaki, 1962)	Taiwan Province
O. h. lindoensis Davis & Carney, 1973	Sulawesi

Davis argued that subspecific status was appropriate for these allopatric populations on the grounds that they are qualitatively identical anatomically and can hybridize in the laboratory producing fertile F_1 and F_2 generations (Wagner & Chi, 1959; Komiya & Kojima, 1959; Moose & Williams, 1963; Davis, 1968). Although detailed studies have revealed small differences between the subspecies (in shell size, shape and sculpture, in electromorphic and antigenic patterns, in fecundity, in growth rates, and in compatibility with various strains of *S. japonicum*), these differences are held to be of minor importance and well within the limits for a polytypic species. Most recent workers have followed Davis' taxonomic recommendation (Mitchell *et al.*, 1981; Cheever *et al.*, 1982; Lee *et al.*, 1982; Sudomo, 1983; Harinasuta, 1984; Kitikoon, 1984). Malek (1980) and a number of general textbooks (Noble & Noble, 1982; Schmidt & Roberts, 1985) are among the few to afford each geographic taxon full species status.

The history of *Oncomelania hupensis* has been explored in remarkable detail by Davis (1968, 1979, 1980) who studied the anatomical, morphological and biogeographical attributes of pomatiopsid snails in South America, South Africa, Asia, Australia and North America. He hypothesized that *Oncomelania* or its immediate ancestor reached Asia on the Indian continental plate during the Miocene. During the concommittent Himalayan orogeny, the snails entered the newly developing Yangtze River system by way of northern Burma and Yunnan and spread to the Pacific coast of China. From there they dispersed to the continental islands of Japan and Taiwan and to the Philippine archipelago and Sulawesi. A late Miocene or Pliocene radiation occurred on Japan and gave rise to two endemic genera (*Blanfordia* and *Fukuia*), to *Cecina* which subsequently spread to western North America, to *O. minima* of Japan (the only other living species of *Oncomelania*), and to the stock which spread to North America as *Pomatiopsis* (Davis, 1979, 1982).

Oncomelania hupensis is therefore viewed as a taxon that arose, spread, and differentiated during the last 6 million years. The subspecies restricted to the islands of Japan, Taiwan and the Philippines were presumably derived independently from the mainland of China; the Sulawesi taxon was presumably derived secondarily from Philippine snails. The extent of the subsequent differentiation within and between the various geographic subspecies has, as noted above, been well documented, and although the precise age of each subspecies is still unknown the general pattern of their phylogeny appears clear.

The relative recency of the evolution of *Oncomelania hupensis* coupled with its sexual mode of reproduction suggests that, if this species is genetically variable, measures of interpopulation allozyme differentiation can be used to test the above hypotheses. This study consequently had three main aims. The first was to establish levels of intrapopulation biochemical genetic variability and the optimal protocol for their resolution. The second aim was to begin an exploration of interpopulation genetic variation with a view to testing Davis' historical hypotheses. This pilot study focuses on *O. h. quadrasi* of the Philippines as its fragmented range serves as a model for the species as a whole. We describe the variation within and between snail populations representing four Philippine islands and compare this allegedly derived subspecies with samples of *O. h. hupensis* from the mainland of China. Our third goal was to initiate a study

TABLE 1. Samples of *Oncomelania hupensis* studied.

Sample name	Locality	Collection date	N	Catalog No.*
O. hupensis quadrasi				
Leyte A	Vicob Ck., Palo	6.83	>1000	102
Leyte B	Mandaraq Ck., Palo	6.83	>1000	101
Leyte C	South Main Canal, Palo	6.83	>1000	103
Luzon A	San Isidro, Sorsogon	6.86	200	105
Luzon B	San Agustin, Sorsogon	6.83	50	106
Mindoro	Victoria	6.83	50	100
Mindanao	Mawab, Davao	8.84	500	107
Lab Stock	Palo and Victoria	79 & '82	>1000	–
O. hupensis hupensis				
China A	Guichi, Anhui	4.82	> 500	110
China B	Guichi, Anhui	2.84	> 400	–

*Voucher specimens were placed in the museum at the Center for Applied Malacology and Entomology, Mahidol University, and were catalogued with the prefix MUFS-PHOO-# for Philippine samples and MUFS-CHOO-# for Chinese

of genetic aspects of host-parasite coevolution by comparing the geographic differentiation of *O. hupensis* with that in *Schistosoma japonicum*. Far less is known about the evolution of this interaction than in the cases of African and American schistosomes and their respective intermediate host snails.

We are accordingly particularly interested in estimating the relative degree of interpopulation genetic differentiation. We will do this using Nei's (1978) widely used measure of unbiased genetic distance (D). D is a measure of the number of codon substitutions per locus; it can vary between 0 (when two samples are genetically identical) and infinity (when two samples share no alleles at any locus). Studies of over a thousand other species of animals have revealed a positive relationship between D and the relative divergence of a group of related organisms (Avise & Aquadro, 1982; Thorpe, 1983; Nei, 1987). These surveys show that the D values between conspecific populations were typically less than 0.06. Interspecific genetic distances, on the other hand, were higher; most being in the range $\bar{D} = 0.10–1.05$. Although there is no simple relationship between a D value and taxonomy, it is with these values in mind that we began this pilot study of *Oncomelania hupensis*.

MATERIALS AND METHODS

Oncomelania hupensis quadrasi was characterized on the basis of seven field-collected samples representing four Philippine islands and one laboratory stock of mixed origin (Table 1). *O. hupensis hupensis* was characterized on the basis of a stock derived from two large samples from Guichi (Anhui Province) on the Yangtze River about 400 km west of Shanghai. The field-collected snails were maintained in our laboratory at the Center for Applied Malacology and Entomology, Faculty of Science, Mahidol University, until they were frozen on dry ice, carried to San Diego, and held at −70°C until analysis.

Our electrophoretic techniques are described in general terms elsewhere (Woodruff, 1975; Mulvey & Vrijenhoek, 1981). Individual snails were thawed and quickly homogenized whole in 0.1 ml of grinding solution (0.01 M Tris, 0.001 M EDTA, 0.05 mM NADP, pH 7.0) with a glass rod. The homogenate was centrifuged at 10,000 g for 2 minutes and the supernatant was absorbed onto Whatman no. 3 chromatography paper wicks (3 × 9 mm) and inserted into 12% horizontal gels made of Sigma starch (Sigma Chemical Co., St. Louis, Missouri). Electrophoresis was carried out for 15 hrs. by which time a bromophenol blue marker dye had migrated 100–120 mm anodally. 4–5 slices were then cut from each gel and each slice was stained for a specific enzyme following standard methods (Harris & Hopkinson, 1977; Richardson *et al.*, 1986). Electrophoretic conditions for the resolution of the 21 allozymes reported here are described in Table 2. The esterase substrate was alpha-naphthyl acetate and the peptidase substrate was leucyl-alanine. Snails from different samples were run on each gel to facilitate comparisons: isozymes were numbered, and allozymes were assigned superscript letters a,b, . . . , in order of decreasing anodal mobility of the primary electromorph. Commonly used enzyme ab-

TABLE 2. Electrophoretic buffers giving optimal resolution of proteins in *Oncomelania hupensis*.

Enzyme (E.C. #)	Abbreviation	Buffer*
Acid phosphatase (3.1.3.2)	ACP	TC-2
Aspartate aminotransferase (2.6.1.1)	AAT	TBE, LiOH
Catalase (1.11.1.6)	CAT	AP
Esterase (3.1.1.1)	ES-1	TBE
	ES-3	TBE, LiOH
Glucose-6-phosphate dehydrogenase (1.1.1.49)	G6PDH	TBE, LiOH
Glyceraldehyde-3-phosphate dehydrogenase (1.2.1.12)	GAP	AP
α-Glycerophosphate dehydrogenase (1.1.1.8)	GPDH	TC-2, TC-1
Glucose phosphate isomerase (5.3.1.9)	GPI	TC-1, AP
Isocitrate dehydrogenase (1.1.1.42)	IDH-1	TC-2
	IDH-2	TC-2
Leucine aminopeptidase (3.4.11)	LAP (PEP-2)	TC-1, LiOH
Malate dehydrogenase (1.1.1.37)	MDH	TC-1
Malic enzyme (1.1.1.40)	ME	TC-2, TC-1
Mannose phosphate isomerase (5.2.1.8)	MPI	TC-2
Peptidase (3.4.11)	PEP-1	TBE, LiOH
	PEP-4	TBE
6-Phosphogluconate dehydrogenase (1.1.1.44)	PGD	AP
Phosphoglucomutase (2.7.5.1)	PGM-1	TC-1
	PGM-2	AP
Sorbitol dehydrogenase (1.1.1.14)	SorDH	TBE

**AP.* 0.04 M citrate adjusted with N-(3-aminopropyl)-morpholine to pH 6.0; diluted 1:19 for gels and undiluted for electrodes (15 hr. 80 V). *LiOH.* Solution A: 0.03 M LiOH, 0.19 M borate, pH 8.1; Solution B: 0.008 M citrate, 0.05 M Tris, pH 8.4; 10% A plus 90% B for gel, A for electrode (15 hr. 180 V) *TBE* 0.087 M Tris, 0.086 M borate, 0.001 M EDTA, pH 9.0; diluted 1:3 for gels. 0.5 M Tris, 0.065 M borate, 0.02 M EDTA, pH 8.0; undiluted for electrodes (15 hr., 80 V). *TC-1.* 0.378 M Tris, 0.165 M citrate, pH 6.0; 13.5 ml diluted to 400 ml for gel and undiluted for electrodes (15 hr. 60 V). *TC-2.* 0.188 M Tris, 0.065 M citrate, pH 6.8; diluted 1:9 for gels and 1:5 for electrodes (15 hr. 150 V).

breviations are typeset in capital letters to indicate the protein and in italics to indicate the presumed allele.

The mean number of alleles per locus, the proportion of loci polymorphic, P (a locus was considered polymorphic if more than one allele was detected), and the mean individual heterozygosity, $\bar{H}$ (by direct count), were calculated for each sample. Allozyme frequencies for the polymorphic loci were tested for their agreement with Hardy-Weinberg expectations for a panmictic population by X^2-test where possible and by the Fisher exact test. Population genetic structuring was studied with Wright's (1978) hierarchical F-statistics (F_{IS}, F_{ST} and F_{IT}) calculated for each locus and sample. Genetic distance coefficients (D) of Nei (1978) and the genetic similarity coefficient (S) of Rogers (1972) were calculated and clustered by the UPGMA algorithm. The above analyses were performed with the BIOSYS-1 computer program (Swofford & Selander, 1981).

RESULTS

Consistent and genetically interpretable results were obtained for 21 loci per sample. Allele frequencies are shown for all polymorphic loci in Table 3. (Detailed descriptions of banding patterns and their interpretation are available from the senior author).

In the field-collected snails from the Philippines the mean number of alleles per locus ($\bar{A}$) was 1.1–1.4; Chinese snails were more variable, $\bar{A}$ = 1.8–2.1. The proportion of loci polymorphic (P) was 0.14–0.24 in the field-collected samples from the Philippines and slightly higher (P = 0.33) in the mixed origin laboratory stock. In contrast, the Chinese snails were three times as polymorphic (P = 0.52–0.62) and varied at 8 loci which were fixed or almost fixed in the Philippines. Mean individual heterozygosity also differed significantly between the Philippines and China ($\bar{H}$ = 0.02–0.09 vs 0.19–0.20).

No significant departure from panmixia was detected in the samples studied. Genotype frequencies were in agreement with Hardy-Weinberg expectations in 27 of 29 tests (polymorphic loci/sample) involving field-collected Philippine snails. No special significance is attached to the two cases involving slight heterozygote deficiencies (*Pep-4*) in Luzon A and *Gpi* in Luzon B) as the remaining 2–3 variable loci in each of these samples were in

equilibrium. Similarly, in the case of the more variable Chinese samples, no significant departure from Hardy-Weinberg expectations was detected in 23 of 24 tests. (The remaining case, *Sordh* in China A, also involved a deficiency of heterozygotes; Fisher exact test $P = 0.051$, fixation index 0.43). With only these three minor exceptions among 53 tests we conclude that in nature these snails are outbreeding at random. This conclusion is supported by the finding that Wright's multilocus F_{IS} statistic was 0.089, a value not significantly different from zero.

The five samples from Leyte and Luzon are practically indistinguishable at the 20 loci examined (Table 3). Mindoro snails differ from those on Leyte-Luzon in having different allele frequencies at 5 of 21 loci, the greatest differences involving *Lap* and *Pgm-2*. Snails from Mindanao were characterized by slower *Acp* and *Idh-1* alleles but were otherwise similar to the other Philippine snails. In contrast, the Chinese snails had unique alleles at 14 of the 21 loci examined. Philippine and Chinese snails can be distinguished from one another on the basis of variation at *Aat, Es-1, Es-3, Gap, Gpi,* Me and *Pgm-1*. If subsequent work shows that Philippine Acp^c is, in fact, not homologous with the Chinese allele of similar mobility, then *Acp* may be added to this list of diagnostic loci. These major differences between Chinese snails and the others is underscored by the calculated value of Wright's F_{ST} (0.64), a value significantly different from zero.

We estimated overall multilocus genetic differentiation between these samples using Nei's (1978) unbiased coefficient of genetic distance (Table 4; Roger's *S* values are shown for comparative purposes). Within the Philippines intersample *D* values were in the range 0.001–0.134 and $\bar{D} = 0.036$. *D* between the two Chinese samples was zero. In marked contrast, the China-Philippines comparison yielded $\bar{D} = 0.617$. These various values were used to prepare the phenogram (Fig. 1).

DISCUSSION

Taxonomic Conclusions

This paper is concerned primarily with the detection of genetic differences in related but geographically isolated populations and the interpretation of their significance. We have examined various samples of a geographically widespread polytypic species, *Oncomelania hupensis,* whose range is fragmented on numerous isolated islands. We found practically no interpopulation variation on a single island (Leyte) in the Philippines, minor variation between samples representing four Philippine islands, and great differences between the Philippines and samples from one locality on the Chinese mainland. The unexpectedly high value for Nei's genetic distance between *O. h. hupensis* (China) and *O. h. quadrasi* (Philippines) ($\bar{D} = 0.62$) suggests that these taxa may have actually diverged to the rank of full species. The Chinese *Oncomelania* from Guichi have alleles not found in the Philippines at 14 of 21 loci. Furthermore, there are no alleles shared between both areas at 5 loci. Such evolutionary divergence at more than 20% of the structural genes is unknown within a single sexually reproducing animal species.

Although Selander & Ochman (1983) have correctly pointed out that allelic isozyme variation is irrelevant to the process of speciation in molluscs, there may be a relationship between the degree of genetic differentiation and taxonomy within a group of related populations. For example, a survey of over 7,000 comparisons of conspecific populations of plants and animals found only 2% of the intraspecific *D* estimates exceeded 0.10 (Thorpe, 1983). In contrast, Thorpe found that for 900 comparisons of interspecific *D* values (between congeners) the mean genetic distance was about 0.40 (range 0.03–>1.0). The majority of well-characterized, sexually reproducing mollusc species also have intraspecific genetic distances of less than 0.10 and interspecific distances (between congeners) of more than 0.05 and typically between 0.20–0.60 (Woodruff, in prep.). Genera for which both intra- and interspecific genetic distances are within these limits include *Bankia* and *Teredo* (Hoagland & Turner, 1981), *Biomphalaria* (Mulvey & Vrijenhoek, 1982; Woodruff *et al.,* 1985; Mulvey *et al.,* 1988; Mulvey & Woodruff, in prep.), *Bithynia* and *Parafossarulus* (Chung, 1984), *Bothriembryon* (Hill *et al.,* 1983), *Brotia* and *Tricula* (Woodruff *et al.,* 1986a; Staub, 1988), *Cerion* (Gould & Woodruff, 1978, 1986, 1987; Woodruff & Gould, 1980, 1987), *Crassostrea* (Hedgecock & Okazaki, 1984), *Crepidula* (Hoagland, 1984; Woodruff *et al.,* 1986b), *Cristilabrum, Ningbingia* and *Turgenitubulus* (Woodruff & Solem, in prep.), *Elliptio* (Davis *et*

TABLE 3. Allele frequencies for 16 polymorphic loci in 10 samples of *Oncomelania hupensis* with summary statistics of genetic variability.*

	Leyte			Luzon					China	
Locus/allele	A	B	C	A	B	Mindoro	Mindanao	Lab	A	B
Aat-a									0.92	1.00
b	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.08	
Acp-a	1.00	1.00	1.00	1.00	1.00	1.00	0.08	1.00		
b									0.08	0.10
c							0.92		0.92	0.90
Es-1-a									0.31	0.55
b									0.69	0.45
c	0.82	0.90	0.83	0.92	0.90	1.00	0.51	0.96		
d	0.18	0.10	0.17	0.08	0.10		0.49	0.04		
Es-3-a									0.01	
b									0.97	1.00
c	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.02	
Gap-a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
b									1.00	1.00
Gpi-a		0.01		0.20	0.05	0.30		0.16		
b									0.09	0.10
c	1.00	0.99	1.00	0.80	0.95	0.70	1.00	0.84		
d									0.30	0.45
e									0.56	0.40
f									0.05	0.05
Idh-1a	1.00	1.00	1.00	1.00	1.00	1.00	0.67	0.99	0.02	0.10
b							0.33	0.01	0.98	0.90
Idh-2a									0.02	
b	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98	1.00
Lap-a									0.05	0.10
b					0.02	0.41		0.14	0.56	0.40
c	0.99	0.97	0.96	0.99	0.98	0.40	1.00	0.73	0.31	0.35
d									0.08	0.15
e	0.01	0.03	0.04	0.01		0.19		0.13		
Me-a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
b									1.00	1.00
Mpi-a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.56	0.70
b									0.44	0.30
Pep-4a	0.57	0.76	0.41	0.05	0.25	0.02	0.98	0.11	0.36	0.40
b	0.31	0.23	0.45	0.90	0.75	0.27	0.02	0.55	0.62	0.60
c	0.12	0.01	0.14	0.05		0.71		0.34	0.02	
Pgd-a									0.03	0.05
b	0.99	1.00	1.00	1.00	1.00	0.98	1.00	0.96	0.91	0.95
c	0.01					0.02		0.04	0.03	
d									0.03	
Pgm-1a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
b									0.69	0.60
c									0.31	0.40
Pgm-2a						0.31	0.06	0.25	0.05	0.10
b	0.98	1.00	1.00	1.00	1.00	0.56	0.94	0.69	0.62	0.60
c									0.09	0.10
d	0.02					0.13		0.06	0.19	0.20
e									0.05	
Sordh-a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88	0.85
b									0.12	0.15
$\bar{N}$	75.9	61.1	62.2	56.2	17.4	52.5	81.5	62.2	31.4	9.6
(S.E.)	6.8	5.6	6.8	6.2	1.3	2.2	3.4	4.2	0.4	0.4
$\bar{A}$	1.3	1.2	1. 2	1.2	1.1	1.4	1.2	1.5	2.1	1.8
P	0.24	0.19	0.14	0.19	0.14	0.24	0.24	0.33	0.62	0.52
$\bar{H}$	0.04	0.03	0.04	0.03	0.02	0.10	0.06	0.08	0.19	0.21

*Samples are described in Table 1; $\bar{N}$-mean sample size (and standard error) per locus, $\bar{A}$–mean no. alleles per locus, P–proportion of loci polymorphic (monomorphic loci: *Cat, G6pdh, αGpdh, Mdh-1 & Pep-1* excluded from this table), $\bar{H}$—mean individual heterozygosity

TABLE 4. Matrix of Genetic Similarity and Distance Coefficients*

Sample	1	2	3	4	5	6	7	8	9	10
1 Leyte A	*****	0.986	0.990	0.958	0.973	0.906	0.905	0.943	0.507	0.519
2 Leyte B	0.002	*****	0.980	0.956	0.972	0.903	0.907	0.940	0.498	0.511
3 Leyte C	0.001	0.005	*****	0.965	0.980	0.910	0.896	0.951	0.512	0.524
4 Luzon A	0.018	0.026	0.011	*****	0.983	0.916	0.865	0.957	0.507	0.517
5 Luzon B	0.008	0.013	0.003	0.002	*****	0.908	0.882	0.953	0.513	0.522
6 Mindoro	0.045	0.053	0.040	0.044	0.046	*****	0.819	0.951	0.512	0.527
7 Mindanao	0.061	0.059	0.069	0.104	0.085	0.134	*****	0.854	0.552	0.564
8 Labstock	0.015	0.022	0.010	0.010	0.010	0.014	0.099	*****	0.518	0.530
9 China A	0.648	0.653	0.643	0.635	0.637	0.658	0.530	0.645	*****	0.946
10 China B	0.617	0.622	0.614	0.608	0.609	0.630	0.506	0.616	0.000	*****

*Below diagonal: Nei (1978) unbiased genetic distance; above diagonal: Rogers (1972) genetic similarity.

al., 1981), *Goniobasis* (Chambers, 1980), *Littorina* (Janson, 1987), *Nautilus* (Woodruff *et al.*, 1983, 1987a), *Partula* (Johnson *et al.*, 1977, 1986b,c), *Samoana* (Johnson *et al.*, 1986a), *Sphaerium* (Hornbach *et al.*, 1980), *Triodopsis* (Emberton, 1988), *Uniomerus* (Davis, 1983). Very few molluscan cases have been reported where intraspecific *D* values exceed 0.20. Three exceptions involve *Goniobasis, Sphincterochila* and *Cepaea*. In *G. floridensis* two intraspecific distances were 0.26 and 0.31; these values are notable in that they are four times as large as the maximum intraspecific distances reported in two other species of *Goniobasis* (Chambers, 1980). *Sphincterochila* is most unusual in that genetic distances within species ($\bar{D} = 0.23$, range 0.09–0.41) are higher in some cases than between species ($\bar{D} = 0.36$, range 0.09–0.58) (values recalculated from Nevo *et al.*, 1983; Table 6A). Finally, in the case of *C. nemoralis* the genetic distances between an English and two Italian populations were 0.42 and 0.63 (Johnson *et al.*, 1984). This latter value, based on 20 loci and 13 individuals, is the highest previously reported intraspecific genetic distance. It has not yet been shown, however, that the Italian populations are in genetic contact with those in northwestern Europe (Johnson, pers. comm.; Ochman *et al.*, 1987); it is possible that the Italian population are in need of taxonomic revision. These few cases notwithstanding, the majority of reported intraspecific genetic distances in molluscs are less than 0.10; the China-Philippines distance for *Oncomelania* of 0.62 would certainly be the extreme case. The fact that this value is more than five times greater than the maximum known genetic distance between populations of *Oncomelania* from the Philippines indicates that these Chinese and Philippine snails are so different from one another as to strongly support their recognition as separate species.

Before proceeding further with this taxonomic conclusion it is necessary to reiterate that there is no simple relationship between a *D* value and taxonomic level. Other factors must be considered. Innate variability, mating system, and mode of geographic differentiation and speciation will all affect the rate of genetic divergence in a clade. Although the overwhelming bulk of the evidence (molluscan and non-molluscan) suggests that the observed *D*-value of 0.62 indicates that these populations warrant recognition as full species we do not seek to prove our argument by intimidation. Exceptions to the general are to be expected in this field of science at this time.

It is also appropriate to examine the statistical soundness of the estimates of genetic distance. First, sample sizes, number of loci examined, and the variety of types of enzymes examined are more than adequate to give reasonable estimates of variability and genetic differentiation (Nei, 1987). Samples of more than 8 loci are unlikely to change estimated *D* values by more than 10% (Gorman & Renzi, 1979). Second, and the first conclusion notwithstanding, the single-gel electrophoretic technique employed here gives an underestimate of true variability and genetic distance, typically detecting only 80% of the number of alleles detected when multiple gels and heat denaturization are used (Ayala, 1982; Selander & Whittam, 1983). Third, although the standard errors of our *D* estimates are large in the case of very small values of *D* (e.g., Leyte A–Leyte B $D = 0.002 \pm 0.005$) the errors are relatively smaller when *D* is large. The mean distance between these Chi-

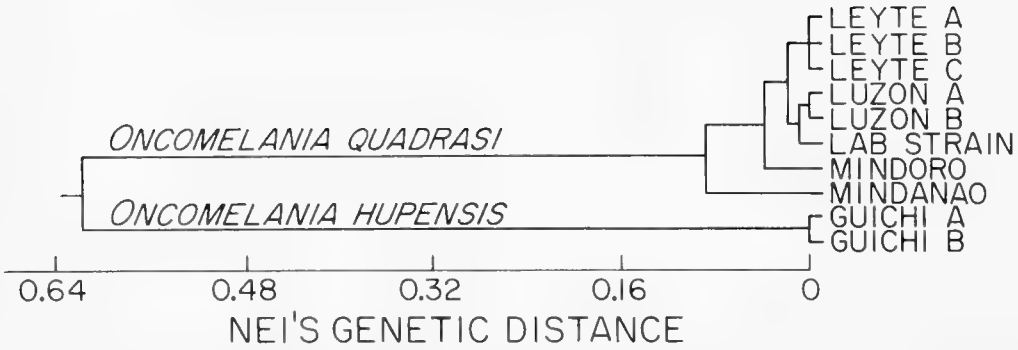

FIG. 1. Phenetic tree based on 21 allozymes, Nei's (1978) unbiased genetic distance and the UPGMA clustering algorithm. The cophenetic correlation is 0.995.

nese and Philippine *Oncomelania* is 0.62 ± 0.20 (Nei *et al.*, 1985). Our preliminary calculation of the China-Philippines *D* is accordingly statistically robust and probably slightly underestimates the true extent of genetic differentiation. (Even greater biochemical differences were detected in a parallel study by Viyanant *et al.* (1987).) The Chinese and Philippine *Oncomelania* examined have experienced about 62 electrophoretically detectable allelic substitutions per 100 loci since their divergence from a common ancestor. We conclude that henceforth *O. h. hupensis* and *O. h. quadrasi* should be treated as full species: *O. hupensis* and *O. quadrasi*.

There is, in addition, a more fundamental reason why we advocate nomenclatorial change. Following Wilson & Brown (1953) we reject the "subspecies" category as useful in defining evolutionary lineages. Similarly, and not denying the great utility of the polytypic species and biological species concepts, we seek now to begin to redefine the various taxa comprising "*Oncomelania hupensis*" in terms of evolutionary species concepts. The biological species concept with its emphasis on reproductive isolation is slowly being replaced with the evolutionary species concept, which places greater emphasis on reproductive recognition (Patterson, 1982; Vrba, 1984) and genetic integrity despite natural or potential hybridization (Woodruff, 1979; Barton & Hewitt, 1985; Templeton, in press).

"One major problem with the exclusive use of the interbreeding community criterion is the tendency on the part of some workers to view speciation as incomplete until sympatry, and then if hybridization occurs to view this as incomplete speciation. This results in two problems with biological species. It makes one feel that he or she is committing heresy if he or she describes an allopatric or parapatric differentiated form as a species. After all, speciation is not "complete." The investigator then sagely refers these perfectly good evolutionary species to a single "polytypic" species composed of two or more subspecies. This invites confusion in two respects. First, it makes for underestimates of the number of independent evolutionary lineages present in a biota. Second, the "subspecies" as an evolutionary lineage will be confounded with the subspecies as a category of convenience—a variant population of an evolutionary species." (Wiley, 1981:28)

As the evolutionary species concept is not yet widely applied to molluscs it may be appropriate to describe it in more detail here. The evolutionary species is a lineage concept that avoids many of the problems of the biological species concept without denying that interbreeding among sexually reproducing individuals is an important component of species cohesion (Wiley, 1981:24). An evolutionary species has a lineage which maintains its integrity from other such lineages and has its own evolutionary tendencies and historical fate. It should come as no surprise that "Many polytypic species composed of discrete and easily diagnosable subspecies are now being broken into species" (Wiley, 1981:34). For further discussions and examples the reader is referred to White (1978), Templeton (1981, 1989), Barigozzi (1982) and Futuyma (1986).

In the present context, *O. hupensis* and *O. quadrasi* are diagnosable without recourse to geographic data. As the differences between the two are not attributable to simple ecophenotypic variation then according to Wiley's

(1981:65–67) criteria for taxonomic decisions involving allopatric populations the two taxa have reached the rank of full species.

Several objections can be raised to this taxonomic revision. First, the existing nomenclature has served us well for many years and emphasizes the undisputed relatedness of the various allopatric populations. Creating a superspecies with 2–6 component species to replace the existing subspecies could obscure this pattern and confuse public health workers. We reject this argument; if anything the recognition of subtle interspecific differences could lead to the development of improved integrated pest management techniques. Second, it can be argued that elevation to species status is contraindicated by the lack of morphological differentiation. Morphological differentiation is, however, a poor indicator of taxonomic status in many groups of sibling species. Numerous examples involving mosquitoes, ascarids and salamanders are reviewed by Bullini (1983); the mean genetic distance between six morphologically and ecologically similar sibling species of *Drosophila willistoni* is $\bar{D} = 0.58$ (Ayala, 1983). Third, the observation that the species, *O. hupensis* and *O. quadrasi*, will hybridize successfully in the laboratory would also imply that they violate the reproductive isolation criterion of the biological species concept. In rebuttal, we argue that the problem lies with an inappropriate species concept. The literature is full of examples of successful hybridization between members of otherwise good species and even genera. An average of 20% of the electrophoretic loci were found to have diverged across a sample of 21 natural interspecific hybrid zones involving various species of animals reviewed by Barton & Hewitt (1983, 1985). It is now clear that semispecies may hybridize in nature without losing their integrity (Endler, 1977; Woodruff, 1979, 1981) and that former decisions to treat allopatric populations as subspecies on the basis of their potentiality for hybridization were founded on a species concept that overemphasized the importance of reproductive isolation. In the present case, however, the evidence does not deal with hybridization in nature—but rather with what occurred under laboratory circumstances where mate choice was constrained. Although this time-honored approach may identify potential reproductive isolating mechanisms it is unlikely to detect characters crucial to specific mate recognition if members of the presently allopatric populations were ever to meet naturally. Finally, it might be argued that any taxonomic revision is premature until all the present subspecies have been reexamined. We reject this argument because the China-Philippines genetic distance is so great; it will be unaffected by the resolution of the taxonomic status of the other alleged subspecies. Furthermore, the proposed revision sets the stage for the testing of a number of interesting biogeographic and evolutionary hypotheses to be noted below.

The recognition of *Oncomelania hupensis* Gredler, 1881, and *O. quadrasi* (Mollendorff, 1895) as species rather than subspecies requires no formal taxonomic revision as both taxa were originally described as species. Although the status of the remaining four subspecies is now open to question, they are undoubtedly in the *O. hupensis* superspecies complex.

Origin of *Oncomelania quadrasi*

The terrestrial and freshwater fauna of the Philippines reached the archipelago, by and large, from the south. The patterns for mammals, reptiles, amphibians and freshwater fish are consistent; these animals reached the Philippines by way of Pleistocene land bridges connecting Sundaland to Palawan or the Sulu Islands and Mindanao (Inger, 1954; Darlington, 1957; Carlquist, 1965; Heaney, 1986). Despite the clear Asian affinities of the Philippine fauna not one species in these groups (excluding recent immigrants) requires a South China Sea crossing. It seems unlikely, however, that amphibious *Oncomelania* conform to this pattern. With the exception of the isolated population in Sulawesi, *Oncomelania* are unknown in southeast Asia, the Malay Peninsula or Indonesia. Passive dispersal across the South China Sea is also problematic as ocean currents and typhoons move in the opposite direction; from the Philippines towards China. Accordingly, we speculate that *Oncomelania* was carried directly from coastal China to the Philippines by migratory birds. The Eastern Asia flyway is well documented (McClure, 1974) and used by numerous wading birds annually. Among the species known to fly between Taiwan and the Philippines are the grey heron (*Ardea cinerea*), cattle egret (*Bubulcus ibis*), great egret (*Egretta alba*), little egret (*E. garzetta*), intermediate egret (*E. intermedia*), black-crowned night heron (*Nycticorax nycticorax*), and sev-

eral bitterns and rails. As these species are associated with *Oncomelania* habitat there is little doubt that eggs, juveniles and even adult snails are occasionally carried around on their muddy feet. The distance between Taiwan and Luzon is about 375 km today but during the Pleistocene when sea levels were lower the maximum water gap (the Bashi Channel between Taiwan and Batan) was less than 100 km. Such distances are short enough to expect high survival rates among any transported snails.

This scenario raises the probability that one or both of the *Oncomelania* species on Taiwan Province (*O. hupensis formosana* and *O. h. chiui*) may be more closely related to Philippine *O. quadrasi* than to *O. hupensis* from Anhui Province. The same may also hold for Japanese *O. h. nosophora* as many of the waders using the Eastern Asia flyway move on from Taiwan Province to Japan. Clearly, a comparison of the genetics of the offshore populations of *Oncomelania* with those on the Chinese mainland will be most illuminating. It will also clarify whether the two subspecies on Taiwan are autochthonous (advocated by Davis, 1967) or represent separate introductions from the continent. At the very least we would expect the occasional dispersal of snails by birds to have a retarding effect on allopatric differentiation.

If *Oncomelania* reached the Philippines by chance over water dispersal we might expect *O. quadrasi* to exhibit reduced genetic variability associated with the founder effect. This is, in fact, what we found: Philippine snails had significantly fewer alleles per locus and only half the proportion of polymorphic loci found in Chinese snails (Table 3). Attractive as the founder effect hypothesis is we doubt it accounts for our observations for two reasons. First, only very severe population bottlenecks ($N<7$) have any significant impact on the variability of a colonist (Nei *et al.*, 1975). Although individual birds may successfully introduce only single snails, it seems likely that repeated introductions would have negated the founder effect. Secondly, the *O. quadrasi–O. hupensis* genetic distance, ($\bar{D} = 0.62$), indicates that the two taxa diverged several million years ago. Using Nei's (1987) conservative arguments, $D = 0.62$ may correspond to a divergence time of about 3 million years. This is time enough, given the very large size of *O. quadrasi* populations, for a full recovery from the reduced levels of genetic variability postulated to characterize early colonists. Although we cannot resolve the problem of reduced variability in *O. quadrasi* relative to Guichi *O. hupensis* we suspect the issue may be somewhat artificial. The appropriate comparison to make is not between *O. quadrasi* and *O. hupensis* from 400 km up the Yangtze but between *O. quadrasi* and its nearest relatives in Taiwan Province or on the adjacent continent. It is most unlikely that the Guichi samples represent this ancestral genome. This underscores the need for further study of geographic variation within Chinese *O. hupensis* as local authorities recognize five subspecies in addition to the two from Taiwan Province (Liu *et al.*, 1981; Lou *et al.*, 1982).

Conclusions

The first goal of the present study was to establish whether *Oncomelania* was variable enough to permit the preparation of a molecular or genetic phylogeny. The samples studied were found to be well suited to this type of analysis based on allozyme variation. *O. hupensis* and *O. quadrasi* differ in their levels of variability ($P = 0.52–0.62$ in the former, $P = 0.14–0.24$ in the latter, $\bar{H} = 0.19–0.21$ vs 0.02–0.10) but neither taxon is unusual, approximate mean levels of variation in amphimictic molluscs are $\bar{P} = 0.47$ (range 0–1.00) and $\bar{H} = 0.14$ (range 0–0.30) (Selander & Ochman, 1983; Nevo *et al.*, 1984). Our preliminary study has revealed up to 14 polymorphic loci per sample—well in excess of the number needed to develop robust molecular phylogenies. In a subsequent report we will characterize the various allelic variants quantitatively so as to facilitate comparative studies in other laboratories.

Our second goal was to explore the extent of genetic differentiation within *Oncomelania quadrasi* in the Philippines. Not surprisingly we found a strong relationship between the degree of geographical isolation and the genetic distance between any two samples. Geographical isolation in the case of *Oncomelania* is related to both geographic distance and saltwater barriers. Thus, the three samples collected within a few km of one another on Leyte are genetically indistinguishable. The Luzon samples, collected 140 km and three islands to the north, are very weakly differentiated from those on Leyte; map distance belies the fact that the snails of Luzon and the almost continuously distributed populations of Leyte-Samar are only separated

today by the shallow (135 m), narrow (<10 km) San Bernardino Strait. Mindoro snails are more differentiated from those of Luzon-Leyte having higher frequencies of certain alleles at *Lap, Pep-4* and *Pgm-2.* The Mindoro sample was collected 250 km from Leyte and 160 km from southern Luzon, and although Mindoro is only 20 km from central Luzon the two islands are separated by a deep and permanent saltwater strait. Mindanao is the most distinctive of the Philippine samples (with a unique *Acp*); the sample was taken 300 km south of Leyte. Today, Leyte and Mindanao are separated by a 30 km wide strait with water 80 m deep. Samples of snails from throughout the archipelago are necessary to fully document the apparent pattern of isolation and distance related differentiation. In addition, future studies will need to relate the alleles found in various Philippine populations to those occuring in, and possibly introduced by migratory birds from China and Japan. We, ourselves, plan to test the hypothesis that the Sulawesi *Oncomelania* are derived from Mindanaoan stock.

Our final goal was to initiate a study of host-parasite coevolution between the snails of the *Oncomelania hupensis* superspecies and the trematodes referred to as *Schistosoma japonicum.* As *O. hupensis* (using this taxon in its traditional sense) is the sole or obligate intermediate host of *S. japonicum* the schistosome could not have established itself in an area until after the snails were introduced. If parasite and host evolve at similar rates we would predict that the genetic distance between source (Guichi, China) and derived (Mindoro, Philippines) populations would be less for the parasite than for the host. This is, in fact, the case: $D = 0.57$ between *S. japonicum* from China and the Philippines (based on 16 loci; Fletcher *et al.,* 1980; Woodruff *et al.,* 1987b; Merenlender *et al.,* 1987) and $D = 0.62$ for *O. hupensis–O. quadrasi.* The relatively large D value between Chinese and Philippine strains of the parasite suggests that the schistosomes were introduced to the archipelago in bird-borne infected snails long before man arrived. This might explain why *S. japonicum* has an unusually wide range of mammalian hosts (Mao & Shao, 1982) compared with most other schistosomes. Clearly, the genetic and evolutionary interactions of *Oncomelania* and *Schistosoma* merit closer attention both at the level of the genetic regulation of compatibility (Yuan, 1958; Yuan *et al.,* 1984; Davis & Ruff, 1973; Chiu *et al.,* 1981; Liu *et al.,* 1981; Lou *et al.,* 1982; Woodruff & Yuan, 1988) and at the population genetic level (Rollinson & Southgate, 1985; Woodruff, 1985). The present preliminary study indicates that some more taxonomic surprises may be forthcoming.

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EARLY SHELL MINERALOGY, MICROSTRUCTURE, AND SURFACE SCULPTURE IN FIVE MYTILID SPECIES

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ABSTRACT

Patterns of early shell microstructure, mineralogy, and exterior sculpture were examined in *Ischadium recurvum*, *Geukensia demissa*, *Brachidontes exustus*, *Mytilus edulis*, and *Modiolus modiolus* (Bivalvia: Mytilidae). In all species, the prodissoconch was aragonitic and had homogeneous microstructure and fine commarginal surface sculpture. An interdissoconch, which also was aragonitic, homogeneous, and with finely sculptured surface morphology, formed in *Ischadium recurvum*, *Geukensia demissa*, *Brachidontes exustus*, and *Modiolus modiolus*. In these four species, secretion of the dissoconch began at a post-settlement stage and was marked on the exterior shell surface by a strong delineation and in fracture section by formation of new shell layers. An interdissoconch was not present in *Mytilus edulis;* formation of the dissoconch in this species was concurrent with settlement.

Key words: shell mineralogy; shell microstructure; shell sculpture; prodissoconch; interdissoconch; dissoconch; mytilid; mussel.

INTRODUCTION

Dramatic changes in microstructure, surface sculpture, and mineralogy occur in the shells of many species of bivalves at the time of settlement (Ansell, 1962; Stenzel, 1964; Taylor *et al.*, 1969; Carriker & Palmer, 1979; Jablonski & Lutz, 1980, 1983; Waller, 1981). The aragonitic prodissoconch, or larval shell, of specimens described to date generally has homogeneous microstructure and fine surface sculpture (Stenzel, 1964; Taylor *et al.*, 1969; Carriker & Palmer, 1979; Waller, 1981). The dissoconch, or adult shell, is aragonitic or bimineralic (calcitic and aragonitic) and usually is comprised of several shell layers and various types of microstructure (Stenzel, 1964; Taylor *et al.*, 1969; Carriker & Palmer, 1979; Waller, 1981). Commarginal and/or radial sculpture ornament the exterior surface of the dissoconch (Ansell, 1962; Carriker & Palmer, 1979; Jablonski & Lutz, 1983; Waller, 1981). In many bivalve families, therefore, a distinct prodissoconch-dissoconch boundary is marked on the exterior shell surface by a change in sculpture and in fracture section by a change in microstructure. In some cases, a change in mineralogy is recognizable also. Carriker & Palmer (1979) presented a detailed examination using scanning electron microscopy of early shell specimens of *Crassostrea virginica* (Gmelin). Waller (1981) included a study of the early shell in his description of the larval development of *Ostrea edulis* Linné. In both oysters, abrupt changes in mineralogy, microstructure, and shell ornamentation were associated with metamorphosis.

The larval-postlarval shell boundary, however, is not clearly defined by such features in all bivalves. An interdissoconch is formed in several mytilid and pectinid species, as well as in a few species belonging to other families (Ockelmann, 1965). This intermediate shell is secreted after the prodissoconch and is structurally distinct from the dissoconch (Jørgensen, 1946; Rees, 1950; Ockelmann, 1965, 1983; Campos & Ramorino, 1980). (Ockelmann (1983) used the term nepioconch for interdissoconch.) Because of similarity in surface morphology of the prodissoconch and interdissoconch, the larval-postlarval boundary is not always obvious in mytilids. The interdissoconch-dissoconch boundary, on the other hand, is marked by a strong delineation in the shell surface. This study examines the surface sculpture, microstructure, and mineralogy of the prodissoconch, interdissoconch, and early dissoconch in five mytilid species.

MATERIALS AND METHODS

Adult specimens of *Mytilus edulis* Linné collected offshore, southern New Jersey; *Ischa-*

TABLE 1. Length (greatest dimension) in μm of the shell stained by Feigl's solution for *Mytilus edulis*, *Ischadium recurvum*, *Geukensia demissa*, and *Modiolus modiolus*. INT = interdissoconch, PRO = prodissoconch.

Species	Range	$\bar{x} \pm$ SD (n = 50)
Mytilus edulis	(PRO) 304–363	333 ± 13.9
Ischadium recurvum	(INT) 507–676	588 ± 32.2
Geukensia demissa	(INT) 641–844	709 ± 42.7
Modiolus modiolus	(INT) 617–811	720 ± 42.1
*Brachidontes exustus**	(INT) 532–718	623 ± 42.5

*For comparison, shell length at the transition from commarginal to cancellate surface sculpture is listed for *B. exustus*.

dium recurvum (Rafinesque) from James River, Virginia; *Modiolus modiolus* (Linné) from Cape Newagen, Maine; *Geukensia demissa* (Dillwyn) from Maurice River, New Jersey; and *Brachidontes exustus* (Linné) from Cabbage Island and Wilmington Island, Georgia, were spawned and reared using standard techniques (see Loosanoff & Davis, 1963; Bayne, 1976). Laboratory cultures of larvae and postlarvae were maintained at temperatures and salinities similar to those at collection sites of adult organisms. Specimens were cleaned for 10 minutes with a 5.25% solution of sodium hypochlorite to remove soft tissues and the periostracum (after Rees, 1950). Shells were rinsed with distilled water, stored in 95% ethanol, and prepared for analysis as follows:

Scanning electron microscopy

Disarticulated valves selected for documentation of patterns of exterior surface sculpture were mounted on silver tape. Additional specimens were mounted for microstructural examination; these were fractured perpendicularly to the hinge line by applying pressure with a dissecting needle near the umbo. All samples were coated with approximately 600 Å of gold-palladium and were photographed with a Hitachi S-450 scanning electron microscope.

Staining to differentiate calcite and aragonite

Mineralogy of the outer shell layer was determined by floating shells in Feigl's solution, with the exterior shell surface contacting the solution, for two minutes at room temperature. The solution differentiates between aragonite and calcite on the basis of different solubilities of the two forms of calcium carbonate (Feigl, 1939; Friedman, 1959; Schneidermann & Sandberg, 1971; Carter, 1980). Generally, a black precipitate indicates aragonite, while lack of a precipitate indicates calcite; however, overexposure or the effect of fine-grained microstructures may confound results (Carter, 1980). The length (greatest dimension) of the stained (aragonitic) region was measured to the nearest 5 μm using a compound microscope.

X-ray diffraction

Mineralogy of the prodissoconch (shell before formation of ligament pit), interdissoconch (shell after formation of ligament pit, but prior to onset of multi-layers), and dissoconch (multi-layered shell) was determined by X-ray diffraction of the whole shell. Samples were analysed with a Siemans Type F Goniometer. Slides were scanned at 2θ values 25–30°, a range that contains the major peaks for aragonite and calcite. Standard aragonite and calcite preparations were analysed for comparison.

RESULTS

In *Mytilus edulis*, a striking boundary separates the smooth exterior surface of the prodissoconch and the distinct, commarginally ridged surface of the dissoconch (Fig. 1). This exterior boundary also marks a transition in microstructure from the homogeneous larval shell to the multi-layered postlarval shell, with a thin, nacreous inner layer and thick, prismatic outer layer (Fig. 2). Results of X-ray diffraction analyses of specimens of *M. edulis* showed that the larval shell is entirely aragonitic; dissoconch valves have recognizable peaks for both aragonite and calcite (Fig. 3A). Feigl's solution formed a black precipitate on the exterior surface of the prodissoconch, while the exterior surface of the dissoconch

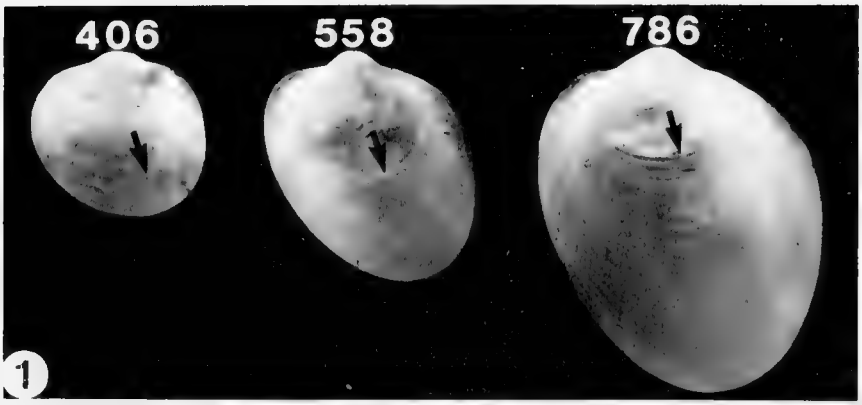


FIG. 1. Scanning electron micrographs of the exterior surface of left valves of *Mytilus edulis* postlarvae. Numbers indicate greatest shell dimension in μm. Arrows mark prodissoconch-dissoconch boundary.

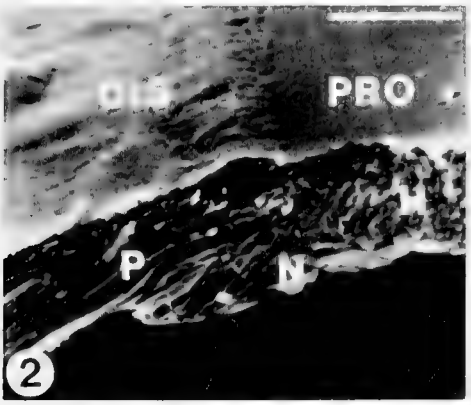


FIG. 2. Scanning electron micrograph of a fractured section of an early postlarval valve of *Mytilus edulis* in the area of transition from prodissoconch to dissoconch. Shell margin is to the left. DIS = dissoconch, H = homogeneous structure, N = nacreous structure, P = prismatic structure, PRO = prodissoconch. Scale bar = 10 μm.

lacked a precipitate. The average length of the stained shell is 333 μm (Table 1).

While shells of examined specimens of *Ischadium recurvum*, *Geukensia demissa*, and *Modiolus modiolus* lack a distinct change in the exterior surface morphology at settlement, a strong border separates the interdissoconch and dissoconch (Figs. 4–6). A shift from commarginal to cancellate surface sculpture occurs at the interdissoconch-dissoconch boundary in *I. recurvum* and *G. demissa* (Figs. 4–5). Both the interdissoconch and dissoconch in *M. modiolus* have commarginal surface sculpture (Fig. 6). X-ray diffraction analyses of specimens of *I. recurvum*, *G. demissa*, and *M. modiolus* indicated that the shell is entirely aragonitic until the dissoconch stage, when the shell becomes bimineralic (Fig. 3B–D). The average length of the aragonitic interdissoconch is 588 μm in *I. recurvum*, 709 μm in *G. demissa*, and 720 μm in *M. modiolus* (Table 1). In all three species, the shell has homogeneous microstructure until the post-settlement transition in surface sculpture and mineralogy, at which point a prominent, nacreous inner layer is secreted (Figs. 7–9). Staining with Feigl's solution revealed that the dissoconch in these three species has a calcitic outer layer. While this calcitic outer layer initially is very thin, its distinct microstructure is recognizable in the early dissoconch (Figs. 10–12). This outer layer is composed of blocky calcite in *I. recurvum* and *G. demissa* (Figs. 10–11) and of prismatic calcite in *M. modiolus* (Fig. 12). An additional, underlying layer of homogeneous aragonite is evident in the early dissoconch of *I. recurvum* and *G. demissa* (Figs. 10–11).

In *Brachidontes exustus*, commarginal shell sculpture is prevalent until a post-settlement stage (Fig. 13). A boundary between commarginal ridges on the interdissoconch and bold, cancellate sculpture on the dissoconch is obvious at an average length of 623 μm (Table 1). A fractured section at this boundary reveals a shift from the single-layered, homogeneous interdissoconch to the dissoconch with a nacreous inner layer and thin outer layer (Fig. 14). The prismatic microstructure of this outer layer is recognizable in

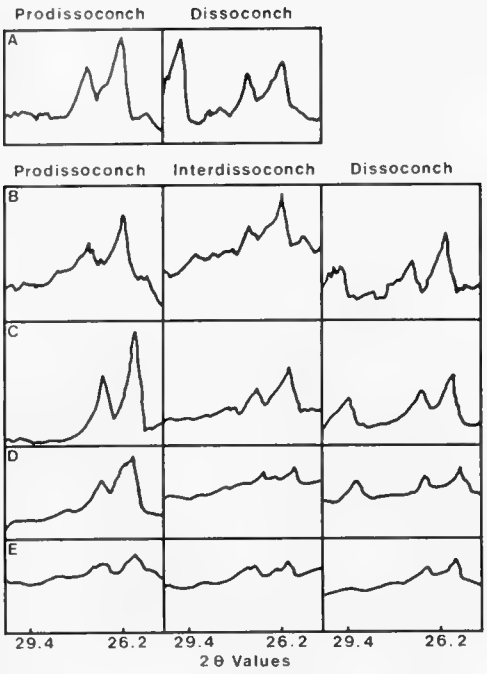


FIG. 3. X-ray diffraction (2θ) scans from 25 to 30° for powdered shell samples. The major K-alpha peak (100%) for aragonite is at a diffraction angle 2θ value of 26.2°; a second peak (52%) is at 27.2°. The major peak for calcite (100%) is at a 2θ value of 29.4°. A. *Mytilus edulis* B. *Ischadium recurvum* C. *Geukensia demissa* D. *Modiolus modiolus* E. *Brachidontes exustus.*

the early dissoconch (Fig. 15). Results of X-ray diffraction analyses and staining with Feigl's solution showed that the outer layer is not calcitic; the dissoconch is entirely aragonitic in this species (Fig. 3E).

DISCUSSION

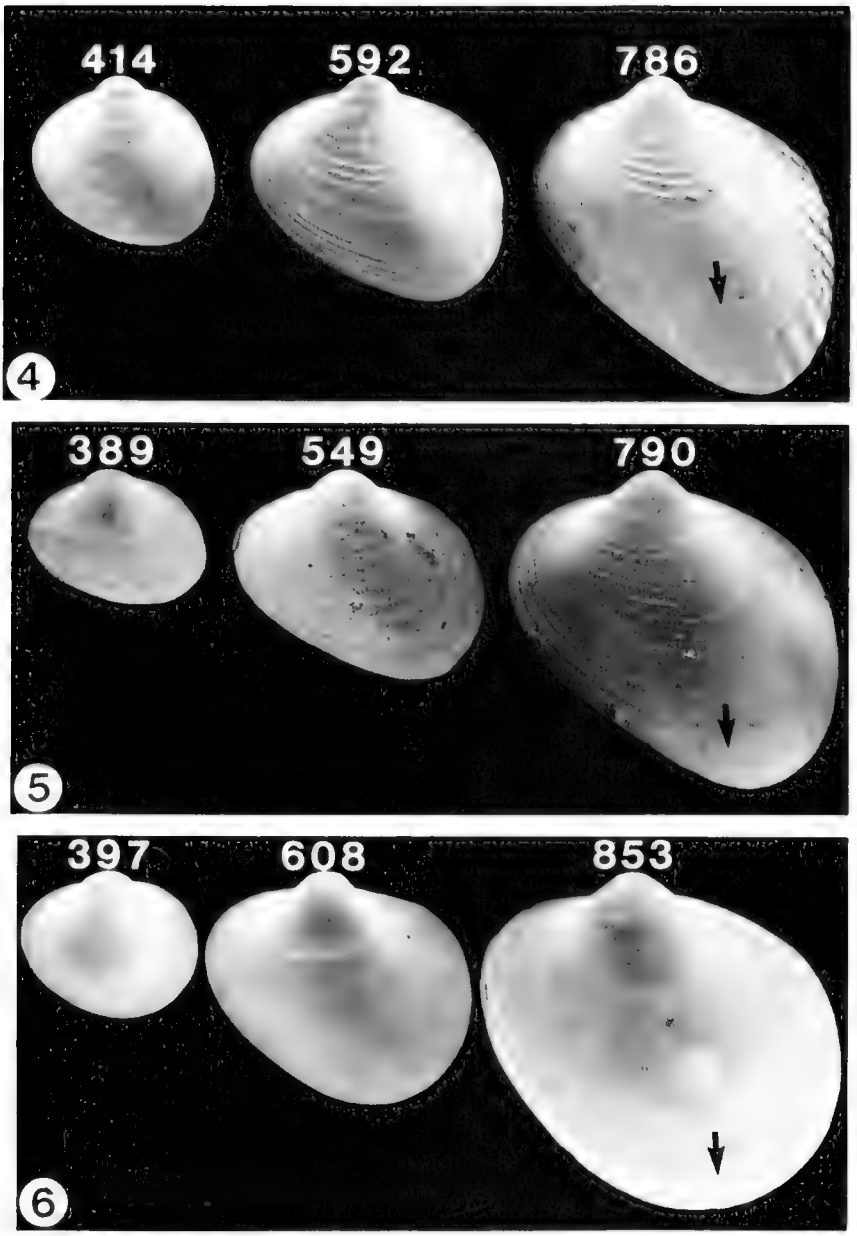
Data on the early shell mineralogy, microstructure and surface sculpture of *Mytilus edulis, Ischadium recurvum, Modiolus modiolus, Geukensia demissa,* and *Brachidontes exustus* are summarized in Table 2. Stenzel (1964) suggested that nearly all species of bivalves have an aragonitic larval shell. The prodissoconch of the five species in the present study is aragonitic and has homogeneous microstructure and fine commarginal surface sculpture. The same features characterize the interdissoconch of *I. recurvum, M. modiolus, G. demissa,* and *B. exustus.*

Ockelmann (1983) stated that most mytilids form an interdissoconch. In the present study, four of the five species examined have an interdissoconch. The prodissoconch-dissoconch boundary in *M. edulis* is well-defined and indicative of settlement. The larval-postlarval border is not obvious in *B. exustus, G. demissa, I. recurvum,* and *M. modiolus* postlarval shells, because of similarity in mineralogy, microstructure, and surface sculpture of the prodissoconch and interdissoconch in these species.

Carter (1980) provided new data on shell mineralogy in several species in the Mytilacea. He examined larval shell mineralogy in 13 species and found that all had an aragonitic larval shell. However, lengths of the larval shell were much greater than previously reported sizes of these species at settlement. For example, the length of the larval shell in *I. recurvum* was reported by Carter as 0.5 mm, but Chanley (1970) indicated the greatest length of a larva with a functional velum was 220 μm for this species. The length of the larval shell of *G. demissa* was reported as 0.7 mm, but Chanley & Andrews (1971) stated that *G. demissa* larvae metamorphosed at lengths ranging from 220 to 305 μm. This aragonitic 'larval shell' was most likely the interdissoconch.

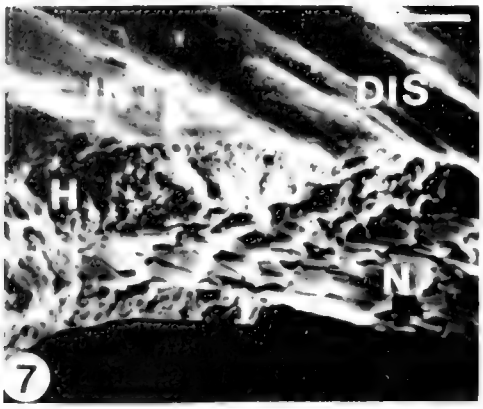
The interdissoconch and dissoconch are more clearly distinguishable because of differences in sculpture and color patterns (Ockelmann, 1983). For example, lack of radial sculpture and color distinguished the interdissoconch from the dissoconch in *Modiolaria marmorata* (Forbes) (Jørgensen, 1946). In *Geukensia* (= *Modiolus*) *demissa,* a sharp delineation at a shell length of approximately 0.65 mm marked a change from a yellow-white or white shell with fine circular ridges to a purple shell with radial surface sculpture (Sullivan, 1948). Campos & Ramorino (1980) described a post-metamorphic transformation from a colorless shell with commarginal striae to a white shell with radial markings in *Brachidontes granulata* (Hanley). Distinct color and surface sculpture characterized the interdissoconch in eight new species of mytilids, including *Modiolus nicklesi, M. pseudobarbatus, M. thorsoni, Musculus koreanus, Rhomboidella obesa, R. malaccana, Xenostrobus mangle,* and *X. balani,* described by Ockelmann (1983).

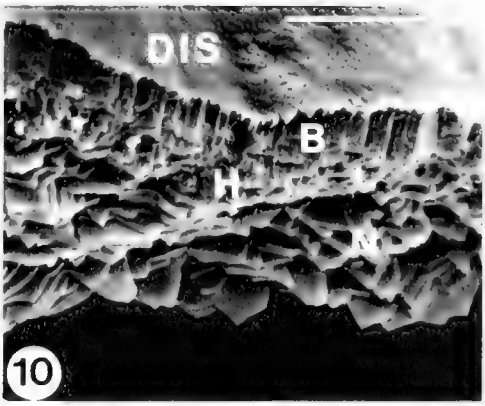
In summary, in all five species an abrupt

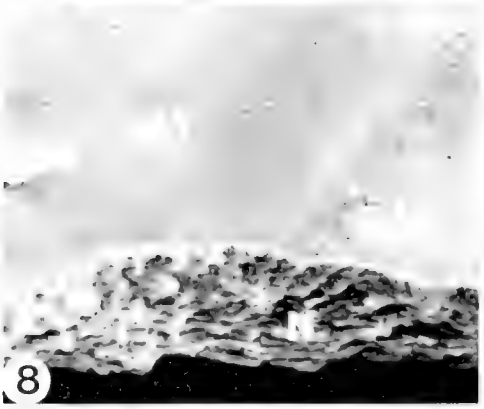


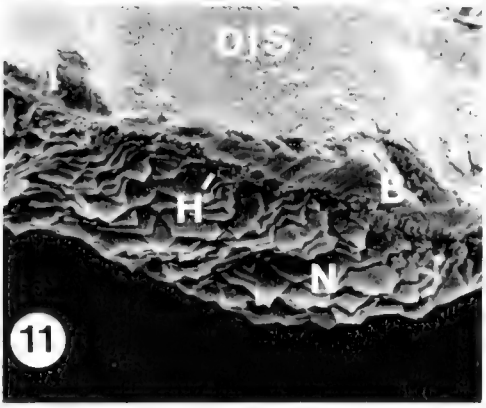
FIGS. 4–6. Scanning electron micrographs of the exterior surface of left valves of *Ischadium recurvum*, *Geukensia demissa*, and *Modiolus modiolus* postlarvae. Numbers indicate greatest shell dimension in μm. Arrows mark interdissoconch-dissoconch boundary. 4. *Ischadium recurvum*. 5. *Geukensia demissa*. 6. *Modiolus modiolus*.

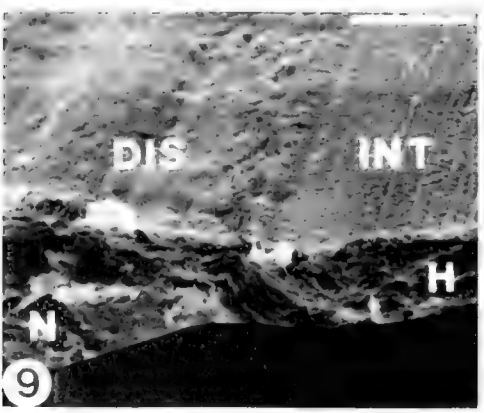
boundary separates the single-layered early shell with homogeneous microstructure from the multi-layered dissoconch. This transition is correlated with settlement in *M. edulis* but occurs at a post-settlement stage in *I. recurvum*, *G. demissa*, *M. modiolus*, and *B. exustus*. *Ischadium recurvum*, *G. demissa*, *M. modiolus*, and *B. exustus* have an aragonitic

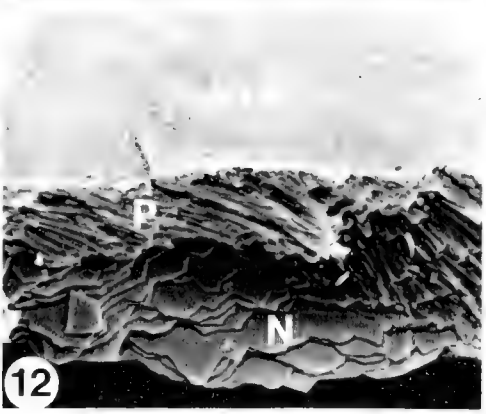












FIGS. 7–9. Scanning electron micrographs of fractured sections of postlarval valves of *Ischadium recurvum*, *Geukensia demissa*, and *Modiolus modiolus* in the area of transition from interdissoconch to dissoconch. DIS = dissoconch, H = homogeneous structure, INT = interdissoconch, N = nacreous structure. Scale bar = 10 μm. 7. *Ischadium recurvum*. Shell margin is to the right. 8. *Geukensia demissa*. Shell margin is to the right. 9. *Modiolus modiolus*. Shell margin is to the left

FIGS. 10–12. Scanning electron micrographs of fractured sections of early dissoconch specimens of *Ischadium recurvum*, *Geukensia demissa*, and *Modiolus modiolus*. B = blocky structure, DIS = dissoconch, H = homogeneous structure, N = nacreous structure, P = prismatic structure. Scale bar = 10 μm. 10. *Ischadium recurvum*. Shell margin is to the right. 11. *Geukensia demissa*. Shell margin is to the right. 12. *Modiolus modiolus*. Shell margin is to the right.

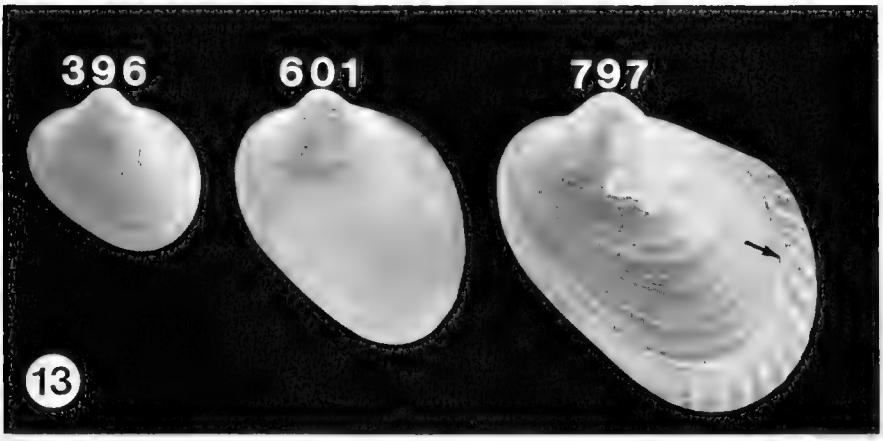


FIG. 13. Scanning electron micrographs of the exterior surface of left valves of *Brachidontes exustus* postlarvae. Numbers indicate greatest shell dimension in μm. Arrow marks interdissoconch-dissoconch boundary.

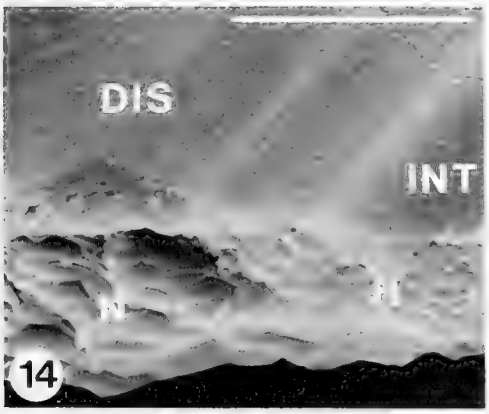


FIG. 14. Scanning electron micrograph of a fractured section of a postlarval valve of *Brachidontes exustus* in the area of transition from interdissoconch to dissoconch. Shell margin is to the left. DIS = dissoconch, H = homogeneous structure, INT = interdissoconch, N = nacreous structure. Scale bar = 10 μm.

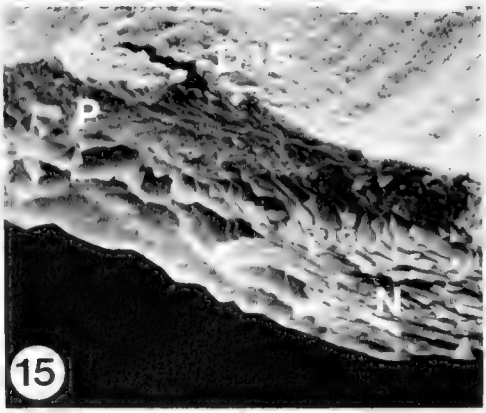


FIG. 15. Scanning electron micrograph of a fractured section of an early dissoconch specimen of *Brachidontes exustus*. Shell margin is to the right. DIS = dissoconch, N = nacreous structure, P = prismatic structure. Scale bar = 10 μm.

interdissoconch with microstructure and surface sculpture patterns similar to those of the prodissoconch of these species. A demarcation in the exterior shell surface occurs between the prodissoconch and dissoconch of *M. edulis* and between the interdissoconch and dissoconch in *B. exustus*, *G. demissa*, *I. recurvum*, and *M. modiolus*. While an outer calcitic layer is not found in the dissoconch of *B. exustus*, definite changes in microstructure and surface sculpture occur at the interdissoconch-dissoconch interface.

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TABLE 2. Mineralogy and microstructure of the prodissoconch, interdissoconch, and early dissoconch in five mytilid species. A = aragonitic, B = blocky structure, C = calcitic, H = homogeneous structure, N = nacreous structure, P = prismatic structure.

Species	Prodissoconch	Interdissoconch	Early Dissoconch
Mytilus edulis	A (H)	absent	C (P), A (N)
Ischadium recurvum	A (H)	A (H)	C (B), A (H), A (N)
Geukensia demissa	A (H)	A (H)	C (B), A (H), A (N)
Modiolus modiolus	A (H)	A (H)	C (P), A (N)
Brachidontes exustus	A (H)	A (H)	A (P), A (N)

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ASPECTS OF THE ANATOMY OF *PLICOPURPURA PATULA* (PROSOBRANCHIA: MURICOIDEA: THAIDINAE), NEW COMBINATION, WITH EMPHASIS ON THE REPRODUCTIVE SYSTEM

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ABSTRACT

The new combination *Plicopurpura patula* (formerly known as *Purpura patula*) is proposed, based on morphological differences in anatomy, radula and egg capsules from *Purpura persica*, the type species of *Purpura*. These characters justify resurrection of the genus *Plicopurpura* Cossmann, 1903.

The reproductive system and its functional implications for *Plicopurpura patula* and other thaidine gastropods receives detailed attention. *Plicopurpura patula* and other thaidine gastropods differ in their reproductive systems from other Muricoidea. *Plicopurpura patula* has a row of posterior seminal receptacles at the dorsal periphery of the albumen gland. Sperm are embedded in the walls of the seminal receptacles and released to fertilize eggs entering the albumen gland. Non-thaidine muricoideans, such as *Nucella*, do not have these posterior seminal receptacles.

Key words: Thaidinae; *Plicopurpura; Purpura;* anatomy; fertilization; reproduction; taxonomy; systematics; morphology.

INTRODUCTION

Little is known about the functional anatomy of thaidine gastropods. Most studies focus on specific aspects of anatomy, such as the alimentary system (Righi, 1964; Wu, 1965; Rajalakshmi *et al.*, 1980, 1981a, 1981b; Shyamasundari *et al.*, 1985), and the reproductive system (Houston, 1976). In particular, radular morphology of thaidines has received considerable attention (for extensive bibliography, see Kool, 1987). Haller (1888) presented a detailed anatomical study of *Concholepas concholepas* (Bruguière, 1789: 252), one of the few well-studied thaidines. Many other anatomical reports exist on a variety of muricoideans closely allied with thaidines, but not monophyletic with them (Kool, Ph.D. dissertation; Kool & Harasewych, in preparation), such as members of the genera *Nucella* Röding, 1798(2):130 (Graham, 1941, 1949; Fretter, 1941; Fretter & Graham, 1962; Harasewych, 1984; Houston, 1976), *Acanthina* Fischer, 1807:174 (Wu, 1985), *Urosalpinx* Stimpson, 1865:58 (Carriker, 1943, 1955; Carriker *et al.* 1972), and *Trophon* Montfort, 1810:482 (Harasewych, 1984; Smith, 1967). Anatomical reports on species of the genus *Rapana* Schumacher, 1817:65, which is monophyletic with Thaidinae Jousseaume 1888:179 (Kool, Ph.D. dissertation; Kool & Harasewych, in preparation), were made by Chukhchin (1970) and Carriker (1981).

The thaidine reproductive system has never been described in any detail; moreover, the mode of fertilization is also unknown. In contrast, the reproductive systems of several *Acanthina* and *Nucella* species, closely allied with Thaidinae, have received considerable attention (Fretter, 1941; Fretter & Graham, 1962; Harasewych, 1984; Houston, 1973; Wu, 1985), but the exact mechanism of fertilization is unknown in these taxa also.

During an extensive anatomical and systematic review of the subfamily Thaidinae, *Plicopurpura patula* (Linné, 1758:739) (commonly known as *Purpura patula* in the literature) was found to be a typical representative of this group. Several living specimens of *Plicopurpura patula* were observed and dissected. This species lives on hard substrates, in low intertidal to shallow, subtidal, high energy environments and feeds on mollusks, such as chitons (Bandel & Wedler, 1987; Clench, 1947:66; 1987:23; Kool, 1987:126) and nerites (Britton & Morton, in press), and also on barnacles (Kool, 1987:126).

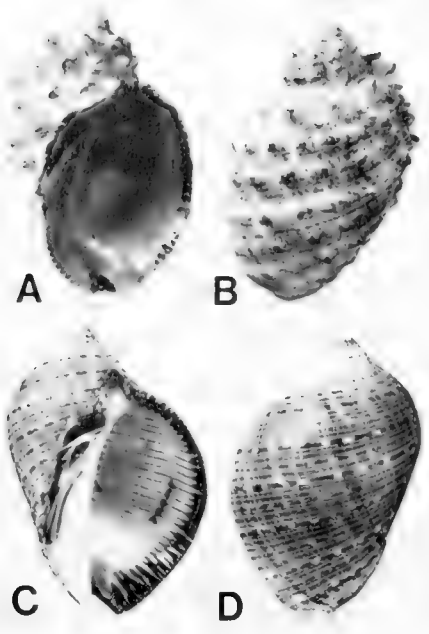

FIGURE 1. Shells of *Plicopurpura patula* (A,B height 53 mm; Isla Mujeres, Mexico; USNM 662235) and *Purpura persica* (C,D; height 60 mm; Nukuhiva Island, Marquesas; USNM 700108). A,C. Apertural view. B,D. Abapertural view.

The anatomical descriptions of *Plicopurpura patula* presented herein, provide insight into both its generic status within Thaidinae, and its functional reproductive processes, such as sperm storage, sperm movement, and fertilization.

MATERIALS AND METHODS

Animals were collected at different times of the year on stone jetties at South Miami Beach, Florida. Live animals were studied at the Smithsonian Marine Station at Link Port, Ft. Pierce, Florida, where they were maintained in finger bowls of sea water, relaxed in a 7.5% $MgCl_2$ solution, and observed and dissected under a binocular dissecting microscope. Snails were preserved in 10% buffered formalin for further dissection and histology. Specimens for histology were embedded in paraffin, sectioned at 7–12 μm and stained with Alcian Blue-PAS-Hematoxylin. Radulae were examined using Zeiss Novascan-30 and Hitachi S-570 scanning electron microscopes following the methodology described by Kool (1987:119). All morphological data on the anatomy of *Purpura persica* are based on personal observations. The anatomical descriptions below all refer to adult snails.

RESULTS

Morphological description of *Plicopurpura patula*

Shell

Shell (Fig. 1A,B) oval in outline, patelli-form, reaching 9 cm in height, and comprising about five strongly shouldered whorls. Protoconch (Fig. 2H-I) of 2.25 whorls, with weak, outward flaring lip and sinusigeral notch; smooth on first whorl, and with tiny, short subsutural plicae on last 1.25 whorls (Fig. 2H-I); last half of protoconch sculptured with microscopic pustules (Fig. 2H-I). Shell spire low; body whorl three-fourths to almost six-sevenths of shell height. Teleoconch whorls sculptured with 7–8 spiral rows of nodules, most pronounced on juvenile specimens, and with four small striae in interspaces. Aperture (Fig. 1A) wide, elliptical, more than three-fourths of shell height. Columella flattened, wide, with acute angle of 45° in lower portion. Apertural lip crenulated, on outer edge blotched with black on and between crenulations on interior side. Pronounced anal canal accommodating posterior siphonal canal. Anterior siphonal canal short and shallow. Shell color grey white to light brown. Columella caramel brown, frequently with sizable dark brown patch on parietal region.

Operculum

Operculum dark reddish-brown, thin, elongate-triangular, with lateral marginal nucleus. Attached surface with edge adjacent to nucleus having narrow, moderately callused, shiny rim.

External anatomy

Animal (Fig. 3) 2–2.5 whorls. Head-foot region, including cephalic tentacles (Fig. 3A, t), nearly solid black. Cephalic tentacles elongate, black, except for white distal tips. Sole of foot yellowish, with several deep and many shallow grooves. Ventral pedal gland com-

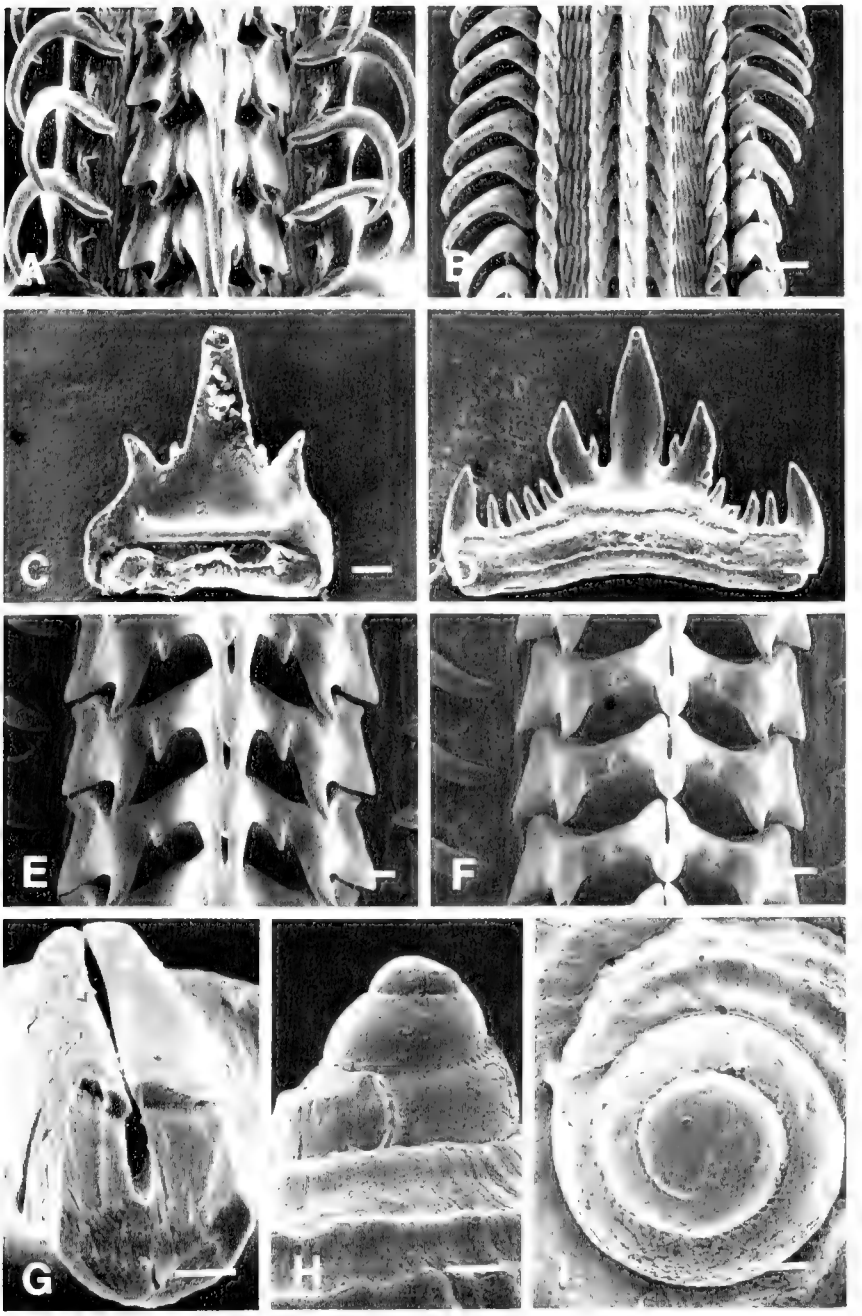

FIGURE 2. Scanning electron micrographs of radulae and protoconchs. A. Radula of *Plicopurpura patula;* scale bar = 25 μm. B. Radula of *Purpura persica;* scale bar = 50 μm. C. Frontal view of rachidian tooth of *Plicopurpura patula;* scale bar = 25 μm. D. Frontal view of rachidian tooth of *Purpura persica;* scale bar = 25 μm. E. Rachidian teeth of *Plicopurpura columellaris;* scale bar = 15 μm. F. Rachidian teeth of *Plicopurpura patula pansa;* scale bar = 25 μm. G. Central cusp of rachidian of *Plicopurpura patula,* broken to show longitudinal slit; scale bar = 10 μm. H. Protoconch of *Plicopurpura patula;* scale bar = 0.2 mm. I. Apical view of protoconch of *Plicopurpura patula;* scale bar = 0.1 mm.

bined with well-developed accessory boring organ, placed centrally, opening close to anterior pedal groove. Mantle edge smooth, slightly crenulated, following aperture contours. Inhalant siphon (Fig. 3A, si) pigmented similar to head-foot and protruding several mm from mantle edge. Pleurembolic proboscis (Fig. 3A, p) semitransparent, with pink odontophores visible.

Mantle cavity

Ctenidium (Fig. 3A, ct) large, about twice the size of osphradium, and straight anteriorly. Both ctenidium and osphradium equidistant from mantle edge. Ctenidial lamellae basically triangular with either concave or convex edges. Base of lamellae longer than lamellar height, especially anteriorly. Base of posterior lamellae shorter than that of anterior lamellae. Number of ctenidial lamellae ranging in number from 170–235 (9–10 per mm). Lamellar support rod protruding slightly beyond lamellar tip but enclosed by lamellar epithelium. Efferent vessel very wide.

Bipectinate osphradium up to one-fifth of ctenidium width. Short portion of base of each osphradial lamella attached to mantle wall. Both pectins symmetrical in overall shape, but right pectin (adjacent to ctenidium) consistently with more lamellae (90–100; 8–9 per mm) than left pectin (70–80). Osphradial lamellae not overlying ctenidial efferent vessel.

Alimentary tract

Pleurembolic proboscis moderately muscular. Left and right accessory salivary glands (Fig. 3C, lasg) usually of equal length, but right accessory salivary gland sometimes shorter. Both accessory salivary gland ducts combining and opening into anterior buccal cavity, close to mouth. Both glands elongate, thin, adjacent to salivary glands (Fig. 3C, lsg), but not intertwined with them. Length of accessory salivary glands about one-third of shell height. Both salivary gland lobes dorsally in buccal cavity, larger than accessory salivary glands and not always clearly separate. Salivary glands of globular appearance, each with one main duct (Fig. 3C, dsg) running anteriorly alongside esophagus, and opening into buccal cavity near mouth. Valve of Leiblein (Fig. 3C, vL) elongate, arising as widening of esophagus. Valve of Leiblein adjacent to, but not embedded in salivary glands. Salivary gland ducts contacting esophagus some distance anterior of valve of Leiblein, but only loosely attached to it. Mid-esophageal gland (Fig. 3C, meg) short, manifested as swelling anterior to connection with gland of Leiblein, weakly developed in some specimens. Connective duct between esophagus and gland of Leiblein (Fig. 3C, gL) equal in diameter to that of esophagus. Esophagus separated from gland of Leiblein or connected with it by connective tissue. Gland of Leiblein caramel brown, spiraling around anterior aorta, thus enclosing it. Whole gland covered with thick, strawlike outer membrane. Posterior duct of gland of Leiblein (Fig. 3C, dgL) narrow, elongate, longer than gland itself and leading into branch of dorsal afferent renal vein. Stomach (Fig. 3C, not labeled) tube-shaped. Posterior two-thirds of stomach having about 10 large folds (Fig. 3C, str); anterior stomach (close to intestine) smooth. Two digestive diverticula (Fig. 3C, dd) at base of long, thin stomach typhlosole (Fig. 3C, stt). Intestinal typhlosole (Fig. 3C, int) thin, but well developed. Rectum widening when alongside capsule gland, narrowing again anteriorly. Anal opening (Fig. 3C, a) small, well defined; distinct anal papilla present (Fig. 3C, ap). Green rectal gland (Figs. 3A,C1,3–5, rg) long, thin, adjacent to rectum and pallial gonoduct. Rectal gland opening into rectum anteriorly.

Radula

Central cusp of rachidian tooth (Figs. 2A,C) about three times the length of lateral cusps, elongate, lanceolate, with slightly widened base. Elongate median slit in central cusp (Fig. 2A,G) beginning at base of central cusp, ending close to tip. Lateral cusps (Figures 2A,C) pointing outward. Inner lateral denticle (Fig. 2A,C) separate from lateral cusp and pointing in opposite direction. Marginal denticles or marginal cusp absent. Lateral teeth (Fig. 2A) strongly curved and thin, equal in length to width of rachidian tooth.

Female reproductive tract

Vaginal opening at distal end of large coiled papilla (Fig. 3C, va). Vagina posterior to anal opening (Fig. 3C, a). From anterior to posterior, pallial oviduct comprising anterior bursa copulatrix (Fig. 3C, bc), capsule gland, ingesting gland (Fig. 3C, ig), and albumen gland (Fig. 3C, ag) with posterior seminal receptacles (Fig. 3C, sr) at dorsal periphery. Anterior bursa copulatrix increasing in size posteriorly

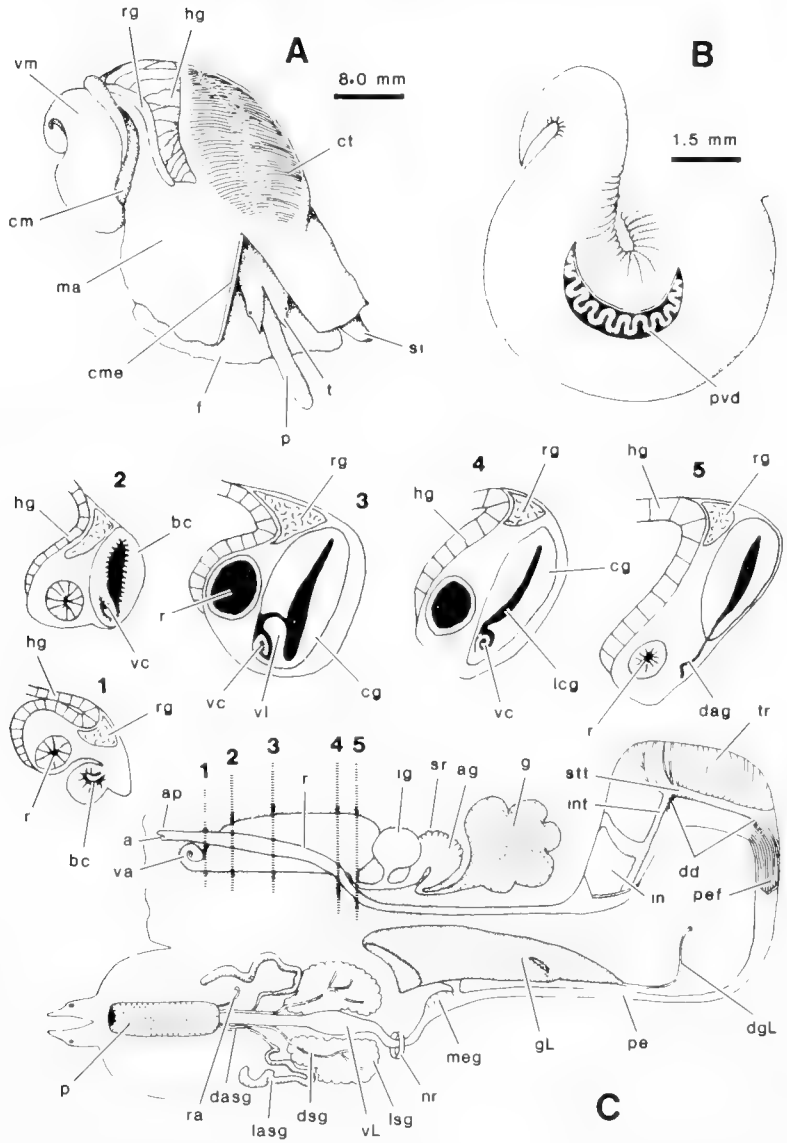

FIGURE 3. Anatomy of *Plicopurpura patula*. A. Male removed from shell with mantle skirt cut longitudinally to expose head. B. Penis. C. Schematic representation of alimentary tract and pallial gonoduct of female specimen. Stomach opened by longitudinal slit from posterior esophagus to beginning of intestine. Structures not drawn to scale. 1–5. Schematic cross sections, indicated by vertical dotted lines, through pallial gonoduct, rectum and hypobranchial gland. a, anus; ag, albumen gland; ap, anal papilla; bc, anterior bursa copulatrix; cg, capsule gland; cm, columellar muscle; cme, cut mantle edge; ct, ctenidium; dag, duct to albumen gland; dasg, duct from accessory salivary glands; dd, digestive diverticula; dgL, posterior duct of gland of Leiblein; dsg, duct from salivary gland; f, foot; g, gonad; gL, gland of Leiblein; hg, hypobranchial gland; ig, ingesting gland; in, intestine; int, intestinal typhlosole; lasg, left accessory salivary gland; lcg, lumen of capsule gland; lsg, left lobe of salivary gland; ma, mantle; meg, mid esophageal gland; nr, nerve ring; p, proboscis sheath; pe, posterior esophagus; pef, longitudinal folds of the posterior esophagus; pvd, penial vas deferens; r, rectum; ra, radular sac; rg, rectal gland; si, siphon; sr, posterior seminal receptacle; stt, stomach typhlosole; t, tentacle; tr, transverse folds of gastric wall; va, vagina; vc, ventral channel; vL, valve of Leiblein; vl, ventral lobe; vm, visceral mass.

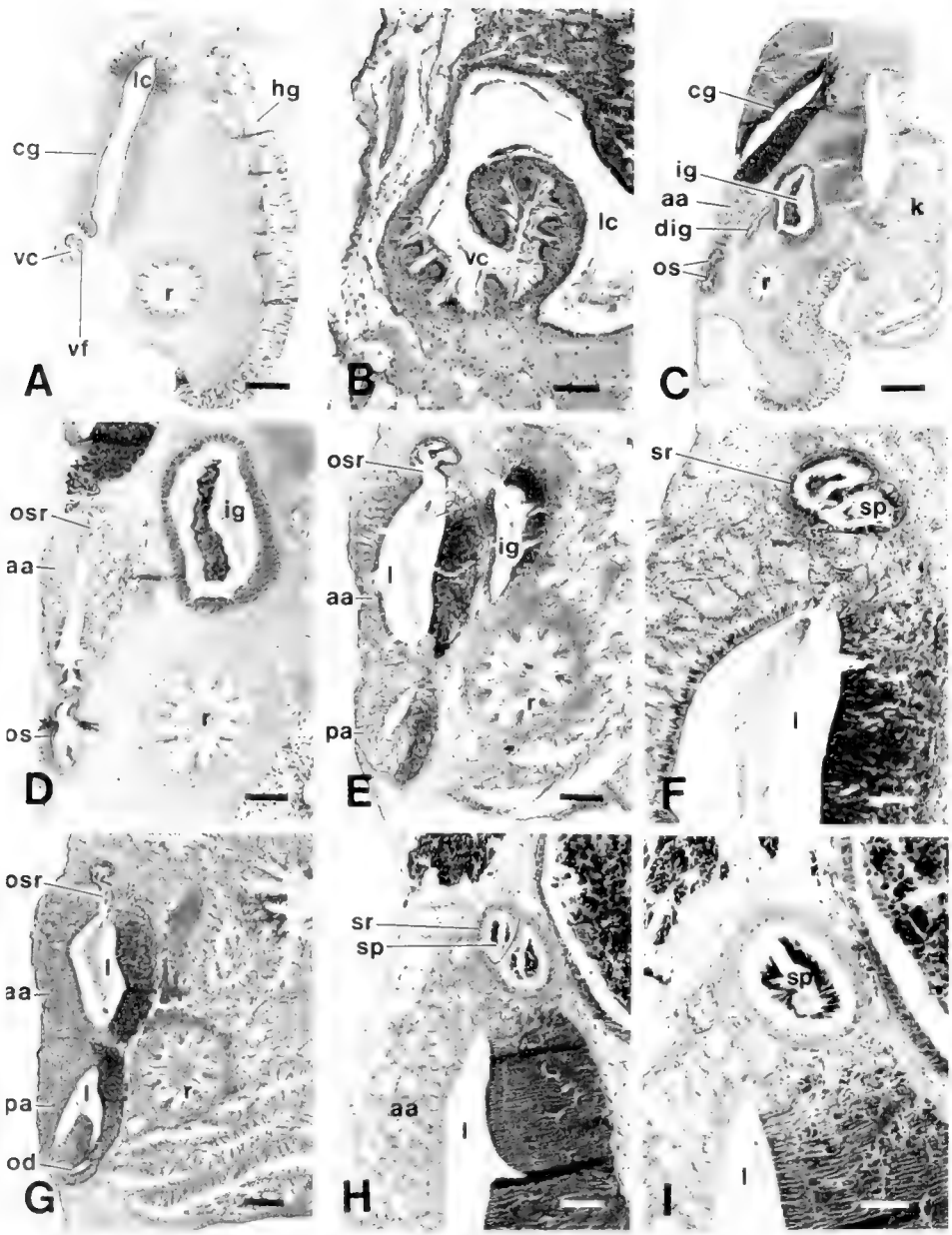

FIGURE 4 Photomicrographs of histological sections through the posterior female reproductive tract viewed head-on, and in antero-posterior sequence. A–B. Through pallial gonoduct. C–I. Through whole snail, posterior of mantle cavity. A and C. scale bar = 1.0 mm. B. Ventral channel and flange; scale bar = 0.2 mm. D,E and G. scale bar = 0.3 mm. F. scale bar = 0.1 mm. I. Seminal receptacle above anterior portion of albumen gland; scale bar = 0.05 mm. aa, anterior (distal) part of albumen gland; cg, capsule gland; dig, duct to ingesting gland; hg, hypobranchial gland; ig, ingesting gland; k, kidney; l, lumen of albumen gland; lc, lumen of capsule gland; od, oviduct; os, ovisperm duct; osr, open connection between albumen gland and seminal receptacles; pa, posterior (proximal) part of albumen gland; r, rectum; sp, embedded sperm; sr, posterior seminal receptacles; vc, ventral channel; vf, ventral flange.

and with many small longitudinal grooves (Fig. 3C1,2, bc). Lumina of anterior bursa copulatrix continuous with lumen of smooth-walled capsule gland (Fig. 3C3,4, cg). Ventral channel of pallial oviduct (Fig. 3C2–4, vc), formed by small, heavily ciliated, scroll-shaped flange with longitudinal folds (Fig. 4B). Ventral lobe (Fig. 3C3, vl) small, present throughout much of capsule gland length (Fig. 3C3,4, cg) and lying over mouth of ventral channel, with lobe tip positioned under left lobe. Capsule gland and rectum (Fig. 3C, r) both embedded in spongy connective tissue, and mantle enveloping both. Ventral sperm channel (Fig. 3C5, dag) dividing posteriorly into two branches: one unciliated, leading into ingesting gland (Fig. 4C, dig); the other ciliated (Fig. 4D, os), leading to albumen gland. Albumen gland a wide tube, folded onto itself, becoming crescent-shaped, often lying on its posterior (proximal) part. Ingesting gland (Fig. 4C–E, ig) a single or double chamber, lying to lower left of posterior (proximal) capsule gland (Fig. 4A,C, cg), extending to left side of albumen gland (Fig. 4E). Dorsal periphery of albumen gland with posterior seminal receptacles (Fig. 4F,H sr) primarily located on anterior (distal) part of albumen gland (Fig. 4E, G, aa). Tiny, rudimentary penis present in all females examined (n = 5).

Male reproductive tract

Sinuous penis (Fig. 3B), tapering distally; sometimes with more extended, flagella-shaped tip, and a less strongly curved base than shown. Penis oval in cross-section, with central, thin, longitudinal, convoluted vas deferens (Fig. 3B, pvd) lying within a larger cavity. Cephalic vas deferens thin, inconspicuous, running from prostate to penis. Prostate gland embedded with rectum in opaque spongy connective tissue. Brown posterior vas deferens, presumably serves as sperm storage area.

Egg capsules

Unknown, but egg capsules of congener *Plicopurpura columellaris* (Lamarck, 1816) are described and illustrated herein (Fig. 5). Each capsule thin walled, fingerlike, elongate, from 7–10 mm in length, with attached thread-like base about one-half of capsule length. Egg capsules examined (ANSP 324406) were deposited on one another, forming ball-like spawn mass of 6 cm in diameter. Dried capsules laterally flattened. Terminal distal plug at tip slightly off-center.

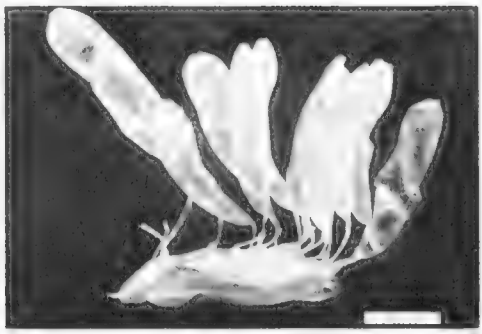

FIGURE 5. Egg capsules of *Plicopurpura columellaris* (near Pachacamac, Peru; ANSP 324406); scale bar = 3 mm.

DISCUSSION

Systematics

The nominal species *patula* has been usually referred to the genus *Purpura* Bruguière, 1789, but differs from *Purpura persica* (Linné, 1758:738) (Fig. 1C,D), the type species of the genus, in a number of morphological characters. Differences in radular morphology are easily discernible in Figure 2: the unique median longitudinal slit in the central cusp of the rachidian tooth of *Plicopurpura* (Fig. 2A) is absent in *Purpura* (Fig. 2B); marginal denticles and a marginal cusp are absent on the narrow rachidian tooth of *Plicopurpura* (Fig. 2C), whereas both cusp and several pronounced denticles are present on the much wider tooth of *Purpura* (Fig. 2D).

Anatomical differences between the two genera occur in the following structures: the vaginal opening of *Plicopurpura* is small, round, and lies at the end of a curled, free papilla, whereas in *Purpura* a slit-shaped vagina lies at the distal end of a thin, short, straight tube; the scroll-like ventral flange in *Plicopurpura* differs much from the less pronounced, hooked flange in *Purpura;* the ingesting gland in *Plicopurpura* consists of one main chamber (Fig. 4C–E, ig), whereas in *Purpura,* it comprises many smaller chambers; the albumen gland in *Plicopurpura* is small and crescent-shaped; in *Purpura,* the albumen gland is large, vertically elongate, and somewhat constricted in the center. Egg

capsules also are markedly different between the two genera: those of *Plicopurpura* are long, narrow and attached by a stalk, whereas those of *Purpura* are short, wide, and have a flat base (Tirmizi & Zehra, 1983:42).

These differences in radular morphology, anatomy, and egg capsule morphology justify the transfer of "*Purpura*" *patula* to a separate genus. The genus *Plicopurpura* Cossmann, 1903, is available for this group; the type species is *Plicopurpura columellaris* (Lamarck, 1816), by original designation. Recent work strongly suggests that the "subspecies" *Plicopurpura patula pansa* Gould, 1853, from the Panamic Province, is a synonym of *P. columellaris* from the same area, as suggested by cross-copulation between individuals of both morphs and a complete series of intergrades (Wellington & Kuris, 1983). The radular morphology of both *Plicopurpura* "*patula*" *pansa* (Fig. 2F; Sabelli & Tommasini, 1979) and *P. columellaris* (Fig. 2E; Sabelli & Tommasini, 1979), is almost identical to that of *P. patula*, which suggests very close relationship between all three taxa. (Radulae are valuable indicators of phylogeny in Thaidinae [Kool, 1987].) Figures 2B and 2D demonstrate that the radular morphology of *Purpura persica* differs in many characters from that of *Plicopurpura* species.

The genus *Plicopurpura* thus appears to contain at most two living species: *Plicopurpura columellaris*, from the Panamic Province, and *Plicopurpura patula* from the Caribbean Province. This decision assumes (a) that *Plicopurpura patula pansa* and *P. columellaris* interbreed and leave viable, fertile offspring; and (b) that two "populations" (in this case, *P. patula* and *P. columellaris*), which are geographically isolated and do not interbreed in nature, are to be regarded as separate species. If copulation between *Plicopurpura columellaris* and its Caribbean relative *P. patula* results in viable and fertile offspring, only *P. patula* would deserve full species status. (For an extensive discussion on nomenclatorial questions concerning closely related taxa from the Panamic and Caribbean faunal provinces, see Coan [1984:163].) Further anatomical and molecular investigations may provide evidence regarding the status of these taxa.

Functional anatomy of female reproductive system

After copulation, sperm is stored in several different parts of the reproductive tract: (a) in the anterior bursa copulatrix (Fig. 3C1,2, bc) and (b) in the posterior seminal receptacles (Figs. 3C, and 4F,H, sr) at the dorsal periphery of the albumen gland. Sperm must enter the albumen gland prior to filling the receptacles, which are in open connection with the lumen of the capsule gland (Fig. 4D,E,G, osr). Sperm were neither found in the ventral channel (Figs. 3C2–4, and 4A,B, vc), nor in the duct to the ingesting gland (Fig. 4C, dig). I have found sperm embedded in the ventral channel of *Nucella emarginata* (Deshayes, 1839:360) and *N. canaliculata* (Duclos, 1832:104), both non-thaidines (Kool, Ph.D. dissertation). Fretter & Graham (1962:334) reported that the duct to the ingesting gland is used for sperm storage in *Nucella lapillus* (Linné, 1758:739), also a non-thaidine (Kool, Ph.D. dissertation). The same authors (1962:337) present both the albumen gland and the capsule gland as plausible sites of fertilization in *Nucella lapillus*, which lacks posterior seminal receptacles. It is more plausible that fertilization occurs in the lumen of the albumen gland of this species, because fertilization of eggs may be obstructed following albumen deposition. Sperm must swim or be transported posteriorly into the albumen gland.

In *Plicopurpura patula* and other thaidines, it is likely that the lumen of the albumen gland is the site of fertilization: sperm are able to contact the eggs soon after leaving the seminal receptacles around the albumen gland. These posterior seminal receptacles may provide a further functional advantage besides short traveling distance to the fertilization site: without them, sperm movement towards the albumen gland would be impeded by eggs moving anteriorly. A "two-way traffic" situation would occur in the duct between the albumen gland and the ventral channel, lessening chances of fertilizing oncoming eggs. If, however, sperm are stored immediately above the fertilization site, only fertilized eggs will move through the duct. This appears to be a more efficient mode of fertilization than that found in non-thaidine muricoideans (*e.g., Nucella*).

I hypothesize that the posterior seminal receptacles around the albumen gland are filled prior to ovulation. Surplus sperm are stored in the anterior bursa copulatrix and when ovulation ceases are moved to the posterior seminal receptacles if these are not already filled to capacity. This ensures fertilization during ovulation and may explain why the posterior

seminal receptacles are filled with sperm in sectioned specimens of this species (n = 3), and almost always appear filled in other thaidines (Kool, in preparation).

The anterior bursa copulatrix may be considered the primary storage area for sperm, as the bursa presumably is filled during copulation; the posterior seminal receptacles may be referred to as the secondary sperm storage area. It is not known how long sperm are stored in any sperm storage area. Questions as to the stimulus of sperm release from the different storage areas also remain unanswered.

SYSTEMATIC CONCLUSIONS

Superfamily: MURICOIDEA Rafinesque, 1815
Family: MURICIDAE Rafinesque, 1815
Subfamily: THAIDINAE Jousseaume, 1888: 179
Genus: *Plicopurpura* Cossmann, 1903:68
Type species: *Plicopurpura columellaris* (Lamarck, 1816:2) (Cossmann, 1903:68; by original designation)
Species: *Plicopurpura patula* (Linné, 1758:739), new combination
Genus: *Purpura* Bruguière, 1789:15
Type species: *Purpura persica* (Linné, 1758:738) (Keen, 1964; by subsequent designation [ICZN Op. 886])

The nominal species "*patula*" has many anatomical and radular characters differing from those of *Purpura persica*, the type species of *Purpura* Bruguière, and is herein placed in the genus *Plicopurpura* Cossmann, the type species of which is *Plicopurpura columellaris*.

The presence of posterior seminal receptacles around at the dorsal periphery of the albumen gland, as found in *Plicopurpura patula*, is a synapomorphy for the Thaidinae. The evolutionary development of this apparently efficient mode of fertilization may have contributed to the thaidine radiation, resulting in a high degree of diversity of thaidine taxa.

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ESTUDIO ANATOMICO DEL SISTEMA NERVIOSO DE *PLATYDORIS ARGO* (LINNEO, 1767) (GASTROPODA, OPISTHOBRANCHIA, DORIDACEA).

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RESUMEN

Se realiza un estudio anatómico del sistema nervioso de *Platydoris argo*. Para ello se describen primeramente los ganglios, así como las comisuras y conectivos que se establecen entre ellos. Seguidamente, para cada ganglio, se describen los nervios y su disposición por el cuerpo. Por último se comparan los datos observados en *P. argo* con los obtenidos por otros autores en otras especies de doridáceos.

ABSTRACT

An anatomical study of the nervous system of *Platydoris argo* has been done. This paper describes, first, the ganglia and the commissures and connectives that are established between them. Following this is a description of the nerves of each ganglion and their distribution throughout the body of the animal, and finally the data obtained on *P. argo* are compared with those observed by other authors about other doridacean species.

Key words: *Platydoris argo*, Doridacea, Opisthobranchia, Gastropoda, anatomy, nervous system.

INTRODUCCION

Las ilustraciones que sobre el sistema nervioso central de los nudibranquios, y opistobranquios en general, aparecen en la literatura, son numerosas. Sobre ellas se han comentado la presencia y localización de los principales ganglios y nervios, sin indicar en la mayoría de los casos el recorrido que cada nervio presenta a través del cuerpo del animal. Sin embargo, son pocos los trabajos que han tratado con cierta profundidad la organización anatómica del sistema nervioso de algunas especies de opistobranquios desde el punto de vista de la descripción anatómica (Hancock & Embleton, 1849, 1852; Russell, 1929; Cervera & García, 1988 entre otros); o bien desde una perspectiva más teórica (Guiart, 1889; Wirz, 1952).

En los nudibranquios, incluso intraespecíficamente, el sistema nervioso no se mantiene invariablemente constante, aunque algunos caracteres sí se presentan más o menos fijos para cada especie. Esto último sucede, fundamentalmente, con el mayor o menor grado de fusión de los ganglios entre sí o la tendencia a presentar, generalmente, los conectivos y comisuras con una determinada longitud. Debido a la variabilidad intraespecífica observada, los datos recogidos en este trabajo reflejan aquellos caracteres presentes en todos los animales empleados, haciéndose además hincapié, en aquéllos que muestran una marcada variabilidad dentro de la especie.

El género *Platydoris* (Fig. 1) tiene una distribución casi cosmopolita y engloba especies de gran tamaño. No obstante, son pocos los datos anatómicos que se conocen de las especies de este género. Aunque Risbec (1956) ya realizase un estudio general de la anatomía de *P. argo*, sin embargo, acerca del sistema nervioso sólo hace muy escasas consideraciones.

El gran tamaño de esta especie, la disponibilidad de ejemplares y su escaso conocimiento anatómico, han determinado su elección para un detallado estudio anatómico del sistema nervioso que permita aportar nuevos datos sobre el sistema nervioso de los doridáceos a la vez que permitir un mayor conocimiento de la especie *P. argo*.

MATERIAL Y METODOS

Los cinco ejemplares de *P. argo* utilizados (de tamaños comprendidos entre 53–68 mm)

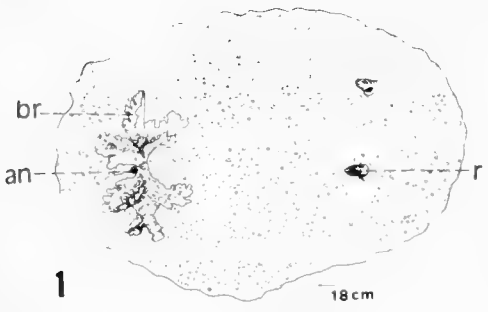


FIG. 1. Vista dorsal de *Platydoris argo.*

se capturaron en Julio de 1981 en el Sur de España (El Campamento, 36° 10′ 42″ N; 5° 23′ W) a 1′5 m de profundidad. Estos ejemplares fueron conservados en formol al 4% y glicerina y también en glutaraldehido con tampón cacodilato. Para facilitar la observación de los nervios, se bañaba el ejemplar, una vez escindido el manto, en una mezcla de formol y azul de metileno, lo cual permitía un mayor contraste entre los nervios y el resto de las estructuras del cuerpo del animal.

De la descripción de los nervios, aquéllos que se originan de los ganglios cerebropleurales son designados con la letra C; los que lo hacen del ganglio visceral, con la V; con una P los que lo hacen de los ganglios pedios; con una G los originados del ganglio genital y una B los que proceden de los ganglios bucales. Aquellos nervios que se disponen de manera simétrica a cada lado del cuerpo e inervan el mismo órgano, se señalan además con el signo +.

DESCRIPCION DE LOS GANGLIOS

Del sistema nervioso central, localizado en el extremo anterior del esófago y sobre el aparato bucal, se distingue una serie de ganglios unidos por medio de conectivos y comisuras (Figs. 2,3).

Los dos ganglios cerebropleurales (gcp) se disponen dorsalmente al esófago y están unidos entre sí por una comisura cerebral muy corta. Del extremo anterior de cada uno de estos ganglios se originan los ganglios rinofóricos proximales (gr) y cerca de la base de estos últimos se localizan los ganglios ópticos (go), bastante reducidos. Algo detrás de los ganglios ópticos, muy próximos al conectivo cerebropedio se sitúan los estatocistos; dorsalmente quedan cubiertos por los nervios de los ganglios cerebropleurales. Del extremo posterior del ganglio cerebropleural derecho, por su superficie ventral, y unido a él a través del asa visceral (av), se dispone el ganglio visceral (gv).

Los ganglios pedios (gp) están localizados más ventralmente respecto a los ganglios cerebropleurales, a los cuales se unen mediante los conectivos cerebropediales. Ambos ganglios pedios se unen entre sí a través de una doble comisura: la comisura pedia (cp), inserta en los extremos posteriores de cada ganglio; y la comisura parapedia (cpp), más fina que la anterior, cuyas inserciones están en las porciones medio anteriores de los ganglios, en sus superficies ventrales. La doble comisura y el asa visceral bordean el esófago (e) envueltos por una vaina.

Debajo del extremo anterior del esófago y adosados a la superficie posterodorsal del aparato bucal se encuentran los dos ganglios bucales (gb), unidos entre sí por medio de una comisura bucal (cb) muy corta, y a la superficie ventral de los ganglios cerebropleurales, por su porción anterior, mediante los dos conectivos cerebrobucales (ccb). La unión de estos conectivos a los ganglios bucales se realiza a través de los nervios B2+ dispuestos lateralmente a cada ganglio (ver más adelante en nervios de los ganglios bucales). Los conectivos cerebrobucales, inmediatamente después de diferenciarse de los nervios B2+, se dirigen ventralmente a cada lado del saco radular para seguidamente girar bruscamente y dirigirse verticalmente hasta los ganglios cerebropleurales (Fig. 3).

De cada ganglio bucal parte un conectivo bucogastroesofágico (cbg) que lo une a cada uno de los ganglios gastroesofágicos (gge). Cada uno de estos conectivos tiene distinta longitud, siendo el conectivo izquierdo más corto que el derecho. Se ha observado, además que esta longitud varía de un animal a otro, aunque en los casos examinados el conectivo izquierdo es más corto que el derecho.

INERVACION DE CADA GANGLIO

Ganglio rinofórico proximal (Fig. 4)

De cada ganglio (gr) parte un largo nervio dirigido a cada rinóforo. En la base de éste, el nervio rinofórico presenta un sensible aumento de su diámetro para seguidamente re-

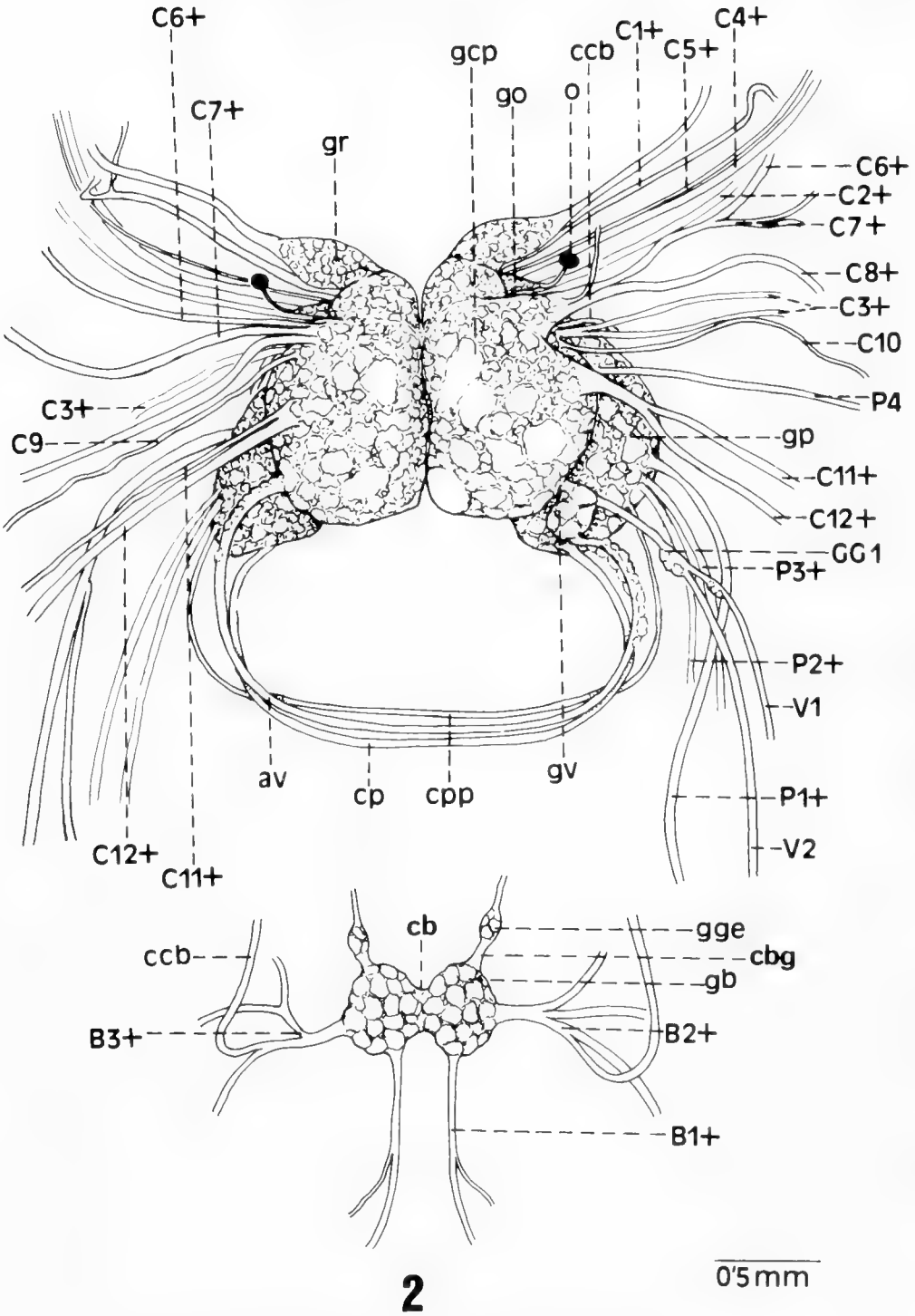


FIG. 2. Vista dorsal de las concentraciones ganglionares circunesofágicas.

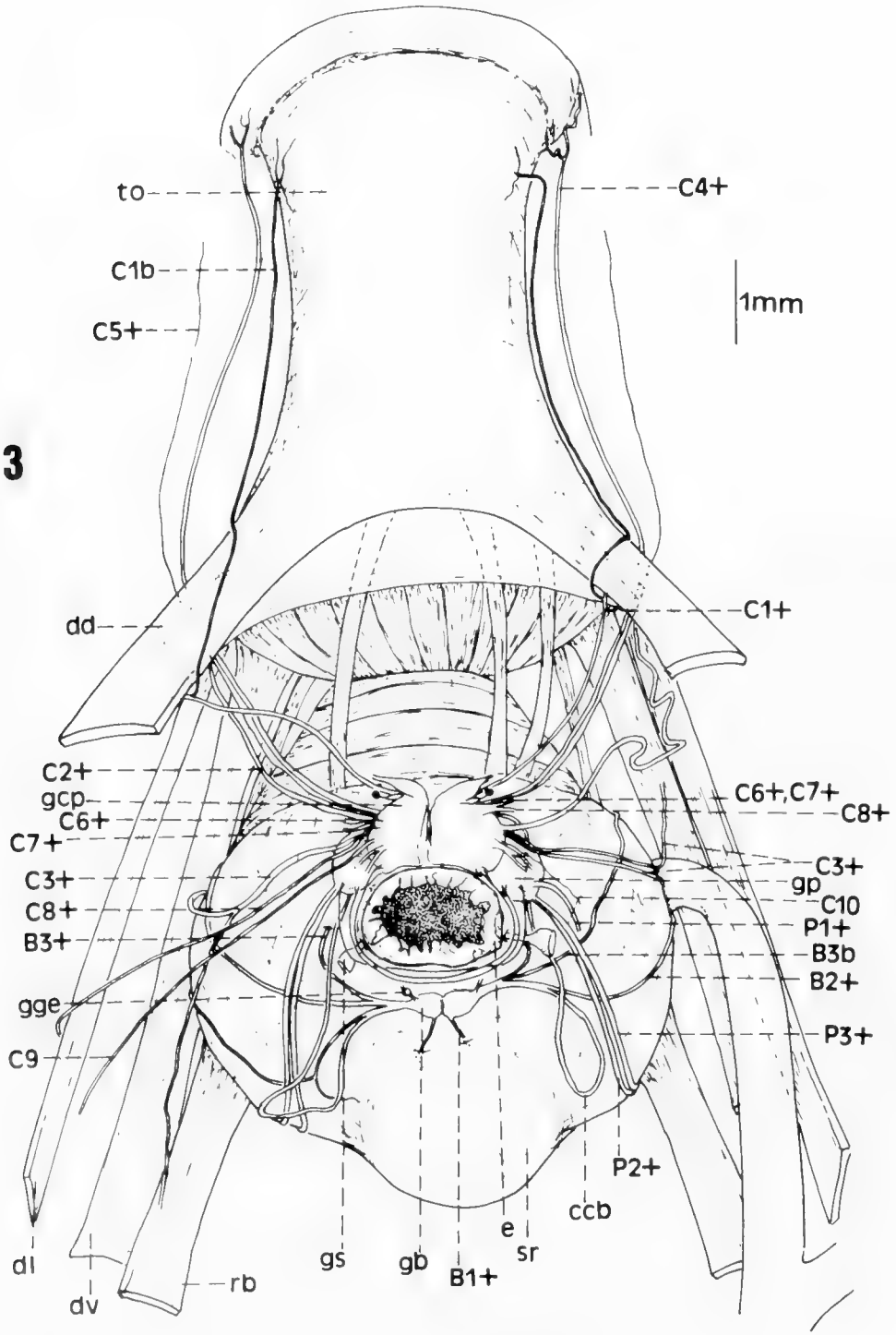


FIG. 3. Disposición de los nervios por el aparato bucal. Vista dorsal.

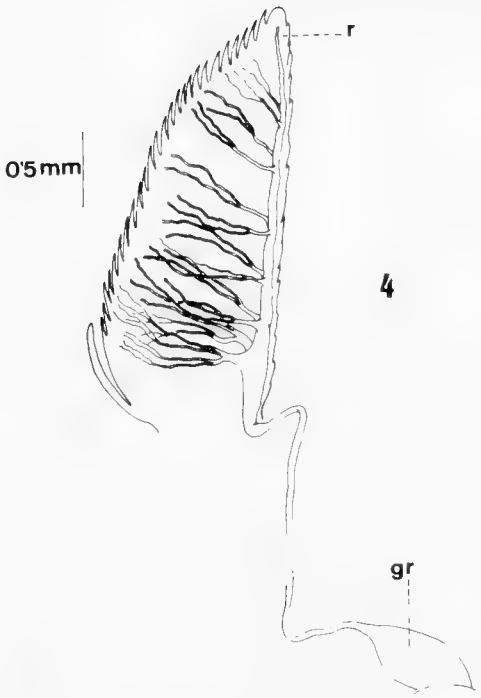


FIG. 4. Inervación del rinóforo.

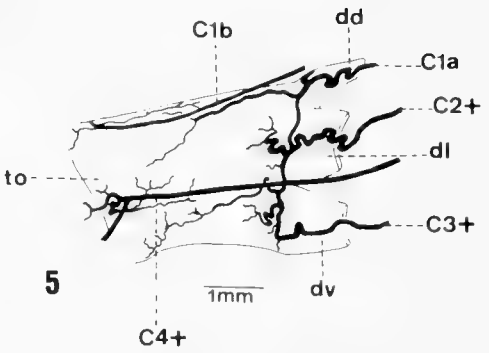


FIG. 5. Inervación de la porción anterior (tubo oral) del aparato bucal.

ducir de nuevo su grosor a medida que penetra en el rinóforo y se acerca a la zona apical de éste. De este tronco nervioso parte, perpendicularmente, una serie de pares de ramas nerviosas que se distribuyen por las láminas rinofóricas. Cada par de ramas nerviosas se diferencian al mismo nivel del eje nervioso y cada una se dirige a cada semilámina (las láminas rinofóricas se encuentran divididas por la mitad dando al rinóforo la apariencia de presentar un surco longitudinal).

Ganglio óptico (Fig. 2)

De cada ganglio óptico (go) parte un nervio muy fino dirigido al dorso del animal. En su extremo se encuentra un pequeño ocelo (o).

Ganglio cerebropleural

C1+ (Figs. 2,3,5). Este nervio parte del extremo anterior de cada ganglio cerebropleural, debajo de los ganglios rinofóricos. Desde aquí se dirige a la superficie ventral del músculo dilatador dorsolateral (dd) del tubo oral (to). En esta zona el nervio se bifurca: una de las ramas formadas (C1a) discurre por la cara ventral del músculo citado anteriormente hasta que éste se inserta en el tubo oral, desde aquí el nervio se divide prolongándose una de las ramas a lo largo del tubo oral mientras que otra rama inerva la porción posterior del tubo oral dorsalmente. De esta zona parte un conectivo que une el nervio C1+ con el C2+. La segunda rama formada a partir de C1+ (C1b), más fina que la anterior se dirige a cada lado del tubo oral hasta el extremo anterior de éste donde se ramifica inervando dicha zona.

C2+ (Figs. 2,3,5). El origen de este nervio se encuentra en la superficie ventral del ganglio cerebropleural, algo más posterior que el origen de C1+. C2+ se dirige al músculo dilatador lateral (dl) del tubo oral, continuando su recorrido junto a dicho músculo hasta la inserción de éste en el tubo oral. Desde aquí continúa su recorrido, ramificándose y penetrando entre las capas musculares del tubo oral. Se encuentra unido por medio de conectivos tanto a C1+ como a C3+.

C3+ (Figs. 2,3,5). El origen se sitúa junto al de C2+. Desde aquí bordea el aparato bucal hacia el músculo dilatador ventrolateral (dv) del tubo oral para seguirlo hasta el extremo posterior del tubo oral. Antes de llegar a éste, el nervio se divide dirigiendo una rama a lo largo del músculo retractor bucal (rb) hasta su origen. En el nervio del lado derecho esta división se da muy próxima al ganglio (Fig. 3).

Al llegar al tubo oral C3+ se ramifica inervando dicha región; una de las ramas nerviosas se dispone longitudinalmente por el tubo oral, ramificándose por éste.

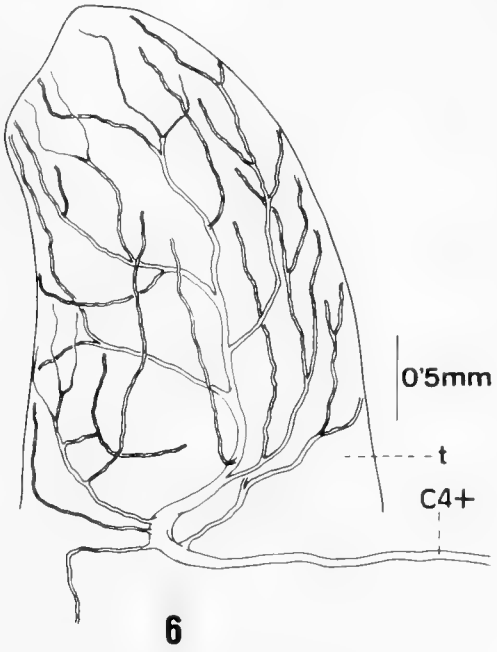


FIG. 6. Inervación del tentáculo oral.

Tanto C1+, como C2+ y C3+ presentan marcadas ondulaciones a lo largo del recorrido realizado sobre los respectivos músculos dilatadores del tubo oral, sobre todo al aproximarse a las áreas de inserción de cada uno de estos músculos.

C4+ (Figs. 2,3,5,6,7a,7b). El origen de este nervio se encuentra entre los nervios C1+ y C2+. Desde aquí se dirige al extremo anterior del tubo oral por donde se ramifica. Una rama se extiende por la porción anterior lateral del tubo oral; otra rama inerva el labio externo del animal y zonas próximas; y una tercera se dirige al tentáculo oral (t) por el cual se dispone longitudinalmente emitiendo a lo largo de su recorrido una gran cantidad de ramificaciones que se extienden, sobre todo, próximas a la superficie y dirigidas hacia el ápice de aquél.

C5+ (Figs. 2,7a,7b). Es un nervio muy fino cuyo origen está muy próximo al del nervio C4+. Desde sus orígenes hasta haber sobrepasado ventralmente al músculo dilatador dorsolateral del tubo oral estos dos nervios van juntos, disponiéndose C5+ sobre C4+. Desde aquí ambos nervios se separan. C5+ llega al extremo anterior del cuerpo por donde se ramifica y dispersa. Antes de llegar a esta zona emite algunas ramas muy finas que se disponen por la superficie dorsal de la membrana que envuelve el aparato bucal.

C6+, C7+, C8+. El origen de estos nervios en los ganglios cerebropleurales presenta una gran variación en cada uno de los ejemplares observados; incluso dentro de un mismo ejemplar también se observan algunas diferencias entre cada ganglio. En algunos ejemplares los tres nervios del ganglio izquierdo tienen sus orígenes muy próximos entre sí, aunque mantienen su independencia desde la base. Sin embargo, en el ganglio derecho el origen de los tres nervios es común. Existe un tronco nervioso grueso que parte de la superficie anterodorsal del ganglio. Una división del tronco nervioso diferencia el nervio C8+; seguidamente una segunda división del tronco nervioso da lugar a los nervios C6+ y C7+.

En otros ejemplares observados el nervio C8+ tiene un origen marcadamente diferenciado de los otros dos desde la base, y a su vez C6+ y C7+ se diferencian inmediatamente después de haberse originado el tronco común.

C6+ (Figs. 2,7a,7b). Se dirige, entre los músculos dilatadores dorsolateral y lateral del tubo oral, hacia la expansión periférica del manto donde se introduce. Desde aquí se dirige hacia el extremo anterior del borde del manto. A nivel de la vaina rinofórica el nervio C6+ se divide para dar lugar a dos ramas: una de ellas (C6a) bordea dicha vaina a la vez que se ramifica varias veces distribuyéndose por esta región del borde del manto; una de las ramas así formadas, tras bordear la vaina rinofórica, se dirige en sentido posterior para conectar con el nervio C7+. En el nervio C6+ del lado derecho del animal la rama que bordea la vaina rinofórica correspondiente, está en una posición más adelantada que la del nervio izquierdo; por lo demás el recorrido es semejante. Suponemos que dichas ramas nerviosas deben inervar la vaina rinofórica además de la porción de borde de manto que abarca.

La segunda rama formada por la bifurcación del nervio C6+ (C6b), continúa hacia el extremo anterior del borde del manto emitiendo sucesivas ramificaciones que a su vez se distribuyen por dichas zonas en todas las direcciones. Los nervios C6+ de cada lado, al llegar al extremo anterior del borde del manto conectan entre sí, formando entre los dos nervios una red nerviosa que se extiende por todo aquél. Se han observado algunas ramas nerviosas muy finas dirigidas a la su-

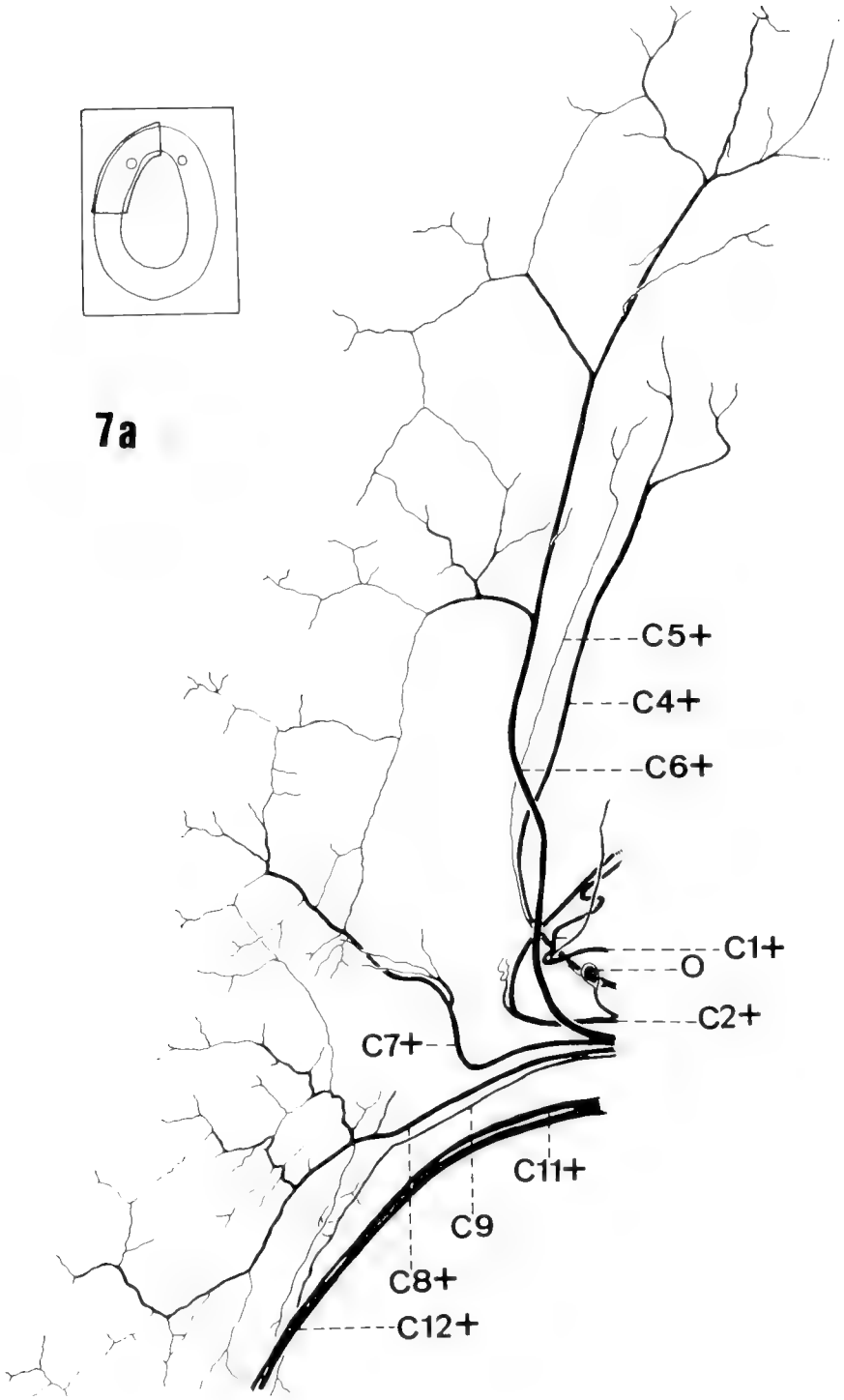
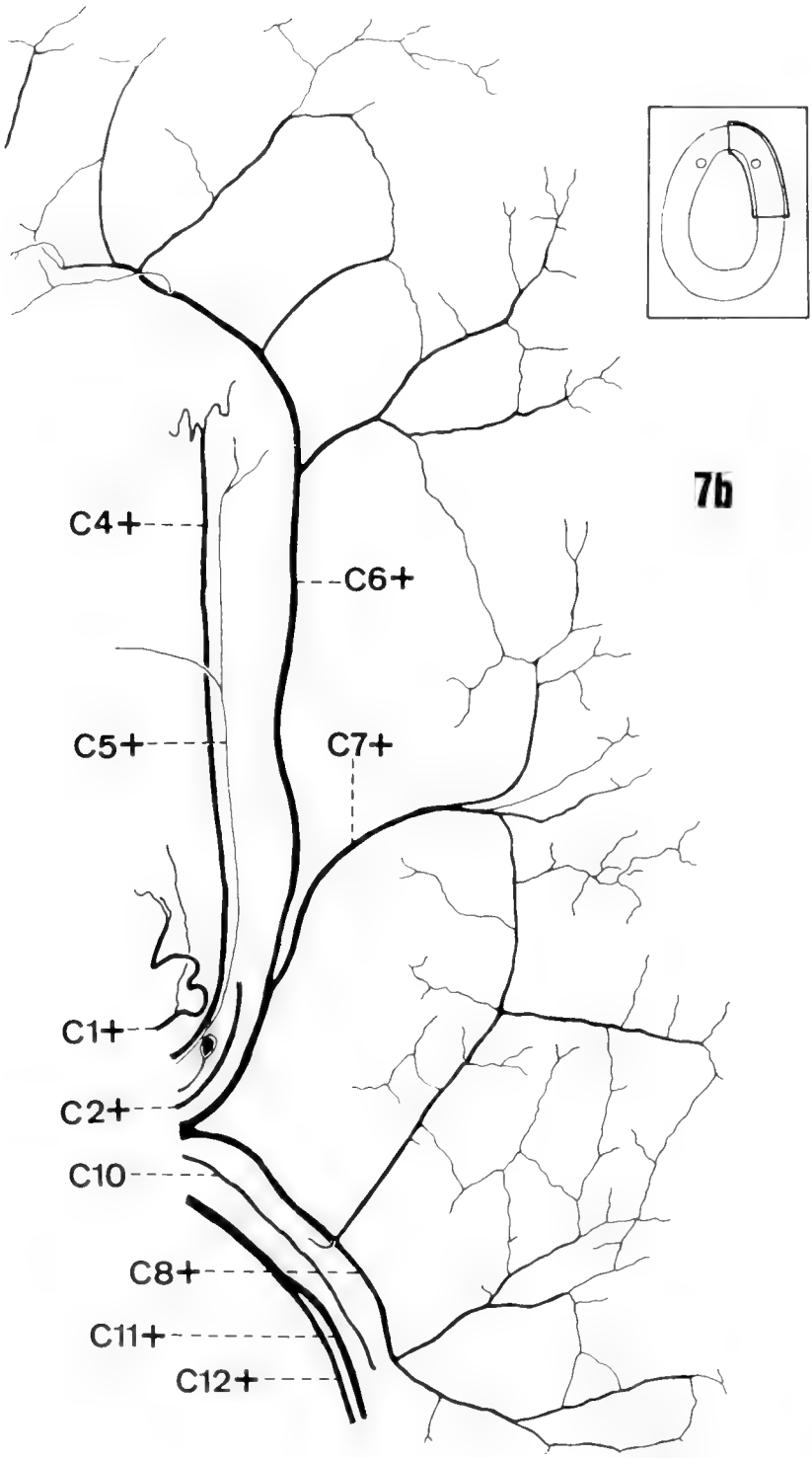
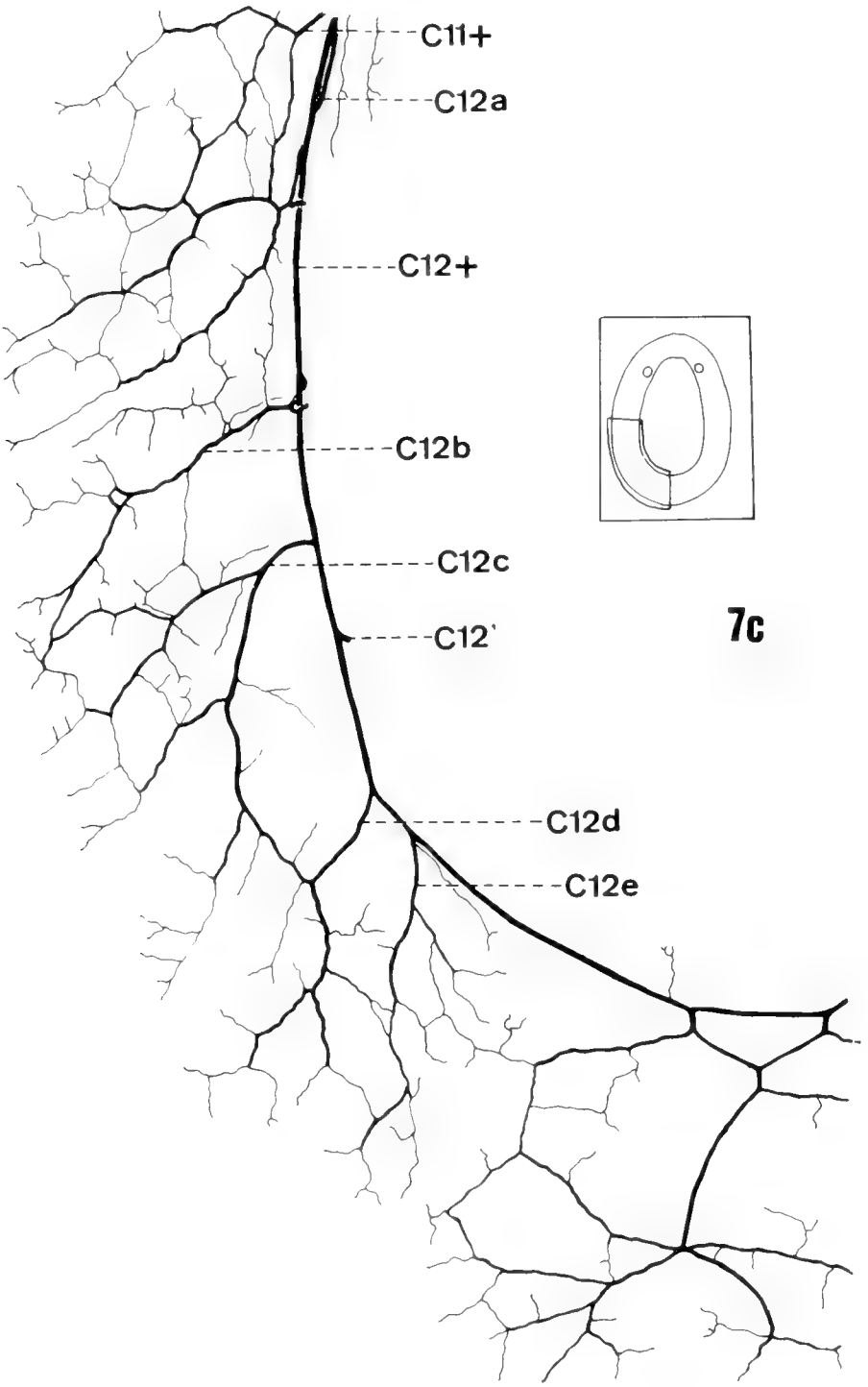
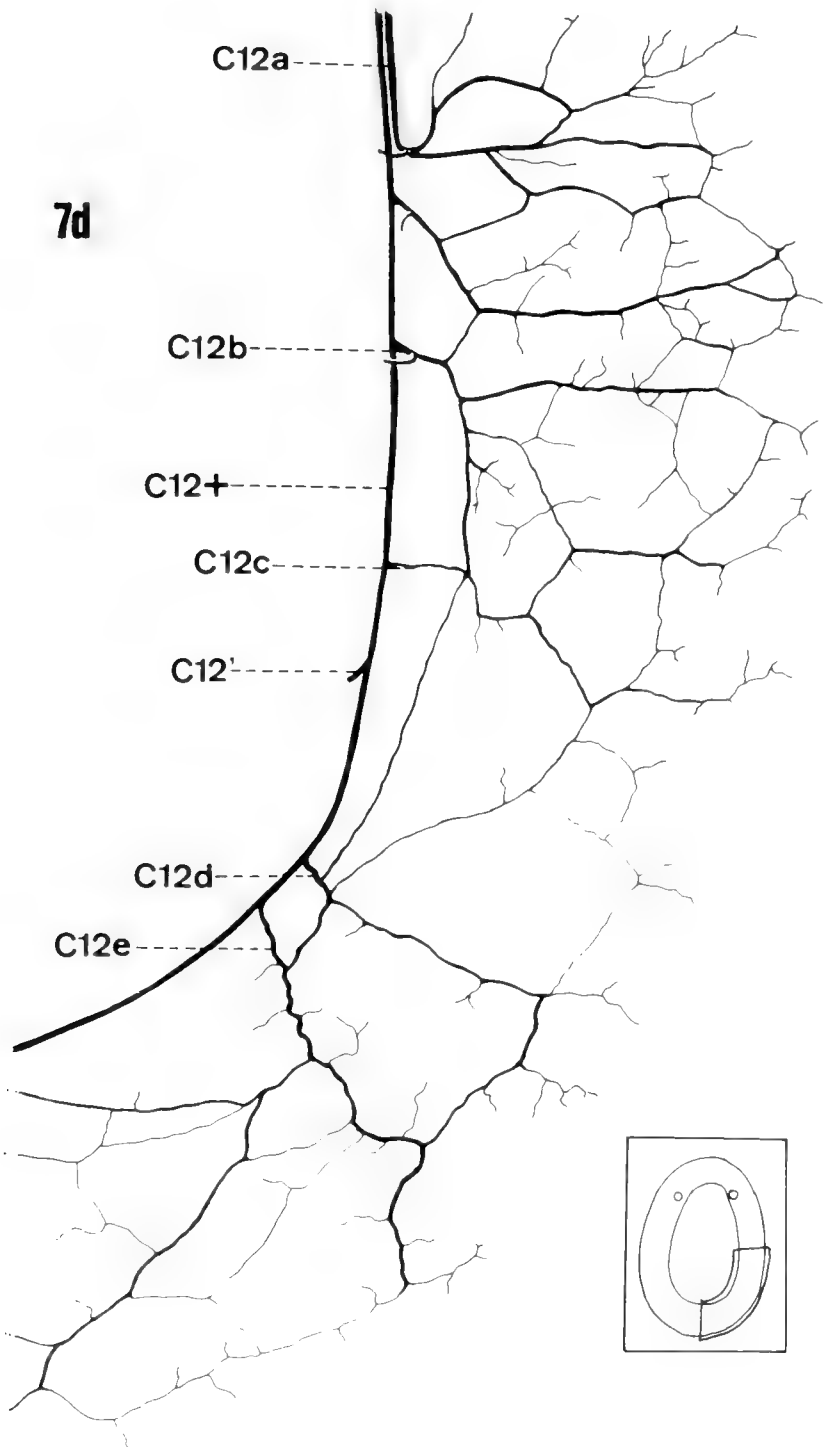


FIG. 7a, 7b, 7c, 7d. Disposición de los nervios cerebropleurales por los bordes del manto.


7b
C4+
C6+
C5+
C7+
C1+
C2+
C10
C8+
C11+
C12+


C11+
C12a
C12+
C12b
C12c
C12'
C12d
C12e
7c


7d
C12a
C12b
C12+
C12c
C12'
C12d
C12e

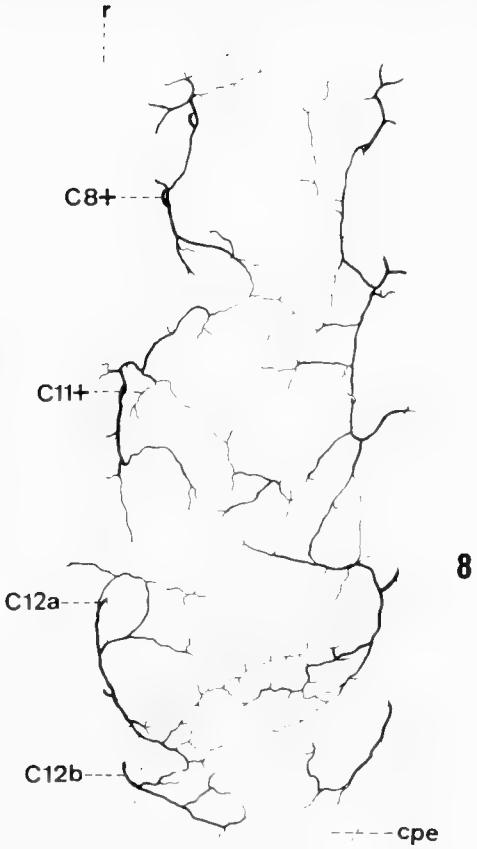


FIG. 8. Disposición de los nervios cerebropleurales por la región dorsal del manto.

perficie dorsal de la membrana que envuelve el aparato bucal.

C7+ (Figs. 2,7a,7b). La porción de la expansión periférica del manto que inerva este nervio es estrecha. Desde su origen se dirige, cruzando dorsalmente el músculo dilatador dorsolateral del tubo oral, hacia el borde del manto. Al penetrar en éste continúa hacia el extremo externo de aquél a medida que se ramifica. Algunas de las ramas actúan de conectivos entre este nervio y los nervios contiguos (C6+ y C8+).

C8+ (Figs. 2,7a,7b,8). Se dirige al borde del manto cruzando dorsalmente el músculo dilatador dorsolateral del tubo oral. Muy próximo a la pared lateral interna del cuerpo emite una rama que va al manto dorsal penetrando en éste e inervando una franja del mismo. Se ha observado un conectivo entre esta rama nerviosa y la que se distribuye por la zona del manto que está al nivel de los rinóforos.

C8+, seguidamente, emite una nueva rama nerviosa dirigida en sentido posterior entre la pared lateral interna del cuerpo del animal y el músculo dilatador lateral del tubo oral. El eje principal de C8+, por su parte, penetra entre la musculatura de la expansión periférica del manto emitiendo inmediatamente otra ramificación, esta vez dirigida en sentido anterior, sobre la que se observa el conectivo entre C7+ y C8+. La rama nerviosa principal del nervio C8+ continúa en el sentido posterior del animal ramificándose por dicha zona. Se observan varias conexiones tanto entre las distintas ramas formadas del mismo nervio como entre C8+ y C11+.

C9 (Figs. 2,7a). Este nervio, observado únicamente en el lado izquierdo, tiene su origen muy próximo al de C8+ aunque algo más posterior. Es un nervio muy fino desde su origen que se dirige en sentido posterior por la superficie lateral interna de la pared del cuerpo sin llegar a entrar en el manto, emitiendo a lo largo de su recorrido algunas ramificaciones. Este nervio solamente se pudo seguir hasta aproximadamente la mitad del cuerpo.

C10 (Figs. 2,7b,9). Es un nervio muy semejante a C9 tanto en grosor como en localización de su origen, aunque en este caso se sitúa en el ganglio derecho. C10 se dirige a las porciones más distales del gonoducto masculino bordeando dorsalmente la vaina penial, de aquí pasa a anastomosar con el nervio genital. Antes de producirse la anastomosis, emite algunas ramificaciones que se distribuyen por la vaina del pene.

C11+, C12+. Estos dos nervios tienen un origen común en la superficie dorsal de los ganglios cerebropleurales. Al igual que ocurriera con los nervios C6+, C7+ y C8+, ambos nervios varían de un ganglio a otro tanto en diferentes ejemplares como dentro de un mismo ejemplar. En el ganglio izquierdo de uno de los ejemplares la diferenciación de ambos nervios surge inmediatamente después del origen del tronco nervioso común (Fig. 2). Sin embargo, en el ganglio derecho la diferenciación de los dos nervios no se realiza hasta haber recorrido el tronco común una cierta distancia. A su vez, este tronco derecho surge en el ganglio a un nivel más anterior que el tronco izquierdo. En otro ejemplar, sin embargo, el tronco nervioso

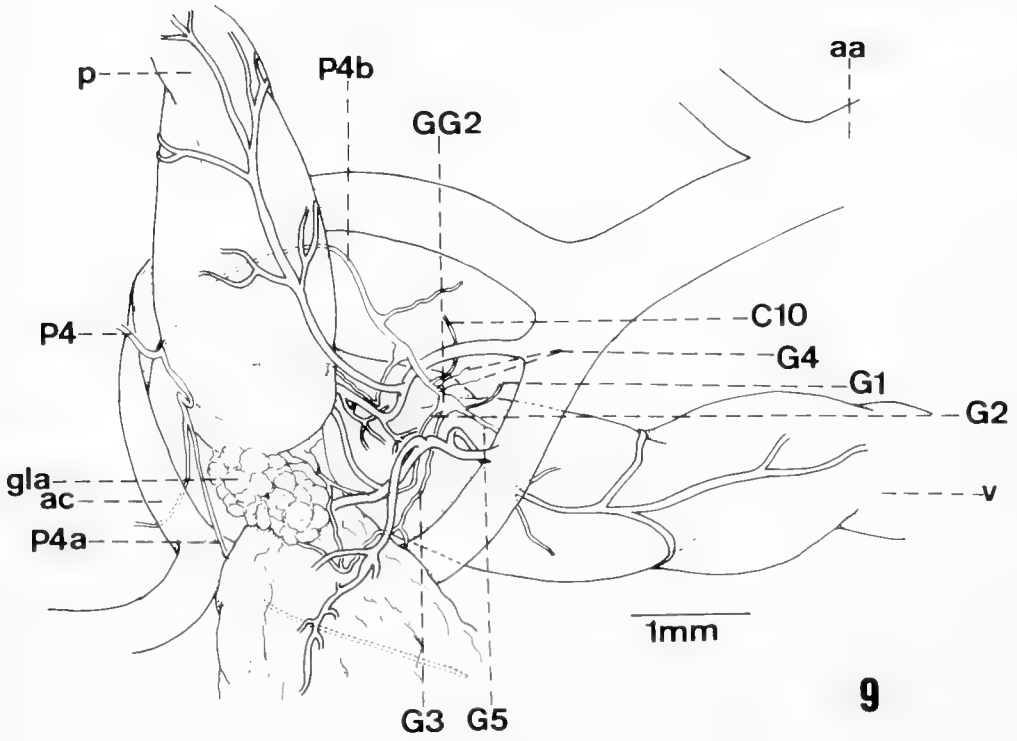


Fig. 9. Inervación de la porción distal de los órganos reproductores.

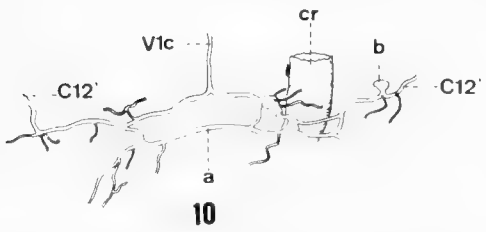


FIG. 10. Conexión de los nervios cerebropleurales C12' de cada lado del cuerpo entre sí y con los nervios viscerales.

común del lado derecho es mucho más largo que el del ejemplar anterior.

C11+ (Figs. 2,7,8). Desde su origen se dirige oblicuamente en sentido posterior hacia la expansión periférica del manto. La entrada del nervio en aquélla se realiza aproximadamente al mismo nivel que el extremo anterior de la glándula digestiva. Justo antes de producirse la entrada emite una rama nerviosa dirigida hacia el manto dorsal, ramificándose sucesivamente para formar la red nerviosa que inerva dicha zona. Presenta conexiones entre la rama anterior y posterior a ella, y entre las dos ramas homólogas. Son frecuentes, al igual que en todas las ramas nerviosas que se distribuyen por el manto, finas derivaciones dirigidas verticalmente hacia las zonas más externas del manto.

Al penetrar la expansión lateral del manto, el nervio C11+ se ramifica sucesivamente, presentando además numerosos conectivos tanto entre las distintas ramas del nervio como con las de los nervios adyacentes. Contribuye así a constituir la red nerviosa que inerva la región dorsal y lateral del manto.

C12+ (Figs. 2,7,8). Desde su origen, este nervio se dirige en sentido posterior, recorriendo la pared lateral interna del cuerpo del animal hasta el nivel de las branquias, donde el eje principal del nervio se introduce por entre la expansión lateral del manto, para desde aquí bordear el penacho branquial (Fig. 1, br) y conectar con el nervio homólogo del otro lado del cuerpo.

En su recorrido se observa una serie de ramificaciones dispuestas por el borde del manto, que constituyen, debido a los nume-

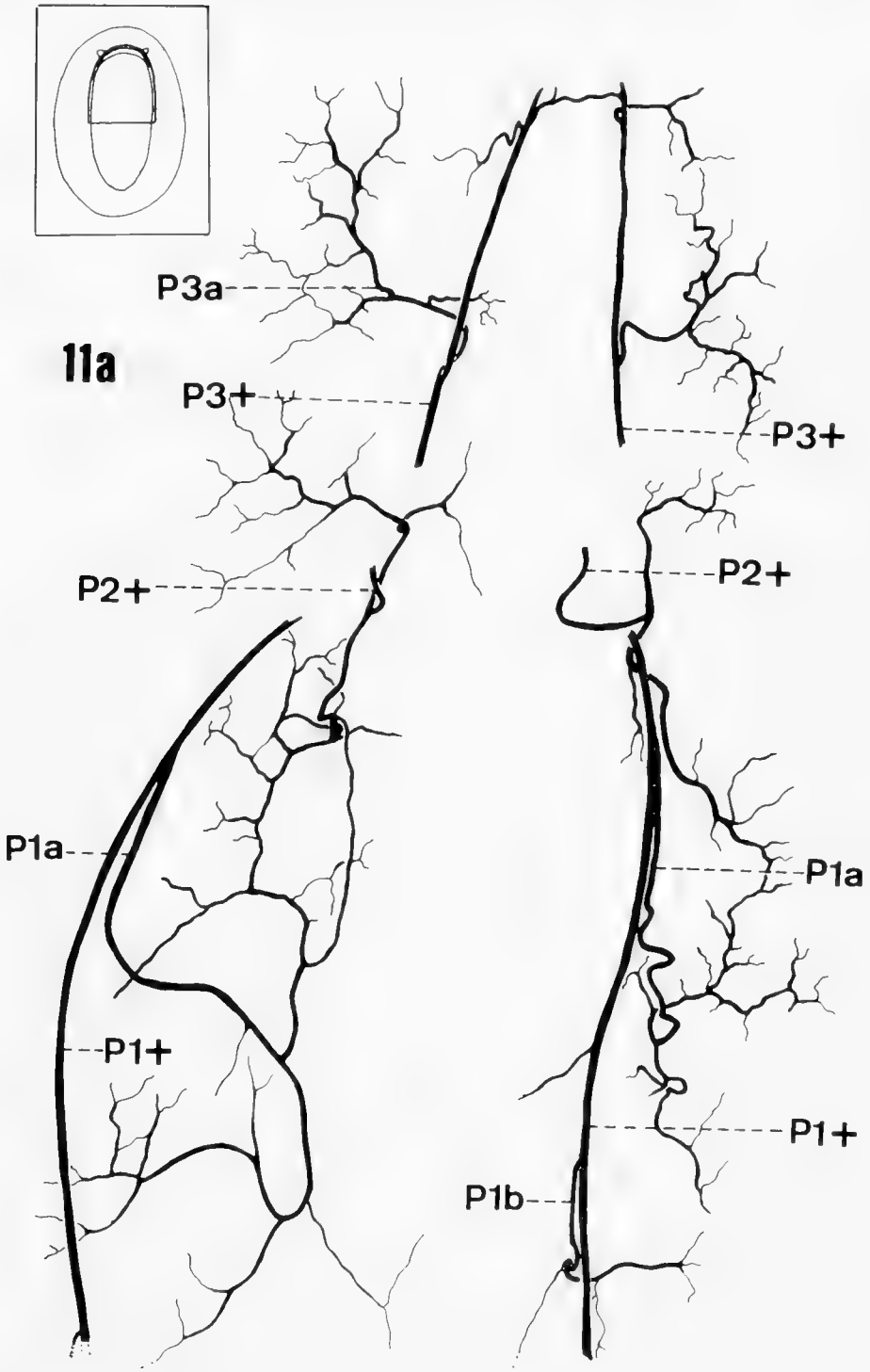
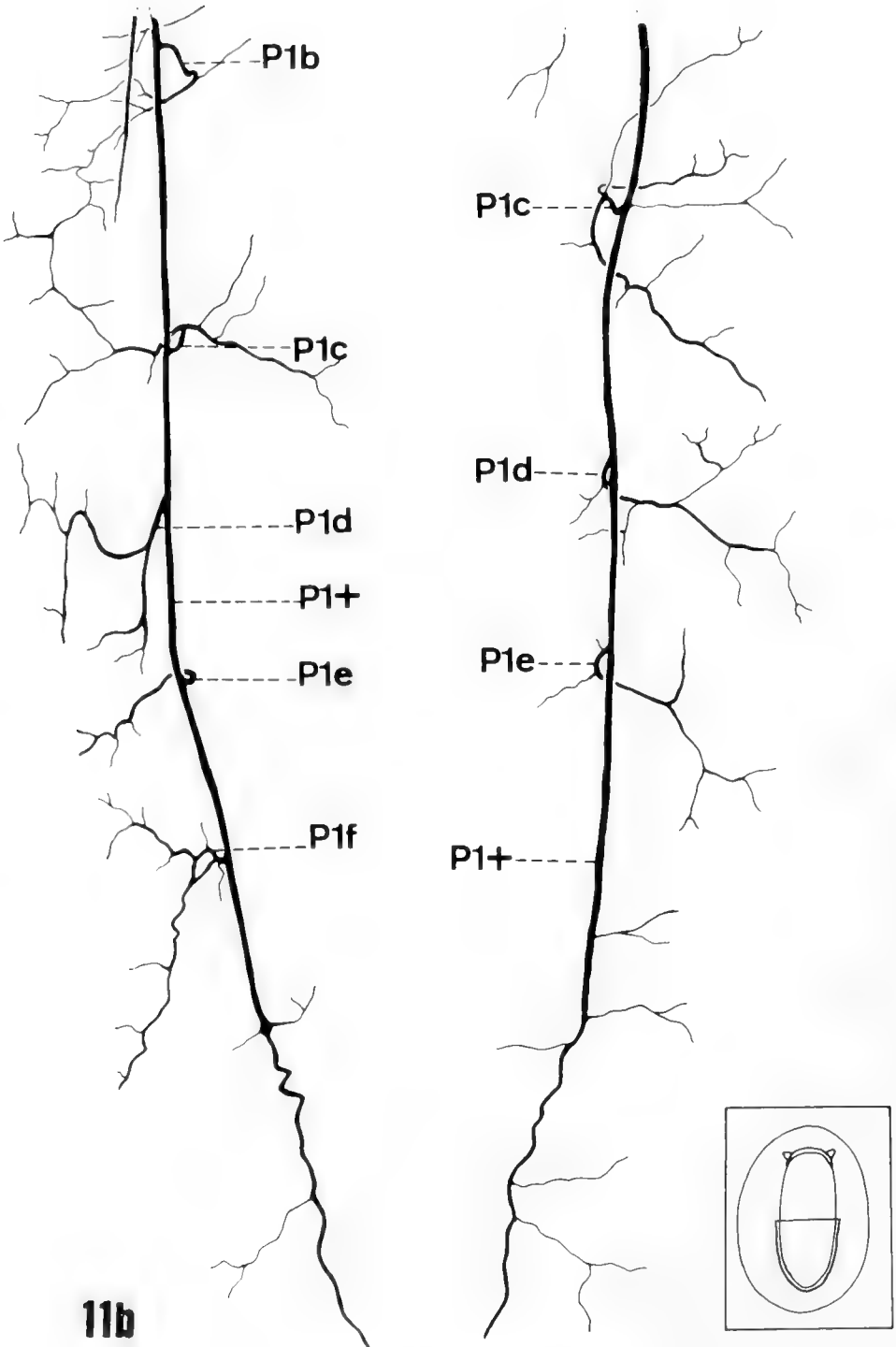


FIG. 11a, 11b. Inervación del pie.



11b

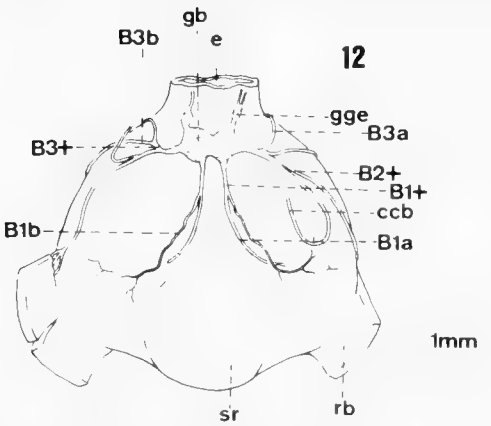


FIG. 12. Vista posterior del aparato bucal.

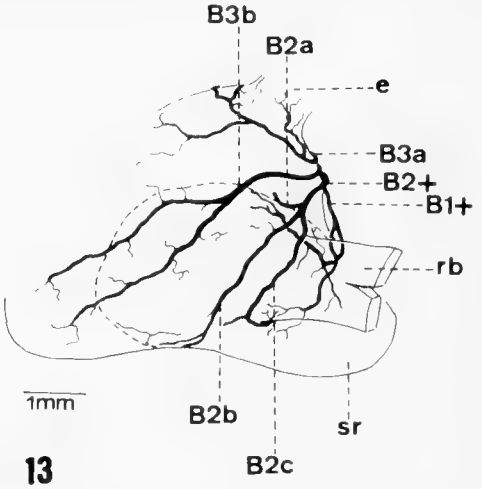


FIG. 13. Inervación de la porción posterior del aparato bucal.

rosos conectivos que presentan, una red nerviosa extendida por todo aquél.

De las ramas formadas directamente a partir del eje principal del nervio, las dos más anteriores (C12a y C12b) emiten, antes de penetrar en la expansión lateral del manto, sendas derivaciones dirigidas hacia la pared dorsal del manto por donde se extienden y ramifican. Se observan algunas conexiones entre las distintas ramas nerviosas y son abundantes además las prolongaciones verticales dirigidas a las capas más externas del manto.

La rama C12a de cada lado se diferencia del eje principal C12+, aproximadamente al nivel del extremo anterior de la glándula digestiva, mientras que C12b lo hace próximo al nivel del extremo anterior del pericardio. Ambas ramificaciones recorren, paralelas al eje principal del nervio, un tramo que varía en longitud de una a otra, siendo marcadamente superior en el caso de C12a.

La tercera rama nerviosa (C12c) originada a partir del eje principal de C12+, aproximadamente a nivel de la mitad del pericardio, entra inmediatamente después de su origen en la expansión lateral del manto. Carece de la derivación vertical dirigida al manto dorsal observada en las dos ramas anteriores. La rama C12c procedente del nervio izquierdo presenta un grosor semejante al de las demás ramas producidas por dicho nervio. No obstante, en el lado derecho, el grosor de C12c es sensiblemente menor.

Aproximadamente al mismo nivel del extremo anterior del penacho branquial, surge una rama de los nervios C12+ (C12′) que rodea la vaina branquial a la cual inerva mediante una serie de finos nervios originados de él y dirigidos al interior de la vaina branquial. De la rama nerviosa se diferencian dos conectivos, uno de ellos (Fig. 10) une el nervio C12+ con el nervio V1c procedente del ganglio visceral; y el otro conectivo recorre el borde anterior del penacho branquial uniendo el nervio C12+ de cada lado (Fig. 10,a). A lo largo de su recorrido este conectivo emite finas prolongaciones nerviosas que se introducen en las branquias. La entrada en cada branquia se realiza entre los vasos sanguíneos eferentes y aferentes principales, y siguen el raquis de la branquia a la vez que emiten derivaciones a cada una de las laminillas branquiales.

La prolongación nerviosa del nervio C12+ del lado derecho presenta algunas variaciones respecto al izquierdo. Las más notables son: la presencia de dos pequeñas concentraciones nerviosas una de ellas próxima al punto de diferenciación de la prolongación nerviosa del eje principal del nervio (Fig. 10, b); y la segunda en el conectivo que une este nervio con A1c de la cual parten finos nervios dirigidos a la cámara renal (cr) (posiblemente sea una concentración ganglionar).

Próximo al nivel del extremo posterior del penacho branquial el eje principal de cada nervio C12+ entra en la expansión lateral del manto. Seguidamente emite un par de ramas nerviosas que a su vez se van a dividir extendiéndose por las zonas posterolaterales de

dicha expansión del manto (C12d, C12e). El eje principal continúa su recorrido hacia el extremo posterior del animal, a través del borde del manto y muy próximo a la vaina branquial. Cerca del eje medio longitudinal del cuerpo del animal, cada uno de los nervios C12+ emite una rama nerviosa dirigida hacia el extremo posterior que, tras un breve recorrido se bifurca dando, por una parte, una rama que conecta con C12e a la vez que emite algunas derivaciones finas, y por otra parte otra rama que actúa de conectivo entre los nervios de cada lado. Al contactar estas dos ramas se dirigen, como una rama única, en sentido posterior, para bifurcarse seguidamente; una de las derivaciones formadas se dirige al borde posterior de la expansión periférica del manto, aunque antes de llegar a éste se divide en cuatro ramas que, dispuestas a modo de estrella se extienden por dicha zona.

Partiendo directamente del eje principal del nervio, se han visto algunas ramas muy finas dispuestas por la vaina branquial.

Ganglio visceral (Figs. 2,10)

Este ganglio (gv) se presenta como un pequeño abultamiento dispuesto en la superficie ventral del ganglio cerebropleural derecho, por su porción más posterior.

Del ganglio visceral parte un tronco nervioso que conecta con un ligero ensanchamiento ganglionar, que hemos identificado como ganglio genital (GG1). Inmediatamente detrás de este ganglio se diferencian dos nervios dirigidos hacia el extremo posterior del cuerpo. Uno de ellos (V2) se dirige, dorsalmente respecto a los órganos reproductores, hasta llegar al nivel del extremo anterior de la glándula digestiva, donde se gira hacia la izquierda y penetra entre esta glándula y los órganos reproductores hasta la superficie ventral de aquélla, por donde se ramifica emitiendo finas derivaciones nerviosas. A lo largo de su recorrido hacia la zona ventral de la glándula digestiva se ha visto en el nervio un pequeño ensanchamiento cuya apariencia externa se asemeja a la que presentan los ganglios, lo cual hace pensar que se trata de alguna concentración ganglionar.

El segundo nervio diferenciado, V1, al llegar al nivel donde la arteria de la glándula sanguínea se diferencia de la arteria anterior, emite una rama muy fina (V1a) que se continúa hacia detrás hasta alcanzar la porción proximal del intestino para disponerse entre éste y la superficie dorsal de las glándulas hermafrodita y digestiva. El nervio V1 continúa junto a la rama formada hasta que ésta se dirige a tales glándulas para, entonces, continuar su recorrido dorsal bordeando el lado derecho del intestino. Desde aquí sigue hasta llegar aproximadamente al nivel en que el ventrículo da lugar al tronco aórtico, donde gira hacia el lado izquierdo del intestino originando otra rama nerviosa (V1b) dirigida al pericardio por cuya superficie se ramifica, hasta llegar a la válvula ventricular (válvula que separa el ventrículo del tronco aórtico). Mientras tanto V1 discurre ventralmente respecto al pericardio siguiendo el borde izquierdo del intestino hasta llegar al nivel de la vesícula renal donde se observa una nueva ramificación. Inmediatamente delante de esta bifurcación hay un pequeño engrosamiento a modo de concentración ganglionar. Una de las ramas formadas, V1c, se dispone en el eje medio longitudinal del cuerpo del animal, ventralmente respecto a la cámara renal y dorsalmente respecto al seno hepático, se dirige hacia el extremo posterior del animal para contactar con los conectivos que lo unirán a cada nervio C12+ (Fig. 10). A lo largo de su recorrido emite algunas ramificaciones muy finas.

La otra rama formada, V1d, se dirige a la vesícula renal ramificándose y extendiéndose por la superficie de ésta; una de las ramas, sin embargo, V1e, recorre parte de la glándula renal para luego entrar en la cavidad pericárdica hasta la aurícula por donde se ramifica.

El nervio V1, mientras recorre el intestino, emite otras ramas nerviosas muy finas que se extienden por la gónada, glándula renal y glándula digestiva.

Ganglio pedio

P1+ (Figs. 2,11). Es el nervio más grueso y de mayor recorrido de los originados en los ganglios pedios (gp). Dorsolateralmente, parte del tercio posterior del ganglio (en el ganglio izquierdo está algo más adelantado) se dirige hacia detrás verticalmente al pie para luego recorrerlo longitudinalmente hasta su extremo posterior. A lo largo de su recorrido los nervios P1+ emiten algunas ramificaciones. La primera de ellas es la más gruesa, P1a, y entra entre la musculatura del pie aproximadamente al mismo nivel que las áreas de origen de los músculos retractores del aparato bucal. Posteriormente a su entrada en el pie se bifurca, una de las ramas se dirige hacia delante para extenderse por esta zona y, a medida que se divide conecta con el

nervio P2+ (Fig. 11). La otra rama va en sentido posterior para distribuirse por el pie hasta el nivel en que se diferencia la segunda rama del nervio P1+ (P1b), lo cual ocurre en un nivel algo más adelantado que el extremo anterior de la cavidad pericárdica.

De cada nervio P1+ parten, además de P1a, cuatro o cinco ramas nerviosas que se introducen en el pie y se ramifican posteriormente, inervándolo. Hacia la última ramificación (P1f), aproximadamente a nivel del borde posterior del penacho branquial, cada nervio P1+ presenta un ligero engrosamiento del que surgen varias ramas nerviosas muy finas. La más gruesa de ellas continúa hacia detrás hasta el extremo posterior del pie.

No se han observado conexiones entre los nervios P1+ de cada lado.

P2+ (Figs. 2,11). Parten del extremo posterior de los ganglios pedios, por su superficie ventral. El origen de estos nervios está al lado del origen de los nervios P3+ (Fig. 3). Desde aquí, ambos nervios rodean el aparato bucal para, una vez alcanzada su superficie ventral, tomar cada nervio direcciones distintas.

Los nervios P2+ se dirigen verticalmente al pie y una vez alcanzado éste se bifurcan, una rama se dirige hacia delante y la otra hacia detrás. Cada una de ellas se ramifica repetidas veces extendiéndose por el pie. La rama posterior, a su vez, conecta con las ramas P1a.

El par de nervios P3+ (Figs. 2,3,11a) se dirige hacia el extremo anterior del pie. Las primeras ramificaciones que parten de estos nervios (P3a) se disponen siguiendo en su recorrido muy próximos a dos vasos laterales de la arteria pedia anterior. Los dos nervios P3+ continúan hacia el extremo del pie por donde se observan algunas conexiones entre los nervios de cada lado del animal.

P4 (Fig. 2). El origen de este nervio se encuentra en la superficie dorsal del ganglio pedio derecho, algo más posterior, aunque bastante próximo al conectivo cerebropedio. No se ha visto ningún nervio semejante en el ganglio izquierdo.

Desde su origen, se dirige hacia la zona más distal del aparato reproductor (Fig. 9). Al llegar a la vaina penial se divide bordeándola. Una de las ramas diferenciadas (P4a), tras bordear la vaina del pene (p) se dirige hacia detrás por la zona de unión del pie con el costado. La otra rama formada (P4b) bordea también la vaina penial, pero en sentido contrario a la anterior. All llegar al espacio que existe entre las vainas del pene y la vagina (v), se observa una serie de engrosamientos, de los cuales el tercero es el de mayor tamaño y de apariencia ganglionar (GG2).

De los dos primeros engrosamientos parten finos nervios dirigidos al atrio común (donde desemboca el pene, la vagina y el oviducto).

Del ganglio genital 2, parten los siguientes nervios (Fig. 9):

G1. Es un nervio muy fino dirigido a la vaina de la vagina, donde se dispone.

G2. Se dirige a la glándula vestibular, por cuya superficie se ramifica. Una de las ramas formadas continúa hasta el atrio genital común.

G3. Su origen está contactando con el del nervio G2. Desde aquí bordea la vaina de la vagina para dirigirse seguidamente a la masa glandular femenina, en la posición más distal de ésta, por donde se ramifica.

G4. Delante del ganglio genital 2, muy próximo al punto de unión del nervio P4b con dicho ganglio, se observan dos nervios muy finos dirigidos hacia la glándula gametolítica. Al lado del origen de estos nervios se produce la conexión del nervio C10 con el P4b.

G5. Junto a G1 se observa un nervio, más grueso que los demás observados del ganglio genital 2, que se distribuye por la masa glandular femenina.

Ganglio bucal

B1+ (Figs. 2,3,12,13). Del extremo inferior de cada ganglio bucal (gb) parte un nervio dirigido hacia el saco radular (sr). De este nervio se distinguen dos ramas principales. La más gruesa e interna (B1a) bordea la región bucal posterior, para llegar al músculo retractor radular superior (este músculo se dispone en la superficie posterior del aparato bucal, a cada lado del saco radular), por donde se ramifica y extiende. Algunas ramificaciones llegan hasta el saco radular y porción posterior de los cartílagos del odontóforo (Fig. 13).

La otra rama nerviosa de B1+ (B1b), más fina y dispuesta más externamente, se distribuye por el músculo protractor bucal superficial, el cual es un músculo fino dispuesto por encima de los retractores bucales (Fig. 12).

Del lado externo de cada ganglio bucal parte un grueso tronco nervioso que rápidamente se bifurca originándose así los nervios B2+ y B3+.

B2+ (Figs. 2,3,12,13). Desde su origen se

dirige hacia las zonas ventrales de la región bucal posterior. De estos nervios se diferencian rápidamente los conectivos cerebrobucales (ccb). Muy próximos a los músculos retractores bucales (rb), los nervios B2+ se dividen dando tres ramas nerviosas. La más superior (B2a) se dirige a los puntos de inserción de los músculos protractores bucales (conjunto de finas bandas musculares dispuestas longitudinalmente entre el extremo posterior del tubo oral y la región bucal posterior), extendiéndose entre ellos. Las otras dos ramas nerviosas penetran entre la musculatura bucal, por detrás del área de inserción de los músculos retractores bucales. La rama nerviosa media (B2b) se dirige hacia la superficie ventral hasta llegar a los músculos aproximador anterior y posterior del cartílago (músculos que unen las dos mitades del cartílago odontofórico ventralmente). Se han observado también algunas ramificaciones finas que, partiendo de B2b, se dirigen hasta el músculo protractor del odontóforo (fino músculo dispuesto longitudinalmente desde la zona de inserción de los retractores bucales hasta el extremo anterior de la región bucal posterior).

B2c llega hasta la superficie ventral de los músculos retractores bucales, donde se divide. Una de las ramas formadas se introduce entre esos músculos, mientras que la otra, algo más gruesa, se dirige hasta los músculos protractores del saco radular.

B3+ (Figs. 2,3,12,13). Localizado más dorsalmente, respecto a B2+, presenta la primera división muy próximo a su origen. Se diferencian así dos ramas nerviosas (B3a, B3b).

La rama B3a, la más dorsal de las dos, se extiende por las zonas más dorsales de la región bucal posterior. B3a se divide varias veces dando por un lado una rama dirigida al esófago (e), hasta la base de las glándulas salivares. Una segunda rama diferenciada desde B3a se extiende por la musculatura superficial dorsal del aparato bucal, llegando incluso a la base del esófago. Por último, una tercera ramificación de B3a se extiende por la musculatura superficial dorsal, llegando algunas de sus ramas al músculo constrictor bucal (grueso músculo que rodea la cavidad bucal interna a modo de esfinter).

B3b recorre el aparato bucal más lateralmente. De B3b surgen dos ramas principales, de las cuales la más superior se extiende dentro de los músculos constrictor bucal y protractores del odontóforo, a los cuales emite finas ramificaciones. La rama inferior penetra en el aparato bucal hasta llegar a la pared bucal.

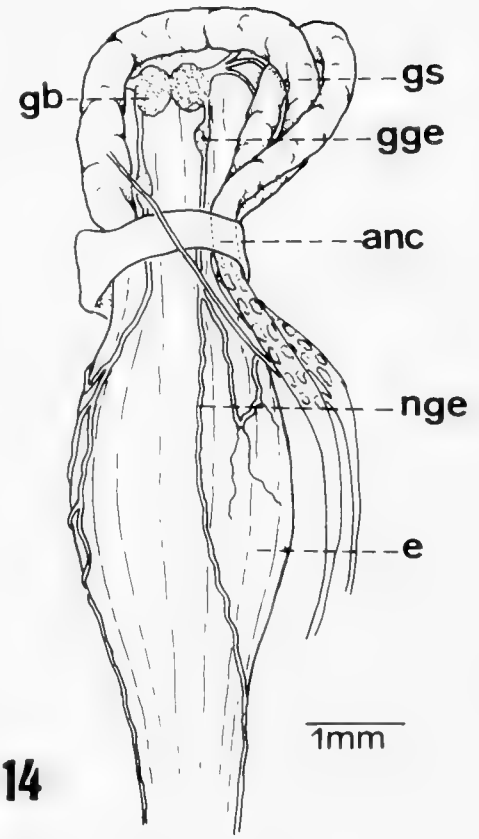


FIG. 14. Inervación del esófago.

Ganglio gastroesofágico (Figs. 2,14)

De cada ganglio parte un tronco nervioso dirigido hacia detrás por la superficie ventral del esófago. Al sobrepasar el anillo nervioso subesofágico estos nervios (nge) se ramifican extendiéndose por todo el esófago (Fig. 14). El eje principal del nervio continúa, sin embargo, hacia detrás, disponiéndose a lo largo de los bordes laterales del esófago hasta llegar al estómago. Una vez en éste, se extiende ramificándose por toda su superficie hasta llegar al ciego gástrico.

DISCUSION

La disposición de los ganglios y principales nervios observados en *Platydoris argo* se co-

rresponde, en líneas generales, con la descrita para otros doridáceos. No obstante, de una comparación más minuciosa de nuestras observaciones, con las consultadas en la literatura para otros doridáceos se pueden resaltar algunos datos de interés. En este trabajo se han omitido caracteres generales, como el acortamiento del asa visceral, la detorsión o la emigración hacia delante y fusión de los ganglios del asa visceral (cefalización y cerebralización, según Wirz, 1952) por haber sido ya tratados con cierta profundidad por diversos autores (Pelseneer, 1894; Guiart, 1901; Russell, 1929; Wirz, 1952; Brace, 1977).

Para la masa ganglionar dorsal en *P. argo* se ha adoptado la denominación de ganglio cerebropleural, debido al grado de fusión que presentan los ganglios cerebroides con los complejos pleurales (complejos constituidos por los ganglios pleural derecho, paleal derecho y supraintestinal y el ganglio pleural izquierdo junto con el paleal izquierdo y subintestinal, los cuales se han ido fusionando desde los órdenes de opistobranquios más inferiores), no diferenciándose externamente más que por la observación de los nervios que de ellos parten y por una ligera constricción en los ganglios. Risbec (1953, 1956) también considera el sistema nervioso central muy concentrado, tanto en los ejemplares de *P. argo* procedentes del Viet-Nam (Risbec, 1956) como de *P. noumeae,* de Nueva Caledonia (Risbec, 1953). Sin embargo White (1950) ilustra el sistema nervioso central de *P. tabulata* donde se observa una clara diferenciación entre el ganglio cerebroide y el complejo pleural.

Aunque en doridáceos en general los ganglios cerebroides y pleurales se encuentran fusionados, el grado de fusión que presentan es variable. Así, se observan especies en las que ambos ganglios están bien diferenciados, como ocurre en el género *Bathydoris* (Evans, 1914; Marcus & Marcus, 1962), o en *Okadaia elegans* (Baba, 1931, 1937), pasando por toda una serie de modelos en los que la diferenciación de las dos porciones ganglionares se produce por una constricción más o menos acentuada de la masa ganglionar. Esta constricción está bien marcada, entre otras especies, en *Onchidoris repanda, O. bilamellata, Acanthodoris pilosa* (Hancock & Embleton, 1852, citadas como *Doris repanda, D. bilamellata* y *D. pilosa* respectivamente) o en *Corambe testudinaria* (Fischer, 1891); algo menos marcada se encuentra en *Archidoris tuberculata, Doris verrucosa* (Hancock & Embleton, 1852, citada la primera especie como *Doris tuberculata*), *Jorunna tomentosa* (Hancock & Embleton, 1852, citada como *Doris johnstoni;* Cervera *et al.,* 1986). Finalmente en otras especies no se observa diferenciación externa entre las dos porciones, por ejemplo en los géneros *Polycera, Goniodoris* (Pelseneer, 1894), *Dendrodoris* o *Doridoxa* (Franc, 1968).

En el caso del género *Doridoxa,* el alto grado de fusión de los ganglios además de la ausencia de branquias y la presencia de un intestino corto, el cual no muestra la curva característica de *Bathydoris* y la mayoría de los eudoridáceos (Minichev, 1970) son caracteres que apoyan la separación propuesta por Tardy (1970) del género *Doridoxa* de la superfamilia Gnathodoridoidea, donde queda incluido *Bathydoris,* y el establecimiento de la superfamilia Pseudoactenoidea que abarca a dicho género.

La localización del ganglio rinofórico proximal de *P. argo,* dispuesto sobre el ganglio cerebropleural y separado de éste por medio de una constricción, es semejante a la señalada en *Phyllidia pulitzeri* por Wägele (1985). No obstante en los doridáceos, en general, estos ganglios pueden estar dispuestos de manera diferente según la especie. Así, hay especies en las que el ganglio rinofórico proximal se sitúa directamente sobre el ganglio cerebropleural sin apreciarse ningún tipo de constricción como ocurre en *Polycera* (Pelseneer, 1894), *Aldisa banyulensis* (García *et al.,* 1986), *Jorunna tomentosa* (Cervera *et al.,* 1986). En otros casos, por el contrario, hay un pedúnculo que separa los dos ganglios, por ejemplo en *Corambe* (Fischer, 1891).

La presencia de un ganglio rinofórico distal, dispuesto en la base de los rinóforos, ha sido descrita para diversas especies de doridáceos, ya sea como un ganglio bien marcado, como indica Baba (1937) para *Okadaia elegans,* o como un engrosamiento del nervio rinofórico en la base de los rinóforos, como es el caso de *Bathydoris* (Evans, 1914). En *P. argo* se ha observado un ligero ensanchamiento del nervio en la base de los rinóforos que podría coincidir con el ganglio rinofórico distal. Hancock y Embleton (1852) sin embargo, no señalan ningún tipo de diferenciación del nervio rinofórico, en sus descripciones anatómicas del género *Doris.*

En doridáceos los ojos suelen disponerse en el extremo de un nervio bien desarrollado,

por ejemplo *Onchidoris bilamellata, Acanthodoris pilosa* (Hancock & Embleton, 1852, citados como *Doris bilamellata* y *D. pilosa,* respectivamente), *Corambe* (Fischer, 1891) y *Phyllidia pulitzeri* (Wägele, 1985). Sin embargo, hay especies en las que los ojos se disponen directamente sobre los ganglios cerebropleurales (por ejemplo *Okadaia elegans;* Baba, 1937), o separados de éstos por un nervio muy corto (*Dendrodoris limbata, Aegires leuckarti, Euplocamus croceus;* Vayssière, 1901). *P. argo* pertenece al primer tipo, con nervios ópticos largos y finos. No obstante White (1950) señala en *P. tabulata* un nervio óptico muy corto.

Modificaciones especiales se observan por ejemplo en el género *Bathydoris,* en el cual Evans (1914) y Marcus y Marcus (1962) señalan la carencia de órganos visuales, quizás motivada por la pérdida de aquéllos, ya que las especies de este género viven a grandes profundidades (hasta 4.400 metros aproximadamente; Marcus & Marcus, 1962.).

De cada ganglio gastroesofágico de *P. argo* parte un tronco nervioso que seguidamente se ramifica para dar lugar el nervio esofágico y el nervio gástrico. En *Archidoris pseudoargus* Rose (1971) observa ambos nervios independientemente desde sus orígenes, además de otros dos nervios pequeños dirigidos hacia la glándula salivar. Por otra parte Hancock y Embleton (1852) en su descripción sobre el género *Doris* señalan tres nervios: uno dirigido a las glándulas salivares, otro hacia el esófago y el tercero que llegaría al estómago. Los dos nervios de *P. argo* se pueden corresponder con los dos últimos señalados por Hancock y Embleton (1852). A las glándulas salivares, como se indicó anteriormente, llega una rama del nervio B3 (B3a).

Del ganglio visceral de *P. argo* sale un único tronco nervioso que emite diferentes nervios dirigidos a los distintos órganos del animal. Hancock y Embleton (1852), sin embargo, señalan un número variable de nervios según la especie; así indican dos nervios en *Onchidoris repanda* y *Acanthodoris pilosa,* tres en *Onchidoris bilamellata* y *Jorunna tomentosa* y cuatro en *Archidoris tuberculata.*

Los nervios que inervan los tentáculos orales en doridáceos están siempre bien desarrollados, incluso Baba (1937) señala este nervio como de los mayores en *Okadaia elegans,* cuyos tentáculos orales son rudimentarios. Baba señala además para este nervio la presencia de un bien marcado ganglio distal del cual los nervios se dirigen hacia el tentáculo oral. En *P. argo,* sin embargo, solamente se ha observado un ligero ensanchamiento del nervio C4+ en la base del tentáculo oral, que vuelve a estrecharse a medida que avanza y se ramifica por el interior de aquél. Además, en *P. argo* el origen del nervio oral es común a otros dos nervios dirigidos al tubo oral y labio externo respectivamente; en *O. elegans,* sin embargo, según Baba (1937), éste parte sólo desde su origen.

Una comparación detallada del origen y disposición de los nervios descritos por Hancock y Embleton (1852) para *Archidoris tuberculata* con los observados en *P. argo* permite relacionar los distintos nervios de la manera siguiente:

Los pares 2°, 3° y 4° señalados por estos autores se corresponden con C1+, C2+ y C4+. El par 5°, por su origen y recorrido coincide con C3+. El par 8° ha sido relacionado con los pares C6+, C7+ y C8+ de *P. argo;* el par 9° se corresponde con el C11+, el 10° par con el C12+. Para estos tres pares de nervios (8°, 9° y 10°) Hancock y Embleton señalan la posible variación que puede existir entre distintas especies. Así en *Doris verrucosa* falta el par 9°; en *Acanthodoris pilosa* el par 8° está dividido dando por una parte lo que en *P. argo* correspondería a C6+ y C7+ y por otra a C8+; los pares 9° y 10° están además presentes. Igual situación ocurre en *Onchidoris bilamellata.*

El par C5+ de *P. argo* debe corresponder con alguna rama del 8° par.

Entre los nervios de los ganglios pedios se puede establecer la relación siguiente: el par 13° correspondería al par P3+ de *P. argo;* el par 14° se asemeja al par P2+ y el par 15° al P1+.

De los nervios de los ganglios bucales la relación se establece entre los pares 16°, 17° y 18° de *Archidoris tuberculata* y B2+, B3+ y B1+, respectivamente, de *P. argo.*

Los nervios genitales en *P. argo* parten de los ganglios genital y pedio del lado derecho. Esta disposición coincide con la que Tardy (1970) señala para los opistobranquios en general. No obstante Russell (1929) sólo indica en *Elysia viridis* el ganglio visceral del cual parte el ganglio genital.

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ABREVIATURAS UTILIZADAS

aa	arteria anterior
ac	arteria cefálica
an	ano
anc	anillo nervioso circunesofágico
av	asa visceral
br	branquia
cb	comisura bucal
cbg	conectivo bucogastroesofágico
ccb	conectivo cerebrobucal
cp	comisura pedia
cpe	cavidad pericárdica
cpp	comisura parapedia
cr	cámara renal
dd	músculo dilatador dorsolateral del tubo oral

dl	músculo dilatador lateral del tubo oral
dv	músculo dilatador ventrolateral del tubo oral
e	esófago
gb	ganglio bucal
gcp	ganglio cerebropleural
GG1, GG2	ganglios genitales
gge	ganglio gastroesofágico
gla	glándula anexa
go	ganglio óptico
gp	ganglio pedio
gr	ganglio rinofórico
gs	glándula salivar
gv	ganglio visceral
nge	nervios de los ganglios gastroesofágicos
o	ojo
p	pene
r	rinóforo
rb	músculo retractor del aparato bucal
sr	saco radular
t	tentáculo oral
to	tubo oral
v	vagina

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BIOACCUMULATION NATURELLE DE MANGANESE ET DE FER DANS LES TISSUS MOUS D'*ANODONTA CYGNEA* (MOLLUSQUE, LAMELLIBRANCHE, METABRANCHIÉ).

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RÉSUMÉ

L'accumulation de manganèse dans les tissus mous du mollusque bivalve *Anodonta cygnea* a été étudiée par les techniques classiques de la microscopie photonique, de la cytochimie et de la microscopie électronique, ainsi que par les procédés modernes de la microscopie analytique (émission ionique secondaire, émission de rayons X et diffusion Raman). Des dosages par spectrophotométrie d'absorption atomique électrothermique ont également été effectués sur les tissus mous, l'eau et les sédiments.

Le tissu conjonctif de la branchie, du manteau et de la glande digestive est le siège principal de l'accumulation du manganèse, en quantités exceptionnellement fortes. Le métal est localisé dans de volumineuses concrétions extracellulaires, où il coexiste avec du phosphore, du calcium, du fer et divers autres métaux, en plus faibles quantités. Le manganèse est sous forme tétravalente Mn^{4+} et le fer dans ses deux degrés d'oxydation Fe^{2+} et Fe^{3+}, ce qui est exceptionnel pour Fe^{2+} dans les tissus animaux. Les concrétions extracellulaires, ou sphérocristaux, sont principalement constituées de phosphate de calcium dans la branchie, mais aucune forme minérale ne peut être mise en évidence dans les deux autres organes. Nous supposons que les métaux y sont piégés sur la composante organique des sphérocristaux et nous émettons l'hypothèse qu'à côté de la fonction bien connue de précipitation de sels minéraux en présence d'une matrice organique, la complexation organo-minérale pourrait constituer à elle seule une autre voie de fonctionnement des sphérocristaux.

Les épithéliums de la branchie, du manteau et de la glande digestive renferment des inclusions minérales, mais ils ne sont pas le site d'accumulation du manganèse. Les cellules de Leydig du tissu conjonctif renferment des concrétions riches en manganèse, mais cette forme d'accumulation intracellulaire est faible en regard de celle des sphérocristaux extracellulaires du même tissu. Enfin, des bactéries sont observées et décrites pour la première fois à notre connaissance, dans les tissus mous de l'anodonte. Ces bactéries contiennent des granulations riches en manganèse. Les procaryotes pourraient donc être la source du métal accumulé dans les tissus mous, et nous suggérons que la réduction du fer Fe^{3+} en fer Fe^{2+} pourrait constituer la source d'oxydation du manganèse. De ce fait, les quantités particulièrement élevées de manganèse stocké dans les tissus mous ne traduiraient pas un métabolisme particulier de ce bivalve, mais celui de la faune bactérienne qu'il héberge. Enfin, il nous paraît important de souligner que la rétention intratissulaire de manganèse et la précipitation de ce métal dans le périostracum sont tout-à-fait indépendantes du dépôt de manganèse en surface des coquilles.

Key words : mineral accumulation ; bacteria ; iron ; manganese ; micro-analysis ; mollusc.

INTRODUCTION

Parmi les nombreuses bioaccumulations naturelles, celle du manganèse est un phénomène répandu chez les invertébrés ; en particulier, parmi les mollusques, les unionidés en stockent des quantités importantes, à la fois dans la coquille et dans les tissus mous (Bradley, 1907, 1910 ; Dubuisson & Van Heuverswyn, 1930 ; Van Heuverswyn, 1930 ; Vinogradov, 1953 ; Ravera & Vido, 1961 ; Merlini, 1962). Dans la coquille, les teneurs peuvent atteindre 560 $\mu g.g^{-1}$, valeurs beaucoup plus élevées que pour les lamellibranches et gastéropodes marins (1,1 à 1,8 $\mu g.g^{-1}$: Segar, Collins & Riley, 1971) ou dulcicoles (30 à 120 $\mu g.g^{-1}$: Merlini *et al.*, 1965). Dans le périostracum, le manganèse

est accumulé dans deux états d'oxydation (Mn^{2+} et Mn^{4+}), à l'intérieur d'une matrice protéique, où il coexiste avec du phosphate de calcium amorphe et du fer trivalent (Swinehart & Smith, 1979). Parmi les tissus mous, ce sont des concrétions renfermant également du phosphate de calcium qui, dans la branchie, stockent l'élément ; elles sont de type sphérocristal et situées dans les espaces extracellulaires (Silverman *et al.*, 1983).

Lors d'une contamination accidentelle, du manganèse ^{54}Mn a été retrouvé non seulement dans les branchies, mais aussi dans le manteau et dans la masse viscérale où il est immobilisé sous une forme peu échangeable, non identifiée (Ravera & Gaglione, 1962 ; Gaglione & Ravera, 1964 ; Merlini, 1969).

Ces données étant dispersées sur plusieurs espèces et ne concernant pas tous les organes, il nous a paru important de rechercher dans une espèce, *Anodonta cygnea*, les différents sites tissulaires et cellulaires d'accumulation du manganèse et d'en déterminer la forme chimique d'immobilisation, ainsi que celle des éléments qui l'accompagnent.

MATÉRIEL ET MÉTHODES

Les spécimens d'*Anodonta cygnea* ont été récoltés à différentes périodes de l'année (octobre, décembre et avril), dans un étang proche de Villers-Cotterêts (région parisienne) et dans le lac Léman ; des prélèvements d'eau et de sédiments ont été effectués simultanément. Des fragments des différents tissus, prélevés et fixés extemporanément, ont servi à l'étude histologique de tous les animaux, ainsi qu'aux dosages du manganèse pour les spécimens de Villers-Cotterêts.

Les dosages du manganèse et du fer ont été effectués sur l'eau, les sédiments et les tissus, par spectrophotométrie d'absorption atomique électrothermique, à l'aide d'un appareil Perkin-Elmer 370 B, muni d'un passeur automatique d'échantillons AS-1 et d'un programmateur pour four, HGA 2100. La minéralisation réalisée par le mélange acide nitrique-acide chlorhydrique (1 : 1) a été suivie d'évaporation à sec et de reprise du résidu par l'eau distillée. Le volume injecté était de 20 µl et pour les deux éléments le cycle d'atomisation a été programmé selon les recommandations du constructeur.

Les fragments destinés à l'étude histologique en microscopie photonique ont été fixés pendant 24 h. par le mélange de Carnoy (éthanol absolu, chloroforme, acide acétique : 6/3/1), inclus à la paraffine et coupés à 5 µm. Cette fixation étant faite en *milieu anhydre,* il n'y a pas lieu de craindre les inconvénients qui résulteraient de l'immersion dans un acide. Les fragments destiné à l'étude cytologique en microscopie électronique ont été fixés pendant deux heures dans le glutaraldéhyde à 1 % en solution tamponnée à pH 7 par le cacodylate de sodium, et inclus à l'epon-araldite ; les pièces destinées au seul examen morphologique ont, en outre, été post-fixées au tétroxyde d'osmium, et les coupes ont été colorées à l'acétate d'uranyle. L'observation des coupes de 100 nm d'épaisseur environ a été faite à l'aide d'un microscope Philips 300.

Les coupes à la paraffine destinées à l'examen morphologique en microscopie photonique, ont été colorées à l'hémalun picro-indigocarmin. La recherche histochimique des sels de calcium, a été réalisée sur coupes à la paraffine par la méthode de von Kossa, celle du fer ferrique (Fe^{3+}) par la réaction de Perls, celle du fer ferreux (Fe^{2+}) par la méthode au bleu de Turnbull et celle du manganèse (Mn^{2+}) par la méthode de réduction d'un complexe d'argent de Mac Nary (*in* Ganter et Jollès, 1970). La recherche des carbonates a été effectuée par la méthode du dégagement gazeux (Bunting, *in* Pearse, 1972), celle des radicaux protéiques par la tétrazoréaction de Danielli et celle des polysaccharides par la méthode à l'acide periodique Schiff (APS). Enfin, les radicaux anioniques des polysaccharides ont été mis en évidence par le bleu alcian à pH 2,5, et les urates par les méthodes de Schmorl et de Gomori.

L'analyse moléculaire (Delhaye & Dhamelincourt, 1975 ; Ballan-Dufrançais, Truchet & Dhamelincourt, 1979 ; Truchet, 1982, 1984) a été effectuée sur coupes à la paraffine étalées sur lame de verre sans collage, à l'aide d'une microsonde Jobin-Yvon MOLE 77 dans les conditions suivantes : Laser : Spectraphysics, argon ionisé, 514,5 nm, 100 mW en sortie du laser. Collecte de flux : objectif 100 à sec Leitz, d'ouverture numérique égale à 0,90. Résolution spectrale : 7 cm^{-1} environ. Une puissance laser plus élevée (300–350 mW) permet de détruire par incinération locale les substances fluorescentes liées à la matière organique sans modification de la phase minérale. Des échantillons témoins de

TABLEAU 1.—Eléments détectés par microanalyse X, sur coupes à la paraffine. Le nombre de croix indique l'intensité des signaux X

	Eléments majeurs				Autres éléments						
	P	Ca	Mn	Fe	Mg	Al	S	Cl	K	Zn	Ba
BRANCHIE											
Concrétions du tissu conjonctif	+ + + +	+ + + +	+ + +	+ +	±	±	+	+	+	+	+
Baguettes chitineuses	+ + + +	+ + + +	+ +	+	−	−	+	+	±	−	+
MANTEAU											
Concrétions du tissu conjonctif	+ + + +	+ + + +	+ + +	+ +	−	−	+	+	±	+	+
GLANDE DIGESTIVE											
Concrétions du tissu conjonctif	+ + + +	+ + + +	+ + +	+ +	±	−	+	+	±	+	+
Cellules basophiles	+	+	−	±	−	−	+	±	+	±	−
Cellules digestives	+	+	−	±	−	−	+	±	+	±	−

phosphates et de carbonates de calcium et de manganèse ont été analysés dans les mêmes conditions. L'échantillon témoin de phosphate de calcium montre la raie de vibration symétrique P-O, centrée sur 965 cm^{-1}, caractéristique de ce sel, ainsi que les raies de réseau, vers 350 et 600 cm^{-1}. Avec l'échantillon témoin de phosphate de manganèse, la raie est décalée vers 950 cm^{-1}, ce qui permet de distinguer sans ambiguïté les deux sels. En revanche, pour les carbonates, la raie de vibration symétrique C-O est centrée sur 1088 cm^{-1}, quel que soit le cation (Mn ou Ca).

L'analyse élémentaire par émission ionique secondaire (analyse ionique) a été effectuée sur des coupes à la paraffine étalées sur or, à l'aide d'un appareil Cameca SMI 300 dans les conditions suivantes : bombardement: O_2^+, 7 μA, très défocalisé pour éviter les effets de charge. Diaphragme de la lentille à émission : 200 μm. Bande passante : 12 eV. Résolution en masse : $m/\Delta m = 300$ environ (Truchet, 1982).

L'analyse par émission de rayons X (microanalyse X), en spectrométrie dispersive en longueur d'onde, a été effectuée sur des coupes à la paraffine et sur des coupes de matériel inclus dans l'epon-araldite. Pour les coupes à la paraffine, l'analyse a été réalisée à l'aide d'une microsonde Cameca MS 46, sous 15 à 20 kV de tension d'accélération, avec un courant d'échantillon de 40 nA, une sonde de 1 μm environ de diamètre et avec les cristaux monochromateurs KAP (Phtalate acide de potassium) et PET (Pentaérythritol). Pour les coupes ultrafines (épaisseur 100 nm environ), l'analyse a été pratiquée à l'aide d'une microsonde Cameca MBX, type CAMEBAX, sous 45 kV de tension d'accélération, avec un courant d'échantillon de 150 nA, une sonde de 500 nm de diamètre environ et les cristaux PET, TAP (Phtalate acide de thallium) et LiF (Fluorure de lithium) (Ballan-Dufrançais & Martoja, 1971 ; Quintana & Halpern, 1983).

RESULTATS

Données morphologiques et de microanalyse

L'analyse X sur coupes à la paraffine ayant montré que les organes impliqués dans l'accumulation du manganèse étaient la branchie, le manteau et la glande digestive (Tableau 1), l'étude a été restreinte à ces organes. Le rein, en particulier, est dépourvu de structures d'accumulation de manganèse.

Dans la branchie, les cellules épithéliales renferment un petit nombre de granulations opaques aux électrons (environ 0,06 μm). L'analyse X y révèle surtout du calcium et du phosphore, ainsi que du magnésium, de l'aluminium, du soufre, du chlore et du zinc en moindres quantités ou en traces ; le manganèse ne s'y rencontre que rarement (Tableau 2). Dans le tissu conjonctif branchial, de très nombreux sphérocristaux sont situés en po-

TABLEAU 2. Eléments détectés par microanalyse X sur coupes ultrafines. (valeurs extrêmes arrondies et exprimées en nombre de coups enregistres pendant 50 secondes; NS = non significatif)

		Eléments majeurs				Autres éléments						
		P	Ca	Mn	Fe	Mg	Al	S	Cl	Cu	Zn	Ba
BRANCHIE												
	Granulations des cellules épithéliales	645 5800	45 1725	NS 560	NS NS	NS 555	35 165	30 70	NS 80	NS NS	NS 220	NS 100
tissu conjonctif	Sphérocristaux extracellulaires	1055 90880	1760 85600	395 33060	65 6370	20 1455	NS 520	65 80	120 245	NS 110	45 330	30 600
tissu conjonctif	Sphérocristaux intracellulaires	855 40570	1890 59635	225 18675	20 2540	NS 325	NS 45	NS 20	35 80	NS NS	NS 105	NS 400
tissu conjonctif	Microgranules des pseudopodes des cellules de Leydig	1385 2910	625 2185	325 420	NS 155	NS 90	50 70	70 285	100 130	NS NS	NS 45	NS 150
	Baguettes chitineuses	38870 53890	37570 43230	18280 25560	3210 7850	770 845	20 250	NS 35	NS 25	60 150	NS 30	135 190
MANTEAU												
	Inclusions dans les cellules épithéliales	450 3320	30 950	NS NS	NS NS	NS 310	NS 130	NS 135	NS 55	NS NS	NS 370	NS NS
tissu conjonctif	Sphérocristaux extracellulaires	620 49910	245 39280	140 14165	NS 4160	NS 655	NS 125	40 145	35 375	NS NS	NS 485	NS 260
tissu conjonctif	Microgranules des pseudopodes des cellules de Leydig	1500 2290	400 1160	255 570	NS 480	NS 140	NS 135	20 140	25 190	NS NS	NS 25	NS 60
GLANDE DIGESTIVE												
	Sphérocristaux extracellulaires	740 56290	280 31560	310 16490	180 9995	30 1135	65 245	NS 140	NS 405	NS 85	NS 550	NS 265
BACTERIE												
	Intacte (granulations)	2060	1265	445	230	50	60	70	160	155	NS	120
	En cours de lyse (granulations)	1050	315	NS	NS	NS	NS	20	55	NS	NS	NS

sition extracellulaire, au sein d'une matrice organique riche en fibres de collagène (Fig. 1a). Les sphérocristaux isolés sont de petite taille (0,03 à 1,7 μm) ; on y observe fréquemment des strates très opaques aux électrons (Fig. 1c). Beaucoup fusionnent pour donner de volumineux amas (Fig. 1 b) ; certains de ces amas peuvent dépasser 10 μm. L'analyse X montre qu'ils renferment beaucoup de phosphore, de calcium et de manganèse ; il s'y ajoute en moindres quantités ou en traces, Mg, Al, S, Cl, K, Fe, Cu, Zn et Ba (Tableaux 1, 2). L'analyse moléculaire par diffusion Raman fournit un seul pic, centré sur 970 cm^{-1}. Aucune autre raie n'étant observée dans les spectres, la diffusion Raman ne révèle donc qu'une composition à base de phosphate de calcium (Fig. 2). Les cellules à prolongements amiboïdes, ou cellules de Leydig, dessinent un réseau tridimensionnel enserrant étroitement les sphérocristaux à strates très contrastées, s'associant parfois pour former, comme ceux des espaces extracellulaires, de volumineux amas pouvant atteindre 1,4 μm. Ces sphérocristaux intracellulaires ont la même composition que les autres concrétions du tissu conjonctif (Tableau 2). Les pseudopodes renferment de petites inclusions, ou microgranules, de 0,03 μm environ, de structure homogène, et où l'analyse X révèle les mêmes éléments que ceux des sphérocristaux. Enfin, les baguettes chitineuses, situées sous l'épithélium, sont très riches en phosphore, calcium, manganèse et fer. Elles renferment également du magnésium, de l'aluminium ainsi que des traces de soufre, de chlore, de potassium, de cuivre, de zinc et de baryum (Tableaux 1, 2).

Dans le manteau, les cellules épithéliales renferment des inclusions minérales, plus nombreuses dans l'épithélium palléal interne. Ces inclusions contiennent surtout du calcium et du phosphore, auxquels s'ajoutent du magnésium, de l'aluminium, du soufre, du chlore et du zinc, mais pas de manganèse. Dans le tissu conjonctif de cet organe, comme dans celui de la branchie, des sphérocristaux extracellulaires se développent au sein d'un réseau tridimensionnel complexe, formé par les pseudopodes des cellules de Leydig (Fig. 1d). Les alvéoles ainsi créées communiquent entre elles et contiennent toujours quelques fibres de collagène ; les pseudopodes renferment des microgranules de 50 nm environ (Fig. 1d). Dans les cristaux extracellulaires, les éléments majeurs sont le phoshore, le calcium, le manganèse et le fer ; il s'y ajoute un peu de magnésium, d'aluminium, de soufre, de chlore, de zinc et de baryum. Les microagranules n'émettent, pour tous ces éléments, que de faibles signaux X, mais compte tenu de leur petite taille, ces signaux correspondent à des concentrations élevées notamment en manganèse (Tableau 2). Aucun pic n'apparaît sur les spectres Raman des sphérocristaux extracellulaires du manteau, ni à la fréquence des carbonates (1088 cm^{-1}). La région allant de 1400 à 1600 cm^{-1}, ainsi que la région des vibrateurs C-H vers 2800–3000 cm^{-1} ne montrent chacune qu'un faible massif indifférencié.

Dans la glande digestive, les deux types cellulaires des tubules (cellules basophiles et cellules digestives) ne renferment que très peu d'inclusions minérales, pauvres en calcium et en potassium, alors que le tissu interstitiel en est très chargé. Dans ce tissu, les cellules de Leydig sont entourées d'un enchevêtrement de fibres. Comme pour le tissu conjonctif de la branchie et du manteau, c'est dans le réseau des pseudopodes que se situent de nombreux cristaux extracellulaires. Ils ne fusionnent que très rarement et sont ordonnés en longues travées. Ils contiennent du phosphore, du calcium, du manganèse et du fer, auxquels s'ajoutent les éléments habituels : Mg, Al, S, Cl, K, Cu, Zn et Ba (Tableaux 1, 2). Comme pour le manteau, l'analyse par diffusion Raman ne révèle ni carbonates, ni phosphates.

L'analyse par émission ionique a été effectuée sur les quatre organes : branchie, glande digestive, rein et manteau. Elle confirme l'accumulation de manganèse par les différentes concrétions, et révèle en outre le métal, en concentrations inaccessibles à l'émission des rayons X, dans l'ensemble des tissus. En particulier, dans le rein, où il n'y a pas de structures d'accumulations, l'émission ionique décèle du manganèse sous forme diffuse, conservé par la fixation (Figs. 3, 4).

Des procaryotes, de 1 à 4 μm sur 0,5 μm environ, occupent les espaces intercellulaires du tissu conjonctif de la branchie, du manteau et de la glande digestive. L'existence d'une paroi et d'un mésosome permet d'affirmer qu'il s'agit de bactéries (Fig. 1e). Elles abondent dans les anodontes de Villers-Cotterêts, et semblent moins nombreuses dans celles du lac Léman. Elles renferment des granulations opaques aux électrons, riches en manganèse et en calcium (Tableau 2). Certaines sont situées dans le cy-

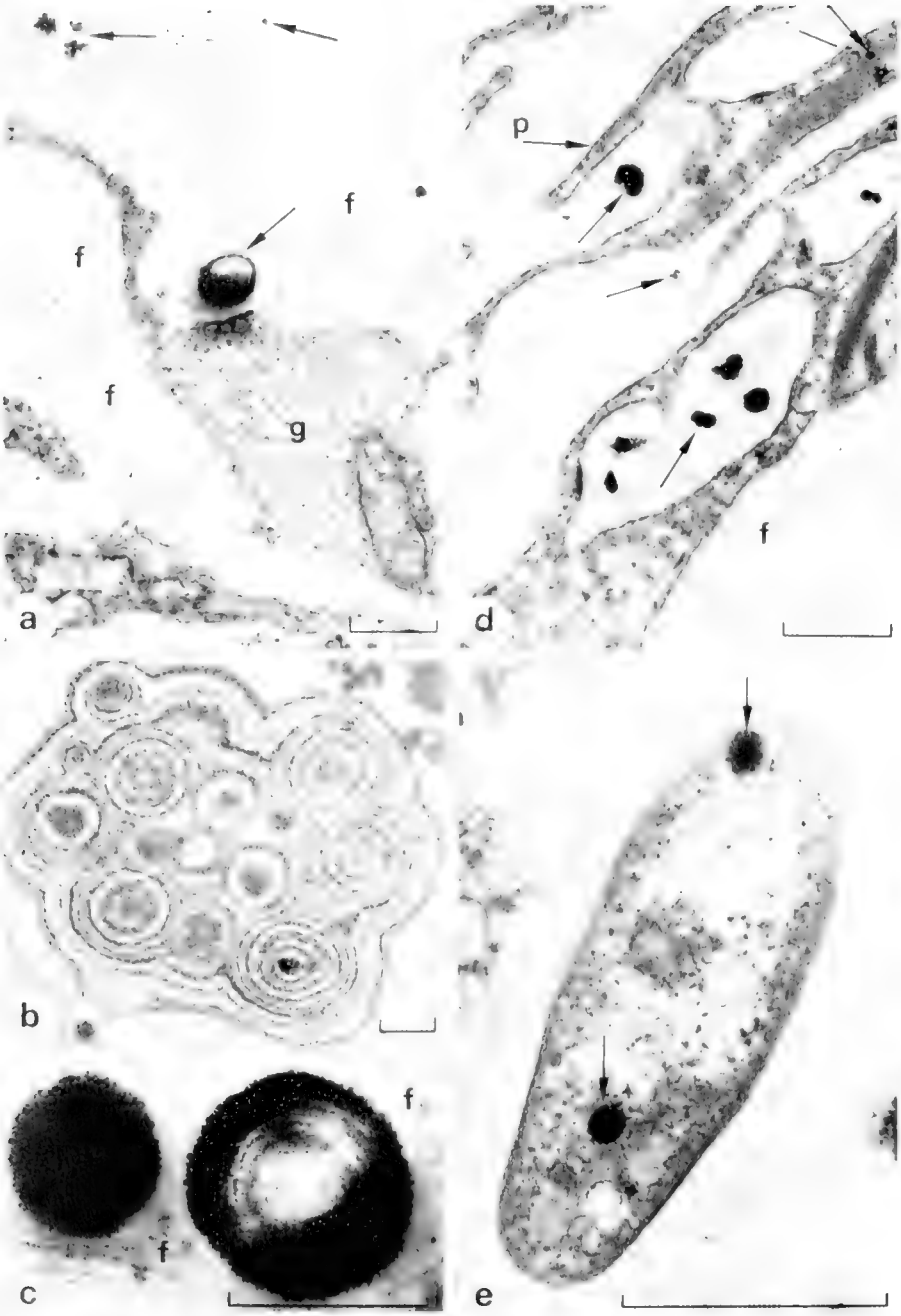


FIGURE 1 Aspects ultrastructuraux. a. Tissu conjonctif branchial; sphérocristaux de tailles variables (flèches) situés dans les espaces extracellulaires et entourés de fibres de collagène. b. Tissu conjonctif branchial; sphérocristaux confluents; caractéristiques de la branchie. c. Tissu conjonctif branchial; sphérocristaux isolés. La dissolution partielle qui intervient fréquemment lors de la confection des coupes met en évidence la stratification. d. Tissu conjonctif palléal. Observer les sphérocristaux extracellulaires situés dans le réseau des pseudopodes des cellules de Leydig (flèches simples) et l'un des microgranules intracellulaires (flèche double). e Bactérie. Noter les granulations opaques aux électrons (flèches), riches en manganèse. f: fibres de collagène; g. rosettes de glycogène; p: pseudopodes de cellules de Leydig. Échelle 0,5 µm.

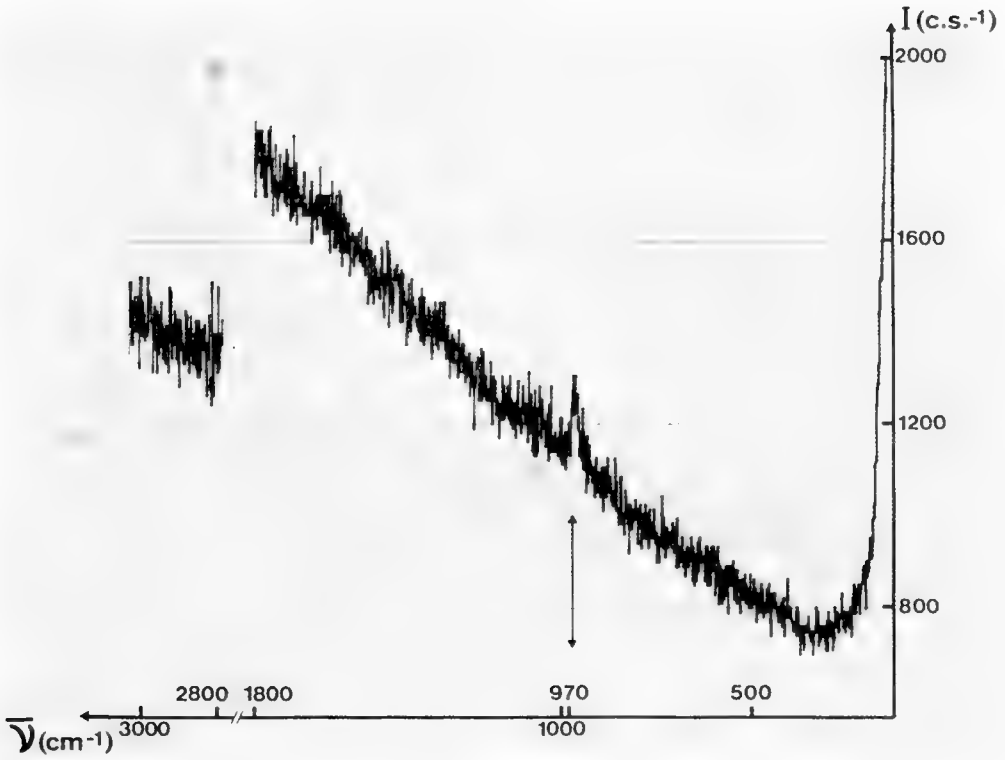


FIGURE 2. Spectre Raman d'un groupe de sphérocristaux du tissu conjonctif branchial. Seule, la vibration symétrique P-O, caractéristique du phosphate de calcium, fournit un signal.

toplasme des cellules de Leydig ; quelques-unes sont morphologiquement intactes, mais la plupart présentent des figures de lyse qui suggèrent une digestion intracellulaire. Au cours de cette lyse, les granulations disparaissent progressivement, ainsi que les signaux X du manganèse et du calcium.

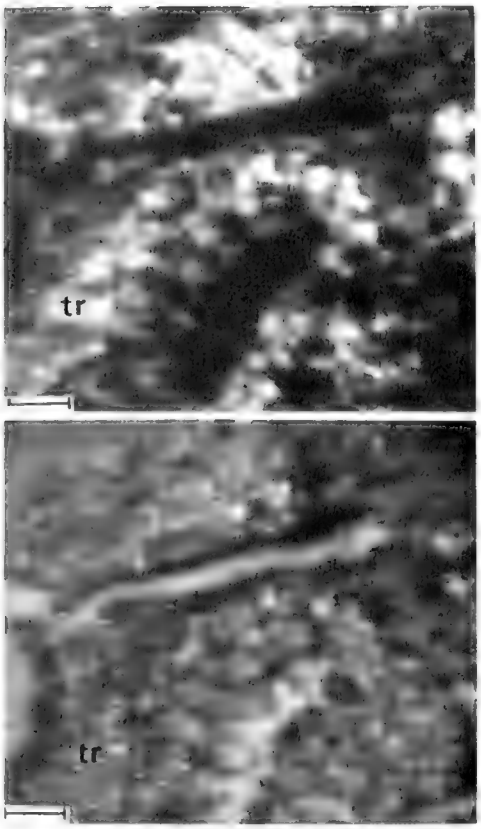
Données cytochimiques

Lors de la recherche des degrés d'oxydation du fer (Fig. 5) et du manganèse, les concrétions et les baguettes chitineuses des branchies réagissent positivement à la fois à la réaction de Perls et à celle au bleu de Turnbull ; la seconde est d'ailleurs beaucoup plus intense dans les cristaux du tissu conjonctif de la glande digestive et du manteau que dans ceux des branchies. La réduction du ferricyanure de potassium est imputable à Fe^{2+} et non à Mn^{2+}. En effet, les essais réalisés sur des sels dissous montrent que la réduction par Mn^{2+} produit un complexe insoluble de couleur brune, qui ne peut être confondu avec le bleu de Turnbull ; or, sur les coupes histologiques, le produit de la réaction est d'un bleu pur. On doit donc conclure à la coexistence, dans les sphérocristaux et dans les baguettes chitineuses de la branchie, des deux degrés d'oxydation du fer.

La faible importance du manganèse bivalent est confirmée par la réaction de réduction du complexe d'argent qui ne donne de résultats positifs que dans de rares cristaux du tissu conjonctif de la glande digestive. C'est donc certainement du manganèse tétravalent qui est stocké dans la majorité des sphérocristaux, conjointement avec les formes bivalentes et trivalentes du fer.

La réaction de caractérisation du calcium confirme la similitude de localisation de cet élément avec les autres métaux (Fig. 5).

Dans aucun des trois organes, ni la tétrazoréaction de Danielli, ni celle de caractérisation des carbonates ne donnent de résultats positifs. La réaction à l'APS n'est que faiblement positive dans les trois tissus. Dans la branchie, la réaction de mise en évidence



FIGURES 3,4. Analyse par émission ionique du rein fixé au Carnoy. 3) Image de la distribution du $^{23}Na^{+}$ (intensité du courant ionique: 5.10^{-15} Ampères; temps de pose: 20 secondes). 4) Image de la distribution du manganèse, $^{55}Mn^{+}$ (10^{-16} A, 15 mn). Les parties en blanc ou grisé correspondent aux zones d'émission. Deux tubules rénaux (tr) sont bien reconnaissables sur les clichés; la distribution du manganèse est très uniforme. Échelle 20 µm.

des polyanions par le bleu alcian est intensément positive, mais elle est négative pour le manteau et la glande digestive. La recherche des urates est également négative, ce que confirme, sur les spectres Raman, l'absence des raies caractéristiques (Fig. 2).

Dosages

Manganèse : les dosages par spectrophotométrie d'absorption atomique montrent des teneurs très élevées (jusqu'à 22 $mg.g^{-1}$) en manganèse pour les trois organes (Tableau 3) ; en revanche, l'eau et les sédiments en sont pauvres : 50 $ng.g^{-1}$ pour l'eau et 80 à 800 $\mu g.g^{-1}$ pour les sédiments. Le facteur de concentration des tissus peut donc atteindre 4.10^5 par rapport à l'eau, et 300 par rapport aux sédiments.

Fer : les teneurs en fer, bien qu'importantes dans les tissus (Tableau 3) n'atteignent pas des valeurs aussi élevées que pour le manganèse. Le facteur de concentration par rapport à l'eau peut cependant atteindre 10^4. En revanche, les sédiments peuvent être plus riches en fer que les tissus de l'anodonte.

DISCUSSION

Chez *Anodonta cygnea,* les teneurs en manganèse atteignent, dans les tissus mous, des valeurs exceptionnellement fortes qui font de cet animal l'un des principaux concentrateurs du métal. Cette accumulation, qui concerne la plupart des organes, n'intéresse toutefois pas le rein, ce qui oppose ce lamellibranche dulcicole à d'autres espèces connues pour stocker le métal (Hignette, 1979 ; Eisler, 1981).

Le principal site d'accumulation du manganèse consiste en volumineuses concrétions, situées dans le tissu conjonctif ; leur structure interne en strates concentriques est celle de sphérocristaux. Ils sont en position extracellulaire, et non dans des vacuoles comme pour d'autres mollusques *(Pomatias)* (Vovelle & Grasset, 1979). Ces sphérocristaux extracellulaires ont déjà été décrits chez *Anodonta* et des espèces voisines par Istin et collaborateurs (Istin, 1970) dans le manteau, et par Silverman *et al.* (1983, 1985, 1987a) dans la branchie. Leurs tailles très diverses suggèrent qu'ils se forment sur place ; il n'est pas exclu que les fibres de collagène qui les entourent puissent servir de trame de nucléation (Mann, 1983).

L'analyse élémentaire révèle que le manganèse est associé de façon constante à du phosphore, à du calcium et à du fer, ainsi qu'à quelques autres métaux, en moindres quantités. Des résultats semblables ont également été obtenus chez *Ligumia* par Silverman *et al.* (1983), mais sur culots de centrifugation. Nos analyses par émission X, effectuées *in situ,* montrent que tous ces éléments coexistent dans les mêmes sphérocristaux. Les teneurs en fer, bien que fortes, n'atteignent pas le caractère exceptionnel de celles du manganèse. Le stockage de fer est assez fréquent dans divers groupes zoologi-

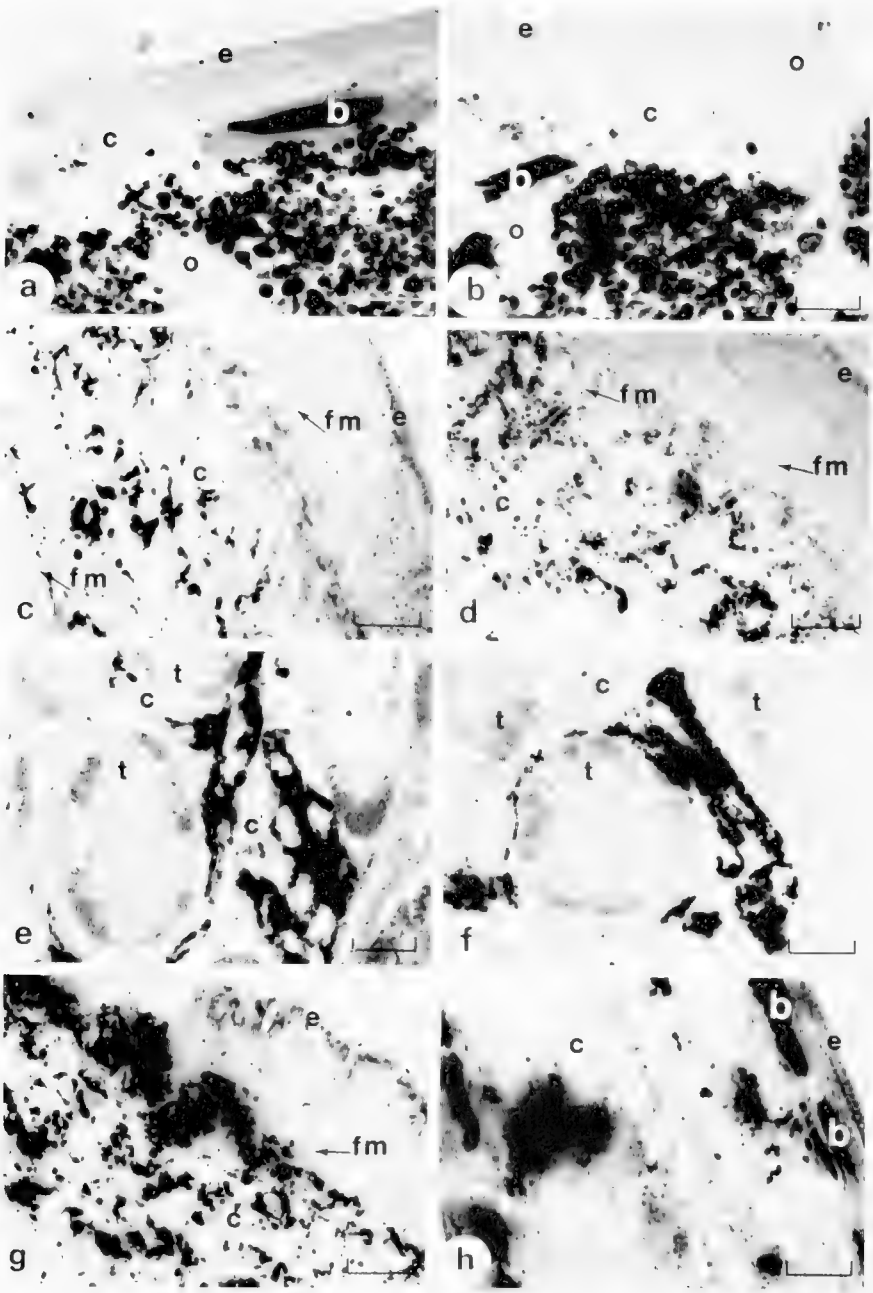


FIGURE 5. Localisation cytochimique du fer et du calcium. a. Branchie; réaction du fer ferreux. b. Branchie; réaction du fer ferrique. c. Manteau; réaction du fer ferreux. d. Manteau; réaction du fer ferrique. e. Glande digestive; réaction du calcium. f. Glande digestive; réaction du fer ferrique. g. Manteau; réaction du calcium. h. Branchie; réaction du calcium. Noter, pour ces différents tissus, l'abondance du fer ferreux et sa localisation identiques à celles du fer ferrique et du calcium. b: baguettes chitineuses; c: conjonctif; e: epithelium; fm: fibre musculaire; o: ostium; t: tubule de la glande digestive. Echelle 100 μm.

TABLEAU 3.—Détermination, par spectrophotométrie d'absorption atomique électrothermique des teneurs en manganèse et en fer de différents tissus d'*Anodonta cygnea*, de l'eau et des sédiments. (valeurs extrêmes exprimées en $g.g^{-1}$ de masse fraiche des tissus)

		Mn	Fe
ANODONTA CYGNEA	branchie	$4.10^{-4} - 2{,}2.10^{-2}$	$3{,}3.10^{-5} - 9{,}6.10^{-4}$
	manteau	$1{,}8.10^{-3} - 1{,}8.10^{-2}$	$5{,}9.10^{-4} - 6{,}5.10^{-4}$
	glande digestive	$2.10^{-3} - 1{,}7.10^{-2}$	$5{,}8.10^{-4} - 9{,}5.10^{-4}$
EAUX		$5.10^{-8} - 7.10^{-8}$	$10^{-7} - 1{,}5.10^{-6}$
SEDIMENTS		$8{,}1.10^{-5} - 7{,}7.10^{-4}$	$9{,}7.10^{-5} - 2{,}1.10^{-3}$

ques, mais l'originalité de l'espèce *Anodonta cygnea* réside dans la localisation extracellulaire des sites d'accumulation, et dans l'existence de quantités importantes de fer bivalent, Fe^{2+}.

Par ailleurs, nos observations concernant le stockage de quantités importantes de manganèse dans les tissus mous de l'anodonte, et celles de Swinehart et Smith (1979) dans le périostracum, montrent que cette bioaccumulation est différente du simple dépôt de métal à la surface de la coquille (Allen, 1960), et il convient même d'opposer ces deux modes d'accumulation du manganèse par les mollusques.

Dans la branchie, nous confirmons que le phosphore et le calcium associés au manganèse précipitent, au moins en partie, sous forme de phosphate de calcium (Silverman *et al.*, 1983). Mais, en ce qui concerne le rôle des concrétions, nos observations sont en désaccord avec l'interprétation de ces auteurs (Silverman *et al.*, 1985, 1987 a, b) ; ayant observé une disparition des sphérocristaux lors de l'incubation des larves glochidium, ils attribuent aux sphérocristaux de la branchie une fonction de réserve de calcium, destiné à la synthèse de la coquille des larves. Pour notre part, nous n'avons pas observé de diminution de la quantité de concrétions dans la branchie d'anodontes incubant des larves glochidium. Mais, n'ayant pas étudié ce phénomène de façon systématique, nous ne sommes pas en mesure d'interpréter cette divergence.

Le manganèse accumulé dans les concrétions se trouvant sous forme tétravalente, il ne peut précipiter sous forme de phosphates, ou alors ceux-ci ne représentent qu'une faible fraction de tout le métal stocké. Cette interprétation est confirmée par l'absence de la raie caractéristique du phosphate de manganèse sur les spectres Raman des sphérocristaux de la branchie. En raison de sa couleur noire, la combinaison MnO_2 doit également être rejetée, les sphérocristaux étant incolores ou légèrement orangés. La forme chimique de stockage du manganèse n'est donc vraisemblablement pas un sel minéral.

Dans les sphérocristaux du manteau, la spectrométrie Raman ne décèle aucune forme minérale ; la région des vibrations C-H ne révèle qu'un massif indifférencié et peu intense. La réaction cytochimique des protéines, des polyanions et celle des carbonates sont également négatives. Dans les concrétions de ce tissu, c'est donc à la fois le manganèse et le calcium qui sont présents sous une forme chimique autre qu'un sel minéral. Or, selon Istin et coll. (Istin & Girard, 1970 ; Istin, 1970), les sphérocristaux du manteau sont constitués de carbonate de calcium. Ces auteurs démontrent clairement le rôle du pH, celui de la pression partielle de CO_2 dissous et la fonction de l'anhydrase carbonique dans les mouvements du calcium, ainsi que la localisation de ce métal et de l'enzyme au niveau des concrétions ; en déduire que celles-ci étaient constituées de carbonate de calcium semblait donc légitime. Nos résultats montrent que les sphérocristaux du manteau représentent bien une réserve de calcium, mais qu'ils ne renferment pas de sels minéraux ; les formes de stockage du manganèse et du calcium restent donc également à déterminer dans cet organe. Il convient alors d'envisager, au moins pour ces éléments majeurs des sphérocristaux, un piégeage sous forme organique. La présence de composés puriques, caractérisés dans le tissu conjonctif d'autres mollusques (Martoja, 1974 ; Ballan-Dufrançais, Truchet & Dhamelincourt, 1979) est exclue,

en l'absence des raies caractéristiques de ces composés dans les spectres Raman. En revanche, la composante mucopolysaccharidique, riche en polyanions, peut complexer de nombreux métaux. A cet égard, les résultats négatifs de la réaction des radicaux carboxylés et sulfatés pourrait s'expliquer par l'abondance de ces cations, bloquant tous les sites réactifs. De même, le résultat négatif de la tétrazoréaction de Danielli indiquerait une saturation des radicaux libres et des groupements prosthétiques des protéines. Le phosphore détecté par analyse élémentaire, et qui ne peut provenir de phosphates, appartiendrait également à la composante organique, peut-être sous la forme d'acide onuphique ou d'un de ses dérivés (Pautard & Zola, 1968). Enfin, l'absence de vibrateurs C-H, toujours intenses sur les spectres Raman lorsque les bioaccumulations sont riches en matière organique (Truchet, 1982), pourrait indiquer une substitution des protons par d'autres éléments électropositifs. Ainsi, la trame organique des sphérocristaux serait en mesure d'assurer à elle seule la fonction d'accumulation de métaux, et jouerait un rôle plus important qu'on ne le supposait jusqu'alors. Dans cette hypothèse, il existerait donc deux mécanismes de rétention des métaux par les sphérocristaux : une complexation par la trame organique et une précipitation minérale, ces deux mécanismes pouvant jouer séparément ou simultanément ; le premier caractérise les concrétions du manteau et de la glande digestive, le second celles de la branchie. Mais, même dans cet organe, les fortes teneurs en Ca et Mn des baguettes chitineuses traduiraient également une forme d'association d'éléments minéraux avec une composante organique, qui pourrait être la trame de conchyoline.

En ce qui concerne les teneurs en manganèse, les inclusions minérales intracellulaires des épithéliums du manteau en sont très pauvres ; les cellules épithéliales ne sont donc le siège d'aucune accumulation de manganèse et, lors du transit, le métal reste sous forme soluble. De même, le manganèse n'est pas accumulé dans les lames basales, ce qui l'oppose au calcium (Istin, 1970 ; Istin & Masoni, 1973). Enfin, les cellules de Leydig en renferment des quantités appréciables, tant dans les sphérocristaux des corps cellulaires (branchies) que dans les microcristaux des pseudopodes. Toutefois, les quantités de manganèse accumulé dans ces cellules sont faibles par rapport à celles qui précipitent dans les sphérocristaux extracellulaires des tissus conjonctifs du manteau, de la glande digestive et de la branchie.

Les quantités importantes de manganèse stockées par l'anodonte pourraient représenter un catabolite d'enzymes du mollusque. Dans cette éventualité, il devrait suivre la voie classique d'excrétion par le rein, or, on ne trouve pas de concrétions à manganèse dans cet organe. Ceci n'exclut pas une éventuelle excrétion sous forme non précipitée ; nos résultats d'analyse ionique vont d'ailleurs dans ce sens. Par ailleurs, l'hypothèse de l'origine enzymatique du métal n'explique pas la coexistence de Mn et Fe, à des degrés d'oxydation inhabituels (Mn^{4+} et Fe^{2+}). Nous suggérons donc une intervention du métabolisme des bactéries intratissulaires. L'implication de bactéries dans l'accumulation du manganèse du périostracum a d'ailleurs déjà été envisagée par Swinehart et Smith (1979), mais ces auteurs n'avaient pu démontrer l'existence de ces microorganismes. Nos observations mettent en évidence une flore bactérienne commensale ou symbiotique, ainsi que son aptitude à stocker du manganèse qui est libéré lors de la lyse bactérienne. Bien que nous n'ayons pu préciser leur abondance il est possible que ces bactéries, comme celles qui assurent la précipitation du manganèse dans les nodules polymétalliques des fonds océaniques, tirent leur énergie de la réacton d'oxydation $Mn^{2+} \rightarrow Mn^{4+}$ (Ehrlich, 1976) ; chez l'anodonte, l'oxydant ne serait pas l'oxygène, mais le fer trivalent. Ce mécanisme original s'ajouterait à ceux qui ont déjà été décrits chez ces animaux (Holwerda & Veenhof, 1984). Si notre hypothèse se trouve confirmée par des études ultérieures, en particulier celle d'autres bivalves concentrateurs de manganèse extracellulaire (*Unio, Ligumia* etc.), il sera établi que les aspects chimiques très singuliers du stockage de Mn et Fe des unionidés sont la conséquence, non de caractères métaboliques particuliers à ces mollusques, mais du métabolisme des bactéries qu'ils hébergent.

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ABSTRACT

The accumulation of manganese in the soft tissues of the bivalve mollusc *Anodonta cygnea* has been studied by photon and electron microscopy, by classical cytochemistry and by the modern methods of analytical microscopy (secondary ion emission, X-ray emission and Raman diffusion). Contents of Mn and Fe in the soft tissues and in water and sediments were quantified by electrothermal atomic absorption spectrophotometry.

The main tissues accumulating manganese are the connective tissues of digestive gland, mantle and gills. Mn is concentrated in extracellular concretions, in great amounts, together with P, Ca, Fe, and some other metals in lesser quantities. Manganese is in the Mn^{4+} state, iron in the two oxidation forms, Fe^{2+} and Fe^{3+} ; the Fe^{2+} is less common in animal tissues. The concretions in the gills are made predominantly of calcium phosphate but, in the concretions of the other organs, no mineral form is detected. This led us to consider the binding of the metals with the organic part of the spherocrystals. Our hypothesis is that, as well as classical mineral precipitation within an organic matrix, the spherocrystals might be in another form of organo-metallic complexation. The two might exist separately or together.

The epithelial cells of gills, mantle and digestive gland also contain mineral inclusions, but these have little manganese ; thus, these cells are not the place of manganese accumulation. Leydig cells present in connective tissue contain other intracellular concretions, rich in manganese. This intracellular Mn accumulation is not very important compared to what is extracellular in the same tissue.

We observed and described bacteria in the soft tissues of this mollusc, for the first time to our knowledge. These procaryotes contain Mn concretions. We suggest that Mn may originate, at least in part, from the activity of these bacteria. In this connection, the reduction of Fe^{3+} to Fe^{2+} leading to the oxidation of Mn^{2+} to Mn^{4+} might be a source of their energy and explains the great quantity of the unusual divalent form of the iron. Thus, the exceptionally high content of manganese in soft tissues would not be correlated with a particular metabolic pathway of the mollusc, but to its procaryotic biota. Our results emphasize the total independence between the accumulation of manganese in soft tissues and in the periostracum, and its deposition at the surface of molluscs shells.

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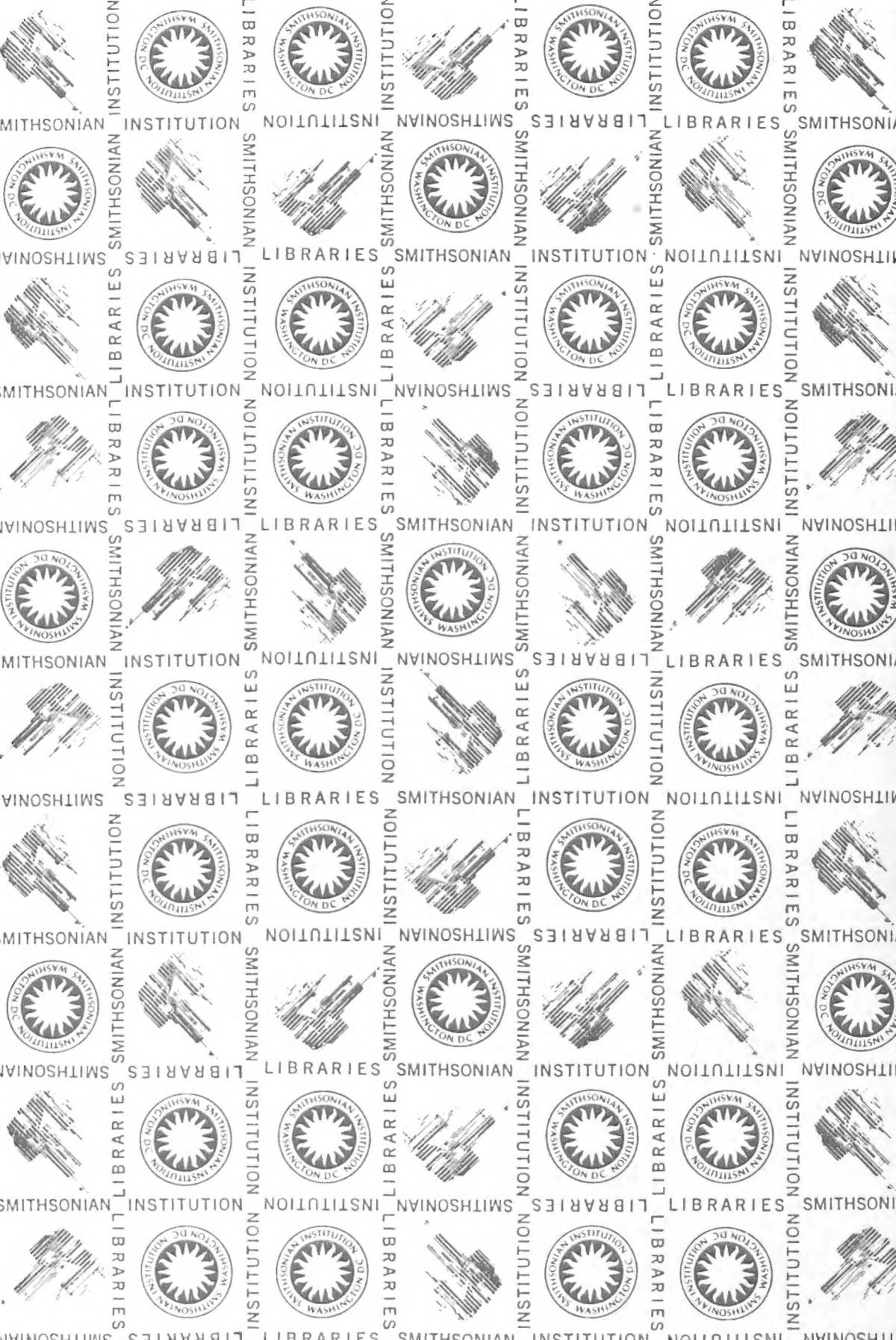
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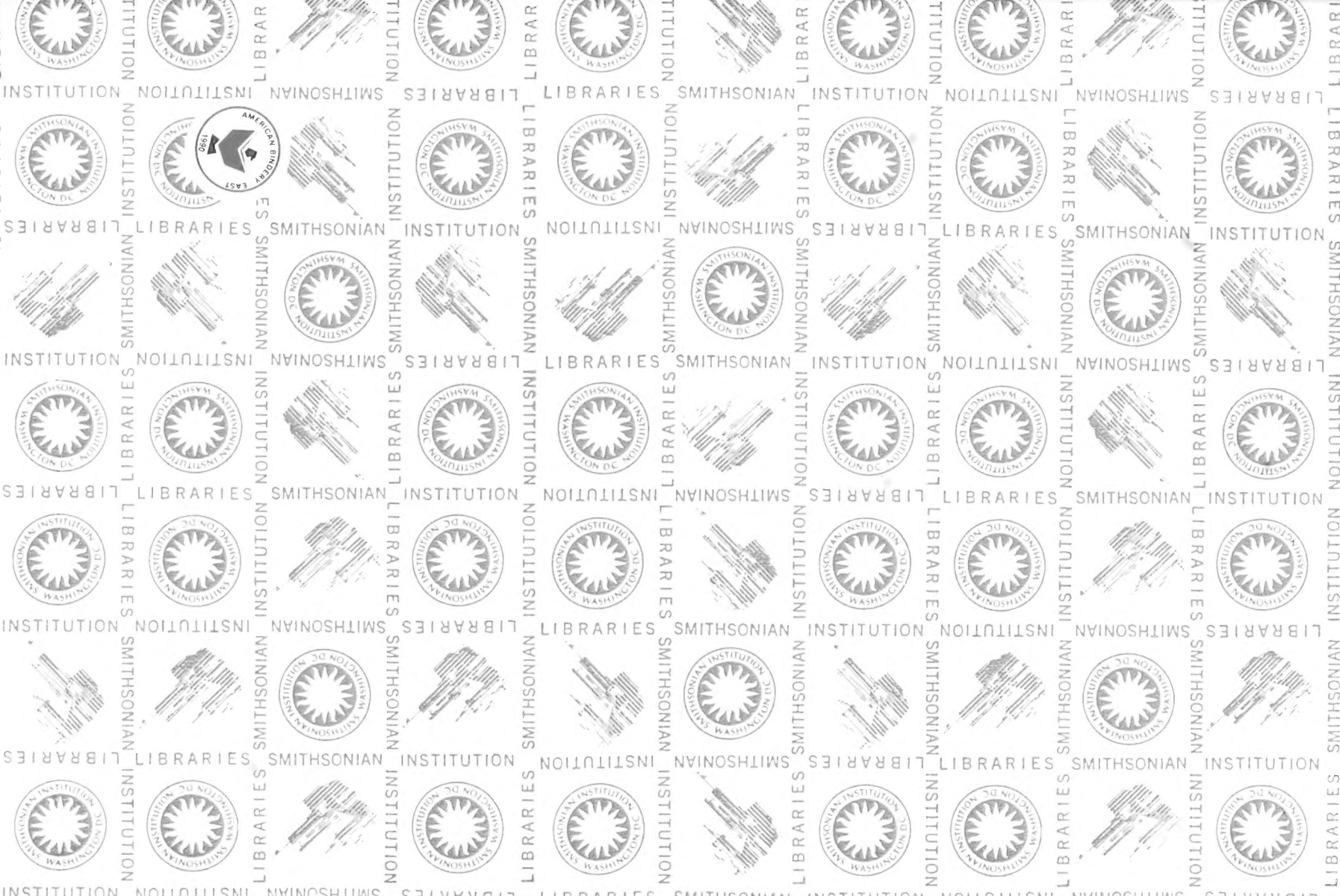
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