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GASTROPOD SHELL FORM, BREAKAGE, AND REPAIR IN RELATION TO PREDATION BY THE CRAB *CALAPPA*[1]

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ABSTRACT

The crab *Calappa hepatica* (L.) attacks most of its gastropod prey by peeling, a process of chipping away the outer shell wall piece by piece in a spiral direction, beginning at the outer lip. Laboratory studies in Guam showed that 33% to 91% of attacks by *Calappa* were unsuccessful, depending on the prey species. A thick outer lip prevents *Calappa* from peeling many adult *Strombus gibberulus*. Thin-lipped *Rhinoclavis* spp. are protected from lethal peeling by a varix located 240° around the body whorl from the outer lip. *Terebra* may be protected by its very small aperture.

The frequency of lethal breakage was studied in collections of "dead" shells from localities in the tropical Pacific. Postmortem breakage, as inferred from damage in drilled shells, was a minor contributing factor to observed frequencies of lethal breakage. The incidence of lethal breakage in the field was highly correlated with attack success of *Calappa* in the laboratory.

Breakage-induced shell repair is common in tropical Pacific gastropods. The incidence of repair is highly correlated with the failure rate of *Calappa* in the laboratory. Spearman rank correlations between the frequencies of repair and lethal breakage were significant and positive within three of the four species and genera examined. Repair is a reliable indicator of the effectiveness of the shell as protection against lethal breakage, but it cannot be used as a reliable measure of the intensity of lethal breakage.

Key words: predation; shell geometry; *Calappa*; tropical Pacific; sublethal injury.

INTRODUCTION

Shell breakage in molluscs is important both as a cause of death and as an agent of selection (Vermeij, 1978, 1979). The extent to which breakage is important in selection with respect to shell architecture is likely to vary within and between species both geographically and temporally. Some species experience breakage principally as a cause of death, whereas individuals of other species commonly survive breakage-inducing attacks when the shell has reached a sufficient (critical) size.

In general, a shell may be broken either by crushing or by peeling. By crushing I mean that a shell as a whole is compressed between two apposing surfaces, such as the jaws of a vice or of a spiny diodontid porcupinefish, the pharyngeal bones of labrid fishes, and the massive crusher claws of many crabs (Liem, 1973; Vermeij, 1976, 1977, 1978; Zipser & Vermeij, 1978; Brown et al., 1979; Palmer, 1979; Yamaoka, 1978). Gonodactylid stomatopod Crustacea, which use the base of the second maxillipeds as a kind of hammer, also practice a form of shell crushing (Caldwell & Dingle, 1975). Experiments with various crushing predators and measurements of gastropod shell strength show that a compact spherical shape (low spire, small aperture), thick outer shell wall, strongly thickened outer lip, sturdy external sculpture, tight coiling, and certain features of crystal microstructure are effective deterrents against crushing (Papp et al., 1947; Kitching et al., 1966; Currey & Kohn, 1976; Vermeij, 1976, 1978; Hughes & Elner, 1979; Palmer, 1979; Vermeij & Currey, 1980). When prey approach or exceed the critical size for a particular predator, damage may be confined to the lip, or it may be evident only as toothmarks or as broken elements of sculpture on the shell's exterior (Vermeij, 1976; Zipser & Vermeij, 1978). Crushing predators are most diversified in shallow hard-bottom communities, especially in the tropics (Vermeij, 1978; Palmer, 1979).

Lip-peeling is the second type of gastropod shell breakage, in which the body whorl is

[1]Contribution no. 146 from the University of Guam Marine Laboratory.

broken away piece by piece, beginning at the outer lip, and continuing in a spiral direction until the soft parts are exposed. Well-known lip-peeling predators include spiny lobsters (Palinuridae) (Randall, 1964; Vermeij, 1978) and sand-dwelling crabs of the genus *Calappa* (Shoup, 1968; Miller, 1975; Vermeij, 1978; Vermeij et al., 1980).

Little is known about shell characteristics that prevent peeling predators from being successful shell-breakers. Traits that might be expected to protect gastropods against peeling include a thick lip, a narrow or very small aperture that is difficult to penetrate, and the ability to retract the edible soft parts far back into the shell (Vermeij, 1978). Retractility and aperture size are frequently associated with spire height. Species able to withdraw the soft parts far into the shell and away from the vulnerable outer lip are usually high-spired and have a small aperture (Hamilton, in prep.). Because calappids are widespread in soft-bottom habitats throughout the shallow-water marine tropics, an understanding of the shell characteristics that prevent successful peeling would contribute to the interpretation of geographical and temporal patterns in shell form of soft-bottom gastropods. The data that are required include observations on *Calappa*'s attacks in the laboratory, estimates of the incidence of breakage-induced mortality in field populations of gastropods, and proof that gastropods in the field can survive attacks by *Calappa* and other shell-peeling predators. If *Calappa* were always successful in peeling a gastropod, there would be no selection between shells with and without traits that prevent lethal peeling. In this paper, I present the three kinds of data, with the following additional questions in mind: (1) what is the relation between shell form and susceptibility to lethal lip-peeling? (2) How are the incidences of lethal breakage and of breakage-induced shell repair (unsuccessful predation) related? (3) How much local variation exists in the incidence of repair independent of shell form?

MATERIALS AND METHODS

Crabs—*Calappa hepatica* (L.)—of various sizes were kept individually in aquaria with running ambient sea water and with enough coarse sand on the bottom for the crabs to bury completely. Each crab was offered a representative range of sizes of several prey species in order to establish the size of the largest individual of a given species that could be killed by a particular crab. Prey were removed as soon as they had been attacked or eaten (usually within a few hours). At any one time, a crab had a choice of several species of prey. All trials were carried out at the University of Guam Marine Laboratory at Pago Bay, Guam, in the summer of 1975 and from January to April, 1979.

Estimates of the importance of lethal breakage as a cause of death were made from collections of "dead" shells (those no longer occupied by a gastropod) from several shallow-water soft-bottom sites in the tropical Pacific. All available intact and broken shells, including apical fragments, were collected by hand. They were later measured for length to the nearest millimeter, and examined for signs of the cause of death. It was impossible to estimate the size of shells when only the apex was available. For the present analysis, only pieces and shells 5 mm or longer were collected and examined.

Breakage was considered to be lethal to the gastropod if the extent of injury was equal to or greater than the damage known to be fatal to the same species in laboratory trials with *Calappa* and other predators (see also Zipser & Vermeij, 1978). If lip breakage was less extensive than would be necessary to kill an individual, it was assumed not to have caused that individual's death. Because many dead shells are sublethally broken, it is easy to overestimate the importance of breakage as a cause of death.

Sometimes, shells are "lethally" broken while they are occupied by hermit crabs (Bertness, 1980) or when empty. Although it is difficult to assess the extent of this artifact, a lower limit of its contribution to the observed number of lethally broken shells can be obtained by examining drilled shells. Drilling by gastropods appears to be a cause of death for gastropods but not for hermit crabs. Any "lethal" breakage found in drilled shells must therefore have occurred after the death of the original snail. A minimum estimate of postmortem "lethal" breakage, f_p, is thus the frequency of lethal breakage in drilled shells (number of broken drilled shells, n_{bd}, divided by number of drilled shells, n_d).

Taking the postmortem artifact into account, I define the frequency of lethal breakage as follows:

$$f_b = (n_b/n)(1 - f_p) \qquad (1)$$

where n_b is the number of lethally broken shells (drilled and undrilled) and n is the total number of shells in the sample.

The frequency of lethal breakage as defined here is a reliable index of the contribution of breakage as a cause of death of snails only if there is no bias in the preservation or collection of intact and broken shells. I suspect that observed frequencies of lethal breakage are conservative estimates of the importance of breakage to overall mortality. Waves and currents may selectively remove fragments and small whole shells from the population of vacated shells. Diodontids and other predators may crush shells so completely that the apical fragments are unsuitable as abodes even for competitively subordinate hermit crabs. Some hermit crabs preferentially shun damaged shells (Abrams, 1980; M. D. Bertness, personal communication). Probably the most credible estimates of the frequency of lethal breakage come from high-spired shells from sandy or muddy environments where transport by waves and currents is limited. The frequencies in this paper are from such environments. The material was collected from the Pacific coast of Panama in the summers of 1976 and 1978, and at Indo-West-Pacific sites from January to July, 1979. "Dead" shells were always collected from the intertidal zone or slightly below, and never at the strand line above high water mark. The species composition of the dead shells was always similar to that of the living molluscs. Dead shells were analyzed only when living representatives of the species in question were also found.

Frequencies of shell repair were studied in detail at seven Western Pacific localities and at one Eastern Pacific site. All intact living and dead shells were inspected for signs of breakage-induced shell repair. Depending on shell shape, repairs were counted on 1, 2, or all whorls of the shell with the aid of magnification. Tiny repaired lip-chips were ignored. In a given sample of intact shells, the frequency of repair was defined as the number of scars per individual. This definition differs slightly from that of some earlier workers (Robba & Ostinelli, 1975; Raffaelli, 1978; Elner & Raffaelli, 1980), who used the number of repaired shells divided by the total number of shells in a sample as their index of repair. The latter index can vary only between values of 0 and 1, whereas the index used in the present paper can take on any value greater than zero. Neither measure takes into account differences between individuals in the number of scars.

RESULTS

Predation by *Calappa*—The outer lip of the shell is the first line of defense of a gastropod against peeling by *Calappa*. In previous work with the gastropods *Nassarius luteostoma* (Brod. & Sow.) and *Strombina clavulus* (Sow.) in Panama, I observed that a small (27.1 mm wide) *Calappa convexa* Saussure was capable of breaking lips up to 1.35 mm in thickness; however, lips thicker than 1.6 mm remained intact (Vermeij, 1978). In the present study, I offered 9 *Strombus gibberulus* L. with lips 1.5 mm to 2.3 mm thick to a 75.1 mm wide *C. hepatica*. None of the 3 *S. gibberulus* with a lip thicker than 2.0 mm was peeled. When a 41.3 mm wide *C. hepatica* was offered 7 *Clypeomorus bifasciatum* (Sow.) ranging in lip thickness from 1.0 mm to 2.3 mm, and 4 *Cerithium columna* Sow. with lips 1.1 mm to 1.4 mm thick, only the five shells with lips less than 1.6 mm thick were peeled at least 180°. *Calappa* thus appears to be prevented from peeling by a thick outer lip.

In a few instances, a thick lip did not prevent lethal breakage. Two shells each from among 14 *Strombus mutabilis* Swainson and 20 *S. gibberulus* that were lethally broken by *Calappa* were peeled from the anterior end or from a hole punched in the dorsum behind the outer lip. In these four shells, the thick lip was circumvented.

A breach in the outer lip is no guarantee that a gastropod will be killed by *Calappa*. The high-spired cerithiids *Rhinoclavis aspera* (L.) and *R. fasciata* (Brug.) have thin adult lips (0.5 to 0.9 mm and 0.3 to 0.6 mm respectively), and more than 90% of attacks by *Calappa* resulted in peeling; however, only 25% of the 64 peels of *R. fasciata,* and 16% of peels of *R. aspera* (n = 25) were lethal. In every instance of unsuccessful predation, peeling extended through circa 240° of angular distance from the outer lip to the first varix, but not beyond this externally and internally thickened structure. Successful peeling requires that the first varix be breached. Similar requirements hold for species of *Cerithium,* whose outer lip is usually thicker (1.0 to 2.1 mm) than in *Rhinoclavis*.

Terebra affinis Gray (lip 0.3 to 0.6 mm thick) has no thickened varices, but attacks on this species are usually unsuccessful. Of the

22 individuals attacked by seven crabs, only two (9.1%) were killed. Although more than 360° of shell must be peeled away in order to expose the soft parts, most unsuccessful peels extended only between 90 and 240°. It is unclear why peeling was discontinued. Possibly, the external tooth of the right claw is too large to fit into the small aperture of the *Terebra* as the outer shell wall is stripped back.

In order to kill *Strombus gibberulus*, *Calappa* must peel through an angular distance of at least 105° if the attack is initiated at the outer lip, as it usually was. Of 39 adult *S. gibberulus* that were attacked, 13 (33%) were killed. The others had small scratches on the outer lip, but the damage was not substantial enough to require repair. Only 8 of 31 juveniles (26%) were broken lethally, and 5 (16%) suffered nonlethal shell injury. The remaining 18 individuals were not attacked, perhaps because they were able to escape from *Calappa*. There was no indication that the sharply serrated operculum was an effective deterrent against crabs.

Broad-apertured naticids (*Natica, Polinices*) were frequently peeled more than 180° by *Calappa*, but the damage required to kill these snails is slight. *C. hepatica* 6 to 8 cm in carapace width can extract a portion of the body of *Polinices tumidus* (Swainson) (shell length 27 to 32 mm) without any shell damage or only a 10° peel. Extensive peeling probably results in a greater yield of flesh.

As a rule, larger predators can kill larger prey. Table 1 shows, however, that this is not always the case with *Calappa hepatica*. *Rhinoclavis fasciata* has a critical size (shell length) of about 30 mm for crabs ranging in carapace width from 33 to 78 mm. Several crabs greater than 65 mm in width were unable to kill *R. fasciata* as small as 24 mm in length. The 30.2 mm *R. fasciata* killed by a large (78.2 mm) *Calappa* would have survived in spite of peeling had a hole not been pierced through the spire. Other *R. fasciata* offered to this crab ranged from 28.5 to 33.7 mm in length, and survived in spite of being peeled back to the first varix. Peeling alone, therefore, proved to be more effective for small crabs than for large ones, perhaps because the external tooth on the claw was too large for the apertures of many high-spired and coniform shells.

Predation on *Strombus gibberulus* was more conventional; larger crabs were able to

TABLE 1. Critical lengths of prey species for several individuals of *Calappa hepatica*.

Prey species	Crab (mm)	N	Prey shell lengths		
			Range given	Largest killed	Smallest not killed
Rhinoclavis aspera	42.6	7	20.7 to 38.1	31.6	20.7
	44.0	7	26.5 to 38.5	30.5	26.5
R. fasciata	33.3	14	18.0 to 33.6	29.8	26.8
	41.3	15	23.8 to 29.5	28.0	24.5
	62.8	10	23.0 to 35.4	30.0	30.5
	67.0	6	24.2 to 32.0	—	24.2
	74.9	4	24.3 to 33.1	—	24.3
	75.1	9	23.8 to 35.6	—	23.8
	78.2	7	28.5 to 33.7	30.2	28.5
Terebra affinis	33.3	7	16.3 to 32.9	—	16.3
	41.3	6	23.8 to 33.6	23.8	24.2
	67.0	9	23.8 to 39.0	—	23.8
	78.2	7	18.7 to 35.3	—	18.7
Strombus gibberulus	33.3	7	15.0 to 27.2	—	15.0
	41.3	12	16.7 to 34.2	—	16.7
	54.9	13	27.4 to 40.0	39.0	27.4
	67.0	7	27.8 to 37.6	27.8	30.0
	74.9	10	18.6 to 46.7	31.0	31.7
	75.1	17	22.8 to 42.2	34.2	34.2
	78.2	7	29.9 to 46.7	35.1	29.9
	80.2	10	27.4 to 43.6	43.6	29.1

N = Number of prey offered.

kill larger prey, either by breaching the lip or by initiating a peel from the anterior end or from a dorsal hole. Substantial individual differences were evident both in the prey and in the crab predators, so that the correlation between crab width and critical prey length is relatively weak (Table 1). Differences among predators of the same species in the ability to break shells have been noted previously for other crabs (Zipser & Vermeij, 1978), and may be the rule among shell-breaking predators.

Although learning is likely to be important in a crab's prey-handling behavior, no evidence of its effectiveness was found in this study. I saw no tendency for the critical size of any gastropod species to increase with the duration of the crab's captivity, nor did crabs inflict progressively less damage to prey that they could not kill.

Lethal Breakage—Lethally broken shells are common in soft-bottom environments (Appendix A). Postmortem "lethal" breakage, as inferred from the incidence of breakage in drilled shells, was unimportant in most samples. The frequency of lethal breakage exceeded 0.10 in drilled shells only in one of the 6 samples in which there were at least 10 shells with a drill-hole. No postmortem breakage could be detected in 3 of the 6 samples. No estimates of postmortem breakage could be obtained for samples of *Strombus* spp. and for many species of *Nassarius* because of the low incidence of drilling.

A relationship between the frequency of lethal breakage and shell architecture was clear at only one site (Venado Beach). Species with thick adult lips or narrowly elongated apertures had significantly lower frequencies of breakage than did species with thin lips and broad apertures. At the Indo-West-Pacific sites, the very high frequencies of breakage in thick-lipped species of *Strombus* destroy any relationship between shell form and breakage. Because most dead broken *Strombus* were recovered as apices, it was difficult to ascertain whether the snails were killed as juveniles or as thick-lipped adults. The present data on *Strombus* confirm and extend those reported earlier (Vermeij, 1979).

Field estimates of the intensity of breakage-induced mortality were highly correlated with the success rate of *Calappa* on the same species in the laboratory. Success rates decreased in the order *Strombus gibberulus, Rhinoclavis fasciata, R. aspera, Terebra affinis.* The species were ranked similarly with respect to the incidence of lethal breakage at 4 localities in Guam (Appendix A).

Shell Repair—The incidence of breakage-induced shell repair in a sample of shells is influenced by the following factors: (1) the likelihood that a shell will be attacked; (2) the probability that an attack will be successful; and (3) the probability that an unsuccessful attack will result in lip breakage requiring repair. The second and third factors depend on shell shape, strength, and size. The first may also be related to shell characteristics, especially if a shell-breaking predator learns to avoid prey that are of an unsuitable size or shape.

The work with *Calappa* suggests that varices, high spires, and large size may be effective in preventing attacks by *Calappa* from being successful. A thickened lip not only repels an attacker, but obviates the need for repair if an unsuccessful attack leaves the lip intact. Larger shells should as a rule have higher frequencies of repair than smaller ones because they have been exposed to shell-breaking agents for a longer time (that is, scars accumulate), and because the probability that an attack is successful decreases as the shell grows larger.

An analysis of complete samples of intact shells (individuals of all sizes belonging to a given species) shows that repair is less common among species with thickened adult lips than among those in which the lip remains relatively thin (Appendix A). This difference is significant at Wom Village ($p < 0.003$, Mann-Whitney U-Test) and Pujada Bay ($p < 0.05$). At Dodinga Bay, the 4 lowest frequencies of repair (out of a total of 7) are those of the 4 thick-lipped samples). The only thick-lipped species sampled in sufficient quantity at Tumon Bay (*Strombus gibberulus*) ranks third lowest among 9 species. At Venado Beach, there is no significant difference between thick-lipped and thin-lipped species. Comparisons among thin-lipped species reveal no patterns in the incidence of repair with respect to aperture shape, presence or absence of an umbilicus, or height of spire.

Of the 10 samples in which frequencies of repair can be estimated in more than one size class (Table 2), 8 show a rise in repair with increasing length, 1 (*Polinices tumidus* from Wom Village) shows a constant incidence of 0, and 1 (*Rhinoclavis vertagus* (L.) from Boear, Aru Islands) shows a mixed pattern. The tendency for repair to increase in frequency in larger shells was also found for

TABLE 2. Shell repair in relation to shell length.

		Frequency of repair in size clsses							
		5–9 mm		10–19 mm		20–29 mm		30–39 mm	
Species	Loc	n	f	n	f	n	f	n	f
Rhinoclavis aspera	Tb							23	.61
	Ac					12	.083	26	.23
R. fasciata	Tb			9	.11	38	.16	18	.56
	Bi			15	0	72	.097	11	.091
	Cl			48	.33	107	.36	67	.55
R. vertagus	B			8	0	17	.059	74	.19
Cerithium coralium	Db			26	.27	11	.82		
Modulus catenulatus	Vb			37	.27				
Polinices tumidus	Wv	13	0	16	0				
P. uber	Vb			12	.083	10	.20		
Natica chemnitzii	Vb			12	.58	9	.67		
Strombus labiatus	Wv					28	.036		
	Pu					19	.13		
S. gibberulus	Pu							11	0
Pyrene versicolor	Db			12	.31				
	Pu			12	.50				
Strombina clavulus	Vb					11	2.00		
Nassarius globosus	Db			9	.44				
	Tg			31	.033				
N. bicallosus	Db	10	.40	25	.96				
N. spp.	Db			10	.60				
N. luridus	Tg			11	.091				
	Wv			22	.27				
N. distortus	Pu			10	1.50				
N. subspinosus	Pu			41	.27				
N. callospira	Wv			10	.10				
N. pullus	Wv			13	.078				
N. quadrasi	Wv			13	.23				
N. luteostoma	Vb					14	.071		
N. pagodus	Vb					13	.46		
N. versicolor	Vb			12	.50				
Olivella volutella	Vb			15	.20				
Cancellaria jayana	Vb			11	.55				
Pilsbryspira aterrima	Vb			17	.47				
Gemmula graeffei	Db					13	.78		
Vexillum exasperatum	Pu			22	.23				
V. spp.	Tb					12	.33		
Imbricaria spp.	Pu			11	.27				
Conus coronatus	Pu			17	.35				
C. arenatus	Pu					13	.31		
C. muriculatus	Wv			14	.14				
C. ximenes	Vb					10	.20		
Terebra anilis	Wv							10	1.10
T. affinis	Tb					13	1.38		
T. elata	Vb					9	.44		
T. spp.	Vb			13	1.54				
Otopleura mitralis	Tb			10	.20				
Pyramidella dolabrata	Tb					10	.20		

Key:
n Number of shells
f Frequency of repair

Localities:
Ac Alupang Cove, Guam, April, 1979
B Boear Island, Aru Islands, Irian Jaya, July, 1979
Bi Bangi Island, Agat, Guam, February, 1979
Cl Cocos Lagoon, Guam, February, 1979
Db Dodinga Bay, west coast Halmahera, Indonesia, July, 1979
Pu Pujada Bay, Mindanao, Philippines, July, 1979
Tb Tumon Bay, Guam, January to April, 1979
Tg Tagbilaran, Baclayon, Bohol, Philippines, July, 1979
Vb Venado Beach, Panama, summers of 1976 and 1978
Wv Wom Village, Papua New Guinea, June, 1979

TABLE 3. Shell repair, breakage, and attack success in relation to shell length in *Rhinoclavis fasciata* from Cocos Lagoon, Guam.

Length (mm)	Repair		Breakage		Attack success
	n	f	n	f	
10.0 to 14.9	12	.33	12	.58	1.75
15.0 to 19.9	36	.33	36	.44	1.33
20.0 to 24.9	28	.43	25	.28	0.58
25.0 to 29.9	79	.33	59	.092	0.19
30.0 to 34.9	55	.51	49	0	0
35.0 to 39.9	12	.75	12	.083	0.13

Attack success: Number of fatal attacks divided by number of scars on living and dead shells in given size class.
n Number of individuals (living and dead)
f Frequency of repair or breakage

many Recent species of Terebridae (Vermeij et al., 1980) and for *Conus sponsalis* Hwass (Zipser & Vermeij, in press).

Because larger prey are more immune to predation by peeling than are smaller individuals, increasing shell length should be associated with a decline in the incidence of lethal breakage as well as with an increase in the number of repairs per individual. The only field sample that was large enough to test this prediction was that of *Rhinoclavis fasciata* from Cocos Lagoon, Guam (Table 3). The data show that the frequency of fatal breakage falls steadily as shell length increases. Moreover, the ratio of lethal attacks to total number of attacks also decreases, as expected. Only one shell longer than 30 mm sustained a fatal attack. This result is consistent with the laboratory observation on *Calappa*, which indicated that this crab is unable to kill *R. fasciata* greater than 30 mm in length.

The frequency of repair in the field is highly correlated with the failure rate of *Calappa* in the laboratory. Among the 4 species studied in detail at Guam, the incidence of scars increases in the order *Strombus gibberulus, Rhinoclavis fasciata, R. aspera, Terebra affinis* (Appendix A). This ranking is identical to that for increasing failure rate.

There appears to be considerable local intraspecific variation in the frequency of repair. This is well illustrated in Guam (Appendix A, Table 2), where repair varies by a factor of 6 in such species as *Strombus gibberulus* and *Rhinoclavis fasciata*. The possibility that these variations in repair reflect intraspecific differences in the amount of shell-breaking

TABLE 4. Spearman rank correlation between frequency of repair and frequency of breakage.

Group	n	Correlation
Rhinoclavis spp.	7	+0.73
Strombus gibberulus	9	+0.75
Terebra spp.	6	+0.61
Nassarius spp.	11	−0.22

n Number of samples

mortality is analyzed in Table 4, in which rank correlations between the frequency of fatal breakage and the frequency of repair in complete samples are given for several species and genera. There is a significant positive correlation at the 0.05 level between repair and lethal breakage in *Rhinoclavis, Terebra,* and *Strombus gibberulus,* but in *Nassarius* the correlation is negative and not significant.

DISCUSSION

Ecologists have often assumed that the incidence of unsuccessful predation (shell repair in the present study) is correlated with, and is therefore a measure of, the proportion of an animal population that is killed by damage-inducing predators (Shapiro, 1974; Raffaelli, 1978; Schall & Pianka, 1980). Schoener (1979) presented a theoretical analysis of tail-breaks in lizards. He showed that the proportion of individuals with injured tails is a measure of predator inefficiency under certain conditions (continuous reproduction, size-independent probability of encounter between prey and agent of mortality, size-independent rate of mortality, injuring agent the only cause of death). When other causes of mortality are introduced, the proportion of injured individuals in the population may sometimes be correlated with the intensity of predation, depending on the temporal distribution of reproduction.

It is difficult to apply Schoener's theoretical work to breakage in molluscs, because breakage-induced mortality and the probability of encounter with shell-breaking agents are strongly dependent on gastropod prey size (see also Hughes & Elner, 1979; Elner & Raffaelli, 1980). Moreover, very little is known about the reproductive schedules of tropical gastropods. Comparisons of my laboratory work on *Calappa* with field estimates of repair and lethal breakage suggest a very high positive interspecific correlation between the in-

cidence of repair and *Calappa*'s predatory inefficiency, and an equally strong negative interspecific correlation between the frequency of lethal breakage and crab failure. At the intraspecific and intrageneric level, however, the frequencies of repair and lethal breakage are often positively correlated (Table 4). Rafaelli (1978) came to a similar conclusion. He found that the frequency of repair in Welsh *Littorina rudis* (Maton) was high in areas where agents of breakage (boulders and the crab *Carcinus maenas* (L.)) were abundant, and low in marshes and on cliffs where these agents were rare.

These results suggest that the relationship between lethal and sublethal damage depends not only on the factors considered by Schoener (1979), but also on the relative strengths and abundances of predator and prey. As the strength of predators increases relative to that of the prey, the proportion of successful attacks rises and the frequency of reparable damage falls (Hughes & Elner, 1979; Elner & Raffaelli, 1980). With an increase in predator abundance relative to the prey, both the frequency of repair and the frequency of lethal damage will increase. The incidence of repair is thus correlated positively with the incidence of lethal breakage only if variation in predation is expressed as variation in the relative abundance of predator and prey. Variations in relative predator strength will have the opposite effect of producing a negative correlation between the incidences of lethal and sublethal damage.

Even if a positive intraspecific or intrageneric correlation between repair and lethal breakage exists, as it does in some gastropods, the frequency of repair is not necessarily a reliable measure of the intensity of breakage-induced mortality because the relationship is not linear. I expect that this situation is common with other types of predation as well, and that attempts by many ecologists to equate nonlethal predation with lethal predation are optimistic or erroneous.

Within assemblages of gastropods, the incidence of repair is a reliable guide to the effectiveness of the shell as a protective device against locally prevailing agents of breakage. The high frequencies of repair in many tropical marine gastropods suggests that these species are surprisingly well adapted even against such relatively specialized predators as *Calappa* (see also Currey & Kohn, 1976; Vermeij et al., 1980). The higher the frequency of repair, the greater is the likelihood that selection in favor of traits that protect against lethal breakage is taking place. Although the frequency of repair is not a measure of the magnitude of this selection nor an indication whether selection is stabilizing, directional, or disruptive, the presence of repair is a necessary condition for selection with respect to breakage resistance. A very low incidence of repair means either that shell-breaking agents are rare or that the agents, regardless of their abundance, are usually successful in fatally breaking the shell. A high frequency of breakage-induced scars means that many or most individuals in the gastropod population are exposed to the unsuccessful attacks of potentially lethal agents of breakage, and that characteristics of the shell (together with other defenses that come into play as the snail is subdued) are effective in protecting the gastropod against fatal breakage.

Varices appear to be effective in limiting the extent of peeling. They are characteristic of the shells of many tropical gastropod families, including the Cerithiidae, Potamididae, Cassidae, Bursidae, Cymatiidae, and Muricidae (Linsley & Javidpour, 1980). Most other tropical gastropod families are characterized by regularly spaced axial ribs, which may function in the same way as do varices. These structures are common on hard and soft bottoms alike, probably because sublethal damage by both crushing and peeling agents usually affects only the outer lip and the immediately adjacent parts of the body whorl. Thick-lipped gastropods, which are also very common in the tropics (many Cerithiidae, Strombidae, Cypraeacea, Tonnacea, Muricidae, Columbellidae, Buccinidae, Nassariidae, Mitridae), have solved the problems posed by shell-breaking predators in a somewhat different or complementary way. Their thin-lipped juvenile stages appear to be short compared to the thick-lipped phase during which damage to the lip is often limited to superficial scratches or chips (see Randall, 1964; Spight & Lyons, 1974; Yamaguchi, 1977).

I have often wondered why crabs such as *Calappa* so often attack oversized or otherwise inappropriate prey. This behavior is not only typical of most shell-breaking predatory fishes and crabs, but indeed of most predators from copepods to lions. Although a full review of this interesting topic is beyond the scope of this paper, it is worth noting that the inept behavior of most predators may be responsible for the evolutionary acquisition by

the prey of effective antipredatory adaptations. If predators could increase their success rate and at the same time regulate their populations so as not to overexploit prey, predators could "concentrate" on other adaptational dilemmas. Of course, it is these dilemmas that ultimately regulate the predator's population, so that their solution would eventually lead to the demise of the inept prey. The real world for the predator thus seems to bear little food of the right size or shape, and requires the prey to test less suitable items continually.

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APPENDIX A

Frequencies of repair and breakage in some soft-bottom gastropods from the tropical Pacific.

		Repair		Mortality			
	Locality and species	n	f	n	n_d	n_{bd}	f_b
	Tumon Bay, Guam						
	Rhinoclavis aspera	31	.45	15	0	0	.27
	R. fasciata	67	.26	25	2	0	.44
T	*Strombus gibberulus*	24	.25	53	0	0	.68
	Otopleura nodicincta (A. Adams)	13	.62				
	O. mitralis (A. Adams)	13	.23				
	Pyramidella dolabrata (L.)	14	.29				
E	*Vexillum* spp.	15	.40				
E	*Conus pulicarius* Hwass	11	.18				
	Terebra affinis	25	1.32				
	Cocos Lagoon, Guam						
	Rhinoclavis fasciata	222	.42	208	96	8	.19
T	*Strombus gibberulus*	17	.24	19	0	0	.84
E	*Conus pulicarius*	16	.063				
	Terebra affinis	13	.92				
	Bangi Island, Guam						
	Rhinoclavis fasciata	98	.082	75	28	0	.17
T	*Strombus gibberulus*	12	.083	11	0	0	.81
	Alupang Cove, Guam						
	Pyramidella dolabrata	19	0				
	Rhinoclavis aspera	44	.16	24	4	0	.083
T	*Strombus gibberulus*	30	.033	120	0	0	.72
	Pago River, Guam						
	Rhinoclavis aspera	44	.16	45	22	0	.16
	Terebra affinis	53	.38	53	10	1	.070
	Wom Village, New Guinea						
	Polinices tumidus	27	0				
T	*Strombus canarium* L.	17	.059	12	0	0	.50
T	*S. gibberulus*	15	.067				
T	*S. labiatus* (Röding)	34	.033	35	0	0	.55
T	*S. luhuanus* L.	15	.067				

APPENDIX A (Continued)

	Locality and species	Repair n	Repair f	Mortality n	Mortality n_d	Mortality n_{bd}	Mortality f_b
T	*Nassarius callospira* (A. Adams)	10	.10	24	0	0	.29
	N. luridus Gould	24	.25	61	3	0	.082
T	*N. pullus* (L.)	13	.078				
T	*N. quadrasi* Hidalgo	14	.21	37	2	0	.19
E	*Vexillum* spp.	11	.45				
E	*Conus muriculatus* Sow.	14	.14				
	Terebra anilis (Röding)	10	1.10				
	Bulla ampulla L.	12	.083	21	0	0	.57
	Pujada Bay, Mindanao						
T	*Strombus gibberulus*	23	0	18	0	0	.61
T	*S. labiatus*	27	.11	33	0	0	.24
T	*S. urceus* L.	11	.091				
T,E	*Pyrene versicolor* Sow.	12	.50				
	Nassarius distortus (A. Adams)	11	1.36	21	2	0	.095
T	*N. subspinosus* (Lamarck)	42	.26	47	7	0	.064
E	*Vexillum exasperatum* (Gmelin)	22	.23				
T,E	*Imbricaria* spp.	11	.27				
E	*Conus coronatus* Hwass	17	.35	25	0	0	.12
E	*C. arenatus* Hwass	18	.28				
	Tagbilaran, Bohol						
T	*Strombus labiatus*	11	.091	11	1	0	.27
T	*S. urceus*	30	0	12	10	0	.58
T	*Nassarius globosus* (Quoy & Gaimard)	32	.059	58	8	0	.31
	N. luridus (Gould)	11	.091	15	0	0	.40
E	*Conus coronatus*	13	0				
	Dodinga Bay, Halmahera						
T	*Cerithium coralium* Kiener	37	.41				
	Nassarius bicallosus (E. A. Smith)	35	.75				
T	*N. globosus* (Quoy & Gaimard)	9	.44				
T	*N.* spp.	16	.47				
E	*Vexillum* spp.	11	.81				
T,E	*Pyrene versicolor* Sow.	13	.31				
	Gemmula graeffei (Weink.)	15	.73				
	Motupore, New Guinea						
T	*Strombus gibberulus*	43	0	43	0	0	.46
	Majuro Lagoon, Majuro Atoll						
T	*Strombus gibberulus*	14	0	10	0	0	.30
	Boear, Aru Islands, Irian Jaya						
	Polinices tumidus	10	.10				
	Rhinoclavis vertagus	148	.16	114	23	0	.17
	Beaufort, North Carolina						
	Terebra dislocata (Say)	120	.083	130			.031
	Venado Beach, Panama						
	Polinices uber (Valenciennes)	27	.13				
	Natica chemnitzii Pfeiffer	26	.50				
	Modulus catenulatus (Philippi)	38	.26				
T	*Strombina clavulus*	13	2.23				
T	*Northia pristis* (Deshayes)	10	.40				
T	*Nassarius dentifer* Powys	20	.55	27	13	3	.15

APPENDIX A (Continued)

		Repair		Mortality			
	Locality and species	n	f	n	n_d	n_{bd}	f_b
T	*N. luteostoma* (Brod. & Sow.)	21	.19	70	33	1	.080
	N. pagodus (Reeve)	16	.67	45	0	0	.33
	N. versicolor (C. B. Adams)	42	.19	16	0	0	.13
	Agaronia testacea (Lamarck)	19	.16	11	0	0	.18
E	*Olivella volutella* (Lamarck)	17	.24	64	4	0	.063
	Cancellaria jayana Keen	13	.62	20	0	0	.25
T,E	*Prunum sapotilla* (Hinds)			21	1	0	.048
E	*Conus ximenes* Gray	17	.18				
E	*C. patricius* Hinds	13	.23				
T	*Pilsbryspira aterrima* (Sowerby)	17	.47				
	Terebra elata Hinds	10	.40	14	5	1	.23
	T. strigata Sowerby	10	1.00				
	T. spp.	14	1.71	12	8	1	.15
	Farfan Beach, Panama						
	Terebra cracilenta Li	49	.29	55	11	0	.11

n Number of individuals
f Frequency
n_d Number of drilled individuals
n_{bd} Number of drilled shells with lethal breakage
f_b Corrected frequency of lethal breakage
T thick lip
E elongated aperture

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DIURNAL MOVEMENTS IN POPULATIONS OF THE PROSOBRANCH *LANISTES NYASSANUS* AT CAPE MACLEAR, LAKE MALAWI, AFRICA

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ABSTRACT

Diurnal variation in abundance of *Lanistes nyassanus* Dohrn (Prosobranchia: Ampullariidae) has been hypothesized to be due to directed daily movements up and down the shore at Cape Maclear, Lake Malawi. We tested this hypothesis and collected data on population density, dispersion, size distribution, and growth rates of *L. nyassanus*. Using SCUBA and following marked snails, we found the following. (1) No directed displacement up and down the slope occurs, but the number of snails buried in the sand is significantly higher in the morning than the afternoon. Most movement was crepuscular or nocturnal and averaged 2.8 m each day. Neither distance displaced nor direction of displacement suggest high vagility. (2) Growth, which averaged 1.0 cm/ring for adult snails, appears to be annual. The increment was correlated with increase in water temperature. (3) The life history strategy appears mixed, with a trade-off of predator defense for high growth rate in thin-shelled young and vice versa in adults. The estimated life span is between 5 and 10 years. (4) Fish predation may contribute to explaining: a) the characteristic morphological traits such as shell weight and relative dimensions; b) the skewed size distribution, which is composed of few thin-shelled juveniles compared to heavy-shelled adults; and c) the burying behavior of the snails.

Key words: *Lanistes*; Ampullariidae; Lake Malawi; diurnal movements; vagility; growth rates; fish predation; life history strategy.

INTRODUCTION

Data on the movements of individuals are critical to testing several major ecological and evolutionary hypotheses. Kozhov (1963), for example, suggests low vagility is a major factor in speciation among benthic invertebrates, including gastropods, in Lake Baikal. However, the mark and recapture data required to assess vagility and to test Kozhov's hypothesis are rare.

Prosobranch gastropods in the deep lakes of the rift valley in Africa are a particularly interesting group. The group has radiated in tropical as well as temperate deep lakes; however, tropical regions are richer than temperate areas in species of prosobranchs (G. E. Hutchinson, in press). For example, 26–30 of the 41 prosobranch species (63–73%) in Lake Tanganyika are endemic (Boss, 1978). Prosobranchs in Lake Malawi show a similar pattern. At least 15 of the 19 species reported (79%) are endemic (Mandahl-Barth, 1972). Little information, however, exists on the natural history, distribution, movement or dynamics of these gastropods (Livingstone, 1981; D. H. Eccles, personal communication).

The available information suggests several testable ecological hypotheses with evolutionary implications. For example, daily variations in relative abundance of the largest endemic gastropod, *Lanistes nyassanus* Dohrn (Prosobranchia: Ampullariidae), are observed (Gray, 1980; C. T. and I. Grace, personal communication; personal observation). To explain the observed daily variations in abundance and density, Gray (1980) hypothesizes that *L. nyassanus* exhibits a daily vertical movement up and down the littoral slope.

Our purpose was: (1) to gather basic ecological information for *Lanistes nyassanus* at Cape Maclear; (2) to test Gray's (1980) hypothesis of daily movement, and (3) to provide an initial estimate of population vagility for this gastropod.

LANISTES MONTFORT

The Ampullariidae (=Pilidae) are medium, globose snails characterized by a taenioglossate radula (>10 mm) and a concentric operculum (World Health Organization, 1977).

The pallial cavity is divided into two compartments: the left one with a ctenidium and the other with a pulmonary sac (Mandahl-Barth, 1972; World Health Organization, 1977). During siphonal respiration the left nuchal lobe is drawn out into an incomplete tube (McClary, 1964). Females are oviparous. Males bear a copulatory organ (verge) near the mantle edge.

Ampullariids are distributed in freshwater throughout the warmer regions of the world and are represented by two genera (*Pila, Lanistes*) in southeast Africa (Mandahl-Barth, 1972; World Health Organization, 1977). The genus *Lanistes* Montfort is confined to Africa. Three species, *Lanistes nyassanus, L. ellipticus* and *L. solidus*, occur in shallow areas around Cape Maclear (Fig. 1), Lake Malawi (Gray, personal communication). *Lanistes nyassanus* Dohrn, endemic to Lake Malawi, can be distinguished from its most similar congener, the deeper-occurring *L. nasutus* Mandahl-Barth, by being larger (up to 65 mm compared to 40 mm), heavier, and with a closed umbilicus. Young *L. nyassanus* (up to about 30 mm) have thin shells with a narrow umbilicus (World Health Organization, 1977).

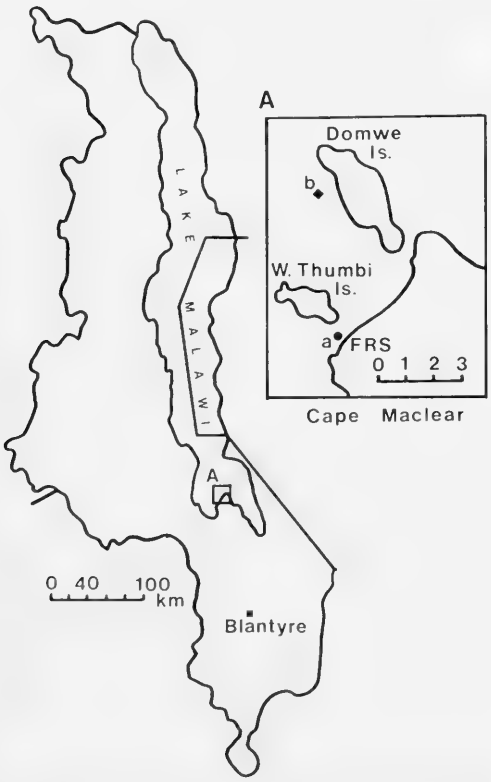

FIG. 1. Map of Malawi with the Cape Maclear region (Inset A), showing the location of our study site (a) at the Fisheries Research Station (FRS) and of the sampling site (b) for temperature profile. North is at the top of the map.

Crowley *et al.* (1964) suggest that there are two distinct habitat types for the lake-dwelling gastropods in Lake Malawi: permanent marshes with *Lanistes ellipticus* and *L. ovum procerus* and lake edge with *Lanistes solidus* and *Lanistes nyassanus* (see also Cantrell, 1979). These species all have heavy shells. *Lanistes nyassanus* is common in the sandy shallow lake edge habitat and the *Potamogeton-Vallisneria* beds of Cape Maclear.

No field data apparently exist on feeding and nutrition of *Lanistes nyassanus*. However, Yonge (1938) suggested that close relatives, *Pila* and nonendemic species of *Lanistes* from Lake Tanganyika, rasp algae from solid surfaces; both have a massive radula with short stout teeth. In Lake Tanganyika these species tend to live on higher plants in estuaries and inlets (Leloup, 1953). At Cape Maclear, Lake Malawi, *L. nyassanus* often faces into the current, with its foot and siphon partially extended and covered with copious mucus. Observations on another ampullariid genus, *Pomacea,* suggest this is a feeding posture. *Pomacea* can evidently feed on protein absorbed on minute particles of organic matter (Johnson, 1952; Cheesman, 1956; McClary, 1964). The material probably includes bacteria and algae (Hutchinson, in press). Ciliary feeding is not unknown among gastropods but is relatively rare among the Ampullariidae (G. E. Hutchinson, personal communication).

METHODS

Our observations were obtained on the sand beach adjacent to the Fisheries Research Station at Cape Maclear (34°50′E, 14°5′S), 12 km W of Monkey Bay, Lake Malawi (Fig. 1), in January 1980, using SCUBA. These data are of three kinds: distribution and density, size and growth, and movement. Surface and 5 m depth water temperatures, which peak in January-February, are available for July 1977 to April 1980 from a nearby sampling station.

The density and distribution of snails were determined in two ways. First, a 30 m transect line was anchored along the 1.5 m depth con-

tour. The number of snails per m^2 on both sides of the line was recorded regularly at 24 hour intervals and occasionally at intermediate intervals. Second, a 128 m^2 permanent grid of 4 rows of 8 quadrats (2 × 2 m each) was created. The rows were also along the slope contour. The first two rows (at 3.25 and at 3.75 m depth respectively) were on open sand. The third and fourth rows (at 4.25 m and at 4.75 m respectively) were in a *Potamogeton-Vallisneria* bed. We recorded the number of snails in each quadrat on 20 Jan. 1980.

We collected snails by swimming horizontal and vertical transects along the shore and collecting every third snail up to 50. Maximum shell height and width were measured (Fig. 2A). We also measured the aperture as an estimate of size since it frequently approximates gastropod biomass more closely than total overall shell dimension, especially if apical wear is common (Fotheringham, 1971; Louda, 1979). Growth increments were curvilinear distance from aperature edge to first growth line, from first to second oldest growth line and, if present, from second to third oldest growth line (Fig. 2B). These were measured at their widest diameter.

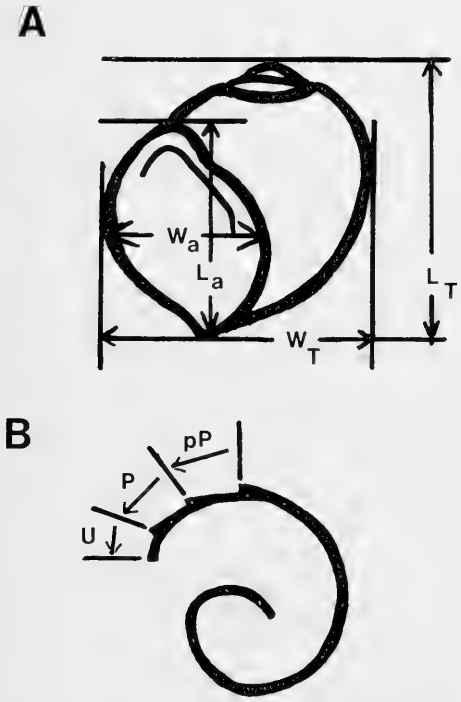

FIG. 2. Diagram of the measurements taken on shells of *Lanistes nyassanus* at Cape Maclear, Lake Malawi. A shows shell dimension where: L_T = total length, or overall height: W_T = total or body whorl width: L_a = aperture length or height: and, W_a = aperture width. B shows growth increment measurements on a transverse section where: pP = pre-penultimate ring, P = penultimate ring, and U = ultimate ring or growth increment.

After the snails were measured, we marked and returned them within two hours to a central stake in a 100 m^2 movement grid at 2.5 m water depth on sand. Snails were marked by gluing a number on the top of the body whorl, epoxying a 10 cm piece of white yarn to the surface between the first and second whorls and putting red enamel paint along the edge of the aperture lip. The yarn allowed detection of buried snails. Recapture success was high (95%) so treatment did not affect survivorship. Specimens of *Lanistes nyassanus* have been deposited in the Peabody Museum, Yale University.

The snails' linear displacement was measured at 4 hr or at 24 hr from the position of last sighting. Each numbered snail's position was marked after every time interval by a numbered flag. The distance between sightings was measured and the flag moved to the new snail position. Displacement from the new position was recorded at the next interval. Four consecutive days of observations were run 16–20 January 1980. Snail activity and position in or on the sand were recorded.

RESULTS

Lanistes nyassanus has been reported at depths from 12 to 28 m (World Health Organization, 1977). This distribution may now be extended up into much shallower water since we were able to study substantial numbers of this species on sand and in adjacent *Potamogeton-Vallisneria* beds at depths of 1 to 5 m at Cape Maclear (Fig. 1). For *L. nyassanus* in shallow water at the high point of the annual cycle of water temperature (McKaye, 1982), the interface of sand and weeds at 3.5 m depth supported the highest densities (Table 1). In the permanent quadrats on sand at 3.5 m, density was 1.3/m^2 (N = 64 m^2). The lowest densities of *L. nyassanus* were in quadrats in the deeper (4.5 m) *Potamogeton-Vallisneria* bed (0.4/m^2, N = 64 m^2). Density on the transect higher on the shore (1.5 m depth) on sand was intermediate (0.95/m^2, N = 60 m^2). Although densities in the morning (1100 hr) appeared to be lower than in the

TABLE 1. Density of *Lanistes nyassanus* (Number per m^2) at Cape Maclear, Lake Malawi, in January, 1980.

Time of Day	N	$\bar{X}$	S.E.	95% C.I.
Line Transect at 1.5 m depth, 30 × 2 m				
1100	60	0.9	0.13	0.64–1.16
1100	60	0.9	0.15	0.62–1.22
1800	60	1.0	1.10	0.82–1.22
Permanent Grid at 3 to 5 m depth,[1] each row 8 × 2 × 2 m quadrats				
Above Weeds	64	1.3	0.20	0.88–1.68
In Weed Bed	64	0.4	0.07	0.21–0.49

[1]Rows 1 and 2, above the weeds, were centered at 3.25 m and at 3.75 m depth respectively. Rows 3 and 4, in the *Potamogeton-Vallisneria* weed bed, were centered at 4.25 and at 4.75 m depth respectively.

early evening (1800 hr) as originally suggested, the difference was not significant (Table 1).

The heavy-shelled *Lanistes nyassanus* were smaller (Table 2: 4.2 cm) than suggested usual (6.1–7.0 cm: World Health Organization, 1977). The largest individual we observed had a shell 5.1 cm high. The hypothesis that *L. nyassanus* is a continuously breeding population with the expected normal size distribution must be rejected. The size distribution was skewed toward large, adult snails at this time of year (Fig. 3). The smallest individual positively identified as *L. nyassanus* was 3.1 cm high. In addition, 3 thinner-shelled individuals, which measured 1.0 and 2.0 cm high and were most likely *L. nyassanus* juveniles, were found among the roots of the *Potamogeton-Vallisneria* bed at 5 m.

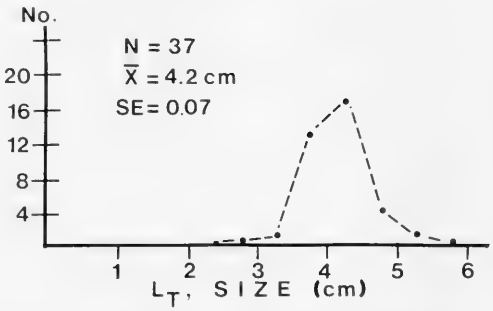

FIG. 3. Size distribution of marked *Lanistes nyassanus* Dohrn at Cape Maclear, Lake Malawi; frequency plotted at midpoints of 0.5 cm size classes, where L_T is the overall shell height.

Penultimate growth increment was not correlated with shell size, either overall or aperture height ($r = -0.03$ and 0.02, respectively, $N = 35$ for each), eliminating the hypothesis that growth was a function of individual size. The 5 smallest adult snails ($\bar{X} = 3.5$ cm height, S.E. = 0.11), in addition, had a penultimate increment of 1.2 cm (S.E. = 0.31) which was statistically equal to the penultimate increment of 1.3 cm (S.E. = 0.28) for the 5 largest snails ($\bar{X} = 4.8$ cm, S.E. = 0.10). Thus adult growth was independent of overall adult shell size. There was a marginally significant correlation ($r = 0.41$, $0.10>p>0.05$) between the penultimate growth ring and the pre-penultimate growth ring for the 18 heavy-shelled *L. nyassanus* with a third growth line (see Fig. 2), lending support to the hypothesis that individuals may vary in their growth rates.

TABLE 2. Size and growth parameters for *Lanistes nyassanus* and the relationships among them (N = 37 heavy-shelled adult snails) at Cape Maclear, Lake Malawi, in January 1980.

	N	$\bar{X}$	S.E.	Correlation Coefficients
Maximum height	37	4.2	0.07	0.86*, 0.79*, 0.86*, 0.03
Maximum width	37	4.4	0.06	0.82*, 0.07, 0.06
Aperture height	37	3.5	0.05	0.36, 0.41
Aperture width	37	2.6	0.04	
Growth increment				
Ultimate (Recent)	37	0.5	0.05	
Penultimate	37	1.0	0.09	
Pre-Penultimate	20	1.0	0.09	

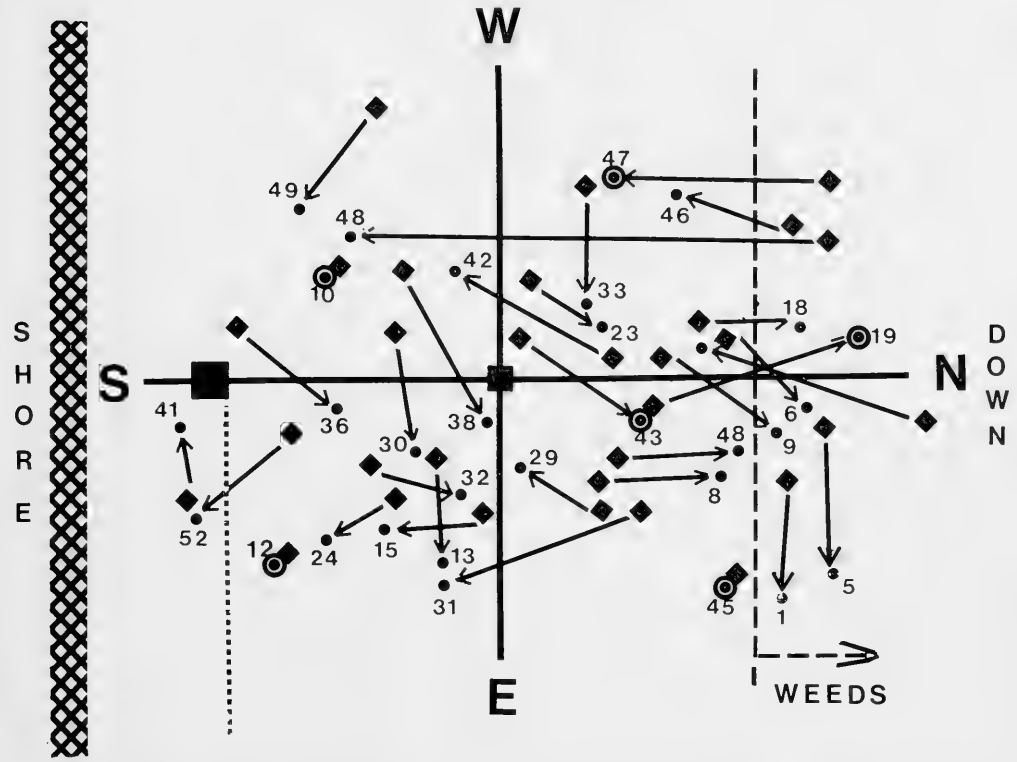

FIG. 4. Movement data for the third day of the consecutive 24-hour observations, 18–19 January 1980, taken at 1630 hr, showing original position (◆), end position after 24 hours (●), and direction of displacement (→) for numbered *Lanistes nyassanus* on the movement grid at Cape Maclear, Lake Malawi. Buried individuals were circled. The dashed line to the right is the upper edge of the *Vallisneria* bed at 3 m depth and the dashed line to the left represents the position of the 30 m transect line at 1.5 m depth.

Movement, as estimated by linear displacement over a 4 hr period (Fig. 4), was greater in the late afternoon-early evening than in the morning or early afternoon (Fig. 5). In January, the average distance displaced from the previous sighting in 4 hr increased as light intensity increased and then decreased from 0830–1230, to 1230–1630 and to 1630–2030 (with dusk by 1730 and dark by 1830 at 3 m depth). In addition, the proportion of marked snails which were buried decreased from morning to evening (Fig. 5). Burial into the top layer of sand occurred at the time of day when snail density tended to decrease (Table 1) and less movement occurred (Fig. 5). Diurnal burial is an alternative hypothesis to explain the pattern of lower densities of snails in the morning than in late afternoon.

Daily displacement over 4 days in January averaged 2.8 m/day (Fig. 6). Snail displacement over the first 24 hr period, which was

FIG. 5. Distance displaced (●) and proportion of marked *Lanistes nyassanus* which were buried (◆) after 4 hr, by time of day, in January 1980; am = 0830 to 1230 hr, pm = 1230 to 1630 hr, and eve = 1630 to 2030 hr, data plotted at midpoint of observation period.

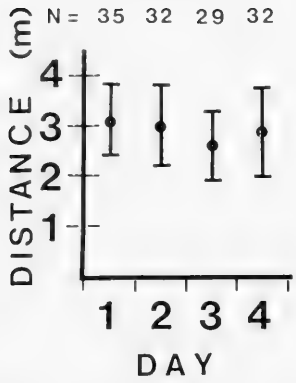

FIG. 6. Mean distance displaced over 24 hr, on 4 consecutive days of observation on marked *Lanistes nyassanus* recorded at 1630 hr, 16–20 January 1980, at Cape Maclear, Lake Malawi: N = the number of snails sighted among the 37 marked individuals available.

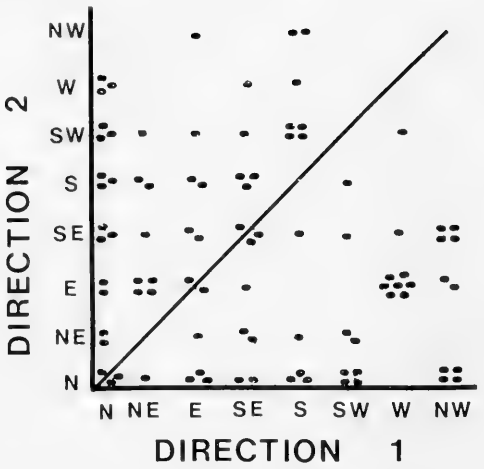

FIG. 7. Direction of consecutive 24 hr displacements per individual using all pairs of displacements for marked *Lanistes nyassanus* in January, 1980, at Cape Maclear, Lake Malawi.

hypothesized to represent a period of extra movement in response to either disturbance or concentration of snails at the central stake, did not differ from that on subsequent days (Fig. 6). The average distance that marked adult snails were displaced daily was remarkably consistent (Fig. 6).

Movement was random in direction. When the direction of a second displacement is compared to the direction of the first displacement no pattern emerges (Fig. 7). The one pattern discernable in any of the daily movement data was downward orientation in the first 24 hr period, i.e. after handling (Table 3). These data were only marginally significant (χ^2 = 3.46, d.f = 1, $0.10>p>0.05$). Gray's (1980) hypothesis of general directed movement up and down the slope must be rejected.

A related alternative hypothesis, that individual snails show patterns of movement, must also be rejected. A positive association of consecutive displacements would support the hypothesis that individual snails were consistent either in moving or in sitting. A negative association would imply a long move is generally followed by a short one and vice versa. No correlation exists (Fig. 8). In addition, there was no correlation ($r = -0.13$, N = 33) between individual adult snail shell size and mean distance displaced per day over the 4 days of observations (Fig. 9). These data support the hypothesis that large and medium snails move equally. Although the five smallest marked snails ($\bar{X}$ = 3.5 cm) had farther daily displacements ($\bar{X}$ = 3.1 m, S.E. = 0.89) than did the five largest ($\bar{X}$ = 2.3 m, S.E. = 23), this difference was not significant (t = 0.87, 4 d.f.).

DISCUSSION

Information on the density of these snails is of interest for at least two reasons. First, the data provide an estimate of numbers and dis-

TABLE 3. Direction of linear displacement by *Lanistes nyassanus* over 24 hr observation periods, at 1630 hr on 16–20 January 1980, at Cape Maclear, Lake Malawi.

Time (Hrs)	N (Snails)	Downslope quadrants			Upslope quadrants			One-way χ^2
		NW	NE	Total	SE	SW	Total	
24	35	15	8	23	4	8	12	3.46
48	32	10	9	19	9	4	13	1.12
72	29	7	8	15	10	4	14	0.03
96	32	4	10	14	14	4	18	0.50

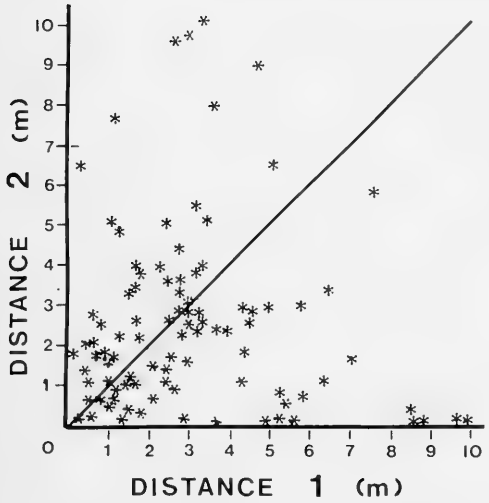

FIG. 8. Distance of consecutive 24 hr deplacements, using all pairs of displacements for marked *Lanistes nyassanus* in January, 1980, at Cape Maclear, Lake Malawi.

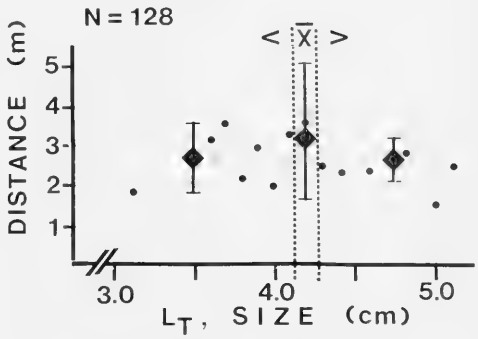

FIG. 9. Average distance displaced over 24 hr for marked *Lanistes nyassanus* by size category in January, 1980, at Cape Maclear, Lake Malawi, where ● = mean values for 0.2 cm size classes and ◆ = average (with 95% C.I.) for snails less than, equal to, or greater than the mean size of the adult population.

tribution of this African prosobranch endemic to Lake Malawi. Second, shells of *Lanistes nyassanus* are an important spatial resource for an endemic, shell-dwelling cichlid fish and play an indirect role in the community dynamics of the sand-dwelling fishes of Lake Malawi (McKaye, 1978).

One factor, which is also of interest in understanding the ecology of *Lanistes nyassanus*, is the rate at which shells become available through patterns of *L. nyassanus* survivorship and mortality. If snail growth occurs during the period of warm water, as is suggested by an ultimate growth increment equal to half the penultimate increment at the middle of the warm water period, turnover time is on the order of 5–10 yr. The variation in this rough estimate is dependent on the rate of juvenile development to 3.0 cm size. Adult life appears to average from 3 to 6 growth periods minimum; 55% of our sample had four or more growth rings, probably representing 5–9 adult years, and the mean was 3.3 distinct rings per snail.

Overall size and relative shell dimensions suggest *Lanistes nyassanus* is morphologically adapted for disturbed habitats such as exposed open coasts of a large lake (G. E. Hutchinson, in press). First, it is large and heavy-shelled. Second, the short spire (Burch, 1968), and broad shell (Cain, 1977), with a short height compared to shell width (H/W = 0.95), and a low ratio of height to aperture (1.20), are typical of exposed area snails. Third, the relatively large (3.5 cm) and especially broad (2.7 cm) aperture (W/H = 0.74) is comparable to measurement of another gastropod on exposed shores, *Goniobasis livescens* (Wiebe, 1926).

Longterm, persistent fish predation pressure may be a contributing factor to the heavy-shelled morphology of *Lanistes nyassanus* and to the occurrence of shallow-water 'thalassoid' endemic gastropods in deep, ancient lakes like Lake Tanganyika. A large and heavy marine-like shell, especially with a closed umbilicus, is an effective antipredator defense (Wright *et al.*, 1967; Vermeij, 1975; Vermeij & Covich, 1978; Calow, 1978). The heaviest freshwater gastropods occur in relatively shallow water in the ancient lakes; G. E. Hutchinson (in press) cites several examples and suggests that these are K-selected (MacArthur & Wilson, 1967). In addition, they may represent adaptation over a long period of coexistence to intense fish predation. The heavier shells of the shallower-water species of *Lanistes*, especially associated with the conspicuousness of *L. nyassanus* on the sand in shallow water, suggest that predation by molluscivorous, sand-dwelling cichlid fish may have been a significant selective pressure. This interpretation is further supported by the skewed size distribution of *L. nyassanus* and by the occurrence of the thin-shelled juveniles in a more heterogeneous and protected microhabitat, the roots of the weed bed.

Freshwater snails, even iteroparous species, have a tendency toward relatively rapid growth and early reproduction when compared with marine species. This life history strategy is usually accompanied by a thin shell relative to marine gastropods (Vermeij, 1975; Calow, 1978). There may, thus, be a tradeoff for rapid development over predator defense. This hypothesis is difficult to assess with respect to *Lanistes nyassanus* but it is consistent with our data. Juvenile *L. nyassanus* appear to grow rapidly. A search, even in protected microhabitats, produced only a few relatively large (1.0–2.0 cm height) juveniles at the middle of the annual cycle.

Adult growth increments are less than those estimated for the young. The adult ratio of the mean penultimate growth ring to average body whorl diameter is 0.23, about one-fifth. Penultimate and pre-penultimate increments each represent approximately 10% of the shell circumference of the adult body whorl. The smallest adult (3.1 cm) had a 6.1 cm circumference up to the first growth line. Thus, the annual increment for the juvenile phase, depending on the length of its juvenile life, must have been: 6.1 cm/yr if the juvenile period was 1 year, 3.0 cm/yr if the juvenile period was 2 yr, or 2.0 cm/yr if the first growth phase was 3 yr. In any case, a 2.0–6.1 cm growth increment represents more rapid growth than the 1.0 cm mean growth increment of adult snails. *Lanistes nyassanus*, thus, may present a mixed life history strategy: a juvenile period with exceptionally rapid growth, during which susceptibility to predation is extremely high, and an adult period with slower growth during which predation mortality is decreased by the development of a heavy shell. Consequently we hypothesize that habitat heterogeneity is critical for early survival and subsequent recruitment of *L. nyassanus*.

The movement data were originally gathered to test Gray's (1980) hypothesis that *L. nyassanus* moves into deeper water during the early hours and into shallower water in the afternoon and evening. No directed displacement was observed over 24 hr periods. Direction of displacement over 4 hr at different times of day was also random. Gray's (1980) hypothesis must be rejected for our study. Density, which averaged 0.95 snails/m^2 at 1.5 m depth, did not vary significantly between morning, afternoon and evening periods. The original observations of fewer snails in the morning than in the afternoon, are best explained by the burial of adult snails into the top layer of sand. Burial was significantly higher during the morning than during the later afternoon or evening. Since burial is shallow, meter by meter counts by a SCUBA diver include buried snails. Yet, these individuals would not be recorded by an observor near the surface. Therefore the diurnal pattern does exist, but it is into and out of the sand rather than up and down the slope. We suggest that these snails bury to escape from visual predators.

The consistency of the average distance displaced per day, the random orientation and the persistence of tagged individuals in one general 100–150 m^2 area provide the main components of an estimate of vagility hypothesized by Kozhov (1963) to be significant in gastropod speciation. There is no evidence of high vagility in our data. The distance displaced at this time of year averaged only 3 m and was nondirectional. Movement during the breeding season, which we expect to occur in August-September as the water begins to warm, needs to be examined. Dependence of juvenile survival on habitat heterogeneity should provide strong selection for directed movement toward the *Potamogeton-Vallisneria* beds for mating and egg-laying. Our observations also suggest vagility between the adjacent habitats, i.e. weeds and sand occur at a relatively high rate and in random sequence.

The lack of directionality of the displacement observed is particularly interesting in light of the morphological capability of the family for air breathing; they have the option of branchial respiration via utilization of the pulmonary sac. Some, such as *Pila* species, are known to leave the water at night to forage (Prashad, 1925). We did find increased movement in the late afternoon and evening. We did not find an upslope, landward directional displacement as would be expected if the hypothesis that these snails move onto land at night to feed is correct.

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ENVIRONMENT AND SHELL SHAPE IN THE TAHITIAN LAND SNAIL *PARTULA OTAHEITANA*

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ABSTRACT

A multivariate analysis was performed on data from Crampton's 1916 study of the arboreal snail *Partula otaheitana* in 50 valleys of Tahiti Nui, Society Islands. Populations in wetter, more shaded valleys tended to have shells more elongate in shape with a higher percentage of banding and with a reduced apertural barrier than populations in drier, more sunlit valleys. The correlations accounted for 20% of the variance in the three shell characters mentioned. Causative factors of these variations remain to be investigated.

Key words: *Partula*; snail; shell shape; environment; Tahiti; evolution.

INTRODUCTION

Cain & Sheppard's (1950) landmark discoveries of some mechanisms of natural selection in shell coloration of the land snail *Cepaea nemoralis* explained some of the geographic variation in one seemingly non-selected polymorphism. Another well-studied land snail group, the partulids, however, remains enigmatic by its possession of apparently neutral characters.

The family Partulidae is hypothesized (Kondo, 1973) to have invaded the Society Islands in three waves, the last of which (genus *Partula*) probably has reduced its predecessors (genus *Samoana*) to near extinction. *Partula* now dominates in upland valleys of the more westerly Society Islands. The genus is isolated by a hierarchy of barriers: onto individual islands by the Pacific Ocean; into specific valleys by dry or devegetated hogback ridges radiating from the center of each volcanic island; and into partially to totally discrete populations within valleys by disrupted patches of vegetation. *Partula* represents a complex array of species, subspecies, morphs, and individual variants.

H. E. Crampton, attracted by partulid diversity and variability, produced classic conchological studies on the partulids of Tahiti, Moorea, and the Mariana Islands (Crampton, 1916, 1925, 1932). Portions of his extensive data have been reanalyzed subsequently (Ludman, 1947; Bailey, 1956). His initial genetic investigations, in which he compared the shells of adult snails with the shells of embryos dissected from their uteri (*Partula* is ovoviviparous), have been greatly augmented by recent hybridization experiments and field observations of Moorean species (Clarke & Murray, 1969, 1971; Johnson, Clarke & Murray, 1977; Murray & Clarke, 1966, 1968a, 1968b). They have shown that several shell characters, namely size, color, banding, and sinistrality of two species of *Partula* from Moorea are highly heritable. Lipton & Murray (1979) compared courtship patterns of the two species and found evidence of behavioral reproductive isolation.

It remains moot whether shell characters of *Partula* are subject to natural selection. Crampton, one of the first American scientists to teach Mendelism, was convinced that the extensive variation in *Partula* resulted solely from mutation and genetic drift and was unaffected by natural selection (Crampton, 1932). His reasoning was that: (1) habitat is uniformly humid tropical; (2) predation is absent, except by rats introduced by man into coastal areas where *Partula* is scarce to absent anyway; (3) the particular plant type which provides the decaying vegetation upon which *Partula* feeds is "a matter of indifference" (Crampton, 1916); and (4) different species of *Partula* occurring micro-sympatrically, even on the same leaf, in any given valley exhibit different shell morphologies and color patterns. Crampton's theory of random, unselected variation in *Partula* has been supported by Huxley (1942) and contested by

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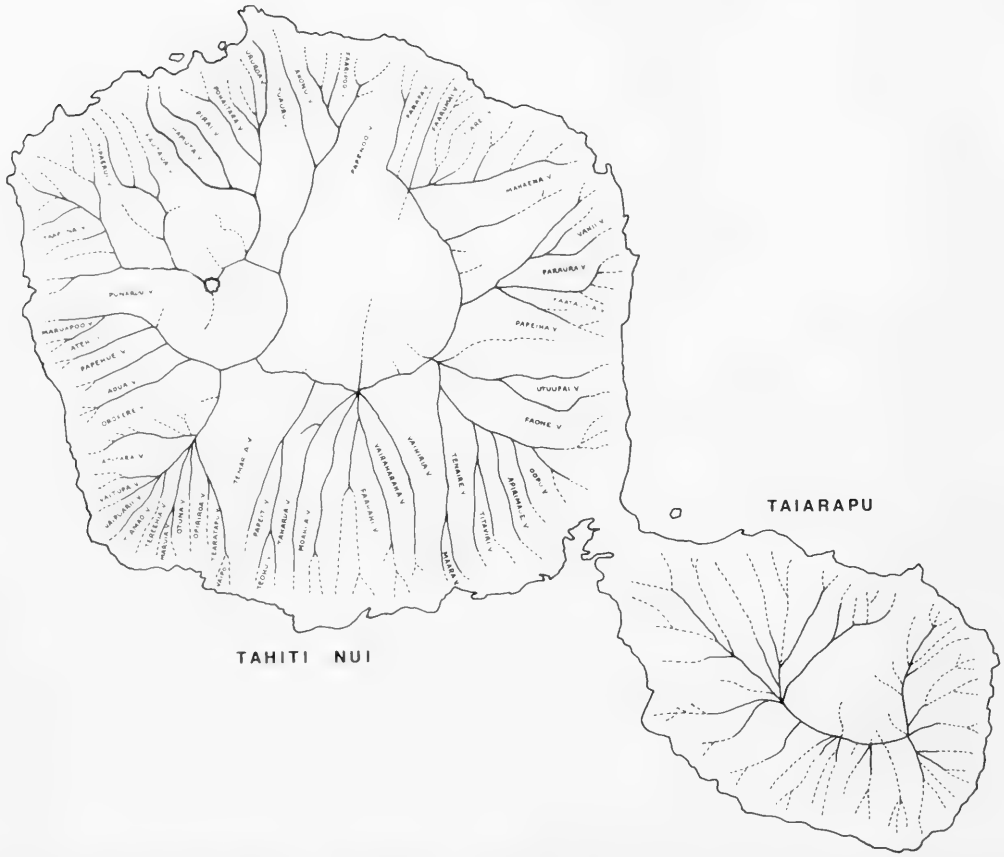

FIG. 1. Tahiti, Society Islands. Lines represent ridge tops. The 50 labeled valleys were those used in this study (from Crampton, 1916).

Cain & Sheppard (1950) and Ford (1964). The majority of attempts to correlate environmental variables with shell characteristics of *Partula* (Clarke & Murray, 1971; J. Murray, 1979, personal communication) have been unsuccessful. A single trend was discovered among the *Partula* of Moorea: there is a clear negative correlation between shell length and altitude. This paper presents evidence of a different nature, based on a multivariate analysis of Crampton's (1916) data, of a linear relationship between general environmental trends (other than altitude) and shell characteristics of *Partula otaheitana* on Tahiti Nui (Great Tahiti).

Crampton recognized six species of *Partula* on Tahiti. Of these, only *P. otaheitana* approaches ubiquity in distribution. This species is endemic to Tahiti, abundant, and exhibits a wide range of shell morphologies and colorations. Crampton divided *P. otaheitana* into eight subspecies whose geographical distributions are mapped in Fig. 2. Sympatry of the nonhybridizing "subspecies" *affinis* and *rubescens* in northern valleys makes Crampton's designations immediately suspect. Indeed, Lundman (1947) proposed promoting *rubescens* to full species rank based on his reanalysis of Crampton's data. However, because Clarke & Murray's (1969) hybridization experiments on *Partula* of Moorea resulted in lumping rather than splitting Crampton's species, and because no additional data have been published concerning the taxonomy of *P. otaheitana,* I shall adhere to Crampton's designations.

The most obvious physical environmental variables that might exert selection pressure on *Partula otaheitana* are temperature and humidity. *Partula* is absent from drier regions

FIG. 2. Distributions of eight subspecies of *Partula otaheitana* on Tahiti, according to Crampton (1916).

of the coasts and from deforested ridgetops, where direct sunlight produces greater temperature fluctuations. It is common to abundant in more shaded areas of consistently high humidity and lower temperature fluctuations, particularly in remaining patches of native vegetation (Crampton, 1916; A. Solem, personal communication). *Partula* is active generally under conditions of saturated humidity; much of the time the animal rests with the shell aperture sealed to the undersides of leaves (Crampton, 1916). Ideally one should have records of temperature and humidity for each area sampled. Since such data do not exist for Tahiti, it is possible only to estimate general trends on the island for those meteorological variables most heavily influencing temperature and humidity, namely insolation and rainfall.

Average insolation in valleys of Tahiti Nui varies according to slope, aspect, shadowing by adjacent ridges, and cloudiness. Variation in all these factors is considerable due to the steeply pitched terrain. Rainfall differs markedly according to position on the island, valley side, elevation, and season. Moisture retention varies drastically with vegetation cover. Much of the moisture carried by the nearly constant southeast trade winds precipitates on the windward side, resulting in about 635 cm per year on the windward coast and an estimated but inadequately measured 2590 cm per year on the highest slopes. The central peak produces a distinct rain shadow, so the leeward coast averages only about 200 cm per year (Crampton, 1916). Thus, average rainfall would vary on Tahiti Nui according to both elevation and horizontal deviation of the mouth of the valley. Given these trends, the relative average insolation and rainfall can be estimated for any given valley on Tahiti Nui because of its circular shape, single central highland area, and regular series of radiating valleys (Fig. 1). Taiarapu (Tahiti Iti) was excluded from analysis for the sake of simplicity.

MATERIALS AND METHODS

Crampton's field parties collected *Partula otaheitana* during four visits: February-March, 1906, June-July, 1907, June-August, 1908, and unspecified months in 1909. A total of 18,509 living adult snails were taken from 50 valleys. Crampton recorded the following set of shell data for each specimen: shell length, shell width, aperture length, aperture width. He calculated the ratios shell width/length, aperture width/length, and aperture length/shell length for each specimen, and also recorded whether the shell was sinistral or dextral, banded or unbanded. He classified each shell into one of five categories according to the degree of development of the parietal apertural barrier (1 = no barrier, 5 = very large barrier), and into one of several categories according to shell color.

Crampton's original measurements of individual shells could not be located. He published only the mean and standard deviation for each shell variable for a given subspecies in a given valley. With eight subspecies occurring, sometimes sympatrically, in 50 valleys, this results in 62 subspecies-valleys for Tahiti Nui. I was able to calculate % sinistral and % banded for each subspecies-valley from Crampton's tables of those percentages broken down according to color form. Crampton used a different color scale for each subspecies, so I standardized all mean color index values to the same scale he used for *P. o. otaheitana*: 1 = lightest, 4 = darkest. To illustrate the procedure by way of example, the sample of *P. o. sinistrorsa* in Tenaire Valley (Crampton, 1916, table 176) consisted of 907 adult shells of which 26.9% were classified by Crampton in the color form *apex,* 25.7% in *cestata,* and 47.4% in *phaea.* Comparing the colors of representative shells pictured in Crampton's (1916) plates, I judged that *apex* corresponded to a value of 1.0 on the *P. o. otaheitana* scale, *cestata* corresponded to 3.4, and *phaea* corresponded to 4.0. (These judgments must show subjective bias, but as long as the bias is consistent

TABLE 1. Fifty valleys of Tahiti Nui: environmental indices and means of shell variables for each subspecies-valley.

Valley name	Valley smallness	Insolation index	Low rainfall index	Subspecies	% Sinistral	% Banded	Darkness	Shell length (SL)	Shell width (SW)	Shell W/L × 100	Aperture length (AL)	Aperture width (AW)	Aper. W/L × 100	AL/SL × 100	Barrier size
Fautaua	1	28	178	*otaheitana*	55.1	0.5	2.70	19.10	10.99	57.44	9.83	7.72	78.47	51.41	2.71
Hamuta	3	43	172	*amabilis*	63.1	2.0	2.72	18.33	10.71	58.41	9.56	7.50	78.23	52.05	3.09
Pirai	2	38	165	*amabilis*	100.0	1.9	1.28	18.28	10.67	58.30	9.46	7.37	77.90	51.69	2.90
Pohaitara	3	33	159	*amabilis*	100.0	0.0	2.85	17.56	10.48	59.62	9.26	7.19	77.68	52.59	2.64
Ururoa	3	10	150	*amabilis*	99.7	0.9	3.54	17.45	10.36	59.34	9.21	7.14	77.54	52.66	2.65
Tuauru	2	5	146	*rubescens*	100.0	0.0	2.25	19.50	10.55	54.00	9.70	7.22	75.12	49.38	1.12
Tuauru	2	5	146	*affinis*	0.8	26.2	3.28	17.38	10.32	59.32	9.32	7.31	78.39	53.49	2.86
Ahonu	2	19	140	*rubescens*	100.0	0.0	1.96	20.66	11.32	54.64	10.28	7.77	75.44	49.66	2.28
Ahonu	2	19	140	*affinis*	2.0	2.0	2.68	17.01	10.06	59.01	9.01	6.93	76.77	52.82	2.48
Faaripoo	3	8	130	*affinis*	0.0	1.9	3.27	16.73	9.90	59.06	8.97	6.81	75.83	53.51	2.08
Papenoo	1	22	122	*rubescens*	100.0	0.0	2.19	20.38	11.30	55.37	10.33	7.70	74.53	50.66	2.48
Papenoo	1	22	122	*affinis*	4.4	0.0	2.73	16.62	9.70	58.21	8.91	6.64	74.33	53.49	1.52
Farapa	3	21	113	*affinis*	0.0	23.7	2.71	17.82	10.29	57.74	9.42	7.22	76.56	52.73	1.14
Faarumai	4	25	106	*rubescens*	100.0	0.0	2.50	20.54	11.86	57.66	10.76	8.21	76.45	52.24	1.00
Faarumai	4	25	106	*affinis*	0.0	3.9	3.32	15.54	9.27	59.58	8.17	6.42	78.40	52.52	2.42
Tiarei	3	38	97	*rubescens*	100.0	0.0	1.77	19.98	11.65	58.30	10.27	7.90	76.90	51.30	1.07
Tiarei	3	38	97	*affinis*	0.0	10.6	3.00	16.22	9.30	57.14	8.37	6.37	76.04	51.44	2.47
Mahaena	2	72	80	*rubescens*	100.0	0.0	2.50	18.58	11.17	60.00	9.87	7.57	76.17	53.00	3.67
Mahaena	2	72	80	*affinis*	2.8	4.3	3.30	16.70	9.70	58.06	8.76	6.83	77.98	52.35	3.01
Vahii	4	55	64	*affinis*	0.0	0.7	3.50	16.65	9.93	59.48	9.01	6.97	77.33	53.95	2.38
Paraura	2	79	56	*rubescens*	100.0	0.0	1.75	19.15	10.84	56.68	9.79	7.67	78.41	51.06	1.98
Paraura	2	79	56	*affinis*	0.0	19.5	3.49	16.31	9.66	59.12	8.66	6.67	77.01	52.95	2.00
Faatautia	4	76	48	*affinis*	0.0	25.0	2.50	16.18	9.37	57.92	8.23	6.63	79.42	50.75	3.42
Papeiha	2	72	43	*rubescens*	100.0	0.0	1.87	20.13	11.29	56.18	10.19	7.82	76.82	50.59	1.01
Papeiha	2	72	43	*affinis*	0.0	8.6	2.50	16.85	9.78	58.02	9.01	6.74	74.67	53.35	2.61

Utuufai	3	82	28	*rubescens*	100.0	0.0	2.50	19.95	11.22	55.70	10.26	7.50	73.70	51.10	1.00
Utuufai	3	82	28	*affinis*	0.0	21.1	2.50	16.66	9.80	58.69	8.79	6.72	76.35	52.61	3.28
Faone	3	63	16	*affinis*	0.0	12.2	2.50	16.49	9.70	58.73	8.68	6.67	76.77	52.53	3.13
Oopu	4	18	7	*rubescens*	100.0	0.0	2.04	22.83	12.89	56.51	11.53	8.67	75.20	50.50	1.00
Oopu	4	18	7	*affinis*	0.0	8.3	4.00	16.76	9.83	58.89	8.89	6.74	75.84	52.86	2.42
Apirimaue	2	14	0	*rubescens*	100.0	0.0	2.50	16.75	10.30	60.50	8.70	7.30	83.50	51.50	1.00
Apirimaue	2	14	0	*affinis*	0.0	0.0	4.00	17.75	10.13	57.17	9.07	6.80	74.50	51.00	1.67
Apirimaue	2	14	0	*sinistrorsa*	100.0	93.0	3.39	19.39	10.70	55.10	9.60	7.18	74.80	49.40	1.00
Titaviri	2	6	10	*sinistrorsa*	68.6	53.1	3.46	19.75	10.91	55.17	10.13	7.57	74.78	51.26	1.08
Tenaire	2	3	7	*sinistrorsa*	41.2	25.7	3.03	19.10	10.68	55.84	9.82	7.45	75.90	51.36	1.03
Maara	4	0	20	*sinistrorsa*	65.5	55.2	3.58	18.00	10.31	57.12	9.13	7.07	77.47	50.71	1.11
Vaihiria	1	6	13	*sinistrorsa*	100.0	91.4	3.54	18.27	10.23	55.95	9.24	7.09	76.58	50.49	1.00
Vairaharaha	1	19	25	*sinistrorsa*	100.0	21.1	3.89	18.09	10.12	55.89	9.25	7.05	75.98	51.08	1.00
Faarahi	2	6	34	*sinistrorsa*	99.6	35.5	3.82	20.10	11.00	54.70	10.08	7.67	76.08	50.07	1.09
Moaroa	2	11	53	*sinistrorsa*	100.0	39.4	3.80	18.64	11.34	55.38	9.46	7.22	76.32	50.70	1.32
Taharua	2	8	58	*sinistrorsa*	99.1	36.3	3.82	18.57	11.36	55.75	9.49	7.22	75.91	51.03	1.36
Teohu	4	7	60	*sinistralis*	100.0	72.5	3.45	17.26	9.92	57.37	8.88	6.76	76.11	51.34	2.68
Papeiti	1	16	65	*sinistralis*	100.0	44.0	3.74	16.67	9.34	55.93	8.55	6.54	76.34	51.20	2.89
Temarua	1	25	77	*sinistralis*	99.6	65.2	3.67	16.85	9.56	56.70	8.75	6.70	76.54	51.81	3.06
Vaipoo	4	8	69	*sinistralis*	100.0	79.0	3.60	17.01	9.67	56.83	8.86	6.72	75.75	52.02	2.90
Tearatapu	4	3	73	*sinistralis*	100.0	50.0	3.75	16.70	9.71	58.20	8.88	6.85	77.13	53.17	2.93
Opiriroa	4	0	80	*sinistralis*	100.0	73.7	3.63	18.21	9.96	54.58	9.24	7.05	76.14	50.63	2.97
Otuna	4	7	83	*sinistralis*	100.0	68.3	3.66	18.55	10.29	55.40	9.56	7.26	75.92	51.38	2.92
Maruia	4	12	83	*sinistralis*	80.6	60.5	3.70	18.02	10.28	57.02	9.34	7.20	77.08	51.72	1.59
Tereehia	2	21	87	*sinistralis*	53.8	90.8	3.55	17.61	9.86	55.89	8.97	6.78	75.60	50.85	1.63
Tiamao	4	32	89	*sinistralis*	54.3	94.2	3.52	17.81	10.15	56.90	9.22	6.92	74.99	51.64	1.46
Vaipuarii	3	50	93	*sinistralis*	100.0	78.9	3.61	18.62	10.58	56.71	9.63	7.26	75.27	51.60	1.04
Vaitupa	3	73	97	*crassa*	100.0	7.5	3.80	17.17	9.68	56.31	9.07	6.77	74.58	52.75	2.37
Atitara	3	85	103	*crassa*	99.4	11.7	3.40	16.72	9.71	58.02	9.02	6.77	75.04	53.85	2.74
Orofere	2	71	111	*crassa*	100.0	4.6	3.70	17.54	10.47	59.63	9.47	7.14	75.35	53.92	3.28
Aoua	3	66	118	*crassa*	100.0	0.0	2.50	17.16	9.95	57.88	9.13	6.92	75.63	53.11	2.76
Papehue	3	68	124	*crassa*	100.0	0.0	2.50	17.84	10.11	56.45	9.27	6.96	74.96	51.90	1.64
Atehi	4	69	131	*crassa*	100.0	68.5	3.90	17.23	9.99	57.95	8.90	6.91	77.50	51.63	1.59
Maruapoo	4	77	133	*crassa*	100.0	12.6	3.90	17.00	9.95	58.42	8.77	6.85	77.94	51.48	3.01
Punaruu	1	88	143	*crassa*	100.0	2.9	3.90	17.19	9.99	58.11	9.08	6.74	74.13	52.74	2.79
Taapuna	2	70	152	*crassa*	100.0	2.2	3.50	18.06	10.47	57.91	9.66	7.15	73.88	53.41	2.42
Tipaerui	2	12	170	*lignaria*	2.6	32.6	3.00	18.00	10.46	58.08	9.47	7.44	78.61	52.53	3.34

across all samples, this method is justifiable.) Multiplying each score by the appropriate percentage, then adding the wieghted scores, I calculated a mean color index of 3.03 for *P. o. sinistrorsa* in Tenaire Valley. For banded shells, color was scored for the unbanded portion of the shell only. I called the color index 'darkness.'

Because of the many morphs, color could have been coded as more than one variable. However, Murray & Clarke (1976a, 1976b) have shown that, for two Moorean species of *Partula*, all genetic loci determining shell color are so tightly linked that they are best considered a single "supergene." On the working assumption that the same applies for *P. otaheitana,* color was restricted to a single variable.

Thus, with an average value for every shell variable for every subspecies-valley, a 62 × 11 data matrix was filled (Table 1, columns 6–16). There are several problems with these data. In order to evaluate differences among valleys with regard to mean shell variables, the samples should be comparable in number of individuals, area sampled, location within each valley, and vegetation cover. Unfortunately, those conditions do not apply to Crampton's collections. Sample size ranged from 2 for *P. o. rubescens* in Faaripoo Valley to 988 for *P. o. amabilis* in Pirai Valley. Crampton gave no indication as to either the area collected or the time spent collecting in each valley. Presumably both varied greatly depending on terrain, vegetation cover, number and identity of collectors, weather, local abundance, etc. Dates of collection ranged from February to August, but since *Partula* shows determinate growth (reaching a final adult size), variation in the date of collection seems irrelevant.

Despite imperfections, the data were considered worthy of analysis for several reasons. First, the general exploratory nature of this study permitted some leeway in experimental design. Second, although the errors introduced by noncomparable samples would obscure any clinal gradients in means of shell variables, the number of valleys was probably sufficiently large (50) that the mean of those errors would approximate zero, thereby leaving the clines unbiased. Third, the fact that samples were taken differently in each valley would similarly tend to iron out errors due to a dry spot in a wet valley or *vice versa.* Finally, it is unlikely, even in Crampton's time, that any sampling regime on Tahiti Nui could avoid all of the same deficiencies. Choosing a sample site or sites in each valley large enough to yield sufficient numbers of snails but small enough to avoid extraneous variation due to environmental gradients, interpopulational differences, and step clines would have been difficult then and impossible today due to habitat destruction.

Lacking actual meteorological data, I devised indices for relative insolation and relative rainfall. For relative insolation, the main axis of each valley was drawn by eye on Crampton's (1916) map of Tahiti Nui. The angular deviation, in degrees, of the axis from a north-south line constituted the insolation index for that valley (Fig. 3A). Thus the values ranged from 0 for north-south oriented valleys receiving the least sunlight (ignoring the effect of shading by the central peak) to 90 for east-west oriented valleys receiving the most sunlight. This variable was named 'insolation.'

For relative rainfall, the deviation of the mouth of each valley from the southeast was calculated. The angle at which a line drawn from the estimated mouth of the valley to the estimated center of the island subtended a northwest-southeast line constituted the rainfall index for that valley (Fig. 3B). Thus the values ranged from 0 for highest rainfall to 180 for lowest rainfall. The variable was named 'low rainfall.'

Size of each valley might mediate the effects of insolation and rainfall on temperature and humidity. Wider valleys might be warmer due to increased insolation. While it is logical to think that wider valleys would be drier, their greater extent of water-retaining flatlands retain runoff longer and hence they could be more humid (Crampton, 1916). Crampton categorized each valley according to size on a scale of 1 to 4 ranging from widest to narrowest valleys (Fig. 3C). I named this variable 'valley smallness.'

Obviously, these three enviromental indices (Table 1, columns 2–4) are gross approximations which ignore the wide range of insolation and rainfall present within any given valley. Nevertheless, their use can be justified by arguments similar to those used in justifying the use of mean shell measures for each subspecies-valley. First, in a search for general trends, some incertitude of data is permissible. Second, although the indices theoretically measure gradients occurring at sites with identical elevation, valley side, aspect, and vegetation, deviations of individual sites from the means of these criteria would tend to

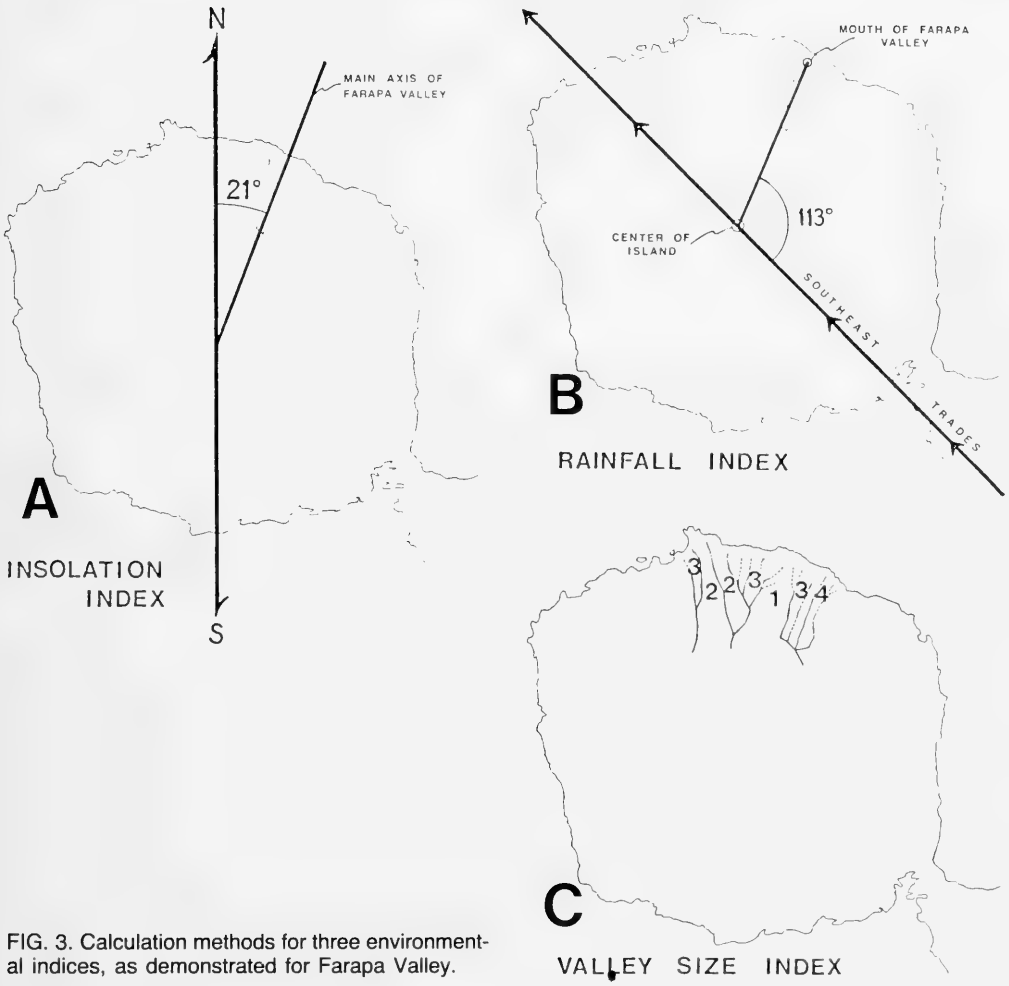

FIG. 3. Calculation methods for three environmental indices, as demonstrated for Farapa Valley.

cancel each other out, leaving the gradients obscured but unbiased. Finally, climatic selection on snail populations is probably effected by an unknown mix of longterm average trends in insolation and rainfall (mean selection) and occasional cataclysms (crisis selection), neither of which is easily measured. It may well be that the derived indices are the best estimates both of long-term trends and of cataclysms, short of setting up and monitoring a weather station in each valley at prohibitive expense.

All statistical analyses employed BMDP-77 computer programs (Dixon & Brown, 1977). For the sake of simplification, the 11 shell variables were subjected to a factor analysis (Tables 2 and 3), a method which combines each group of highly intercorrelated variables to form a new single variable (factor). For example, the four measurements shell length and width, and aperture length and width, were highly intercorrelated, and therefore they were combined, each appropriately weighted, to form a single factor which I named 'shell size.' The total factor analysis resulted in condensing 11 shell variables to seven shell factors (Table 2). These were used as new and more independent variables for further analysis. The relationship between the three standardized environmental indices and the seven standardized shell factors was investigated by two different procedures. First, each shell factor in turn was regressed on the three environmental indices by stepwise linear regression. In order for this test to be valid, the data must conform with several

TABLE 2. Rotated factor loadings of seven named factors extracted from 11 shell variables of *Partula otaheitana*. The seven factors explain 98.6% of the variance. Loadings <0.250 were replaced by zero. Oblique (D-Quartamin) rotation was used (see Table 3).

	Shell size	Shell squatness	Aperture roundness	% Sinistral	% Banded	Barrier size	Darkness
Aper. width	1.011	0.0	0.0	0.0	0.0	0.0	0.0
Aper. length	0.960	0.0	0.0	0.0	0.0	0.0	0.0
Shell width	0.957	0.0	0.0	0.0	0.0	0.0	0.0
Shell length	0.839	0.0	0.0	0.0	0.0	0.0	0.0
AL/SL	0.0	0.925	0.0	0.0	0.0	0.0	0.0
Shell W/L	0.0	0.804	0.315	0.0	0.0	0.0	0.0
Aper. W/L	0.0	0.0	0.984	0.0	0.0	0.0	0.0
% Sinistral	0.0	0.0	0.0	1.004	0.0	0.0	0.0
% Banded	0.0	0.0	0.0	0.0	1.000	0.0	0.0
Barrier size	0.0	0.0	0.0	0.0	0.0	0.998	0.0
Darkness	0.0	0.0	0.0	0.0	0.0	0.0	1.000

TABLE 3. Correlation matrix of shell factors of *Partula otaheitana*. Mean correlation = 0.239 (not significant), standard deviation = 0.157.

	Shell size	Shell squatness	Aperture roundness	% Sinistral	% Banded	Barrier size	Darkness
Shell size	1.000						
Shell squat.	−0.361	1.000					
Aper. round.	−0.115	0.180	1.000				
% Sinistral	0.441	−0.381	−0.127	1.000			
% Banded	−0.161	−0.374	−0.097	0.195	1.000		
Barrier size	−0.440	0.442	0.124	−0.210	−0.159	1.000	
Darkness	—0.438	0.065	−0.171	0.003	0.500	0.035	1.000

assumptions (Poole, 1974). The assumption of linear relationships between all shell-environment pairs was judged not violated based on visual examination of bivariate scattergrams. The assumption of orthogonality of predictor variables (environmental indices), a very commonly violated assumption (Green, 1979), was considered not violated on the basis of the low, non-significant correlations among the 3 indices, as shown below.

	Insolation	Low Rainfall	Valley Smallness
Insolation	1.000		
Low Rainfall	0.111^{ns}	1.000	
Valley Small.	0.008^{ns}	0.056^{ns}	1.000

The assumption of normality of each variable was tested by calculating kurtosis: a variable having a kurtosis value ≤3.00 was considered normally distributed. The only variable violating this assumption was the factor 'shell size,' which had a kurtosis value of 4.47. According to Green (1979), regression is a sufficiently robust technique that such minor violation is tolerable, so long as the distribution of the variable is strongly unimodal, as was the case in this instance. Second, a canonical correlation analysis was performed between the set of shell factors and the set of environmental indices. The assumptions of canonical correlation are satisfied by meeting the assumptions of regression.

RESULTS

The seven shell factors were easily named by examining their loading patterns (Table 2), which are relative measures of the contribution of each variable to the additive construction of each factor. The intercorrelations among the new shell variables (factors) were low (Table 3), indicating their relative independence.

Only three of the seven shell factors were

TABLE 4. Summary of stepwise regressions of seven shell factors on three environmental indices. Degrees of freedom were 1, 60 for F-to-enter. Significance levels are: *($p < 0.05$), **($p < 0.01$), ns ($p > 0.05$).

Shell factor	% Variance explained by regression	Initial F-to-enter values		
		Low rainfall	Insolation	Valley smallness
Shell size	0.0	0.026ns	1.731ns	0.547ns
Shell squatness	19.8	5.822*	9.091**	1.532ns
Aper. roundness	0.0	0.080ns	0.506ns	1.047ns
% Sinistral	0.0	0.174ns	0.075ns	0.155ns
% Banded	20.9	5.042*	10.847**	1.689ns
Barrier size	22.9	11.697**	5.933*	0.327ns
Darkness	0.0	1.549ns	2.769ns	0.744ns

significantly predicted by the environmental indices (Table 4). Neither shell size, aperture roundness, % sinistral, nor shell darkness was predicted. Shell squatness and apertural barrier size were both positively predicted by low rainfall and insolation. Insolation was most important in predicting shell squatness, whereas low rainfall was most important in predicting barrier size. Percent banded was negatively predicted by low rainfall and insolation, with insolation the most important. Each of the three significant regressions explained about 20% of the variance of its dependent variable. Valley smallness had no value as a predictor of any of the shell factors.

A single significant canonical variable was extracted which expressed a correlation coefficient of 0.71 between the two sets (Table 5). Strong positive loadings of insolation and low rainfall correlated with strong positive loadings of shell squatness and barrier size and with strong negative loading of % banded.

TABLE 5. Canonical variable loadings for set of environmental indices *vs.* set of shell factors. The single significant ($p < 0.001$) canonical variable gives a correlation coefficient of 0.710 between the two sets.

	First set
Insolation	0.809
Low rainfall	0.674
Valley smallness	−0.059
	Second set
Shell size	−0.189
Shell squatness	0.620
Aper. roundness	−0.071
% Sinistral	−0.010
% Banded	−0.648
Barrier size	0.646
Darkness	−0.357

The results of stepwise regression and canonical correlation were entirely complementary. Together they suggest that populations of *Partula otaheitana* in wetter, more shaded valleys tend to have shells more elongate, with a higher percentage of banding, and with lesser development of a parietal barrier than populations from drier, more sunlit valleys. This trend is depicted in exaggerated form in Fig. 4.

DISCUSSION AND CONCLUSIONS

Several aspects of this study may obscure a true relationship between environment and shell characters. First, as previously discussed, all variables used were averaged or indirectly estimated for each entire valley. Second, there were almost certainly a large number of other variables influencing shell characters (see Jones *et al.*, 1977, for a discussion of this problem with regard to *Cepaea*), including predation. For example, in contrast to Crampton's conclusion of zero predation above coastal elevations, Kondo (1973) mentioned a specimen of *Samoana burchi,* a species no less heavily shelled than *Partula otaheitana,* collected at 1250 m on Tahiti Iti (where *P. otaheitana* also occurs), the apical half of which "had been eaten by some animal." A. Solem (personal communication) reports extensive predation on *Partula* by rats throughout Tahiti. Third, the two "subspecies" *P. o. rubescens* and *P. o. affinis,* which are sympatric in 11 northeastern valleys, exhibit dissimilarities which may be interpreted as character displacement related to reproductive isolation mechanisms. *P. o.*

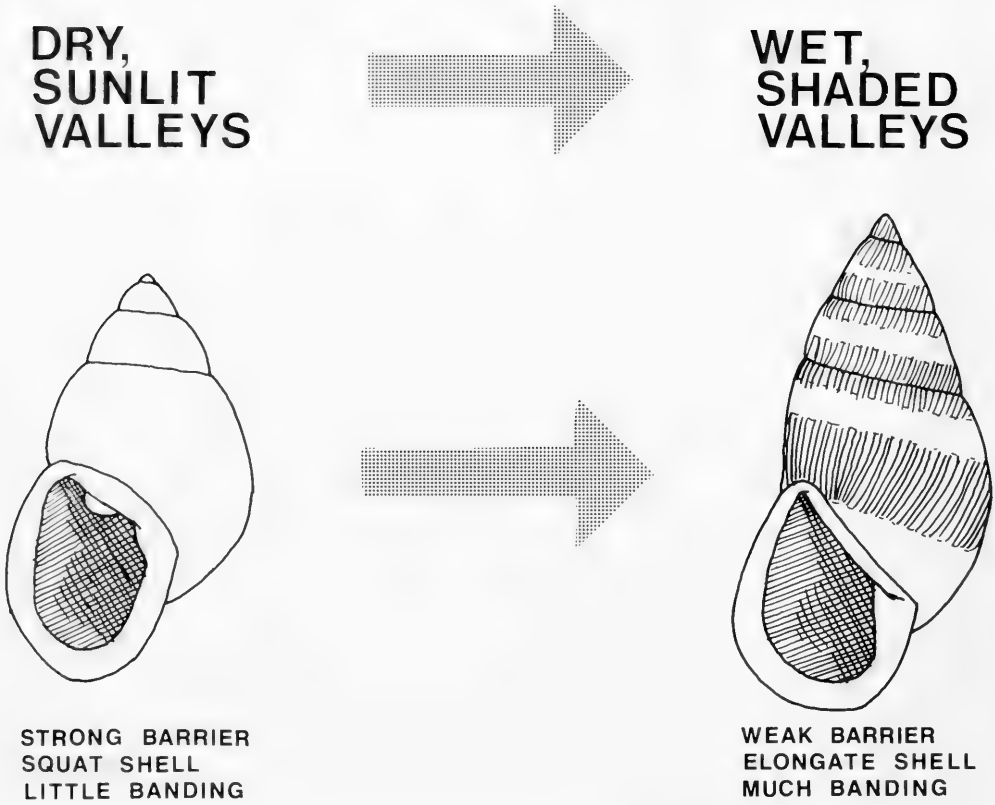

FIG. 4. Relational trends between enviromental indices and shell factors of *Partula otaheitana* on Tahiti Nui. Drawings exaggerate the trends by an approximate factor of 5.

rubescens is exclusively sinistral and never banded; *P. o. affinis* is predominantly dextral, sometimes banded (maximum 25%) and consistently smaller than *P. o. rubescens* (Crampton, 1916). Lipton & Murray (1979) found that opposite direction of coiling in *P. suturalis* is correlated with incompatibilities in mating behavior that "may result in partial reproductive isolation of dextral and sinistral animals." Whatever the cause of divergence between *P. o. rubescens* and *P. o. affinis,* the result is to obscure environmental correlations. Whether these are species, semispecies, or systematically confused remains to be determined.

It is noteworthy that, in spite of these obscuring aspects of the data, two of the three environmental indices explained (in a statistical sense) 20% of the variation in three of the shell factors. Correlation, of course, does not imply causation. As Sokal (1978) put it, "Phenotypic distributions are believed to reflect the distribution of selective forces through evolutionary history, but while undoubtedly true in general, this is quite difficult to demonstrate in a specific case. . . . Although causal links between such distributions are frequently assumed, . . . they are by their nature very difficult to prove." With that air of caution, it is worthwhile to examine whether shell characters of *Partula otaheitana* which are correlated with an environmental trend could feasibly have been caused by that trend, based on related evidence.

Apertural barriers in land snails have been assumed to function as protection against small invertebrate predators (Solem, 1976) or as aids in forming effectively placed mucous membranes to prevent desiccation (Emberton, unpublished). The apertural barrier of *Partula otaheitana* never reaches sufficient size to be very convincing as a block to predators (Fig. 4), yet the possibility cannot be ruled out that it discourages attack by unknown invertebrate predators or parasites. Mucous membranes are formed only around

the periphery of the aperture when the snails seal to the undersides of leaves, therefore the barrier is unlikely to be adaptive in this context.

Both shell squatness and low incidence of banding in some desert snail species have been construed as adaptations to dry, sunny conditions (Yom-Tov, 1971; Schmidt-Nielsen *et al.,* 1977). *Partula* is found only under humid, shady conditions. Such conditions, however, are relative. *Partula* may be sufficiently sensitive that slightly less humid regimes exert selective pressures similar in kind but not degree to those experienced by desert snails.

Another hypothesis to explain some of the trends depicted in Fig. 4 was suggested by Alan Solem. Incremental shell growth to the edge of the aperture can occur only while the snail is extended, but thickening of the shell at any growth stage and size increment of the apertural barrier in adults can occur while the snail is retracted, sealed to a leaf, and quiescent. Snails in drier valleys would spend a greater percentage of time in quiescence than snails in wetter valleys. Hence, assuming that new shell is formed by both active and quiescent snails, those in drier valleys could have more squat, less elongate shells with a thicker apertural barrier as a growth byproduct. This hypothesis may invoke non-selected variation to explain phenotypic trends, and could be tested. For example, newly-born siblings could be reared under different humidity regimes. It is relevant that Pollard (1975) demonstrated a negative correlation between rainfall and shell thickness in *Helix pomatia* in a range of collections from southern England.

Are the same phenotypic trends evident in other species of *Partula*? *Partula hyalina,* the next most widely distributed Tahitian *Partula,* has not been analyzed by the methods of this paper but appears to have a distribution of shell shapes similar to that herein demonstrated for *Partula otaheitana.* Based on a total of 463 measured adult shells, Crampton concluded that "(shells) in the north are somewhat shorter . . . far broader . . . (and) much stouter than the southern (forms) . . . [although] the relations mentioned are not invariable by any means." *Partula hyalina,* a species with an "unusually distinctive" phenotype, unlike other Tahitian *Partula*, is widespread on other islands (Crampton, 1916).

Partula clara, a species of much more restricted range, on the other hand, showed the opposite phenotypic distribution. Shells of the southern quadrant of Tahiti Iti "on the whole are the stoutest group" (Crampton, 1916). It may be relevant that Crampton deduced *P. clara* had been a rare species which had, within 50 years, profusely expanded its range and numbers.

The other three species of Tahitian *Partula* were highly restricted in range.

Conchological trends in Moorean *Partula* are also inconsistent. Lundman (1947), when mapping Crampton's data, discovered a cline in *Partula taeniata* on Moorea which approximates the trends reported here for *P. otaheitana* on Tahiti Nui. Shells of *P. taeniata* were larger and longer in the northeast, broader in the extreme north, narrower in the south; aperture width/length graded from small in the southeast to large in the northwest; barrier size graded from absent in the south to weak in the north. Nearly all shells were dextral, and no trends in banding were apparent. On the other hand, *P. suturalis,* sympatric with *P. taeniata,* showed opposite, though weaker clines in shell size, length, and width, but it showed the same clinal trend as *P. taeniata* in aperture width/length. Dextral shells of *P. suturalis* occurred in the southeast, sinistral in the northwest and northeast.

These conflicting trends lead to two possible interpretations. First, the correlations between environment and shell shape occur fortuitously as a byproduct of clines in shell shape produced by restricted gene flow, for example. Or, second, that the correlations occur by way of direct causal relationship between environment and shell shape which can be masked or overridden by rapid migration, interspecific competition, etc. Choosing the correct one of these alternatives would be a difficult if not impossible task, but could have highly fruitful results. For example, it might be found that the *degree* to which environment and shell shape were correlated for a given species of *Partula* would indicate relatively how long that species had occupied its present range.

That the relationships between shell variables and environment in *P. otaheitana* on Tahiti Nui were invisible to Lundman's (1947) method of mapping characters speaks well for the power of the multivariate tests used in this paper. Green (1979) emphasized the value of multivariate analysis as an exploratory tool to detect basic trends in data. He recommended using more than one technique in order to safeguard against the pitfalls inherent in any one. The greater the number of dependent

variables in a series on which stepwise regression is performed, the greater the chance of concluding a significant regression exists when in fact it does not. Significance levels of the three significant regressions in Table 4 are probably low enough ($p < 0.01$, $p < 0.001$, $p < 0.001$) to assure that this error has not been made. The regression results are further reinforced by the clearcut results of canonical correlation analysis (Table 5). Because canonical correlation maximizes the correlation between two sets of variables, it may introduce spurious relationships or exaggerate real ones (Pimentel, 1979). Stepwise regression guards against those dangers, especially because it indicates the strength of the relationship as percent explained variance.

To return to the original question of whether shell characters of *Partula* are subject to natural selection, the question remains unanswered by this analysis. Nor does it answer the question of whether the shell characters are adaptive (whether selected or not). At most, this analysis raises the possibility that adaptation and natural selection have played a role here. The distribution of partulid phenotypes is a fascinatingly complex one; the key to its understanding doubtless lies neither in the extreme non-selectionist view of Crampton (1916) nor in a dogmatic adaptationist program (as criticised by Gould & Lewontin, 1979), but somewhere in between.

Partula otaheitana can still be found in moderate to high densities at middle to upper elevations on Tahiti (A. Solem, personal communication). Ecological fieldwork on these contemporary populations would provide both a valuable update to Crampton's work and a more empirical test of the trends detected in this analysis. *Partula* on Tahiti presents the same opportunity of an approachable natural experiment in evolution that first attracted Crampton over 70 years ago.

The evolutionary relationships among species of *Partula* on Moorea are now reasonably well understood, thanks to over 15 years of exemplary work by Murray, Clarke, and coworkers (for a review, see Murray & Clarke, 1980). Moorea and Tahiti are successive volcanoes formed over the same hot-spot and separated by movement of the Pacific Plate. Moorea is 1.65 ± 0.13 million years old, whereas Tahiti Nui is only 0.65 ± 0.22 million years old (Dymond, 1975). In many respects, then, Tahiti is a younger, less-eroded version of Moorea. Building a data base on Tahitian *Partula* comparable to that already existent for Moorean *Partula* would allow valuable and unique insights into the relationship between geomorphology and organic evolution.

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PLANKTONIC LARVAE OF NEW ENGLAND GASTROPODS. V. *BITTIUM ALTERNATUM, TRIPHORA NIGROCINCTA, CERITHIOPSIS EMERSONI, LUNATIA HEROS* AND *CREPIDULA PLANA*

Catherine Thiriot-Quiévreux[1] and Rudolf S. Scheltema[2]

ABSTRACT

The veliger larvae of *Bittium alternatum, Triphora nigrocincta, Cerithiopsis emersoni, Lunatia heros*, and *Crepidula plana* from plankton at Woods Hole, Massachusetts, U.S.A., are described for the first time. Characteristics are given to distinguish veligers of *Crepidula plana* from those of *C. fornicata*. Photomicrographs taken with a scanning electron microscope show details of the larval shells, and the living veligers of two species were drawn extended.

Key words: plankton; veliger larvae; New England; gastropods; *Bittium; Triphora; Cerithiopsis; Lunatia; Crepidula*.

INTRODUCTION

The larval stages of most prosobranch gastropods of the New England region (U.S.A.) are still unknown. Among the 42 species of prosobranch gastropods listed in the preliminary Woods Hole check-list (Russell-Hunter & Brown, 1964: 139–146), the mode of development for only 31 has been determined. Nineteen are known to have an indirect development with a planktonic larva, twelve develop directly without a planktonic stage, while the mode of development of the remaining eleven species is still unknown. Actually, the check-list of Russell-Hunter & Brown contains only the most common prosobranch species, less than half of those occurring in the Woods Hole region.

Veligers of 13 among the 19 Woods Hole species known to have planktonic larvae have been described thus far in published accounts. These include *Acmaea testudinalis* (Müller, 1776) (Kessel, 1964); *Anachis avara* (Say, 1822) (A. H. Scheltema, 1969); *Anachis translirata* (Ravenel, 1861) (Scheltema & Scheltema, 1963, as *A. avara*; A. H. Scheltema, 1969); *Crepidula fornicata* (Linné, 1758) (Werner, 1955); *Lacuna vincta* (Montagu, 1803) (Lebour, 1937); *Littorina littorea* (Linné, 1758) (Thorson, 1946 and references therein); *Nassarius* (*Ilyanassa*?) *obsoletus* (Say, 1822) (R. S. Scheltema, 1962); *Nassarius vibex* (Say, 1822) (R. S. Scheltema, 1962); *Nassarius trivittatus* (Say, 1822) (Scheltema & Scheltema, 1965). In addition there are illustrations of the larval shells of four other species, *viz. Caecum pulchellum* Stimpson, 1851, *Seila adamsi* (H. C. Lea, 1845), *Cerithiopsis greeni* (C. B. Adams, 1839), and *Mitrella lunata* (Say, 1826) (Thiriot-Quiévreux, 1980). The external morphology of the larvae of the six remaining species with planktonic development is known only from unpublished data or not at all. Thus, there remain even among the 42 common prosobranchs (including those for which the mode of development is still unknown) probably 15 to 20 undescribed planktonic veliger larvae.

Although the prosobranch larvae of the Western North Atlantic are poorly known, the same is also true for other regions of the world (*vide* Robertson, 1974, for a summary of regional studies). Only in restricted localities such as Plymouth, England (Lebour, 1937) the western Baltic (Thorson, 1946), Banyuls-sur-Mer on the French Mediterranean coast (Thiriot-Quiévreux, 1969, 1972), the Bay of Naples (Richter & Thorson, 1975), the North Sea (Fretter & Pilkington, 1970), off the South Island (Otago) New Zealand (Pilkington, 1976) and in Kaneohe Bay, Hawaii (Taylor, 1975) are the gastropod veligers well enough known that illustrations, summaries and descriptive keys are available for identification of larvae. Yet an understanding of the population dynamics and ecology of species with plankonic stages requires first of all that the larvae be recognizable in the plankton. It is the goal of the present series of papers to

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describe veliger larvae of all common gastropods in the Woods Hole region so that they may be identified in plankton samples.

METHODS

Three methods can be used to identify and describe gastropod veligers. The first of these is to collect spawn from known females held in the laboratory and to grow their larvae through complete development (e.g. R. S. Scheltema, 1962; Christiansen, 1964; Struhsaker & Costlow, 1968). The second method is to collect larvae from the plankton and to rear them through metamorphosis to a juvenile stage that can be identified (e.g. Lebour, 1937; Thorson, 1946; Thiriot-Quiévreux, 1967a, 1967b, 1969). Finally a third method which has sometimes been successfully used is the comparison of unknown larval shells to the protoconch of identified juvenile or adult shells (e.g. R. S. Scheltema, 1971b, 1972; Thiriot-Quiévreux, 1974, 1975; Thiriot-Quiévreux & Rodriguez Babio, 1975). Previous contributions to this series have used the first method of rearing larvae from spawn. However, there are certain gastropod species that will not readily deposit eggs in the laboratory either in adequate numbers or with sufficient regularity or whose egg capsules are unknown and hence cannot be collected in the field. Because of the difficulty in obtaining their spawn, most of the larvae newly described here were taken from the plankton and reared in the laboratory.

Since the first paper in this series (R. S. Scheltema, 1962), scanning electron microscopy has revolutionized the study of gastropod larval shells (Fretter & Pilkington, 1971); Robertson, 1971; Thiriot-Quiévreux, 1972, 1980; Richter & Thorson, 1975). In the present contribution we have included micrographs of the larval shell of each species discussed using a Stereoscan 4 Scanning Electron Microscope (Cambridge Scientific Instruments).

The laboratory work was done in the Woods Hole region; samples were taken at the mouth of Hadley Harbor and the northwest entrance of Woods Hole channel. Plankton tows were taken with a net of 1/3-meter diameter and a mesh of 240 μm. Samples were diluted to a convenient volume in a large finger bowl and subsamples were viewed under a binocular stereoscopic microscope (Wild M-5); all gastropod veligers were removed with a pipette. Larvae were placed in small 3-cm petri dishes and periodically fed *Isochrysis* or *Phaeodactylum* grown for this purpose in unialgal cultures. Shells to be used for scanning electron microscopy were kept up to 3 months in neutral 95% ethyl alcohol.

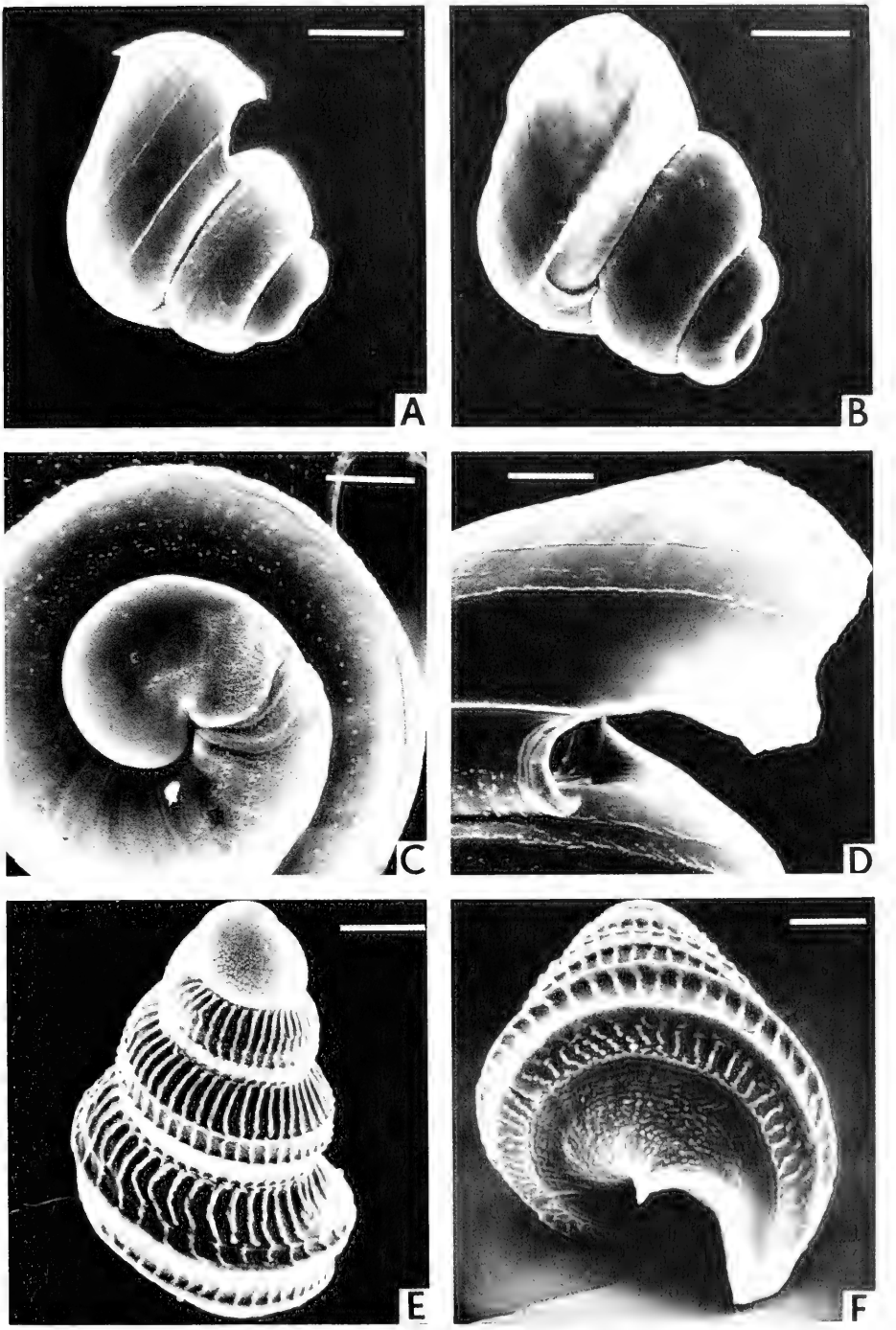
Previous descriptions of larval species within the same genus often aid in the later identification and description of closely related larvae. Each of the five species described here for the first time belong to genera for which other larval species are already known. This knowledge has aided in their identification.

DESCRIPTIONS OF LARVAE

Bittium alternatum (Say, 1822)
(Figs. 1A–D, 4B)

Shell: Dextral; 350 μm long in fully-developed veliger; 2-1/2 whorls; transparent, dark amber to brown; sutures of spire somewhat darker than rest of shell, columella dark brown; body whorl terminating in a rectangular protuberance or "sinusigerous" lip (Figs. 1A, D; Fig. 4B) resembling that in *Bittium reticulatum* (Thorson, 1946; Thiriot-Quievreux, 1969, 1974). With the scanning electron microscope the embryonic shell at the apex of the spire has a single smooth whorl (Fig. 1C); the second whorl has many small pustules particularly numerous near the sutures, and a single fine spiral granulated thread which decorates the lower part of the spire (Fig. 1A); on the body whorl of the larval shell are three

FIG. 1. Scanning electron micrographs of larval shells of *Bittium alternatum* (A–D) and *Triphora nigrocincta* (E–F). A) *B. alternatum*—larval shell showing spiral cords on body whorl. Scale = 100 μm. B) *B. alternatum*—shortly after metamorphosis. The edge of the sinusigerous lip is visible. Scale = 100 μm. C) *B. alternatum*— apical view of larval shell. Scale = 40 μm. D) *B. alternatum*—detail of body whorl showing sinusigerous lip. Scale = 40 μm. E) *T. nigrocincta*— larval shell showing embryonic whorl and typical axial and spiral ribs. Scale = 100 μm. F) *T. nigrocincta*—larval shell showing base of body whorl. Scale = 100 μm.


A
B
C
D
E
F

characteristic spiral cords (also seen with the optical microscope); the first is very marked and found on the upper half of the body whorl; the second and third are very fine and are on the lower part of the body whorl (Figs. 1A, D). After metamorphosis the postlarval shell growth completely surrounds the rectangular beak and two median spiral ridges are formed (Fig. 1B).

Soft parts: Velum bilobed, lobes circular and colorless; foot with dark pigmentation; operculum transparent; digestive gland greenish; two black eyes; two cephalic tentacles (Fig. 4B).

Remarks: This species is said to be replaced by *Bittium varium* (Pfeiffer, 1840) from Maryland and southward to Florida, the Gulf of Mexico and the West Indies to Brazil (Abbott, 1974, as *Diastoma*). The larva of the southern species is described by Thiriot-Quiévreux (1980) and differs in that it lacks the 3 spiral cords of *B. alternatum*.

Triphora nigrocincta (C. B. Adams, 1839)
(Figs. 1E, F)

Shell: Sinistral, 600 μm long when fully-developed; amber-brown; the specimens collected and examined had four whorls and appeared ready to metamorphose; embryonic portion of shell with 1 1/4 whorls, ornamented with closely spaced pustules which are denser at the periphery than in the center of the embryonic whorl; post-embryonic larval whorls with strong axial costae and one and then two spiral keels; the lower concave portion of the body whorl of the larval shell has a zone of axial threads followed by lines of tubercles which form concentric rings (Fig. 1F). The threads are most regular on the columella at the aperture.

Soft parts: Velum colorless; pigmentation of foot black with white patch on base.

Remarks: This species is apparently closely related to *Triphora perversa* (Linné, 1758) of Europe. Indeed, it sometimes is regarded as a subspecies of the latter (Johnson, 1934: 107; Abbott, 1974: 111). The larval shell also resembles superficially those described as *Triphora perversa* Thorson, 1946; Fretter & Pilkington, 1970; Thiriot-Quiévreux & Rodriguez Babio, 1975; Richter & Thorson, 1975). However, the European *Triphora perversa* is apparently a complex of four distinct species; the species described here most closely resembles *Triphora adversa* (Bouchet & Guillemot, 1978: 350, figs. 15–16). The systematics of the family Triphoridae in the Western Atlantic are clearly in need of further study. Larvae of the Triphoridae are known to be widely dispersed throughout the North and equatorial Atlantic and it is likely that at least some species will prove to be amphi-Atlantic in their geographic range (R. S. Scheltema, 1971a).

Cerithiopsis emersoni (C. B. Adams, 1838)
(= *subulatum* "Montagu" of authors)
(Figs. 2A–D)

Shell: Dextral; length 700 μm in fully-developed larvae; whorls ornamented with small tubercles arranged more or less in transverse rows (Figs. 2A, B); axial ribs at the end of the embryonic whorl. Later whorls lack transverse rows of tubercles but have instead regularly spaced opisthoclinal axial ribs; the periphery of the body whorl is characterized by a strong spiral thread (Figs. 2C, D). At the stage close to metamorphosis, the shell is dark red brown and has five whorls.

Soft parts: Early planktonic larva has colorless bilobed velum, roundish foot without pigmentation, transparent operculum, and dark pigmentation on head. Late larva with velum slightly tetralobed and with fine edge of red-brown pigment. Small dots of dark pigment appear on head, the anterior part of body and foot.

Remarks: Spawn was obtained from animals held in the laboratory; in the natural environment it is found on stones, sponges, and bryozoa. Egg masses convex, circular and gelatinous, 1.85 mm in diameter, smooth and very transparent at time of spawning; the mass rapidly becomes opaque from small debris which the adult places upon it. The eggs are resilient, yellow, inside a membrane and about 150 μm in diameter. An egg mass contains about 50 eggs. The emergence of larvae from the egg mass occurs after 15 days at room temperature (ca. 20–23°C.). When the larva escapes it has a brown shell with 1-1/4 to 1-1/2 whorls (Fig. 2A).

The species of *Cerithiopsis* here described differs from British species in details of ornamentation (Fretter & Pilkington, 1970) but resembles the protoconch of a Pliocene specimen of *Cerithiopsis emersoni* illustrated by Olsson & Harbison (1953, pl. 49, fig. 1).

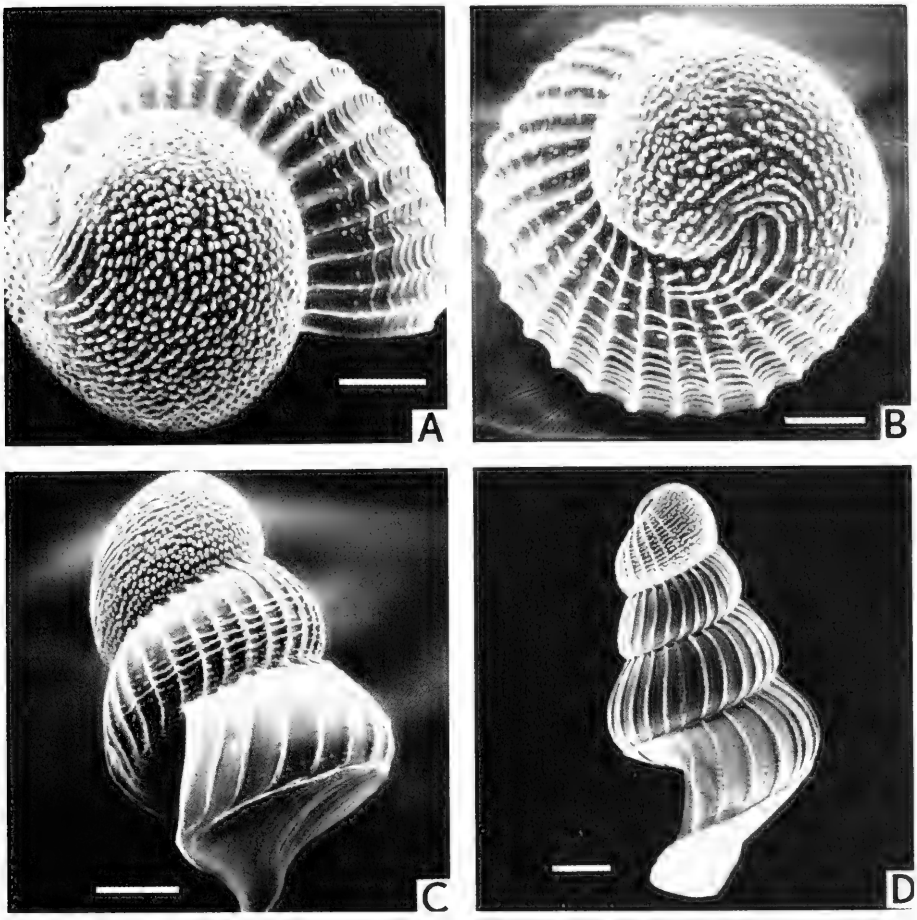


FIG. 2. Scanning electron micrograph of larval shells of *Cerithiopsis emersoni*. A) Larval shell at the time of emergence from egg capsule, apical view. Scale = 40 μm. B) Larval shell during early planktonic stage, apical view showing initial embryonic whorl. Scale = 40 μm. C) Shell of intermediate-stage larva about midway in development. Scale = 100 μm. D) Larval shell at time of metamorphosis. Scale = 100 μm.

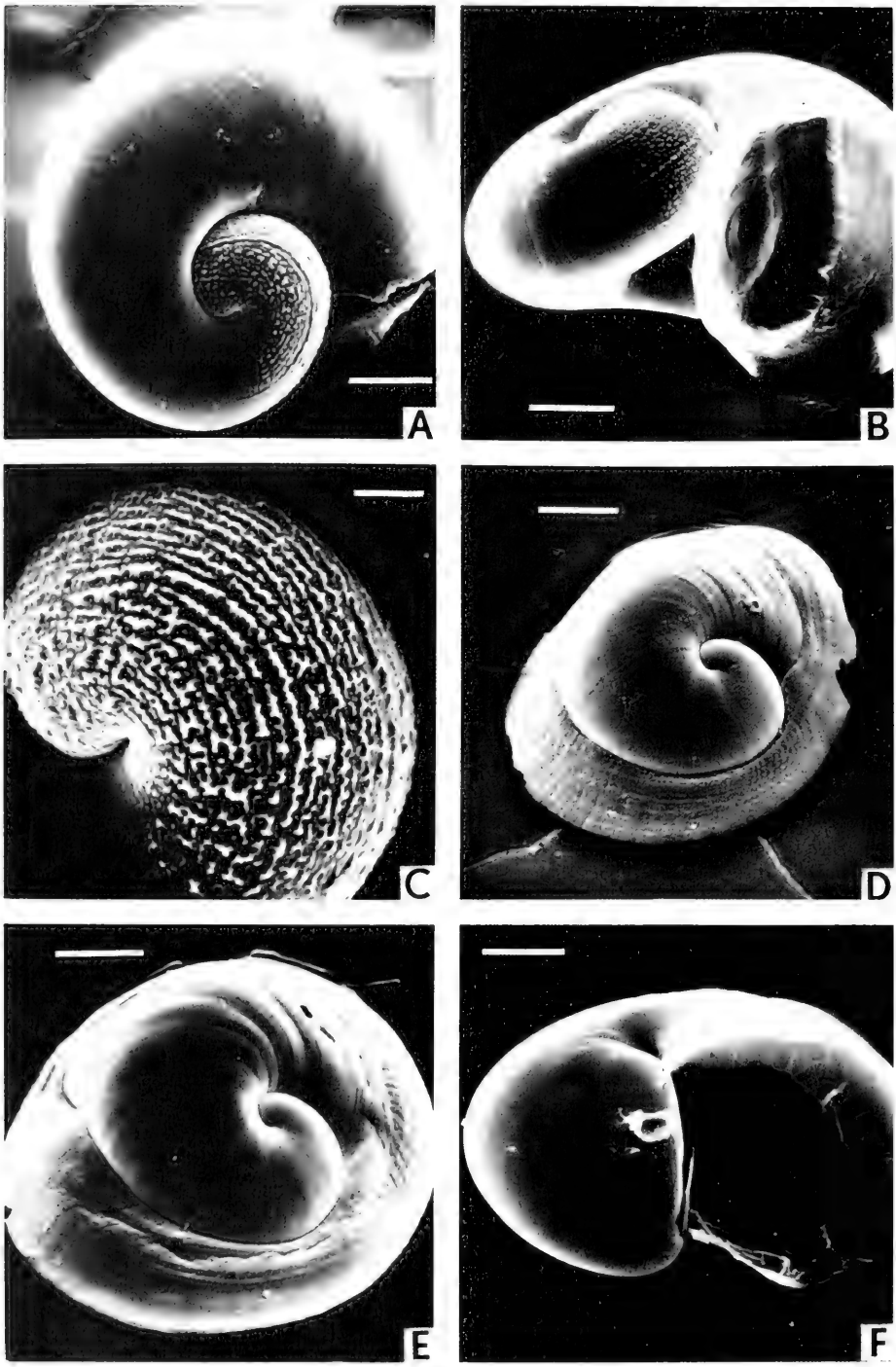
Lunatia heros (Say, 1822) (Figs. 3A–C, 4A).

Shell: Dextral; length at settlement 800 μm; globose, holostomatous; attaining 1-1/2 whorls at metamorphosis (Fig. 3A) with distinct umbilicus. There is an opaque spiral operculum. Embryonic portion of shell has granular spiral lines which are more or less continuous and that appear as fine threads under light microscope; the remaining portion of the larvel shell is smooth.

Soft parts: Velum is four-lobed (Fig. 4A). The lobes are slender and flexible when development is completed; they have a fine darkly-pigmented edge and elongated dark red pigment spots at their extremities. Dark pigmentation occurs above the mouth between velar lobes.

Remarks: This species can be readily distinguished from other naticid larvae in the Woods Hole plankton by the spiral threads on the embryonic shell and the pigment spots on the end of each velar lobe. In all other commonly occurring forms the embryonic shell does not have these spiral lines.

The complete development has been previously studied and described from larvae grown from egg collars deposited by snails in the laboratory (Dacy, 1965). The egg collars are described by Giglioli (1955). Development


A
B
C
D
E
F

in laboratory culture was completed in 31–54 days; the average shell length "from the edge of the aperture to the opposite side when the larva was lying on its side" was 792 μm at the time of settlement (range 660–940 μm).

Lunatia heros larvae are remarkably similar to those of the European species *Natica alderi* Forbes [= *N. poliana* Chiaje, *N. pulchella* Risso, *N. nitida* (Donovan), *N. intermedia* Philippi] illustrated by Thorson (1946: 218, fig. 130) and Fretter & Pilkington (1970: 17, figs. 20A–D).

Crepidula fornicata (Linné, 1758)
(Figs. 3E, F, 4F–H).

Shell: About 760 μm in length at settlement; colorless; transparent; the embryonic portion smooth. As growth progresses the apical end of embryonic shell becomes overlain by the next succeeding whorl (Fig. 3F, Figs. 4F–H); shell more convex than *Crepidula plana* (see below); axial growth striae are distinct.

Soft parts: Velum bilobed; dark pigment along edge and with a variable number of yellow or yellow-green spots over its surface. Elongated yellow spots on mantle and bottom surface of foot; black pigmentation on head, foot and intestine; digestive gland translucent yellow.

Remarks: The veliger of this species is described by Werner (1955) and by Fretter & Pilkington (1970, 1971). There are three species of *Crepidula* common in the New England region, viz. *C. fornicata, C. plana* and *C. convexa*. Only the former two have planktonic larvae; in *Crepidula convexa* development is completed within the mantle cavity. Since larvae of *C. fornicata* and *C. plana* are easily confused with one another both are considered here even though the former has already been described elsewhere.

Crepidula plana Say, 1822 (Figs. 3D, 4C–E).

Shell: 650 μm at settlement; quite transparent as in previous species; final whorl of completely developed larva is less swollen and consequently not so convex or globose as *C. fornicata*. Embryonic shell at apex of larval shell not overlain by the succeeding whorl as in *C. fornicata* (compare Figs. 4C–E with Figs. 4F–H). Growth striae of embryonic and also later larval shell numerous and distinct. Teleoconch growth shows very fine spiral and axial striae forming a more regular pattern than in *C. fornicata*. Bandel (1975) described the shell of the newly emerged larva.

Soft parts: Velum bilobed with darkly pigmented edge; yellow spots on velum sometimes absent; head and foot with yellow spots, intestine with diffuse dark pigment; kidney easily seen through shell owing to dark pigmentation.

Remarks: Larvae of the two species of *Crepidula* described above are most reliably distinguished from one another by their shells. The shell of *C. fornicata* is convex as a result of growth in which the first (i.e. embryonic) whorl is slightly overlain by the immediately succeeding whorl whereas the shell of *C. plana* is more nearly flat (i.e. not convex) owing to the fact that the growth of the first post-embryonic whorl more or less is in the same plane as the embryonic whorl, i.e. the post-embryonic whorl does not conspicuously overgrow the embryonic shell. Less reliably the two are distinguished by pigmentation: yellow spots on mantle and a dark pigmented intestine in *C. fornicata*; no pigment on the mantle or intestine and dark kidney in *C. plana*.

ACKNOWLEDGEMENTS

We acknowledge with thanks the assistance of Jan A. Pechenik in the collection of field samples. Algal cultures for the work were maintained by Isabelle Williams to whom we are much obliged. Dr. Robert Robertson of the Academy of Natural Sciences of Philadelphia was helpful in a number of ways and allowed us free use of the collections in his

FIG. 3. Scanning electron micrographs of *Lunatia heros* (A–C), *Crepidula plana* (D) and *Crepidula fornicata* (E–F). A) *L. heros*—intermediate-stage larval shell showing spiral threads on embryonic whorl, a character distinguishing this species from others of the genus in the Woods Hole region. Scale = 100 μm. See also Fig. 4A. B) *L. heros*—larval shell at stage approximately the same as A, profile view showing characteristic operculum. Scale = 100 μm. C) *L. heros*—detail of embryonic whorl showing beaded nature of spiral threads. Scale = 40 μm. D) *C. plana*—larval shell at time of metamorphosis. Scale = 200 μm. See also Figs. 4C–E. E) *C. fornicata*—larval shell at time of metamorphosis. Scale = 200 μm. See also Figs. 4F–H. F) *C. fornicata*—larval shell at intermediate stage of development—profile view. Scale = 100 μm.

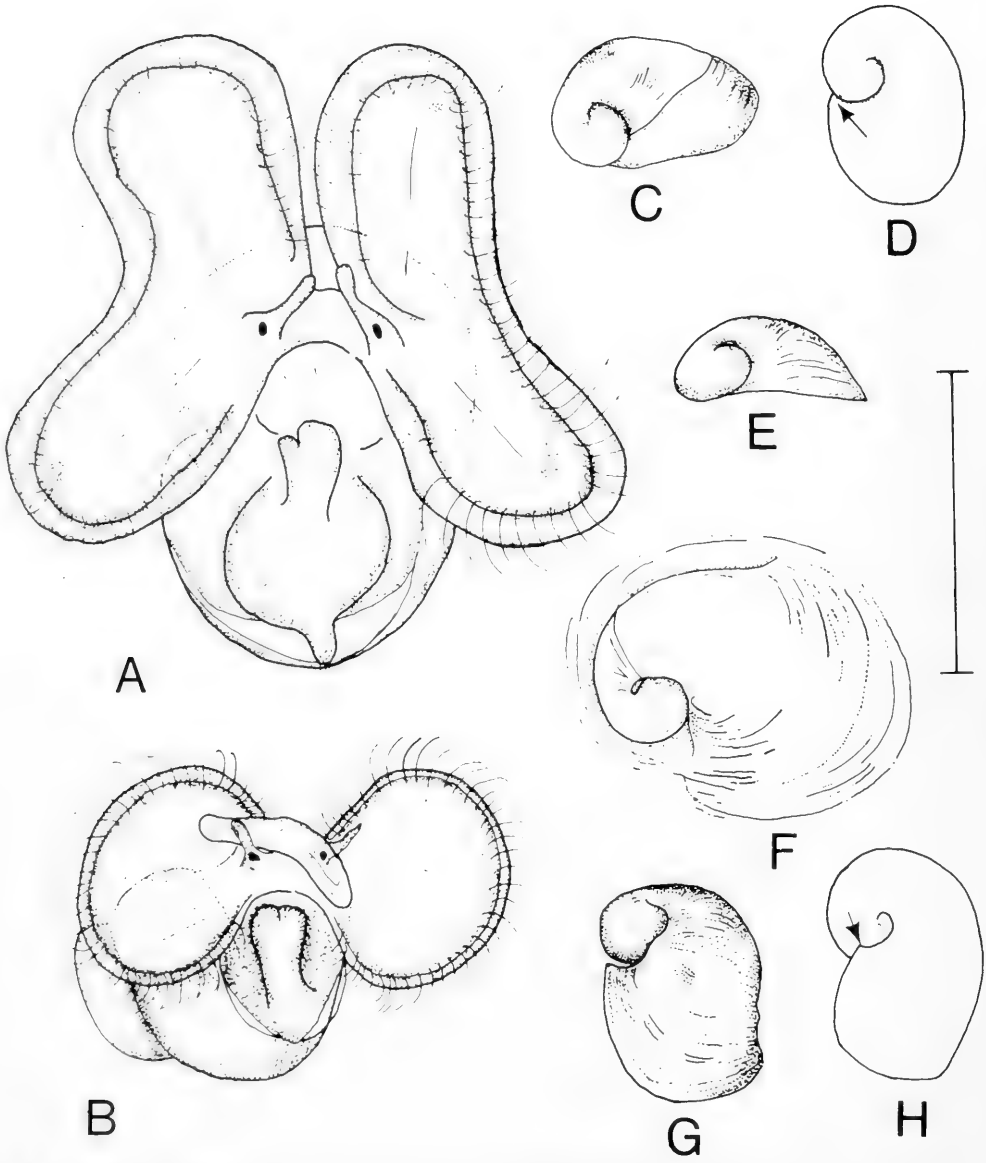


FIG. 4. A) Swimming veliger of *Lunatia heros* at an intermediate stage; the propodium is only partly developed. The velar lobes have not reached their definitive proportions; each will grow longer and the pigmented spots will increase in size and intensity before settlement. B) Swimming veliger of *Bittium alternatum*. The velum lacks coloration; the mesopodium is darkly pigmented. The shell terminates in a broad rectangular protuberance, the sinusigerous lip. The larva shown is at a stage shortly before metamorphosis; development of the foot is almost complete. C) *Crepidula plana*—shell fifteen days after release from female and just after settlement; compare with shell of *C. fornicata*, F below. D) *C. plana*—outline of settled larva showing growth of second whorl; compare with *C. fornicata*, H below. E) *C. plana*—settled specimen showing coiling; compare with *C. fornicata* below. Post-larval growth results in "flat" adult form through coiling in a single plane. F) *Crepidula fornicata*—shell thirty-six days after release from female and recently settled, showing apex; compare to C above. G) *C. fornicata*—twenty-nine days after release of veliger from female, showing embryonic whorl partly overlain by succeeding whorl; see also F and H. The more or less helical growth results in a higher more nearly convex adult shell than in *C. plana*; compare E and G. H) *C. fornicata*—settled larva showing growth of spire; overgrowth of succeeding whorl shown by arrow; compare with D above. Scale = 1 mm, applies to Figs. C–H only.

care. Alison Stone Ament kindly provided us with larval shells of *Crepidula fornicata* and *C. plana* grown in culture in connection with her own research. These reared larvae confirmed differences between the two species found in plankton samples. A Stereoscan 4 Scanning Electron Microscope (Cambridge Scientific Instruments) was made available by the Centre Océanologique de Bretagne at Brest, France. This research was supported in part by a grant from the U.S. National Science Foundation 0CE73-00439A02. This is contribution number 4098 of the Woods Hole Oceanographic Institution.

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REPRODUCTION IN A PERIPHERAL POPULATION OF *CYRENOIDA FLORIDANA* (BIVALVIA: CYRENOIDIDAE)

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ABSTRACT

Reproductive physiology of a marginal population of the bivalve *Cyrenoida floridana* (Dall) was studied in an attempt to determine the extent of adaptation to temperature conditions at the geographical range limit.

Results of the study indicate that the marginal population had extremely low spawning success, and that a second reproductive cycle was initiated in the fall but never completed. The second reproductive cycle is completed by populations in the ancestral parts of the range where winters are less severe. The disruptive effect of immigrant genes on adapted complexes are postulated to be responsible for the lack of reproductive adaptation exhibited by the population.

Key words: reproduction; Bivalvia; *Cyrenoida*; peripheral populations; geographic range expansions.

INTRODUCTION

Increase in a species' range may be mediated by a number of factors. First, the species may be introduced into an open habitat by unpredictable events. In this category one can include small-scale, random colonizations of islands (or habitat islands) by species propagules (MacArthur & Wilson, 1967; Diamond, 1969; Simberloff & Wilson, 1969, 1970; Williams, 1972; Simberloff, 1976) or large-scale, not necessarily random, invasions mediated by plate tectonics (Simpson, 1965; Mayr, 1970; Patterson & Pascual, 1972; Davis, 1979). Second, a species may expand its range over contiguous territory in response to ameliorating environmental conditions. This category would include range fluctuations brought about by periods of glaciation (Webb, 1969; Stehli & Wells, 1971; Clarke, 1973). Third, ranges can be increased by accidental or purposeful introductions by man (Elton, 1958). Hesse et al. (1951), however, point out that extension of the distributional range is also influenced by certain intrinsic attributes of the species, including genetic variability, physiological tolerance, and mode of reproduction and dispersal. Such intrinsic limitations may restrict the ability of a species to expand its distributional range regardless of opportunities presented.

Grant & Antonovics (1978) mention that marginal populations provide suitable units for the determination of processes involved in range extensions. In the same vein, marginal populations allow for the identification of factors limiting expansions of range. These factors may be related, among others, to physiological tolerance, geographic barriers, resource availability and biological interactions such as competition and predation (Crisp & Southward, 1958; Kinne, 1970; Jackson, 1972, 1974; MacArthur, 1972). Once the presence or absence of such factors has been established, it may be possible to predict the extent of future range expansions. For instance, increase in range within ecological time can be predicted for a species whose peripheral populations show no evidence of physiological stress—such as drastic population fluctuations, reproductive difficulties and growth stunting (Stearns & Sage, 1980) or biotic interference such as competitive or predatory limitation (Crisp & Southward, 1958).

Reproductive data gathered at a range margin can be used as a sensitive measure to ascertain the extent of adaptation of the peripheral population to local conditions. Timing of initiation of gametogenesis and spawning together with percentage of individuals spawned in the peripheral population can be compared with similar data from central populations to determine if the peripheral population is responding to novel environmental cues or whether the response remains essentially similar to that of central populations.

This study focuses on certain maladaptive

aspects of the reproductive cycle of a peripheral population of the intertidal bivalve *Cyrenoida floridana* (Dall) located on Canary Creek Marsh near Lewes, Delaware, U.S.A. Following an initial range description by Dall (1896), in which the bivalve was limited to Florida and southern Georgia, several range expansions have been reported. These reports trace the progress of the species from its ancestral range through the Chesapeake and Delaware Bays (Johnson, 1934; Wass, 1972; Leathem et al., 1976). Also, J. P. E. Morrison (National Museum of Natural History) collected the bivalve from a number of localities along the east coast from 1952 to 1954, including its northernmost limit in Cumberland County, New Jersey (unpubl. data). This relatively rapid range expansion seems coincident with, and can probably be attributed to, the construction of the Intracoastal Waterway. This series of canals probably also provides avenues of dispersal for juveniles to peripheral areas subsequent to local extinctions caused by periods of severe climate.

METHODS

The study site, Canary Creek Marsh, is located northwest of the town of Lewes, Delaware, and has been described by Gallagher (1971), Sullivan (1971) and Elliot (1973). The largest percentage of the marsh surface is covered by halophytic plants, and *Cyrenoida floridana* is found among the roots of this vegetation, buried to a depth of about 1 cm. Highest densities of the bivalve occurred at a level of 1.4 m above Mean Low Water in soils covered with a thin layer of filamentous algae which improved moisture retention (Kat, 1978).

Sampling of the marsh surface at locations with highest bivalve densities was conducted weekly from June to December, followed by a bi-weekly schedule from January to April. The samples were washed over two sieves with 2.4 and 0.4 mm meshes, and bivalves thus obtained were relaxed with magnesium chloride and fixed in Bouin's solution. This fixative is mildly acidic and dissolved the shell. Specimens measuring from 3.5 to 4.5 mm were embedded whole in paraffin, sectioned at 6–8 μm and stained with Harris' hematoxylin and Eosin Y (Humason, 1972).

Attempts were made to find populations north of Lewes at locations described by Morrison (unpubl. data). No cyrenoidas were found at any of these locations, even though Morrison's field notes indicated relatively high population densities.

RESULTS

Cyrenoida floridana is a simultaneous hermaphrodite: both types of gametes are produced concurrently during the entire reproductive life of an individual. Male and female sex cells are found in the same gonadal follicle, although there is a suggestion that spermatogenic cells are more numerous in the dorsal regions of the gonads.

Four major gonadal stages can be recognized in *C. floridana*: gametogenic, mature, spawned, and resorptive (Table 1). During early stages of gametogenesis the follicle walls are thick and compact, and the interior of the follicles present a disorganized picture, containing oogonia, spermatogonia, partition cells, pycnotic cells and amoebocytes. The follicle walls become progressively thinner as gametogenesis continues and the gonad expands in volume. Pycnotic cells and amoebocytes eventually disappear.

Both oogenesis and spermatogenesis are gradual processes. The progressive stages of spermatogenesis can be recognized by the successive predominance of spermatocytes, spermatids, and finally spermatozoa. Similarly, a number of characteristic changes occur in the appearance of the oocytes as they mature. At first, the nucleus is small, ranging

TABLE 1. Reproductive state of *Cyrenoida floridana* on Canary Creek Marsh, April, 1976 to March, 1977 (n = 125). Individuals classified according to predominant gonadal condition.

Month of Year	Resorptive or indifferent	Gameto-genic	Mature	Spawned
J	+			
F	+	+		
M		+		
A		+		
M		+		
J		+	+	
J		+	+	+
A			+	+
S	+		+	
O	+	+		
N		+		
D	+			

from 8 to 12 μm in diameter. The nuclei of small oocytes stain darker than their cytoplasm (Fig. 1). As the oocyte matures, the nucleus expands in diameter to measure about 25 μm, and the chromatin in the nucleus disperses so that the cytoplasm stains darker than the nucleus (Fig. 2).

The mature state of oogenesis occurs when large numbers of oocytes project into, or lie free within the lumina. Spermatogenic regions contain spermatids superimposed by clusters of spermatozoa. The follicle walls are generally thin, and follicle cell nuclei widely dispersed.

The spawned stage is easily recognized by the presence of spawned ova in the intralamellar brood pouches. Total spawning was not observed in any individual, as many gametes were retained within the follicles after spawning had occurred. Spawning to any degree was only observed in 9% (n = 65) of the bivalves collected during July, August and September.

The method of fertilization was not directly observed in *C. floridana*, but it is probable that, upon detection of spermatozoa in the water column, oocytes are cleared from the follicles into the lamellar brood chambers where fertilization takes place. Fertilization may also be partially internal. This was evidenced by the observation of embryos developing within the gonadal follicles, which indicates incomplete spawning of a fertilized egg. Whether this internal fertilization results from self-fertilization is unknown; however, a large number of spermatozoa which had undergone the acrosome reaction in response to mature oocytes (Dan & Wada, 1955; Popham, 1974) were found within the follicles from early June to the middle of September.

The resorptive stage is characterized by changes in the appearance of the gametes as well as the presence of amoebocytes within the follicles. Oocytes undergo autolysis during which the nucleus becomes almost transparent, followed by convolution and rupture of the nuclear membrane. Those oocytes attached to the follicle wall also show signs of cytolysis, during which light areas appear in the yolk. As these changes take place, amoebocytes invade the follicles and phagocytize the remaining gametes.

The reproductive cycle described above was repeated twice during the period of ob-

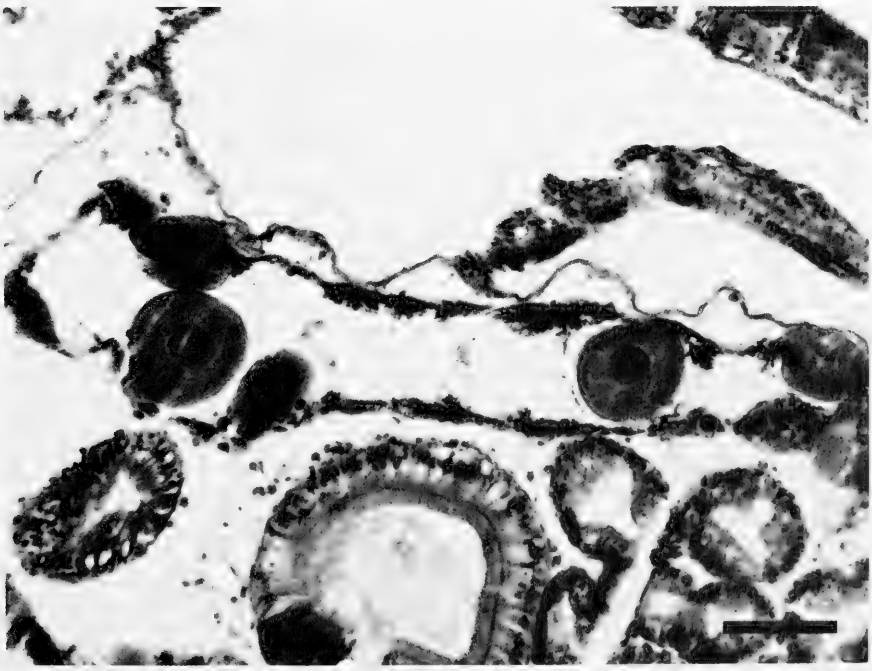

FIG. 1. Immature oocytes with nuclei which stain darker than the yolk. Note immature oocytes and spermatocytes attached to the follicle walls (scale bar = 100 μm).

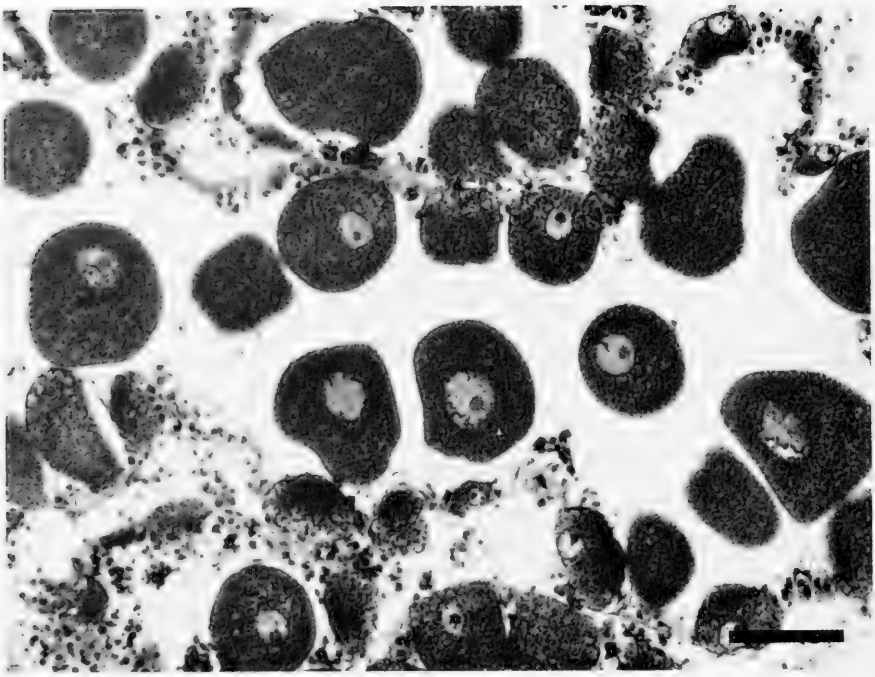

FIG. 2. Mature oocytes with nuclei which stain lighter than the yolk (scale bar = 100 μm).

servation. The first cycle began during the late winter, continued through spring and summer, and ended with resorption in early fall. The second cycle began with gametogenesis during the middle of fall, but maturity of the gonadal products was not reached, and resorption took place during the late fall. Spawning thus occurred only once, even though two cycles were initiated.

The second gametogenic cycle is of considerable interest because of its lack of completion. Most oocytes produced were irregular (Fig. 3), and cytolysis was evident in the larger oocytes after a short period of development. The follicle walls remained thick.

DISCUSSION

The low success of spawning observed in the peripheral population of *Cyrenoida floridana* (9%) could have been caused by several factors such as low density of individuals (Mayr, 1970; Soulé, 1973), unreliable temperature cues (Kinne, 1963; Korringa, 1957; Van der Schalie and Berry, 1973), an adverse effect of lowered temperatures on normal gametogenesis, and the possibility that self-fertilization caused reduced fertility (Paraense, 1959; Gee & Williams, 1965).

More interesting is the observed interruption of the second gametogenic cycle, which constitutes strong evidence of reproductive maladaptedness of the population. Reproductive physiology of *C. floridana* has not been reported from other parts of its range, but examination of individuals collected near Brunswick, Georgia, in December and St. Marks, Florida, in July revealed developing juveniles in the demibranchs indicating completion of the first and second reproductive cycles in central populations (Table 1). In addition, Subrahmanyam et al., (1976) mention that recruitment of juveniles takes place twice on Florida marshes. From these observations it can be concluded that the bivalve spawns twice in ancestral portions of the range, while it is limited to a single spawning in Delaware: a rather common observation for wide-ranging species (e.g. Stauber, 1950; Loosanoff & Nomejko, 1952; Butler, 1955; Prosser, 1955; Korringa, 1957; Kinne, 1970; Jackson, 1974; Frank, 1975). The difference between this study and previous studies of

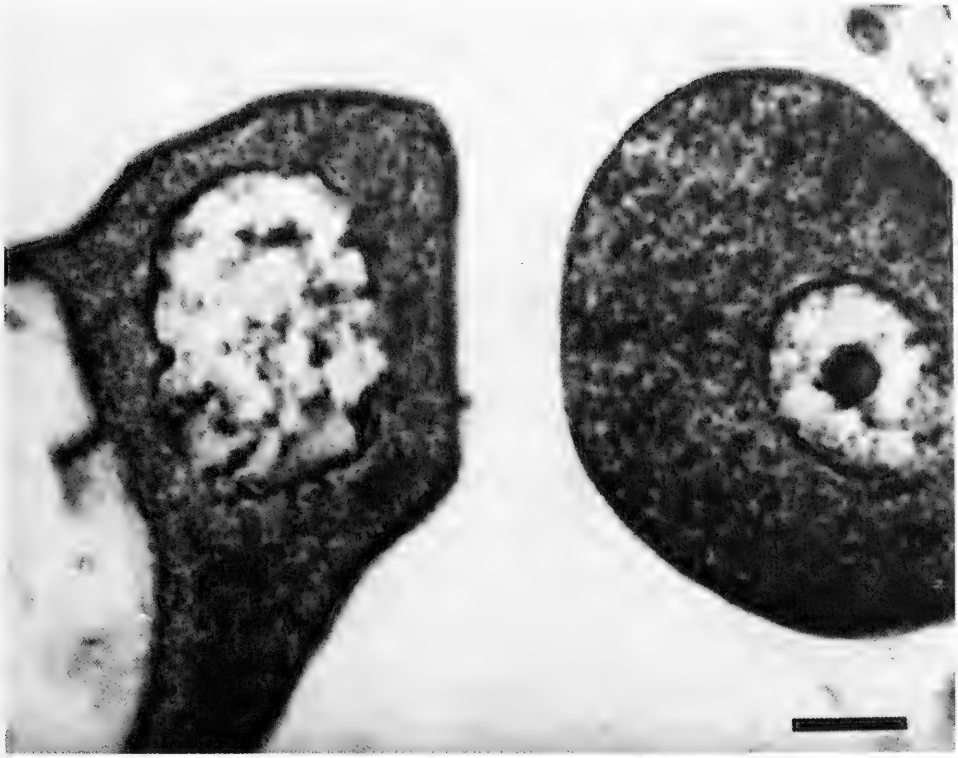

FIG. 3. Irregular oocytes produced during the interrupted second reproductive cycle. The oocytes both show signs of karyolysis during which the nuclear membrane becomes convoluted (right), then ruptures, followed by disappearance of the nucleolus (left) (Scale bar = 20 μm).

latitudinal variation in reproduction lies in the observation that a second reproductive cycle is still *initiated* by populations of *C. floridana* at the range periphery, but not completed.

The activity of phagocytic amoebocytes determines if wastage of reproductive energy is involved in the second reproductive cycle, and hence if it should be selected against. Purchon (1968) and Yonge (1928) have indicated that amoebocytes may play a role in digestive processes by transferring nutrients from the digestive tract to epithelial cells. While histological sections of the resorptive stage revealed high numbers of amoebocytes outside the follicle walls in addition to those engaged in phagocytosis, there was no evidence of a concentration of these amoebocytes within the digestive system or among epithelial cells. While reproductive material was phagocytized rather than voided through the gonadal ducts as in the oyster, for instance (Galtsoff, 1964), which indicates that some redistribution of energy is likely, wastage of this reproductive energy is nevertheless indicated, as efficiency of transfer along a series of steps is low.

During the severe winter of 1977, the population of *C. floridana* at Canary Creek suffered heavy mortality. A survey of the population taken before and after the winter showed that heaviest relative mortality occurred among the youngest age classes, and that population density decreased from an overall average across the marsh of 285 individuals/m^2 to 8.3 individuals/m^2. Such high mortalities seem common among peripheral populations (Welch, 1968; Gallagher & Wells, 1969), and the absence of populations of the bivalve from areas sampled by Morrison in 1954 suggest that such fluctuations might be repetitive.

Peripheral and marginal populations subject to frequent extinctions can be re-established from two main sources (Slatkin, 1977). Colonists can either be drawn from the central

population at random (migrant-pool model) or originate from refugia near the periphery of the range (propagule-pool model). Marginal populations formed by the migrant-pool model can be expected to contain the same genotypes as found in central populations, and, as such, be ill-adapted to range periphery conditions during severe years assuming limited phenotypic compensation. Marginal populations formed by the propagule-pool model can be expected to be better adapted, since their phenotypes have been subjected to physical selection pressures similar to those at the range margin (well-defined clines are associated with this model). In the case of *C. floridana*, the migrant-pool model seems to best explain the lack of reproductive adaptation to local conditions.

Survivors of catastrophic selection are probably extremely important in the formation of populations exhibiting physiological adaptations to local conditions (Miller, 1956; Lewis, 1962; Harper, 1977). However, survivors may face serious problems in finding mates, especially in sessile species. It has been pointed out that self-fertilization is a probable cause for reduced spawning success, and can consequently be expected to be selected against for this and other reasons in central populations (Paraense, 1959; Moore & Lewis, 1965; Lewis, 1966; Antonovics, 1968). However, as Ghiselin (1974) points out, it is better to self-fertilize than not to fertilize at all. When population densities of adapted individuals (derived from the survivor group) build up to levels where cross-fertilization again becomes possible, those individuals which preferentially cross-fertilize gain a selective advantage by virtue of their higher reproductive success. If, however, this population is flooded by individuals derived from the central population during climatically benign periods, cross-fertilization may reduce fitness by disrupting adapted gene complexes (Levin, 1970; Emlen, 1978).

The extent of the distributional range of a species with little phenotypic (in this case reproductive) compensation is dependent on its capability to differentiate into locally adapted populations (Bradshaw, 1965). If such differentiation is prevented by gene flow and fluctuating selective regimes which may be severe (directional) during some years and mild (non-directional) during others, such species will generally be restricted in distribution, and characterized by fluctuating range boundaries comprised of low-fitness populations. Clines in unisolated species of this kind will tend to be indistinct, both due to the effects of gene flow and selection gradients which migrate over the geographic range of the organism.

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EFFETS DES CONDITIONS D'ÉCLAIREMENT SUR LA CROISSANCE DE *LYMNAEA STAGNALIS* (GASTÉROPODE PULMONÉ)

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RÉSUMÉ

Lymnaea stagnalis a été élevée au laboratoire depuis l'éclosion jusqu'à la mort sous diverses conditions d'éclairement: photophase longue (16/24 h), photophase courte (7/24 h), obscurité, intensité lumineuse variable (1300, 180, 50 et 10 lux), lumières colorées (ultraviolette, bleue, verte, jaune et rouge).

En lumière blanche, l'élevage sous courte photophase se traduit par une augmentation de la longevité; la réduction de l'intensité lumineuse sous longue photophase produit le phénomène inverse. Seules les radiations rouges ont un effet positif sur la durée de la vie.

A intensité lumineuse égale, la croissance de la coquille est nettement augmentée quand l'élevage se déroule sous photophase courte (13.4% en moyenne); ce phénomène est encore plus net quand les animaux évoluent à l'obscurité (23.7%).

La réduction de l'intensité lumineuse ne se traduit par une diminution de la croissance que sous longue photophase.

L'élevage des limnées sous des radiations ultraviolettes, vertes et jaunes modifie peu leur croissance. Par contre sous les radiations bleues la croissance est diminuée alors qu'elle est augmentée sous les radiations rouges.

Dans tous les cas la croissance des coquilles peut être décrite par le modèle de von Bertalanffy et l'étude des paramètres k et t_∞ nous a permis de préciser les effets des différentes modalités d'éclairement.

Mots clefs: *Lymnaea*; croissance; éclairement; photophase; intensité; obscurité; longueur d'onde.

INTRODUCTION

Nous avons constaté chez *Lymnaea stagnalis* (Linn.) d'importants troubles de la croissance et de la fécondité lors d'intoxications à long terme par divers toxiques (Bluzat et al., 1979; Bluzat & Seugé, 1981; Seugé & Bluzat, sous presse). Il nous a paru intéressant de rechercher si des troubles du même ordre pouvaient être provoqués par des facteurs externes.

Dans un premier travail (Seugé & Bluzat, 1982), nous avons envisagé l'influence de la minéralisation de l'eau. Dans le présent article nous examinons les effets des conditions d'éclairement sur la croissance en étudiant successivement: la photophase, l'intensité lumineuse et la qualité de la lumière. Les conséquences de ces différentes condition d'élevage sur la potentiel reproducteur seront envisagées par ailleurs.

Des travaux très anciens indiquent que les limnées ne perçoivent pas les radiations rouges (Graber, 1884 dans Franc, 1968) et n'effectuent pas leur développement embryonnaire sous les radiations vertes (Yung, 1878 dans Franc, 1968).

Nous ne disposons pas d'autres informations relatives à ce sujet en dehors des investigations à court terme de van der Steen (1967) concernant la fécondité et d'un résumé des premiers travaux de Bohlken et al. (1978).

MATÉRIEL ET MÉTHODE

L'animal utilisé est le Mollusque Gastéropode Pulmoné *Lymnaea stagnalis* élevé depuis plusieurs générations au laboratoire dans l'eau de ville (pH: voisin de 7.5; dureté totale: 230 mg $CaCO_3$/litre; 20° ± 0.5 C; aération permanente); l'alimentation est assurée par des feuilles de laitue fournies en quantité suffisante et l'eau est renouvelée chaque semaine. Toutes nos expériences sont conduites avec des animaux groupés dans un bac de verre contenant deux litres d'eau. Trois cents jeunes limnées sont mises

en expérience le jour de leur éclosion; à cinq semaines elles sont mesurées pour la première fois et seuls les 40 individus de plus grande taille sont conservés. Une deuxième sélection est opérée de la même façon un mois plus tard: chaque lot est alors constitué de 12 limnées qui sont maintenues en expérience jusqu'à ce que 50% d'entre elles soient mortes; l'étude de chaque lot est donc arrêtée quand le nombre de limnées vivantes est ≤ 5.

Les élevages sous lumière blanche (tubes Mazda "blanc industrie" TFR/40/BBL) sont réalisés soit en longue photophase, 16/24 heures d'éclairement (séries 16) soit en courte photophase, 7/24 heures d'éclairement (séries 7). Différentes intensités lumineuses, mesurées à la surface des bacs, ont été utilisées: 1300 lux (16 – 1 et 7 – 1), 180 lux (16 – 2 et 7 – 2), 10 lux (16 – 3 et 7 – 3) et 50 lux (16 – 4). Une série d'animaux a été élevée à l'obscurité totale (0) à l'exception de courtes périodes nécessaires à leur entretien. Les élevages sous lumières colorées n'ont été conduits qu'en longue photophase (16/24 h): les gammes de radiations sont obtenues par des tubes lumineux spéciaux entourés d'un filtre de rhodoïd "R.P." de 0.25 mm d'épaisseur. Les tubes et les filtres utilisés sont les suivants: Lumière ultraviolette (U.V.): tubes TFA/4L/5 Mazda; pas de filtre; λ = 380–440 nm; 360 lux. Lumière bleue (B): tubes TF/40 Bleu Mazda; rhodoïd n° 2005; λ = 430–480 nm; 230 lux. Lumière verte (V): tubes TF/40 Vert Mazda; rhodoïd n° 2003; λ = 500–550 nm; 360 lux. Lumière jaune (J): tubes TF/40 Jaune Mazda; rhodoïd n° 222; λ = 550–630 nm; 1300 lux. Lumière rouge (R): TL/40/15 Philips; rhodoïd n° 227; λ = 630–670 nm; 50 lux.

Toutes ces intensités lumineuses ont été mesurées à la surface des bacs; il est en effet impossible d'apprécier l'intensité exacte reçue par chaque limnée.

Chaque mois la hauteur des coquilles est mesurée (Bluzat & Seugé, 1979); nous avons vérifié que la croissance pouvait être décrite selon le modèle de von Bertalanffy, $Tt = t\infty (1-e^{-k(t-to)})$, en construisant la droite de Walford (1946), taille au temps t + 1 en fonction de la taille au temps t; le paramètre k est égal au logarithme népérien de la pente de cette droite et une détermination graphique nous a permis d'évaluer to à −0.25 mois; $T\infty$, la taille maximale spécifique, est calculée d'après l'équation de von Bertalanffy. L'étude statistique des résultats (analyse de variance et test de Student) nous permet d'apprécier le niveau de sécurité des différences observées.

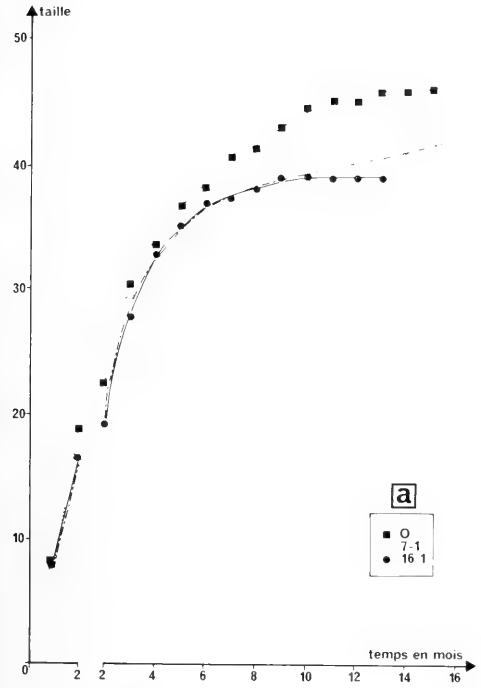
RÉSULTATS

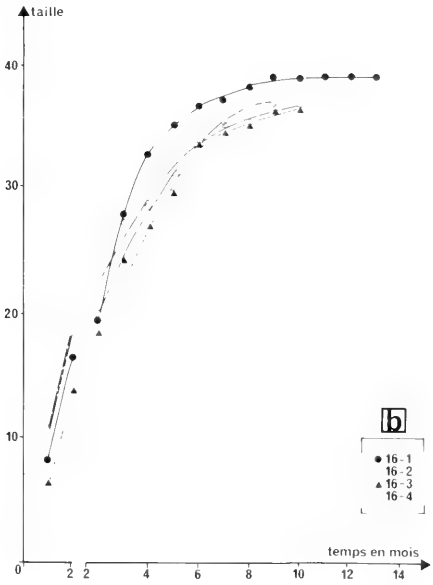
L'évolution de la taille des coquilles est représentée graphiquement dans les Figs. 1 et 2; les données concernant les deux premiers mois sont détaillées dans le Tabl. 1.

La Fig. 1 montre l'influence de la durée de la photophase (1a) ainsi que celle de l'inten-

TABL. 1. Tailles moyennes (T) des coquilles et erreur standard à la moyenne (S. E.), à l'âge de 1 mois (première sélection) et à 2 mois avant (a) et après (b) la deuxième sélection.

Conditions d'éclairage	Age: 1 mois		Age: 2 mois			
			a		b	
	T (mm)	S. E.	T (mm)	S. E.	T (mm)	S. E.
16 – 1	8.17	0.209	16.3	0.393	19.25	0.468
16 – 2	10.52	0.296	19.52	0.357	22.5	0.3
16 – 3	6.37	0.275	13.75	0.592	18.29	0.727
16 – 4	10.17	0.184	18.16	0.219	19.67	0.204
7 – 1	7.59	0.133	16.04	0.321	18.42	0.345
7 – 2	7.76	0.102	16.86	0.25	18.67	0.327
7 – 3	8.6	0.153	18.15	0.306	20.62	0.4
0	7.96	0.352	18.64	0.464	22.45	0.604
U. V.	12.15	0.219	21.37	0.255	23.08	0.286
B.	9.99	0.117	20.61	0.24	22.25	0.332
V.	10.74	0.173	20.74	0.25	22.42	0.254
J.	11.54	0.154	20.7	0.27	22.54	0.258
R.	10.47	0.122	21.55	0.229	22.87	0.132





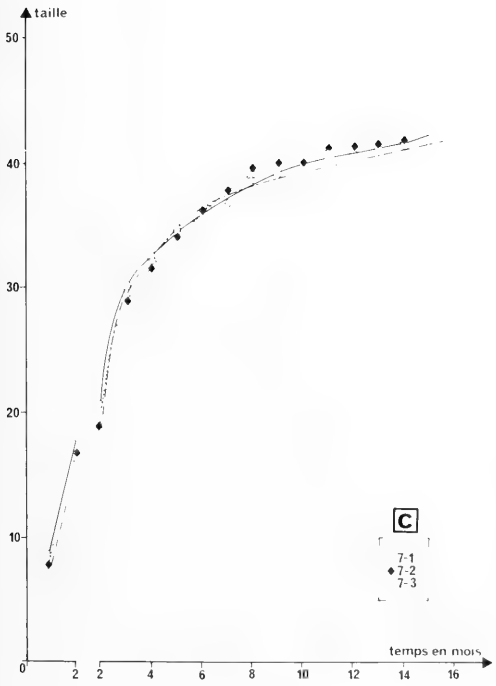


FIG. 1. Croissance de la coquille de *Lymnaea stagnalis* en fonction de la durée de la photophase et de l'intensité de l'éclairement: 1a: photophase (16 heures, 7 heures et 0 heure = obscurité); 1b: intensité lumineuse en photophase longue (16 – 1: 1300 lux; 16 – 2: 180 lux; 16 – 3: 10 lux; 16 – 4: 50 lux). 1c: intensité lumineuse en photophase courte (7 – 1: 1300 lux; 7 – 2: 180 lux; 7 – 3: 10 lux). L'interruption de l'axe des temps à 2 mois correspond à la deuxième sélection: le point de gauche indique la taille moyenne des limnées avant celle-ci; le point de droite précise la taille moyenne des 12 animaux les plus grands maintenus seuls en expérience.

TABL. 2. Effets de différentes conditions d'éclairement sur la durée de vie et sur la croissance des coquilles de *Lymnaea stagnalis*. k et t_∞: paramètres de l'équation de von Bertalanffy; tmx ob.: taille maximale observée et son erreur standard (S. E.); p: niveau de signification de la différence observée soit avec le témoin 16 – 1 soit avec le témoin précisé entre parenthèses.

Conditions d'éclairage	Durée où $12 \geq$ effectif ≥ 10 (mois)	Durée moyenne de vie (mois)	k	p	T_∞	Tmx ob.	S.E.	p
16 – 1	11	13.25	0.478	–	39.25	38.9	0.65	–
16 – 2	9	10	0.404	$p<0.001$	37.07	36.5	0.487	$p\simeq0.01$
16 – 3	6	10.5	0.276	$p<0.001$	38.56	36.3	0.789	$p\simeq0.02$
16 – 4	6	9.25	0.334	$p<0.001$	39.38	36.9	0.695	$p\simeq0.02$
7 – 1	14	17	0.390	$p<0.001$	42.2	42	0.428	$p\simeq0.001$
7 – 2	14	14.75	0.376	(7 – 1) n. s.	41.87	41.67	0.569	(7 – 1) n. s.
				(16 – 2) $0.05<p<0.1$				(16 – 2) $p<0.001$
7 – 3	15	15.5	0.393	(7 – 1) n. s.	42.78	42.67	0.843	(7 – 1) n. s.
				(16 – 3) $p<0.001$				(16 – 2) $p<0.001$
				$p<0.001$				$p<0.001$
0	14	15.5	0.338		46.22	45.95	0.509	
				(7 – 3) $0.001<p<0.01$				(7 – 3) $p<0.001$
U. V.	13	14	0.426	$p<0.001$	39.89	39.8	0.601	n. s.
B	10	11	0.574	$p<0.001$	35.55	35.5	0.666	$0.001<p<0.01$
V	8	11	0.503	$0.05<p<0.1$	38.25	38.1	0.391	n. s.
J	9	11	0.498	$p\simeq0.1$	40.55	40.3	1.534	n. s.
R	11	14	0.454	$0.02<p<0.05$	42.1	42	0.915	$0.01<p<0.02$
				(16 – 4) $p<0.001$				(16 – 4) $p<0.001$

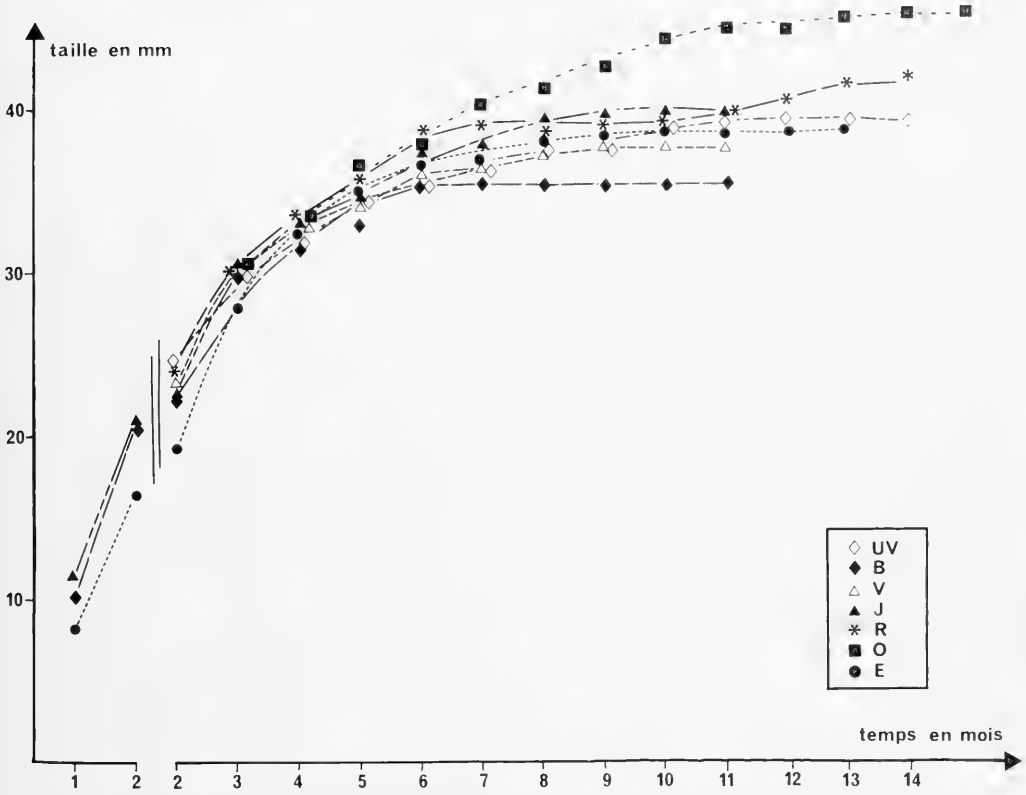


FIG. 2. Croissance de la coquille de *Lymnaea stagnalis* en fonction de la qualité de la lumière (photophase longue); au cours des deux premiers mois la croissance de tous les groupes (sauf E = 16 – 1) est à peu près identique. Abscisse: idem Fig. 1.

sité lumineuse en photophase longue (1b) et en photophase courte (1c).

La Fig. 2 présente l'évolution de la taille des limnées élevées sous diverses lumières colorées et permet la comparaison avec les témoins lumière blanche et les témoins obscurité.

L'étude détaillée de la croissance de ces coquilles permet de conclure que le modèle de von Bertalanffy reste applicable dans tous les cas. Le Tabl. 2 précise la durée moyenne de la vie des animaux de chaque groupe et rassemble les valeurs des paramètres k et $T\infty$ ainsi que celles des tailles maximales observées.

DISCUSSION

Survie

L'élevage de *Lymnaea stagnalis* se révèle possible sous toutes les conditions lumineuses éprouvées. Néanmoins, la durée de l'éclairement, son intensité et la qualité de la lumière modifient, dans un sens ou dans l'autre, l'espérance de vie des limnées (Tableau 2).

A intensité lumineuse égale, une courte photophase augmente nettement la longévité et le résultat obtenu avec les animaux élevés dans une obscurité quasi-totale va dans le même sens. Par contre, il faut noter que les intensités lumineuses faibles (16 – 2) et très faibles (16 – 3 et 16 – 4) sont défavorables à la survie des animaux en photophase longue.

Dans le cas des lumières de couleur la durée de vie est soit normale: ultraviolet et rouge, soit plus courte que celle du témoin lumière blanche (16 – 1): bleu, vert et jaune. Le résultat obtenu avec la lumière rouge est assez paradoxal car, l'intensité lumineuse étant faible (50 lux), une durée de vie relativement courte, du même ordre que celle des groupes 16 – 3 et 16 – 4 (10 mois environ)

était prévisible. La lumière rouge excerce donc probablement une action spécifique positive.

Croissance

En règle générale, nous avons constaté une bonne homogénéité de l'évolution de la taille des coquilles des limnées dans chacun des lots mis en expérience (Tableau 1 et Tableau 2; dans ce dernier seules figurent les S. E. relatives à la dernière taille moyenne calculée). Par ailleurs, il est important de souligner que la densité de population est restée stable (Tableau 2) pendant longtemps dans tous les cas et n'a pas pu influencer significativement la croissance (Forbes & Crampton, 1942). Dans le présent article l'évolution de la vitesse de la croissance en fonction de l'âge des animaux n'a pas été étudiée en détail; le calcul du paramètre k par la méthode de Walford (1946) en rend compte globalement.

Nous examinerons successivement l'influence de la longueur de la photophase, de l'intensité lumineuse et de la qualité de la lumière.

Longueur de la photophase

La Fig. 1a montre que pendant 10 mois la croissance des coquilles est tout à fait comparable dans les groupes 16 – 1 et 7 – 1. Au delà il n'en va plus de même; les animaux élevés en courte photophase ont alors une croissance plus importante: les tailles maximales observées sont très nettement différentes. La comparaison des séries 16 – 2, 7 – 2 d'une part, 16 – 3, 7 – 3 d'autre part conduit exactement à la même conclusion. Le modèle de von Bertalanffy permet de décrire dans tous les cas la croissance des coquilles; l'étude des paramètres k et $T\infty$ montre que leur valeur, pour une intensité lumineuse donnée, dépend de la longueur de la photophase (Tabl. 2).

Bohlken et al. (1978) ont signalé, chez *Lymnaea stagnalis*, que l'élevage sous courte photophase provoquait une croissance plus importante; nos résultats sont donc en plein accord avec celui de ces auteurs.

Intensité lumineuse

La Fig. 1b indique qu'en jour long une forte réduction de l'intensité lumineuse se traduit par une croissance plus faible; la différence est nettement sensible à l'âge de quatre mois.

La Fig. 1c met au contraire en évidence que la réduction de l'intensité lumineuse n'a aucun effet sur la croissance des animaux élevés sous courte photophase: les tailles maximales observées ne diffèrent pas.

L'étude des paramètres k et $T\infty$ de l'équation de von Bertalanffy confirme ces conclusions (Tabl. 2): (1) sous courte photophase les valeurs de k ne varient pratiquement pas quelle que soit l'intensité lumineuse; par contre, sous longue photophase, la valeur de k diminue avec celle de l'intensité I, ces deux variables étant liées par l'équation $k = 0.0975 \log I + 0.1763$ où $r = 0.997$ et $0.01 < p < 0.001$; (2) les valeurs de $T\infty$ sont très voisines des tailles maximales observées à l'exception de deux cas: 10 et 50 lux sous longue photophase (16 – 3 et 16 – 4); ceci souligne qu'une intensité lumineuse très faible sous longue photophase constitue une condition particulièrement aberrante qui perturbe profondément la croissance, comme le montre par ailleurs l'augmentation de la variabilité des tailles (Tabl. 1).

Cas de l'obscurité

Un élevage à l'obscurité pratiquement permanente peut être envisagé comme réalisé à la fois sous une photophase extrêmement réduite et avec un éclairage d'intensité pratiquement nulle. Dans ce cas nous constatons que la croissance est particulièrement importante (Fig. 1a); la taille moyenne maximale observée est de 45.95 mm (42.67 mm chez animaux 7 – 3) et la valeur du paramètre k particulièrement faible (0.338). Ce résultat paraît tout à fait compatible avec celui obtenu sous courte photophase et très faible éclairement et souligne qu'il existe encore une différence nette entre un élevage effectué sous une intensité aussi faible que 10 lux et celui réalisé à l'obscurité. Ces faits démontrent que les photorécepteurs de la limnée sont sensibles à des intensités très faibles; cette conclusion doit être rapprochée de celles de van der Steen (1967) et de Lickey et al. (1977) puisque ces auteurs ont précisé que *Lymnaea* et *Aplysia* perçoivent respectivement des intensités lumineuses de 1 et 2 lux.

Qualité de la lumière

Dans cette expérience nous avons négligé volontairement le rendement énergétique de chacune des gammes de radiations utilisées. Rappelons en outre que, si dans tous les cas la photophase est de 16/24 heures, l'intensité lumineuse n'est pas la même d'une lumière colorée à l'autre.

L'examen des courbes de croissance (Fig. 2) et des tailles moyennes maximales observées indique que seuls deux cas diffèrent significativement des témoins lumière—blanche (Tabl. 2): (1) la croissance est nettement plus faible (Tmx ob = 35.5 mm) sous lumière bleue; (2) elle est indiscutablement plus importante (Tmx ob = 42 mm) sous lumière rouge. Il faut noter toutefois que la croissance semble toujours se dérouler normalement dans la mesure où les tailles maximales spécifiques calculées restent très proches des tailles maximales observées; par contre, les valeurs du paramètre k sont, malgré des variations d'assez faible amplitude, spécifiques de chaque gamme de radiations.

Ces résultats nous permettent de répondre à l'une des questions que nous nous sommes posées: les limnées percoivent-elles les lumières ultraviolette, bleue, verte, jaune et rouge? Dans tous les cas la réponse est indiscutablement positive puisqu'aucun de ces résultats n'est identique à celui obtenu lors de l'élevage à l'obscurité.

La faible croissance observée dans le cas de la lumière bleue pourrait être éventuellement attribuée au niveau assez faible de l'intensité lumineuse (230 lux) mais la comparaison avec les animaux 16 – 2 (180 lux) nous conduit à conclure qu'il n'en est rien et que les radiations bleues exercent un effet dépresseur spécifique sur la croissance.

L'expérience qui s'est déroulée sous la lumière rouge constitue un cas particulier pour deux raisons: (1) la température s'est révélée être en moyenne de 21°C alors qu'elle a pu être maintenue à 20°C dans tous les autres cas, (2) l'intensité lumineuse était particulièrement faible: 50 lux.

Un élevage réalisé sous 16/24 heures de lumière blanche à la température de 22°C (Tmx ob = 39.9 mm et k = 0.478) nous permet de n'attribuer qu'un très faible effet à l'élévation de la température dans l'expérience sous lumière rouge et les résultats obtenus avec le témoin 16 – 4 (16/24 h de lumière blanche—50 lux) soulignent l'existence d'une action positive spécifique des radiations rouges sur la croissance.

L'ensemble de ces expériences montre que la durée de l'éclairement, son intensité et sa qualité agissent sur le déroulement de la croissance, sans doute par l'intermédiaire de divers récepteurs photosensibles; à notre connaissance la littérature scientifique ne nous offre aucun point de comparaison directe.

D'après les travaux de Geraerts (1973, 1975) et Joosse (1975) la croissance de la coquille de *Lymnaea stagnalis* est sous le contrôle d'une hormone (Growth Hormone) secrétée au niveau des L.G.C. (Light Green Cells) des ganglions cérébroïdes. Nos résultats suggèrent que des perturbations de cette sécrétion hormonale pourraient être induites par la longueur de la photophase, l'intensité de la lumière et sa longueur d'onde. Cette hypothèse s'appuie sur les travaux de Wendelaar Bonga (1971) et Roubos (1975) qui mettent en évidence un rythme journalier dans l'activité des cellules neurosecrétrices du cerveau de *Lymnaea stagnalis*. Dans des études antérieures nous avons déjà enregistré d'importants troubles de la croissance provoqués par des insecticides (Seugé et Bluzat, 1981) et des détersifs (Bluzat et Seugé, 1981); il nous paraît donc très vraisemblable que des perturbations du fonctionnement des cellules neurosecrétrices puissent être entraînées par des facteurs abiotiques.

CONCLUSIONS

Le présent travail a mis en évidence que la survie et la croissance de *Lymnaea stagnalis* sont perturbées par: (1) la durée de l'éclairement journalier; (2) l'intensité lumineuse; (3) la longueur d'onde de la lumière.

La croissance des coquilles est plus importante (13.4% en moyenne) quand l'élevage est réalisé sous photophase courte (séries 7 – 1, 2 et 3) que sous photophase longue (séries 16 – 1, 2, 3 et 4); un véritable gigantisme est obtenu quand l'élevage est effectué à l'obscurité: augmentation de la taille de 23.7% (témoin: moyenne des séries 16 – 1, 2, 3 et 4).

La croissance n'est pas affectée par la réduction de l'intensité lumineuse sous courte photophase; une conclusion opposée peut être formulée dans le cas d'un élevage sous longue photophase où la valeur du paramètre k de l'équation de von Bertalanffy est proportionnelle à l'intensité lumineuse.

Par rapport au témoin 16 – 1, en considérant la taille moyenne maximale observée, la croissance n'est pas significativement modifiée par l'élevage sous les lumières ultraviolette, verte et jaune. Par contre, elle est diminuée (8.7%) dans le cas de la lumière bleue et augmentée (8%) dans celui de la lumière rouge. Les valeurs du paramètre k mettent en évidence un effet spécifique de ces radiations.

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ABSTRACT

THE EFFECTS OF DIFFERENT LIGHT CONDITIONS ON THE GROWTH OF *LYMNAEA STAGNALIS* (GASTROPODA: PULMONATA)

J. Seugé and R. Bluzat

Pond snails, *Lymnaea stagnalis*, were reared in the laboratory, from hatching to death, under various light conditions: total darkness, two photophases (16/24 and 7/24 hours), four light intensities (1300, 180, 50 and 10 lux), five lights of different wavelengths (U.V., blue, green yellow and red).

With white light, rearing under short photophase induced an increase in longevity; the decrease of light intensity under long photophase led to an opposite result. The red light had a positive effect on life expectancy.

At constant light intensity, shell growth was clearly increased when rearing was achieved under short photophase (13.4%); this result was even more evident when the snails were kept in darkness (23.7%).

The reduction of light intensity induced a decrease of shell growth only under the long photophase.

The rearing of pond snails under U.V., green and yellow radiations only slightly modified growth. Conversely, under blue light shell growth was reduced whereas it was increased under red light. In all cases, shell growth could be described by von Bertalanffy's pattern; parameters k and T_∞ allowed us to point out the effects of the various lights.

MALACOLOGIA, 1982, 23(1): 63–73

GROWTH IN MARINE GASTROPODS: A NON-DESTRUCTIVE TECHNIQUE FOR INDEPENDENTLY MEASURING SHELL AND BODY WEIGHT

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ABSTRACT

A technique is described for obtaining non-destructive measurements of shell weight and body wet weight in marine gastropods. Shell weight is obtained by weighing whole animals in seawater and using a regression between these values and destructively sampled dry weights of shell. This weight may then be subtracted from whole weight in air, to provide an estimate of body wet weight. Shell weights are more accurately estimated than body weights. However, the mean cumulative error of this technique for estimating body weights is 10.6% for *Thais lamellosa*, 4.9% for *T. canaliculata* and 4.8% for *T. emarginata*. The possible application of this technique to other carbonate skeleton-producing invertebrates is briefly discussed.

Key words: Gastropoda; non-destructive measurement; Mollusca; shells; growth; *Thais*; carbonate skeletons; weight.

INTRODUCTION

Growth in molluscs may be assessed using several different quantities (Wilbur & Owen, 1964), the most common of which is one or more caliper measurements of shell size (Branch, 1974; Frank, 1965; Kenny, 1977; Randall, 1964; Spight, 1974; Yamaguchi, 1977). Other techniques, including laser diffraction (Strömgren, 1975) and total weight (Stickle & Duerr, 1970; Walne, 1958) have also been used to assess mollusc size. A drawback to these measures is that they measure attributes primarily of the shell and only indirectly those of the animal residing within it. Where shell shapes and thicknesses are very similar, such measures of size or weight are usually adequate if animals are growing actively. However, body size changes are not always paralleled by changes in shell size: decreases in body weight due to spawning or starvation will not be accompanied by concomitant decreases in shell size; shell growth may continue in some molluscs in the absence of feeding (Revelle & Fairbridge, 1957: 267; Rhoads & Young, 1970: 163; Zischke et al., 1970); and the spires of gastropod shells can often erode with increasing age (Spight et al., 1974). In these situations, shell size measurements will not provide an accurate estimate of body size.

To circumvent these problems, I have developed a non-destructive technique to separate body growth from shell growth in marine prosobranch gastropods. This technique relies upon two weight measurements: 1) a weight of the whole animal immersed in seawater, which ultimately provides an estimate of shell weight (see Havinga, 1928, and Nishii, 1965, for immersed weight estimates of shell weight in oysters; and Bak, 1973, for application to corals; Lowndes, 1942, has applied a similar technique to a number of invertebrates and vertebrates), and 2) a weight of the entire animal, shell plus body, in air. Subtracting the estimated weight of the shell from the total weight provides an estimate of the body weight, and the animal is still intact. Below, I describe the application of this technique to three species of thaidid gastropods, *Thais lamellosa* (Gmelin, 1791), *T. canaliculata* (Duclos, 1832) and *T. emarginata* (Deshayes, 1839), all inhabitants of North American rocky intertidal shores from Alaska to California (Ricketts et al., 1968).

In essence, this technique takes advantage of the specific gravity differences between shell and tissue. By weighing intact animals in two mediums of differing specific gravity (air and seawater), it is possible to separate the relative contribution of each component to the animal's total weight. It further takes advantage of two convenient attributes of gastropod molluscs: 1) the mantle is not attached to the shell, thus extrapallial fluid is not irrevocably trapped, and 2) it is possible to remove a sub-

stantial amount of the pallial water without damaging the animal. Thus the whole weight may be reduced to shell plus body weight, with a minimal amount of residual extravisceral water.

MATERIALS AND METHODS

'Immersed weight,' or the weight of whole animals in seawater, was obtained by placing them on a 3 cm × 3 cm VEXAR® plastic screen platform, supported by and suspended from a fine copper wire that could be hooked directly to the underside of a Mettler P153 balance. The balance was placed on a stand that straddled the container of seawater in which the animals were to be weighed. By taring the balance to compensate for the weight of the suspended platform, actual weights of the immersed animals could be read with no correction. Snails were introduced individually using a pair of forceps and weights recorded to the nearest 0.001 g.

Because specific gravity differences were being used to separate shell weight from body weight, it was important to ensure that there was *no* air inside the mantle cavities of individuals prior to weighing them under water, otherwise shell weights could have been underestimated. A procedure used to minimize this possibility was to completely immerse the animals for 24–48 hours prior to weighing, since most animals appeared able to clear their mantle cavities of air over this period. Animals were also 'chased' into their shells with the tips of the forceps immediately prior to placing them on the immersed platform; this acted to squeeze much, though probably not all, of any residual air out of the mantle cavity. Air was detected as bubbles released by the withdrawing animal in fewer than 5% of the animals; these individuals were noted.

To obtain non-destructive estimates of shell weights from immersed weights, it was necessary to compute a regression of actual shell weight on immersed weight for all three species. For this purpose, I measured immersed weights for individuals from a size range of all three species. The shells were then broken open using a C-clamp to avoid uncontrolled shattering, and the fragments separated from the body and dried to constant weight at 80°C (Tables 2–4). The slopes of these regressions of destructively sampled shell dry weight on immersed weight (regressions 1–3, Table 1; Fig. 1) were then used to estimate actual shell weight from immersed

TABLE 1. Least squares linear regressions for shell and body weight estimates. Weights are measured in grams. N = number of individuals. See Figs. 1 and 2 for plots of the data for regressions 1–3 and 8–10 respectively.

Regression number	Species	N	Regression equation	R^2
Shell dry weight (Y) from immersed whole weight (X)				
1	*T. lamellosa*	27	$Y = 1.572X + 0.0162$	0.9998
2	*T. canaliculata*	21	$Y = 1.558X - 0.0075$	0.9995
3	*T. emarginata*	19	$Y = 1.530X + 0.0032$	0.9997
Body immersed weight (Y) from body dry weight (X)				
4	3 spp. pooled	16	$Y = 0.202X - 0.0008$	0.9946
Shell dry weight (Y) from corrected immersed whole weight (X)				
5	*T. lamellosa*	27	$Y = 1.598X + 0.0174$	0.9999
6	*T. canaliculata*	21	$Y = 1.600X - 0.0013$	0.9996
7	*T. emarginata*	19	$Y = 1.605X + 0.0017$	0.9993
Ash free dry weight (Y) from estimated body weight (whole wt.–shell wt.) (X)				
8	*T. lamellosa*	27	$Y = 0.1043X + 0.0180$	0.8050
9	*T. canaliculata*	21	$Y = 0.1974X + 0.0141$	0.9117
10	*T. emarginata*	19	$Y = 0.2514X - 0.0029$	0.9846
Log ash-free dry weight (Y) from log shell length (X)				
11	*T. lamellosa*	27	$Y = 2.940X - 5.426$	0.9277
12	*T. canaliculata*	21	$Y = 2.709X - 4.743$	0.9471
13	*T. emarginata*	19	$Y = 3.304X - 5.440$	0.9759

weight of the whole animal for each species. It is clear from the high R^2 values (0.9995–0.9998) that immersed weight can provide a very accurate, non-destructive estimate of actual shell weight.

Some care must be exercised in the application of a single regression to different species, however, since not all the weight of a snail immersed in seawater is due to the shell. The different slopes in regressions 1–3 (Table 1; Fig. 1) reflect differences in the amount of tissue. To assess the contribution of tissue weight to immersed weight, I separated a small number of individuals of all three species from their shells and measured the immersed weight of the bodies alone. These were then dried to constant weight at 80°C. Regression 4 (Table 1) describes how much a given dry weight of tissue weighs when immersed in seawater before it has been dried (dry weights were used here because they are much more accurate than attempting to uniformly towel-dry animals for wet weights in air). If the tissue dry-weight values of Tables 2–4 are multiplied by the slope of this regression, subtracted from the total immersed weights, and these corrected immersed weights (i.e. corrected for the contribution of body weight to immersed weight) regressed against destructively sampled shell dry weights, the differences between the three species disappear (regressions 5–7, Table 1). This is to be expected since the specific gravity of shell material should be essentially the same for all three species. However, since it is only changes in the amount of body weight *relative to the weight of the shell* that will affect the accuracy of the uncorrected estimate of shell weight, and also since the contribution of the entire body to immersed weight is at most 4% (4% for *T. emarginata*, less than 3% for *T. lamellosa* and *T. canaliculata*), such a correction will yield very slight differences in the estimated shell weights. In other words, since the entire body weight of an immersed animal amounts to less than 5% of the total

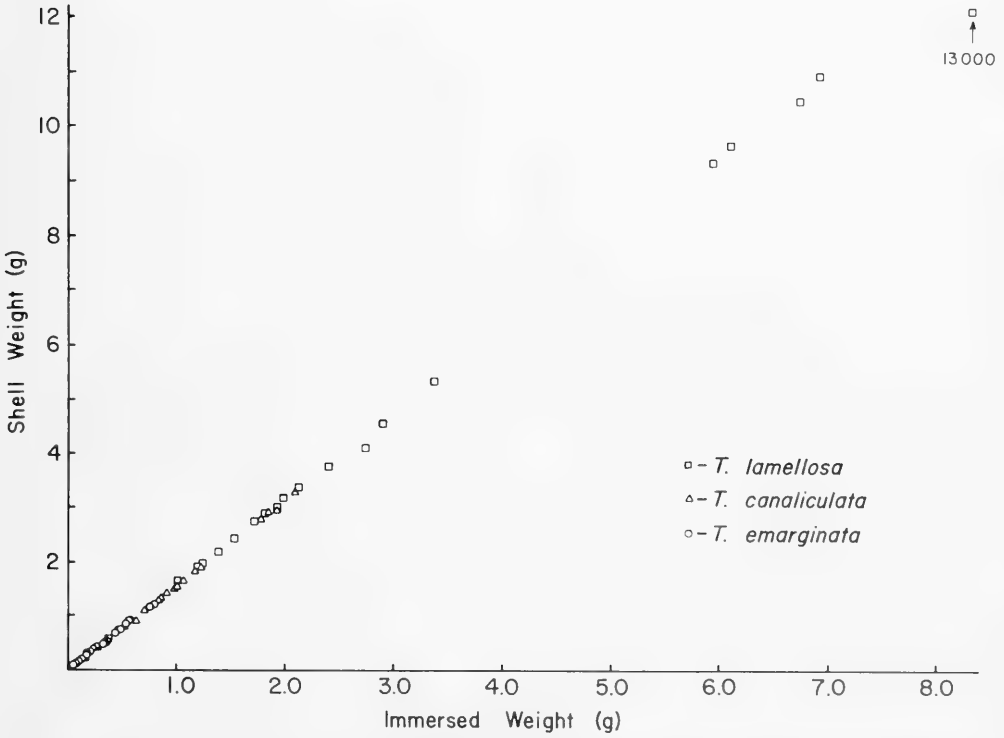

FIG. 1. The relationship between immersed weight of whole animals and destructively sampled dry weight of the shell for three species of *Thais*. The regression equations for these data are in Table 1, regressions 1–3. The high coefficients of determination (Table 1) indicate that shell weight is very accurately estimated from the weight of the whole animal immersed in seawater.

TABLE 2. Whole weights, immersed weights and error analysis for *Thais lamellosa*. Repeat whole weights were obtained on three successive days. Immersed weights were measured immediately prior to whole weights on the last two days. f = no penis. m = penis present.

Shell length (mm)	Sex	Whole weights (g)				Error		Immersed weights (g)			Error		Estimated body wt. (g)		
		1	2	3	Mean	Max.	%	1	2	Mean	Max.	%	1	Cum. error Max.	Cum. error %
58.2	f	17.992	17.661	17.461	17.681	0.261	1.46	8.277	8.272	8.274	0.005	0.06	4.681	0.266	5.68
56.0	f	15.181	14.840	14.702	14.908	0.341	2.25	6.721	6.699	6.710	0.022	0.33	4.453	0.363	9.41
55.1	f	14.506	14.780	14.806	14.697	0.274	1.89	6.666	6.617	6.642	0.049	0.74	4.184	0.323	7.72
53.9	f	13.784	13.047	13.544	13.458	0.737	5.35	6.084	6.103	6.094	0.019	0.31	3.786	0.756	19.97
52.9	m	14.701	15.215	12.956	12.945	0.514	3.50	6.904	6.892	6.898	0.012	0.17	4.025	0.526	13.07
52.0	m	13.154	13.673	12.679	13.169	0.994	7.27	5.923	5.909	5.916	0.014	0.24	3.840	1.008	26.25
41.2	m	7.369	7.224	7.449	7.347	0.225	3.11	3.374	3.364	3.369	0.010	0.30	2.019	0.235	11.64
39.1	m	6.252	6.133	6.214	6.200	0.119	1.90	2.898	2.888	2.893	0.010	0.35	1.667	0.129	7.74
37.0	m	5.478	5.355	5.619	5.484	0.264	4.93	2.395	2.383	2.389	0.012	0.50	1.712	0.276	16.12
36.9	m	4.609	4.548	4.738	4.632	0.190	4.18	2.119	2.109	2.114	0.010	0.47	1.306	0.200	15.31
36.1	f	4.488	4.645	4.654	4.596	0.157	3.50	2.129	2.120	2.125	0.009	0.42	1.239	0.166	13.40
35.3	f	4.169	4.210	4.194	4.191	0.041	0.98	1.996	1.987	1.992	0.009	0.45	1.015	0.050	4.93
35.2	m	4.107	4.126	4.011	4.081	0.115	2.79	1.937	1.930	1.934	0.007	0.36	1.105	0.122	11.04
33.9	m	3.640	3.643	3.671	3.651	0.028	0.77	1.738	1.730	1.734	0.008	0.46	0.893	0.036	4.03
32.6	f	4.014	3.948	3.992	3.985	0.066	1.64	1.831	1.817	1.824	0.014	0.76	1.112	0.080	7.19
32.6	f	3.149	3.108	3.078	3.112	0.041	1.30	1.382	1.377	1.380	0.005	0.36	0.931	0.046	4.94
32.5	f	5.449	5.595	5.553	5.532	0.146	2.68	2.581	2.566	2.574	0.015	0.58	1.449	0.161	11.11
30.5	m	3.037	3.057	3.026	3.040	0.031	1.01	1.526	1.529	1.528	0.003	0.20	0.624	0.034	5.45
28.9	m	2.491	2.483	2.457	2.477	0.026	1.05	1.241	1.242	1.242	0.001	0.08	0.507	0.027	5.33
27.9	f	1.991	1.976	1.960	1.976	0.016	0.81	0.977	0.978	0.978	0.001	0.10	0.439	0.017	3.87
27.7	f	2.351	2.301	2.315	2.322	0.050	2.13	1.149	1.148	1.149	0.001	0.09	0.498	0.051	10.04
27.5	m	2.102	2.110	2.051	2.088	0.059	2.80	1.013	1.015	1.014	0.002	0.20	0.487	0.061	12.53
27.1	m	2.355	2.338	2.355	2.349	0.017	0.73	1.196	1.196	1.196	0.000	0.00	0.455	0.017	3.74
25.9	m	1.969	1.986	1.955	1.970	0.031	1.56	1.004	1.006	1.005	0.002	0.20	0.274	0.033	12.04
17.1	?	0.570	0.570	0.569	0.570	0.001	0.18	0.291	0.292	0.292	0.001	0.34	0.117	0.002	1.71
16.6	?	0.530	0.532	0.522	0.528	0.010	1.88	0.271	0.271	0.271	0.000	0.00	0.100	0.010	10.00
14.0	?	0.300	0.313	0.303	0.305	0.013	4.33	0.156	0.160	0.158	0.004	2.56	0.052	0.017	32.69
					Means	0.177	2.44				0.009	0.30		0.188	10.63

TABLE 3. Whole weights, immersed weights and error analysis for *Thais canaliculata*. Repeat whole weights were obtained on three successive days. Immersed weights were measured immediately prior to whole weights on the last two days. f = no penis. m = average penis. 'm−' = small penis.

Shell length (mm)	Sex	Whole weights (g)				Error		Immersed weights (g)			Error		Estimated body wt. (g)		
														Cum. error	
		1	2	3	Mean	Max.	%	1	2	Mean	Max.	%	1	Max.	%
35.1	f	4.545	4.471	4.505	4.507	0.074	1.63	2.103	2.094	2.099	0.009	0.43	1.217	0.083	6.82
35.0	f	4.110	4.091	4.059	4.087	0.032	0.78	1.848	1.841	1.845	0.007	0.38	1.208	0.039	3.23
33.9	m	4.165	4.112	4.096	4.124	0.053	1.27	1.916	1.913	1.914	0.003	0.16	1.159	0.056	4.83
30.6	f	3.734	3.654	3.594	3.661	0.080	2.14	1.773	1.774	1.774	0.001	0.06	0.885	0.081	9.15
28.9	f	2.573	2.532	2.517	2.541	0.041	1.59	1.170	1.172	1.171	0.002	0.17	0.728	0.043	5.91
28.6	f	2.516	2.477	2.484	2.492	0.039	1.55	1.230	1.232	1.231	0.002	0.16	0.605	0.041	6.78
27.2	f	2.290	2.279	2.277	2.282	0.011	0.48	1.041	1.041	1.041	0.000	0.00	0.737	0.011	1.49
27.0	f	2.292	2.285	2.280	2.286	0.007	0.31	1.059	1.063	1.061	0.004	0.38	0.638	0.011	1.72
26.9	f	2.144	2.144	2.104	2.131	0.040	1.87	0.978	0.983	0.981	0.005	0.51	0.603	0.045	7.46
25.9	m	1.982	1.952	1.950	1.961	0.030	1.51	0.909	0.909	0.909	0.000	0.00	0.557	0.030	5.39
25.9	f	1.886	1.870	1.841	1.866	0.029	1.55	0.838	0.839	0.838	0.001	0.12	0.591	0.030	5.08
25.1	f	1.876	1.865	1.843	1.861	0.022	1.18	0.845	0.848	0.846	0.003	0.36	0.554	0.025	4.51
23.1	m	1.456	1.456	1.449	1.454	0.007	0.48	0.703	0.705	0.704	0.002	0.28	0.351	0.009	2.56
22.3	f	1.329	1.337	1.320	1.329	0.017	1.27	0.632	0.634	0.633	0.002	0.32	0.341	0.019	5.57
21.2	m	1.099	1.094	1.088	1.094	0.006	0.55	0.522	0.523	0.523	0.001	0.19	0.281	0.007	2.49
19.3	f	0.829	0.826	0.812	0.822	0.014	1.69	0.364	0.366	0.365	0.002	0.55	0.257	0.016	6.23
19.3	m	0.937	0.942	0.941	0.940	0.005	0.53	0.467	0.468	0.468	0.001	0.21	0.200	0.006	3.00
16.5	m	0.541	0.541	0.534	0.539	0.007	1.29	0.245	0.248	0.247	0.003	1.22	0.156	0.010	6.41
16.0	f	0.580	0.576	0.573	0.576	0.004	0.69	0.260	0.262	0.261	0.002	0.77	0.177	0.006	3.39
13.6	m	0.340	0.342	0.341	0.341	0.002	0.59	0.165	0.165	0.165	0.000	0.00	0.083	0.002	2.41
13.5	?	0.346	0.349	0.345	0.347	0.004	1.15	0.161	0.164	0.162	0.003	1.86	0.093	0.007	7.53
					Means	0.025	1.15				0.003	0.38		0.027	4.86

TABLE 4. Whole weights, immersed weights and error analysis for *Thais emarginata*. Repeat whole weights were obtained on three successive days. Immersed weights were measured immediately prior to whole weights on the last two days. f = no penis. m = average penis. 'm - ' = small penis.

		Whole weights (g)						Immersed weights (g)					Estimated body wt. (g)		
						Error					Error			Cum. error	
Shell length (mm)	Sex	1	2	3	Mean	Max.	%	1	2	Mean	Max.	%	1	Max.	%
27.2	f	1.991	1.964	1.951	1.969	0.027	1.36	0.800	0.799	0.800	0.001	0.13	0.749	0.028	3.74
25.7	m	1.836	1.829	1.822	1.829	0.007	0.38	0.766	0.765	0.765	0.001	0.13	0.656	0.008	1.22
22.7	f	1.182	1.187	1.159	1.176	0.028	2.36	0.493	0.495	0.494	0.002	0.34	0.422	0.030	7.11
22.3	m	1.339	1.335	1.333	1.336	0.004	0.30	0.581	0.583	0.582	0.002	0.34	0.433	0.006	1.39
22.3	f	1.245	1.245	1.235	1.242	0.010	0.80	0.542	0.543	0.543	0.001	0.18	0.401	0.011	2.74
21.8	m	1.296	1.289	1.274	1.286	0.015	1.16	0.569	0.571	0.570	0.002	0.35	0.397	0.017	4.28
21.2	f	1.104	1.112	1.096	1.104	0.016	1.44	0.455	0.456	0.456	0.001	0.22	0.417	0.017	4.08
20.2	f	0.889	0.886	0.877	0.884	0.009	1.02	0.363	0.363	0.363	0.000	0.00	0.327	0.009	2.75
20.0	f	0.816	0.811	0.804	0.810	0.007	0.86	0.331	0.330	0.331	0.001	0.37	0.304	0.008	2.63
19.9	f	0.779	0.774	0.768	0.774	0.006	0.78	0.322	0.322	0.322	0.000	0.00	0.280	0.006	2.14
18.0	m	0.636	0.635	0.635	0.635	0.001	0.16	0.271	0.270	0.271	0.001	0.37	0.218	0.002	0.92
17.2	m	0.528	0.528	0.519	0.525	0.009	1.70	0.222	0.222	0.222	0.000	0.00	0.180	0.009	5.00
16.9	f	0.583	0.576	0.576	0.578	0.007	1.20	0.244	0.246	0.245	0.002	0.82	0.199	0.009	4.52
15.6	m -	0.420	0.419	0.416	0.418	0.003	0.72	0.172	0.175	0.173	0.003	1.74	0.150	0.006	4.00
15.4	f	0.410	0.408	0.401	0.406	0.007	1.72	0.169	0.168	0.168	0.001	0.59	0.146	0.008	5.48
13.9	f	0.288	0.289	0.285	0.287	0.004	1.38	0.125	0.126	0.126	0.001	0.80	0.092	0.005	5.43
12.3	f	0.231	0.234	0.225	0.230	0.009	3.85	0.105	0.104	0.105	0.001	0.95	0.066	0.010	15.15
11.1	f	0.159	0.162	0.161	0.161	0.003	1.89	0.072	0.073	0.073	0.001	1.67	0.048	0.004	8.33
10.5	?	0.130	0.131	0.129	0.130	0.002	1.53	0.060	0.061	0.061	0.001	1.67	0.031	0.003	9.68
					Means	0.009	1.29				0.001	0.55		0.009	4.77

immersed weight, the fractional differences in body weights among animals with the same shell weight will be much less and hence introduce little error. As the ratio of shell to body weight decreases, however, this error will increase, thus for animals with slight shells this technique may be less accurate.

Whole weight in air ('whole weight' in Tables 2–4) was measured after several preparatory steps that removed most of the extravisceral water. In *Nucella lapillus* (Linné, 1758), this water may account for up to 39% of the total water of an attached animal (Boyle et al., 1979), and unless removed would be included as part of the body wet weight. Also, because animals will retract on their own to varying extents on different occasions, these preparatory steps permitted a much higher level of repeatability.

Snails were first arrayed aperture up on paper toweling in the order in which they were to be weighed. Each animal was then 'chased' back into its shell by stimulating the foot with a small modeling brush. A soft absorbent tissue (e.g. Kimwipe) was subsequently pressed firmly up against the retracted foot with a pair of forceps to squeeze out nearly all the remaining water. The animals were left on the toweling until all the shells in a group were visibly dry (approximately 20–40 min) and then weighed on top of a Mettler P153 balance to the nearest 0.001 g (Tables 2–4). This whole weight corresponded to shell plus tissue wet weight, plus any residual mantle water. The shell weight, as estimated from immersed weight (regressions 1–3, Table 1; Fig. 1), was then subtracted from the whole weight, thus providing an estimate of body tissue wet weight.

A more desirable correlation, though, was

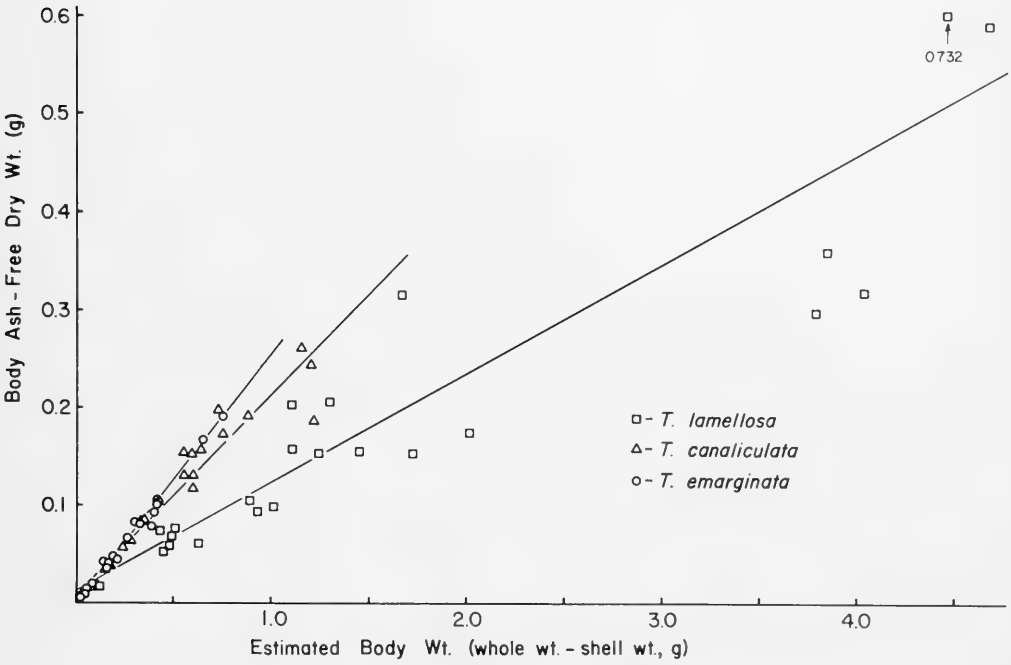

FIG. 2. The relationship between estimated body weight (the weight of the whole animal in air minus the weight of the shell) and destructively sampled ash-free dry weight of the body for three species of *Thais*. The regression equations for these data are in Table 1, regressions 8–10. As indicated by the coefficients of determination (Table 1), this relationship is least accurate for the largest species (*T. lamellosa*, squares) and most accurate for the smallest species (*T. emarginata*, circles). *T. canaliculata* (triangles), an intermediate-sized species, exhibits an intermediate level of variability. Much of the variation about these regressions appears due to differences among individuals in the percent water and percent ash of the body. The regressions of body *dry* weight on estimated body weight (whole weight minus shell weight) and of body *wet* weight on estimated body weight exhibit much less scatter: coefficients of determination (R^2) = 0.8591, 0.9217 and 0.9887 for body dry weight, and coefficients of determination (R^2) = 0.9770, 0.9871 and 0.9989 for body wet weight respectively for the three *Thais* species, *T. lamellosa, T. canaliculata* and *T. emarginata*.

between this value of estimated body weight (i.e. whole weight minus shell weight), and ash free dry weight, since ash free dry weight is a better estimate of metabolizing or metabolizable tissue. Regressions 8–10 (Table 1; Fig. 2) describe these relations for *Thais lamellosa, T. canaliculata* and *T. emarginata* respectively. Again, there is a close correspondence (R^2 values 0.8050–0.9846, Table 1), though not as precise as for shell weight. This two-step weighing procedure thus provides independent, non-destructive estimates of body and shell weight, allowing either to be used to measure growth.

The second step of this process, squeezing the water out of the mantle cavity, may traumatize the animal to some extent. However, in a field monitoring experiment involving all three species (Palmer, 1980), subgroups of each species were either 1) weighed as above in addition to being tagged and measured for shell length, or 2) only tagged and measured. There were no significant differences for any of the species between the proportion of animals recovered from the two treatment groups over the course of the following two weeks or after 2-1/2 months (Table 5), suggesting that the trauma associated with the weighing technique is slight for these species.

Immersed weight on the other hand involves little more disturbance than dislodgment of the animals from the bottom. If they have been immersed for a sufficient length of time prior to weighing, there is no need to force any air out of the mantle cavity and they may be placed directly on the submerged weighing platform. The entire operation, except for the brief transfer, takes place under water.

Another consideration regarding this technique is its repeatability. A comparison of R^2 values from regressions 1–3 with those of regressions 8–10 (Table 1) indicates that the replicability of immersed weights is greater than that for whole weights (Tables 2–4). For *Thais lamellosa* (Table 2) the mean maximum percent error for whole weight is 2.5%. Immersed weights of *Thais lamellosa* vary by less than 0.022 g between successive weighings and are in general much more accurate (mean percent error = 0.38%). For *Thais canaliculata* the mean maximum percent error is 1.15% for whole weights and 0.39% for immersed weights. For *Thais emarginata* these errors are about the same, 1.29% and 0.55% for maximum whole weight error and immersed weight error respectively. Tables 2–4 also indicate what the potential cumulative error might be when estimating body weight as described above.

DISCUSSION

The principal advantage to length as a measure of gastropod size is the comparative ease with which it may be obtained. Caliper measurements of shell length even to an accuracy of 0.1 mm require only a few seconds and with practice are repeatable to 0.2 mm. They are also readily obtainable in the field with a minimum of disturbance to the animals. Weight measurements, on the other hand, to be of sufficient accuracy (and hence utility), almost invariably require that animals be brought back to the laboratory, thereby increasing the disruption of the animal's normal activity as well as requiring additional time to return them to the field.

However, there are several limitations to shell length as a measure of animal size. First, as gastropod shells age, the spires begin to erode. Spight (1974) consistently observed negative "growth" (change in shell length) over the winter in *Thais lamellosa* at Shady Cove which he recognized as being due to spire erosion (see also Spight et al., 1974). Second, as animals increase in size there is a progressively smaller change in length for a given change in body weight so given the limit on resolution of length change imposed by the repeatability of caliper measurements (0.1–0.2 mm), changes in weight will be more readily detected than changes in length. Third, in mature gastropods, where shell growth is almost negligible, body weight may still vary seasonally in association with spawning, reduced activity over the winter or increases or decreases in the food supply. These body weight changes would pass undetected if only shell length is recorded. Finally, if populations differ from each other in shell shape, or if there is shape variation among individuals within a single population, then a given length change in animals of the same initial length will be associated with different changes in body weight.

In the final analysis, the choice of weight or length to measure growth, if not set by logistical constraints, is determined by the kind of information desired. The correlation between log shell length and log body weight is generally high for animals from a single population

TABLE 5. Proportions of marked animals recovered from each of two treatment groups on various days following release at Deadman Island, San Juan Islands, Washington, U.S.A. (48°27′N, 122°55′W). Marked animals were released on 4/26/78. SD = standard deviation. t = t value from paired *t*-tests.

Treatment	Number released	Proportion of released animals recovered												
		Date recovered (month/day)												
		5/4	5/5	5/6	5/7	5/9	5/11	5/12	5/13	5/15	7/16	Mean	SD	t_s
Thais lamellosa														
Length + weight	50	0.02	0.08	0.14	0.02	0.10	0.12	0.18	0.10	0.08	0.22	0.11	0.063	1.17
Length only	29	0.03	0.07	0.10	0.00	0.07	0.07	0.14	0.07	0.03	0.14	0.07	0.044	
Thais canaliculata														
Length + weight	50	0.28	0.42	0.46	0.42	0.40	0.34	0.34	0.36	0.42	0.62	0.41	0.092	0.18
Length only	262	0.28	0.30	0.40	0.40	0.38	0.47	0.47	0.50	0.41	0.50	0.41	0.076	
Thais emarginata														
Length + weight	50	0.62	0.62	0.50	0.70	0.70	0.78	0.80	0.80	0.86	0.80	0.72	0.111	1.70
Length only	139	0.55	0.58	0.58	0.66	0.70	0.80	0.78	0.78	0.69	0.58	0.67	0.095	

TABLE 6. Regressions of body weight change (Y) on shell length change (X). Shell lengths are in mm. Body weights are measured in grams. N = number of individuals. *T. lam.* = *Thais lamellosa*. *T. can.* = *T. canaliculata*. *T. em.* = *T. emarginata*.

Regression number	Species	Length change		N	Regression equation	R^2
		Mean	Range			
1	*T. lam.*[1]	−0.02	−0.5 to 1.6	11	Y = 0.071X − 0.0425	0.1985
2	*T. lam.*[2]	−0.02	−1.0 to 1.8	17	Y = 0.072X − 0.0434	0.3117
3	*T. lam.*[3]	6.43	3.3 to 10.3	27	Y = 0.045X − 0.0024	0.5635
4	*T. lam.*[4]	9.31	4.8 to 12.2	24	Y = 0.057X − 0.0865	0.7540
5	*T. can.*[1]	1.21	−0.3 to 4.1	28	Y = 0.018X + 0.0785	0.1178
6	*T. em.*[1]	1.24	−0.7 to 5.3	40	Y = 0.039X + 0.0061	0.7063
7	*T. em.*[2]	2.01	0.3 to 3.7	9	Y = 0.025X − 0.0019	0.7575

[1]Deadman Island, field growth.
[2]Point George, Lopez Island, field growth.
[3]Collected from False Bay, San Juan Island; grown in cages.
[4]Collected from Turn Rock, San Juan Islands; grown in cages.

(regressions 11–13, Table 1), indicating that length can provide a fairly reliable estimate of body weight. In addition, the correlation between *change* in length and *change* in body weight can also be relatively high for animals in the field and in cages, as long as they are increasing in size (regressions 3, 4, 6 and 7, Table 6; but note regression 5). Consequently, if growth rates are positive and of moderate magnitude relative to spire erosion, and if shell shapes are essentially the same, then length can provide an adequate measure of body size. If populations of different shape need to be compared, a single destructive correlation of body weight on length for each population can permit comparisons between them based on length measurements alone. If, on the other hand, there is a possibility that animals may lose weight and it is important to detect such a loss, or if there are significant differences in the rates of spire erosion among populations being compared, length measurement alone may lead to much lower resolution of growth rate differences.

In principle this technique would be applicable to any organism composed of two major components of differing specific gravity. Weights of the organism first in a medium whose specific gravity is very close to that of one of the components, and then in a second medium which may or may not be of similar specific gravity to the second component, will, via the appropriate regressions, permit independent estimates of both body components. In practice, such separation may be less feasible for other carbonate skeleton-producing invertebrates (sclerosponges, corals, brachiopods, bryozoans, bivalves and echinoderms), since for many the removal of extravisceral water will be difficult to accomplish reliably or without damaging the animal.

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THE OCCURRENCE OF *MERCENARIA MERCENARIA* FORM *NOTATA* IN GEORGIA AND SOUTH CAROLINA: CALCULATION OF PHENOTYPIC AND GENOTYPIC FREQUENCIES

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ABSTRACT

Genotypic and phenotypic frequencies of the *notata* form of *Mercenaria mercenaria* were calculated from data provided by four studies: two natural populations from Georgia, one from South Carolina, and one hatchery brood. Phenotypic frequencies calculated for each study ranged from 0.76% to 2.25%. Gene frequencies calculated by Maximum Likelihood Estimation were 0.04% to 0.11%. There were no significant differences between samples of natural populations. The natural populations and the hatchery brood are not comparable. Research applications using these rare alleles as useful markers for breeding experiments with this valuable commercial species are briefly described.

Key words: *Mercenaria mercenaria* form *notata*; gene frequency estimation; natural occurrence.

INTRODUCTION

The hard clam, *Mercenaria mercenaria* (Linné, 1758), is a commercially important bivalve indigenous to the Atlantic and Gulf coasts of North America. The shell usually is a uniform whitish color which frequently appears tan to dirty gray as a result of sediment stains (Abbott, 1974). *Mercenaria mercenaria* form *notata* (Say, 1822) is a rare variant of *M. mercenaria* distinguished by brown bands or zigzag lines on the surface of the shell. It occurs throughout the range of *M. mercenaria* from the Gulf of St. Lawrence to Florida and the northern Gulf of Mexico (Abbott, 1974).

M. mercenaria notata was designated a subspecies of *M. mercenaria* until Chanley (1961) crossed *M. mercenaria mercenaria* and *M. mercenaria notata* in the laboratory, and genetic analysis of the resulting offspring demonstrated that the *notata* marking is an individual variation controlled by a single gene or by several closely linked genes inherited as a Mendelian unit (Paul E. Chanley, personal communication). The commonly observed *notata* pattern is the heterozygous form and individuals homozygous for the *notata* allele have solid brown bands (Chanley, 1961). These two forms are illustrated in Fig. 1.

METHODS

Literature on the occurrence of the *notata* variant is as scarce as the animal itself. Few papers devoted to the distribution and density of populations of *M. mercenaria* have noted the incidence of *notata* individuals in those populations. Phenotypic and genotypic frequencies of the *notata* variant of *M. mercenaria* were calculated using three studies which recorded the occurrence of the *notata* form in their samples: Eldridge et al. (1976) on the South Carolina coast; Walker et al. (1980), an intensive survey of Wassaw Sound, Georgia; and Humphrey (in prep.) on the Georgia coast. Further information was provided by the analysis of a brood spawned in the Virginia Institute of Marine Sciences Eastern Shore Laboratory from 300 individuals collected in Wassaw Sound.

The samples from South Carolina were collected using patent hydraulic tongs, and those from Georgia were collected either with rakes or by examining quadrats by hand; therefore the collected specimens were at least two years old due to the inability to detect smaller individuals by these methods. In addition to age-related attrition, habitat conditions in these areas tend to disfigure the shells. Individuals are commonly located in anaerobic

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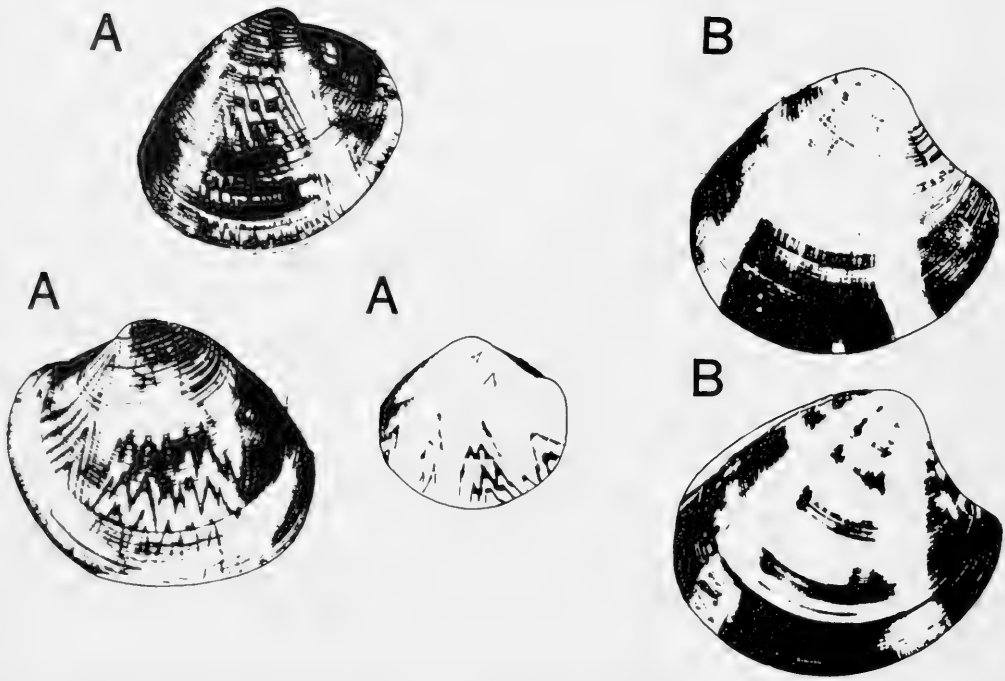

FIG. 1. Heterozygous (A) and homozygous (B) phenotypes of *Mercenaria mercenaria* form *notata* from the southeastern United States; high contrast (Kodalith) photograph showing representative individuals of each phenotype.

mud which tends to blacken the shells, and shell erosion is common. These factors make *notata* harder to detect which leads to the assumption that the percentages calculated for natural populations are minimum estimates.

RESULTS AND DISCUSSION

Eldridge et al. (1976) estimated the frequency of *notata* clams in South Carolina estuaries using the data of Gracy (1974). Out of 11 sites where *notata* was found, 19 of the 1539 individuals were *notata* (1.23%); sample percentages ranged from 0.71% to 2.17%.

In the two studies of clam populations on the Georgia coast, the frequency of the *notata* variant was recorded. Walker et al. (1980) in a survey of clam populations from Wassaw Sound near Savannah, Georgia, examined fifty-seven stations which yielded a total of 2339 clams; of these, 13 samples contained a total of 18 individuals with *notata* markings (Table 1). Frequency of the *notata* variant calculated for this area using all stations was 0.77%; sample percentages ranged from 0.36% to 12.5%. Humphrey (in prep.) took six samples of clam populations from a wider geographic area. One sample was from Wassaw Sound, three were from Sapelo Island (approximately 48 km to the south), one was from Doboy Sound near the south end of Sapelo Island, and one was from St. Simon's Island. The total latitudinal range was approximately one degree (110 km). Of 1881 individuals, 21 (1.12%) were identified as *notata*, sample percentages ranged from 0.29% to 1.73% (Table 2). The hatchery spawned brood contained 234 (2.25%) *notata* individuals in a sample of 10,378; this is higher than the overall percentage in either state.

Gene frequency estimation

The best calculation for the estimation of gene frequencies is the Maximum Likelihood Estimation:

$$p = \frac{A + \frac{1}{2}B}{N}$$

p = gene frequency estimate; A = number of individuals homozygous for the gene estimated by p; B = number of heterozygotes; N

TABLE 1. Samples of *Mercenaria mercenaria* collected from the survey of Wassaw Sound, Georgia. 1977–79 (Walker et al., 1980).

Sample*	N	No. *notata*	% *notata*	Gene frequency (q)
1	13	1	7.69	.0385
2	25	1	4.00	.0200
3	281	1	0.36	.0018
4	174	1	0.57	.0029
5	101	1	0.99	.0050
6	125	2	1.60	.0080
7	82	1	1.22	.0061
8	25	2	8.00	.0400
9	8	1	12.50	.0625
10	71	2	2.82	.0141
11	51	1	1.96	.0098
12	144	3	2.08	.0104
13	12	1	8.33	.0417
Total (pooled)	2339	18	0.77	.0039

*Samples containing *notata*.

TABLE 2. Samples of *Mercenaria mercenaria* collected from the Georgia coast (Humphrey, in prep.).

Sample	N	No. *notata*	% *notata*	Gene frequency (q)
St. Simon Is.	300	2	0.67	.0033
Doboy Sound	346	6	1.73	.0087
Sapelo Is. I	310	5	1.61	.0081
Sapelo Is. II	294	5	1.70	.0085
Sapelo Is. III	348	1	0.29	.0014
Cabbage Is. (Wassaw Sound)	283	2	0.71	.0035
Total	1881	21	1.12	.0056

= total number of individuals in the sample; q = frequency estimate of the other allele; the variance of this estimate:

$$\frac{pq}{2N}$$

is the minimum variance for gene frequency estimation (Speiss, 1977). There were no homozygous *notata* detected in the Georgia studies, although individuals were closely examined. The South Carolina study made no distinction between genotypes of *notata* individuals; therefore, this method of gene frequency calculation, which requires the number of heterozygotes, cannot be used for all three studies. If it is impossible to distinguish one of the homozygous genotypes, the maximum likelihood estimation provides another method for calculation of gene frequencies.

$$p = \sqrt{\frac{A}{N}}$$

p = gene frequency whose homozygote is distinguishable; A = number of individuals of that homozygote; N = total number of individuals in the sample. The variance of this estimate, $\frac{1-p^2}{4N} = \frac{pq}{2N} + \frac{q^2}{4N}$ is larger than the minimum variance by a quantity proportional to q^2 (Speiss, 1977).

For the data in these studies the estimate of q is so small that there is very little difference between estimates; for example the variance (σ^2) of p for the South Carolina study is:

$$\frac{pq}{2N} \times .0000020018 \text{ and } \frac{1-p^2}{4N} = .0000020081$$

For these three studies the gene frequency estimates are the same by either method when calculated to the fourth decimal place.

South Carolina; $p = .9938, q = .0062, \sigma^2 = .0000020$
Wassaw Sound; $p = .9961, q = .0039, \sigma^2 = .0000008$
Georgia Coast; $p = .9944, q = .0056, \sigma^2 = .0000015$
Hatchery brood; $p = .9889, q = .0113, \sigma^2 = .0000005$

If the populations are in Hardy-Weinberg equilibrium then the expected numbers of each genotype can be calculated from the formula:

$$Np^2 + 2Npq + Nq^2 = N$$

For the South Carolina Survey, the values (expected values given in parentheses) are:

1520(1519.97) + 19(18.97) + 0(.06) = 1539(1539.00)

For Wassaw Sound:

2321(2320.79) + 18(18.17) + 0(.04) = 2339(2339.00)

For the second Georgia Survey:

1860(1859.99) + 21(20.95) + 0(.06) = 1881(1881.00)

In none of these studies is the expected occurrence of the homozygous *notata* form equal to one, so it is quite possible that there was an absence of homozygous *notata* rather than a failure to detect them. Chanley (personal communication) has found homozygotes in nature but they are rare. In that case the simple gene counting formula:

$$p = \frac{A + \frac{1}{2}B}{N}$$

can be used. This formula was used to calculate the gene frequencies in Tables 1 and 2.

Heterogeneity between samples was calculated by testing the phenotypic frequencies.

South Carolina:			
	1520	19	= 1539
Georgia (Wassaw Sound):			
	2321	18	= 2339
Georgia coast:			
	1860	21	= 1881
Total:	5701	58	5759

With two degrees of freedom, the chi-square value was 2.35, $0.5 < p < 0.3$. It is not meaningful to calculate expected numbers for the hatchery brood, nor can it be compared to the natural populations, since both the percentage of *notata* and the actual number of individuals that spawned to form the parental generation for this sample are not known.

The *notata* variant is the only morphological character inherited as if controlled by a single gene that has been found in *M. mercenaria*. The uses of such a marker are numerous. For example, one application would be the marking of offspring from controlled matings to determine their subsequent success. Since the allele is rare in nature, releasing *notata* larvae and checking adjacent populations in subsequent years could supply much needed information on larval migration, provided that migration is not affected by this allele. Measures of genetic parameters such as gene frequency and heterozygosity are of particular interest to population geneticists working with electrophoretic alleles because they provide a known morphological marker for comparison with data derived from enzyme markers.

Morphological markers of known genetic basis have many possible uses in practical shellfish research. As more species are successfully reared in hatcheries, use of these markers to identify individuals whose genetic composition results in desirable traits such as rapid growth, increases the efficiency of controlled breeding programs. Newkirk (1980) not only confirmed the genetic control of shell color in *Mytilus edulis* but also found that the gene for this color polymorphism was linked to growth rate.

Research with the genetics of several commercial bivalves has been done (Kraeuter et al., in prep.; Innes & Haley, 1977), but very little work has been done with the *notata* variant of *M. mercenaria*. Study of the natural occurrence of *notata* in conjunction with the many studies done on populations of *M. mercenaria* would not require much effort, and the potential usefulness of this marker makes it desirable to acquire more knowledge about its characteristics in nature.

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LETTERS TO THE EDITORS

ON SIBLING SPECIES AND GENETIC DIVERSITY IN FLORIDA *GONIOBASIS*

Two studies by Chambers (1978, *Malacologia,* 17: 157–162; 1980, *ibid.,* 20: 63–81) are pioneer contributions to the understanding of genetic diversity and relationships in a genus that has always perplexed systematists. *Goniobasis* is confusing because of the numerous described species (ca. 500), their morphological variation within and between populations, and the frequent instances of parallel evolution of shell characters among distantly related taxa. Chambers shows that in some instances genetic diversity exceeds shell differentiation and that taxonomic classification based on isozyme analysis may be more nearly objective than classifications based on shell features. However, three items in these papers need reexamination.

Chambers (1980: 65) identifies a species from the Ichetucknee River as *G. athearni,* which is correct. Earlier (1978) he identified this same population as a sibling of *G. floridensis.* In the more recent paper he shows that *G. floridensis* and *G. athearni* are distantly related genetically within *Goniobasis,* but he continues to regard the Ichetucknee population a sibling species of *floridensis.* Because the Ichetucknee population of *athearni* is distantly related genetically to *floridensis,* they cannot be sibling species. Sibling species are very closely related taxa that have diverged sufficiently genetically to be reproductively incompatible, but are morphologically hardly distinguishable. If one relies solely on shell criteria, there are numerous instances in which unrelated species of *Goniobasis* are hardly distinguishable from each other. Also, there are many instances in which species of *Goniobasis* are barely distinguishable by shells from species of *Pachychilus, Potodoma, Lithasiopsis, Juga, Semisulcospira, Doryssa* (Pleuroceridae), or *Tarebia, Melanoides,* and *Hemisinus* (Thiaridae). Clearly similar shells in these species do not mean that they have not yet diverged in shell characters. More likely, similar shell types have evolved due to similar strong environmental selection factors acting independently on unrelated species. *G. athearni* and *G. floridensis* are not sibling species in light of the genetic data that Chambers presents (1980, Tables I, II). Yet this point is not satisfactorily addressed in the 1980 paper, and most likely it would not be noticed by authors gleaning the literature for examples of sibling speciation. It is also appropriate to note that *G. athearni* had not been found in the Ichetucknee River among numerous collections in the MCZ, UMMZ, and the FSM made there prior to 1970. Chambers' study population was found only at a single station, a roadside park where U.S. Highway 27 crosses the Ichetucknee River. Currently the species has spread widely in the Ichetucknee and has moved downstream into the confluent Santa Fe River. Apparently the Ichetucknee River population was introduced there after 1970. Biogeographic interpretations based on its presence there are highly tenuous.

Chambers (1980: 65) states that he found *G. floridensis* in Holmes Creek and the Choctawhatchee River, but did not find *G. clenchi,* which was reported from these places in earlier literature. However, his illustrations (p. 76; fig. 7 C-D) of specimens from the Choctawhatchee River are typical *clenchi.* This is significant because he identified the Choctawhatchee population as an isozymatically distinct species of "*G. floridensis.*" The error is compounded on pp. 74–75, where he shows that "*G. floridensis*" and *G. dickinsoni* intergrade in shell sculpture in the Chipola River but not in the Choctawhatchee River. The interpretations he gives are that "*floridensis*" and *dickinsoni* are far more diverse genetically than they actually are, and biological speciation has occurred that previously was not recognized. Both interpretations are incorrect. Serious errors could be perpetuated in the scientific literature because of this misidentification.

Finally, Chambers interprets taxonomic diversity in *Goniobasis* by criteria established for the *Drosophila willistoni* group. In this group, semi-species, sibling species, and subspecies are definable on the basis of apparent genetic distances. Such is not the case in *Goniobasis.* Chambers' data give inconclusive results. In some instances (*clenchi-floridensis, clenchi-dickinsoni*) interbreeding does not occur even though genetic distances are much less than between subspecies in

Drosophila. Also a morphologically very distinct geographic subspecies of *floridensis* (Wacasassa River) is not genetically distinguishable from adjacent populations of typical-appearing *floridensis*. In other instances apparently great genetic distances occur between morphologically indistinguishable populations of *floridensis*. Clearly the criteria of genetic distances used in the *Drosophila willistoni* group for defining specific and infraspecific categories are not applicable to taxonomic hierarchies within *Goniobasis*.

The use of genetic distances by Chambers for showing phylogenetic relationships in Florida *Goniobasis* is a major advance in the development of an objective set of criteria for determining phylogenetic relationships. However, the data base he presents is too ambiguous at this point to permit clear-cut application to taxonomic classification and hierarchies in *Goniobasis*.

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SIBLING SPECIES AND GENETIC DIVERSITY IN FLORIDA *GONIOBASIS:* A REPLY

The snails of the genus *Goniobasis* are a quandary to the fastidious taxonomist and a source of fascination to the evolutionary biologist. The complex patterns of geographic variation observed in the Florida *Goniobasis* led me to apply electrophoretic methods (also known as allozyme, isozyme or isoenzyme methods) in evolutionary and systematic studies of these snails (Chambers, 1978, 1980). Although one of the earliest applications of electrophoretic methods to a taxonomic problem dealt with mollusks (Davis & Lindsay, 1967), these techniques are new to many malacologists. I am taking this opportunity to respond to some specific questions that Thompson has raised about the application of these methods in studies of the Florida *Goniobasis*. These questions will be discussed in the same order that Thompson has presented them.

Sibling species

Thompson's definition of sibling species requires that sibling species be very closely related and show little genetic divergence. This definition is not consistent with the term sibling species as it was originally defined (Mayr, 1942) or with its subsequent use in the literature. Thompson's definition is closest to that of Mayr (1969: 411) who defines sibling species as ". . . closely related species which are reproductively isolated but morphologically identical or nearly so." Mayr does not state in that work how "closely related" sibling species must be, but he does in other writings (Mayr, 1942, 1963, 1970, 1976). For example, in Mayr's (1942) work that originally defined sibling species, he states that such species do not need to be phylogenetic siblings (p. 151). In a more recent review (1976: 513–514) Mayr emphasizes that sibling species need not be genetically very similar and that their morphological similarity is not merely because these species are *in statu nascendi* and have not had time to diverge. Isoenzyme studies of sibling species (Webster & Burns, 1973; Ayala, 1975; Nei, 1975: 185; Avise, 1976; Ryman et al., 1979) have confirmed Mayr's view that sibling species may display profound genetic differences. According to Avise (1976: 112), "Morphological similarity of sibling species belies their large genetic differences." Chambers' (1978) use of the term sibling species for the genetically distinct species at the Ichetucknee River is therefore consistent with the original definition of the term and its subsequent usage in the literature.

Thompson should realize from reading Chambers [1978, 1980, and in press (preliminary draft sent to Thompson in September, 1980)] that I recognize that shell features alone can be unreliable for classification and that convergence is common in gastropod shell features. For example, Chambers (1978: 161) specifically noted that the occurrence of sibling species in the Ichetucknee River ". . . perhaps is due to convergent evolution in shell sculptural characters." One does not know for certain whether the Ichetucknee River species are convergent since no fossils are known that would indicate the physical appearance of the ancestors of either species.

Thompson's list of genera that show similarity in shell sculpture are of evolutionary interest but not particularly relevent to a discussion of sibling species since these taxa are mainly allopatric, with the unfortunate exceptions of *Tarebia granifera* Lamarck and *Melanoides tuberculata* (Müller) which have managed to colonize some portions of North America (Dundee, 1974). The sibling species concept is clearly most relevant in areas where sympatric species may be confused with one another. I have observed *T. granifera* in the Ichetucknee River and *M. tuberculata* in Alexander Springs, Lake County, Florida. The shells of these species are not difficult to distinguish from those of the native *Goniobasis* found at these localities.

The large genetic distance between the sibling species in the Ichetucknee River and its significance were discussed in Chambers (1978). Since that work was cited in Chambers (1980) little would have been achieved by repeating that discussion in the latter paper, which was also published in *Malacologia.*

Goniobasis floridensis (Reeve) and the population whose identification was suggested as *Goniobasis athearni* (Clench & Turner) by Chambers (1978, 1980) from the

Ichetucknee River were referred to as sibling species in the 1980 paper because some individuals from the Ichetucknee River are still difficult to safely place in the correct species without electrophoretic identification.

A Recent Introduction at Ichetucknee River?

Thompson's suggestion that the Ichetucknee River population referred to *G. athearni* by Chambers (1980) was recently introduced is a valid alternative hypothesis to that of Chambers (1978), which indicated that the seemingly large geographic distance between the Ichetucknee River population and related forms in the Apalachicola River drainage had parallels in other aquatic organisms, and that the headwaters of these two systems drain adjacent areas in Georgia (Jackson, 1975). The following additional observations on the introduction hypothesis are offered. Both *G. floridensis* and the *G. athearni* form were observed at or near the head of Ichetucknee Springs in 1974; this is 3 km distant from the site of the collection reported on in Chambers (1978). Thompson does not indicate how numerous are the pre-1970 collections of *Goniobasis* from the Ichetucknee River. One 1970 collection (Ohio State Museum no. 336) yielded both species at the site of the Chambers' 1978 study. The only lot in the U.S. National Museum (USNM 515795) consists of three abraded shells collected in 1926 which cannot be identified with any certainty.

Thompson suggests that *G. athearni* was introduced to the Ichetucknee River after 1970 and states, without presenting evidence, that this species has since spread downriver into the Santa Fe River. I collected this species at Blue Springs on the Santa Fe River (Gilchrist Co.) in January 1974. This site is about 20 km upstream from the confluence of the Ichetucknee River and the Santa Fe River. Movement of this species this far upstream between 1971 and 1974 requires rates of locomotion exceeding measured rates for *Goniobasis* (Krieger & Burbanck, 1976; Mancini, 1978). If *G. athearni* was introduced to the Ichetucknee River as late as Thompson states, either these snails have dispersed on their own with extraordinary speed or there must have been a second introduction to account for the species' presence in Blue Spring early in 1974.

Electrophoretic data (Chambers, 1980) indicate that the Ichetucknee River *G. athearni* has four alleles not detected in the Chipola River *G. athearni* sample. These alleles could be used as markers for locating the source population of the Ichetucknee River population if indeed it is a recent introduction. The introduction hypothesis could not be supported if these alleles could not be found in reputed source populations.

Biogeographic interpretations are usually tenuous; they are also an essential part of any study of geographic variation. The introduction hypothesis for the Ichetucknee population is provocative, testable to some extent, and may well prove true; but its formulation does not absolve the researcher from the responsibility of providing prudent biogeographic interpretations such as that suggested by Chambers (1978).

G. floridensis *and* G. clenchi

The population from site 15 (Chambers, 1980) was referred to *G. floridensis* after comparison with the following:

1. The population at Holmes Creek (Vernon, Washington Co.) which was referred to *G. floridensis* by Clench & Turner (1956). Thompson does not cite the earlier literature which, he maintains, reports *G. clenchi* from Holmes Creek. I am not aware of any published records of *G. clenchi* from that stream.
2. Four paratypes of *G. clenchi* (USNM 861587).
3. Description, with figure, of *G. clenchi* (Goodrich, 1924).
4. A lot of *G. clenchi* (USNM 668077) acquired from the Museum of Comparative Zoology, Harvard University.
5. Redescriptions of *G. floridensis* and *G. clenchi* by Clench & Turner (1956). These descriptions are very similar. *G. floridensis* shells are described as having a stronger peripheral cord, more indented suture, and less flattened whorls than shells of *G. clenchi.*

The population sampled at site 15 was variable, but most individuals were closer to *G. floridensis* material and were well within the range of variation of the population at Holmes Creek at Vernon which was identified by Clench & Turner (1956) as *G. floridensis*. It is likely that *G. clenchi* and *G. floridensis* in the Choctawhatchee River Drainage are conspecific since populations referred to those different species overlap in shell characters. Chambers (in press) describes chromosomal divergence between *G. floridensis* from

Holmes Creek and *G. floridensis* in the Chipola River drainage.

Electrophoretic data

Thompson makes several errors and misinterpretations in his discussion of the electrophoretic data bearing on the relationships between *G. clenchi* and *G. floridensis*. Chambers (1980) did not refer to the site 15 population or any other population as "an isozymatically distinct species," but rather has warned against making such judgments based on electrophoretic evidence alone (p. 73) and discussed the conditions under which electrophoretic data are relevant to taxonomic work (pp. 75–76). Contrary to Thompson's assertion, Chambers' (1980) data indicate little divergence between the site 15 sample and other Florida panhandle samples of *G. floridensis*. The interpretations that Thompson attributes to Chambers, ". . . '*floridensis*' and *G. dickinsoni* are far more diverse genetically than they actually are, and biological speciation has occurred that previously was not recognized. . . ," do not appear in Chambers (1980) and are contrary to the views expressed in that paper on *G. dickinsoni* and the Florida panhandle samples of *G. floridensis*. *G. dickinsoni* and *G. floridensis* were, in fact, shown to have diverged very little, which supports an interpretation that these species are closer than recognized in previous literature (Clench & Turner 1956), and not more diverse, as maintained by Thompson. Including the site 15 sample with *G. floridensis* has a negligible effect on the total gene diversity within the complex because that sample, having only one unique allele (*Acph-1*^{100}), was very close to other samples.

Semispecies, sibling species, and subspecies in the *Drosophila willistoni* group were not defined by genetic distances, as Thompson states, but by reproductive relationships (Ayala et al., 1974). Those authors calculated genetic distances in order to measure genetic divergence between samples at varying levels of taxonomic divergence. These data were chosen as a standard of comparison for describing genetic divergence between *Goniobasis* populations because the *D. willistoni* group is one of the best-studied groups where both taxonomic divergence, based on reproductive relationships, and genetic divergence between populations are known. Chambers (1980: 72–73, 75–76) discussed the possible values and shortcomings of these comparisons. Chambers (1980) did not advocate the strict application of these comparisons for making taxonomic decisions but stated that "Electrophoretic methods alone cannot generate classification . . ." and warned that ". . . taxonomic decisions based solely on such comparisons [with *D. willistoni*] will often be erroneous" (p. 72). Chambers (1980: 76) clearly indicated the subjective nature of these comparisons when studying closely related species.

Thompson objects that electrophoretic data (Chambers, 1980) do not distinguish a *G. floridensis* "subspecies" (which apparently has not been described) in the Waccasassa River. This form intergrades with a form with the "standard" *G. floridensis* sculpture pattern in the Wekiva River (Chambers, 1980, fig. 5). Both morphological and electrophoretic evidence indicate that the Waccasassa River "subspecies" is not so "very distinct" as Thompson believes.

One must take care when using the term "phylogenetic" when referring to electrophoretic data. In particular, fig. 3 of Chambers (1980) is a dendrogram that summarizes the genetic distance values in table 2 and is not strictly a phylogeny, a term which is not used in that paper. Conditions that must be met for such a dendrogram to represent a phylogeny are set down in Nei (1975).

The subjectivity of applying of electrophoretic data to taxonomic classification was clearly indicated in the discussion of systematic implications in Chambers (1980). Few sets of data, whether from electrophoretic, karyotypic, or morphological studies, permit "clear cut" determinations of taxonomic groupings of related allopatric populations.

Electrophoretic, karyotypic, and morphological data each give the researcher a view of a different aspect of an organism's biology and should not be viewed as antagonistic to each other. Each data set, however, should be evaluated according to criteria that take into account the theoretical constraints of the particular technique employed. Chambers (1978, 1980) identifies some of these criteria for the application of electrophoretic data to the classification of riverine snails. Given the enormous taxonomic problems presented by *Goniobasis* it would be unwise to discard any set of data that supplies a measure of an important aspect of evolutionary divergence.

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AMERICAN MALACOLOGICAL UNION
SYMPOSIUM: FUNCTIONAL MORPHOLOGY OF CEPHALOPODS

Organized by Clyde F. E. Roper, A.M.U. President
William H. Hulet, Rapporteur
Louisville, Kentucky
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INTRODUCTION TO THE INTERNATIONAL SYMPOSIUM ON FUNCTIONAL MORPHOLOGY OF CEPHALOPODS

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When the broad diversity in morphology of cephalopods is considered, it seems surprising that so little is known about the functional nature of morphological features in this highly evolved group of mollusks. The reasons for this, perhaps, are rooted in the past when the very problems that plague us today were even more unsurmountable: collections of specimens, especially of oceanic forms, were woefully inadequate; maintaining cephalopods alive for behavioral observations was almost impossible, with the occasional exception of *Octopus* and *Sepia*; microscopic and other laboratory instrumentation was primitive or non-existant. With the exception of a short burst of activity in the very late 19th Century and early 20th Century, led by the German master Professor Carl Chun, strikingly few morphological studies exist, and even fewer deal with functional aspects.

In an attempt to begin to alleviate the gap in our knowledge, an international symposium entitled "Functional Morphology in Cephalopods" was conducted during the 46th Annual Meeting of the American Malacological Union held at Louisville, Kentucky (21 July, 1980). The symposium papers, presented by teuthologists with a broad range of research interests, reflect the current activities in functional morphology. Both the papers and the vigorous discussions that followed pointed out that many problems and gaps still exist and suggested new directions in research. Interestingly, a majority of the papers dealt with features of the skin—chromatophores, iridophores, photophores, and epithelium. Other topics covered salivary glands, the mantle complex, and the "kidneys" as a unique habitat for parasites. These studies reflect the significant advances that have been made in the past two decades in our ability to capture and maintain cephalopods alive for observation and experimentation and to explore ever-finer structural details through transmission and scanning electron microscopy. This symposium provided a forum for the beginning of the modern age of functional morphology of cephalopods.

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THE FUNCTIONAL ORGANIZATION OF CHROMATOPHORES AND IRIDESCENT CELLS IN THE BODY PATTERNING OF *LOLIGO PLEI* (CEPHALOPODA: MYOPSIDA)

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ABSTRACT

The tropical arrow squid *Loligo plei* is capable of a wide range of colorful body patterns used for camouflage and visual communication. These *body patterns* are produced by the appearance of different combinations of chromatic and postural *components*. This paper deals solely with the chromatic (color) components, which are broken down into groups of morphological *units* comprising one or two types of *elements*: chromatophores and iridescent cells located in the dermis of the skin. These elements are not distributed evenly across the body surface, but are organized into one of seven different morphological units. The chromatophore elements in *L. plei* are yellow, red or brown and may be arranged into any one of four morphological units. Each morphological unit is described in terms of its distribution across the skin surface as well as the sizes, colors and static morphological array (horizontal and vertical) of chromatophores within the unit. The iridescent cells—iridophores and reflector cells—are reflective elements positioned subjacent to chromatophores. They are arranged into three units that may appear either yellowish-white, pink or green; the horizontal distribution of each unit is described. The seven types of morphological units provide the capability of producing 16 differently shaped and colored components. Each chromatic component is described in terms of the functional morphology of the units that constitute it. In *L. plei,* the expression of chromatic components is a result of (1) the particular static morphological array of elements within differently constructed units, and (2) the selective nervous excitation of those morphological units. The resultant color of each component is postulated to aid in camouflage in shallow water, but in intraspecific signalling it is contrast and configuration of the components (and not color) that are important in transmitting information. Three aspects of sexual dimorphism related to chromatic behavior are demonstrated in *L. plei,* and chromatic expression within the genus *Loligo* is reviewed and compared.

Key words: squid; *Loligo*; skin morphology; chromatophores; iridophores; color change; behavior; camouflage.

INTRODUCTION

Camouflage and visual communication are highly advanced in cephalopods and they have developed a wide range of body patterns to convey this visual information. It is my purpose in this paper to illustrate in *Loligo plei* (Blainville, 1823) how the anatomically fixed elements of pattern and color change—the chromatophores and iridescent cells—are differently organized and distributed in the dermis of certain body areas to produce an assortment of spots, streaks, splotches, etc. used in body patterning. While a great deal of work has been performed on the structure and physiology of the chromatophore organ over the past 150 years, few studies have been aimed at describing the range of body patterns that the chromatophores and iridescent cells produce. Body patterns of selected species of the genera *Octopus* (Cowdry, 1911; Hanlon & Hixon, 1980; Packard & Sanders, 1969, 1971; Packard & Hochberg, 1977; Warren et al., 1974), *Eledone* (Boyle & Dubas, 1981), *Sepia* (Holmes, 1940, 1955) and *Sepioteuthis* (Boycott, 1965; LaRoe, 1970; Moynihan, 1975) have been examined in varying detail. In these studies it has generally been assumed for each species that (1) the anatomical array of chromatophores and iridescent cells is fixed, that is, it is recurrent and constant over the entire body surface, and (2) the appearance of body patterns is produced principally through the selective nervous excitation of groups of differently colored chromatophores. In *Loligo plei* the organization of chromatophores and iridescent cells is not constant; it differs in specific

areas of the body. Therefore, the actual appearance of specific chromatic components results not only from selective nervous excitation of differently colored chromatophores, but from the size and distribution (horizontal and vertical) of chromatophores and iridescent cells on different areas of the body.

To describe the organization of the chromatic elements I have followed the hierarchical classification of body patterning developed by Packard & Sanders (1969, 1971) and Packard & Hochberg (1977), and further clarified by Packard (1982) in this symposium. Essentially they have determined that *body patterns* of cephalopods are produced by different combinations of chromatic, textural and postural *components.* The chromatic components, in turn, may be broken down into morphological and physiological *units* comprising two *elements*: chromatophores and iridescent cells in the dermis of the skin. In this paper I will describe (1) the two elements of patterning in *L. plei,* (2) the functional morphology of seven types of morphological units, and (3) sixteen resultant chromatic components. The remaining aspects of body patterning in *L. plei* will be the subject of a future publication. These aspects include descriptions of postural components, behavioral movements and body patterns as well as the behavior associated with them.

Loligo plei is commonly referred to as the tropical arrow squid and is distributed on the continental shelf and slope on the east coasts of North and South America from North Carolina, throughout the Gulf of Mexico and Caribbean Sea, to Brazil (LaRoe, 1967; Voss et al., 1973; Cohen, 1976; Whitaker, 1978). It is a pelagic, schooling species that is abundant off the Texas coast in depths of 20 to 75 m, and it is commonly caught together with the long-finned squid *Loligo pealei* and *Lolliguncula brevis* (Rathjen et al., 1979). *Loligo plei* was originally described by Blainville (1823), and later made the type species of the genus *Doryteuthis* by Naef (1912). There is disagreement regarding its generic status (see Cohen, 1976), but I will rank *Doryteuthis* as a subgeneric designation and hereafter refer to this species as *Loligo plei.*

MATERIALS AND METHODS

Squids were collected under night lights with soft-mesh dipnets and transported in shipboard seawater tanks to laboratory aquaria. They were maintained in round 2 m diameter tanks or 10 m long raceway systems (Hanlon et al., 1978). Over 500 individuals were observed between 1975 and 1980. Squid survival in both systems averaged 20 days, with some animals surviving up to 84 days. Individuals ranged in size from 3 mm ML (mantle length) to 348 mm ML. The average sizes for squids in this study were 90 mm ML for females and 175 mm ML for males; maximal mantle lengths were 211 mm ML for females and 348 mm ML for males. Most observations and photographs were made through viewing ports built into the sides of the tanks, and the remainder from above the tanks. Close-up observations of the skin were made at 6, 12 and 25× under a Wild stereomicroscope, usually with the squids narcotized in 1% ethanol in seawater. Color videotapes were made through the microscope by adapting the video camera lens to the third viewing eyepiece and lighting the subject with fiber optics. All photographs were taken with flashed light using a Nikon F camera, 24, 55 and 105 mm lenses, and a Nikon bellows unit for high magnification photographs of the skin. Frozen sections of pieces of skin were prepared to confirm the depth distribution of differently colored chromatophores. Small skin patches were excised from live squids, frozen and cut in a Bright Model FS/FAS/M cryostat, then stained with a polychrome (Toluidine Blue and Basic Fuchsin), dehydrated with ethyl alcohol, cleared in xylene and mounted with Eukitt mounting media on glass slides. Frozen sections were viewed and photographed with a Leitz Orthoplan compound microscope. Colors were standardized according to the Pantone Matching System (PMS, 1966) and in the text the PMS color number is given in parentheses.

ELEMENTS OF BODY PATTERNING

The elements of body patterning are the smallest individual morphological entities in the skin that are responsible for displaying color. In *Loligo plei* there are three colors of chromatophores—yellow, red, brown—and two types of iridescent cells—iridophores and reflector cells. The chromatophores produce pigmentary colors of relatively long wavelengths by reflection of light after differential absorption in the pigment cell. The iridescent cells produce structural color of a wide spec-

trum of wavelengths by reflecting ambient light from the iridosomal platelets within the cell. It is the combined effects of all these elements that result in color and pattern. For a more detailed account of how elements produce color the reader is referred to Packard & Hochberg (1977: 193). Their descriptions are based upon *Octopus,* and some important differences in the skin of *Octopus* and *Loligo* should be pointed out: (1) chromatophores of *Loligo* are much larger and fewer per unit area, (2) in general *Loligo* has three color classes of chromatophores—yellow, red, brown—but no black (melanophores) or orange chromatophores as in *Octopus,* (3) in *Loligo* no white leucophores (Packard & Sanders, 1971) have been identified, (4) there are no grooves in the skin of *Loligo* that partition the chromatophores and iridophores into discrete, recognizable skin patches such as those found in *Octopus,* and (5) the skin of *Loligo* is smooth and can not produce the papillae that result in different skin textures in *Octopus.* In summary, the skin of *Loligo* is less differentiated and complex than that of *Octopus*, but the range of body patterns that *Loligo* can produce is nevertheless extensive.

Chromatophores

Many aspects of the morphology, ultrastructure and physiology of the chromatophores of *Loligo* have recently been elucidated in considerable detail by Florey (1966, 1969), Cloney & Florey (1968), Florey & Kriebel (1969), Mirow (1972a) and Weber (1968, 1970, 1973). Chromatophores are controlled by nervous stimulation (Florey, 1966, 1969; Florey & Kriebel, 1969). The neural connections of individual chromatophores are complex yet poorly understood, but it is known that individual chromatophores receive multiple innervation (Florey, 1966, 1969; Weber, 1968, 1970, 1973) and that certain groups of chromatophores may be linked by their musculature (Froesch-Gaetzi & Froesch, 1977). Boycott (1961), Young (1974, 1976, 1977) and Messenger (1979) have contributed to an understanding of some of the neural connections of chromatophores in the brain, but the details of the way the central nervous system controls the chromatophores are by no means clear.

Fig. 1A shows the three basic color series of chromatophores found in *Loligo plei*: yellow (PMS 135), red (PMS 201) and brown (PMS 491). These should be regarded strictly as broad categories of color, since in living squids gradations of all three colors are seen. For instance, there are pink-colored chromatophores (PMS 204) of the same size range and depth as the red, but it is not clear whether these represent a separate color series of chromatophores or nascent red chromatophores with less pigment. An important consideration in color determination is that the quality of light reflected from chromatophores changes as the pigment granules are dispersed or concentrated within the pigment container. For instance, when the chromatophores are only partially expanded, yellow appears more orange (PMS 152), red appears darker red or almost brown (PMS 492) and brown appears almost black (PMS 497). When frozen sections of chromatophores are examined microscopically there is considerable gradation of color among all the chromatophores and distinction between red and brown is often difficult. The pigments of cephalopod chromatophores have not been identified biochemically and the number of color series of chromatophores is unclear (Packard & Hochberg, 1977). For *Loligo,* the general concensus among other workers (Cloney & Florey, 1968; Schelling & Fioroni, 1971; Mirow, 1972a; Weber, 1973; Williams, 1909) and myself is that there are only three color series—yellow, red and brown—with pink being considered in the red series.

Iridophores and Reflector Cells

In contrast to chromatophores, the morphology, ultrastructure and physiology of the iridescent cells of cephalopods are less well understood. Brocco & Cloney (1980) have recently investigated the morphology of *Octopus* reflector cells and compared them with sepioid and teuthoid iridophores. In accordance with their nomenclature the term reflector cell will be used here to describe the uniform iridescence of various eye parts of *Loligo plei*, since presumably their ultrastructure is similar to the reflector cells of the eye parts of *Loligo forbesi* (Denton & Land, 1971) in which the broad surfaces of the reflective platelets face the integument. Iridophores are nonpigmented cells that reflect, diffract and scatter light. They are characterized by their intracytoplasmic platelets of high refractive index and they are distinguished from reflector cells by the orientation of their platelets, which are oriented on edge relative to the surface of the skin. Some aspects of iridophore

morphology in the squids *Loligo opalescens* and *Loligo pealei* have been clarified by Mirow (1972b) but because of complex membrane systems within the iridophore she was not able to construct a three-dimensional model of the cell. The physics of diffraction so thoroughly known in fish platelets (*cf.* Denton, 1970) has not been applied in studies of iridophores in cephalopods (but see Denton & Land, 1971) and therefore the mechanisms of reflection and the functional morphology of the iridophores in producing specific color effects is yet unknown. These aspects are beyond the scope of this work and I will limit my description to a macroscopic visual analysis of the distribution, color and transient appearance of iridophores and reflector cells in living *L. plei.*

There appear to be three expressions of iridescence in *Loligo plei.* First is the bright, smooth reflective layer of reflector cells that is always visible on the eye parts and ink sac. Second is the shingled appearance of iridophore splotches on the mantle and fins. Third is the shingled appearance of a continuous sheet of iridophores on the mantle collar, the dorsal head region and the first pair of arms. Mirow (1972b) described short and long iridophores in *Loligo* but it remains to be determined what their interactions are and which visual effects they produce. It is noteworthy that iridophores are present on the mantle of the hatchlings of *Loligo* (Arnold, 1967; Schelling & Fioroni, 1971; McConathy et al., 1980), but they are not conspicuous and they are more numerous on the ventral surface. Histological sections of skin show that there are also iridophores in the dermis of the lateral (Fig. 9) and ventral surfaces of adult *L. plei* but they are not obvious in the living animals.

CHROMATIC UNITS: THE STATIC MORPHOLOGICAL ARRAY

The chromatic units contain the anatomically fixed elements used in color change, i.e. the static morphological array. The chromatic units involving chromatophores are recognizable to a human observer both in their retracted and expanded states because of the relatively uniform and recurrent distribution of elements within them. The units of *Loligo* are not delineated by skin grooves as in *Octopus,*

FIG. 1. Aspects of body patterning in the tropical arrow squid *Loligo plei.*

A) Standard Discoid Units, the most common and widespread arrangement of chromatophores. Brackets indicate the approximate dimensions of a single morphological unit. Note the considerable overlap among chromatophores of the three colors. The chromatophores are not yet at maximal expansion. From the dorsal mantle of a male, 220 mm ML. Line represents 1 mm.

B) Tentacular stalk (center) and second arm spot (right) of a male squid, 165 mm ML. On the stalk note the Yellow-Brown Discoid Units and the areas devoid of chromatophores. Arrow points to one of the 7–8 spots characteristic of the chromatic component TENTACULAR STRIPE AND SPOTS. The second arm spot is produced by selective nervous excitation of a group of Modified Discoid Units. Note the overlap among all the brown chromatophores. Line represents 2 mm.

C) Frozen sections of a Standard Discoid Unit. Yellow chromatophores lie above reds, and reds lie above browns. From a male squid, 108 mm ML. Line represents 200 μm.

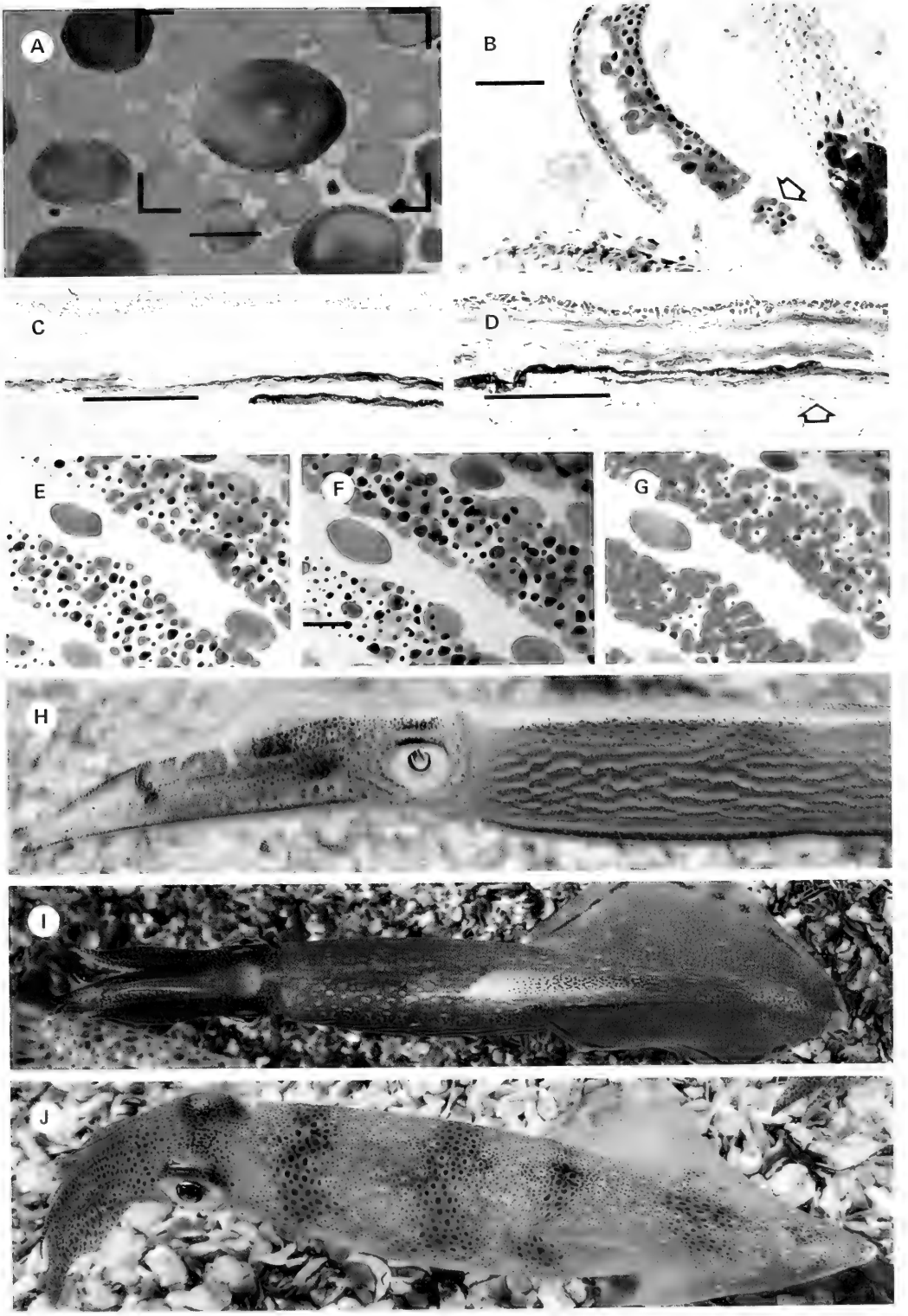
D) Frozen section of a Lateral Flame Unit. Yellow chromatophores lie above browns, and browns lie above reds. Arrow indicates the layer of iridophores deeper in the dermis (see Fig. 9). Purple layer just below the epidermis is an excess of polychrome stain. From a male squid, 108 mm ML. Line represents 200 μm.

E, F, G) Different phases of expansion of the same group of Lateral Flame Units that make up the chromatic component LATERAL FLAME. See explanation in Discussion. From a male squid, 220 mm ML. Line represents 1 mm.

H) Lateral view of a male *Loligo plei,* 201 mm ML, showing the following chromatic components to a nearby squid: TENTACULAR STRIPE AND SPOTS, ARM SPOTS, LATERAL FLAME, MID-VENTRAL RIDGE and DORSAL ARM IRIDOPHORES.

I) Top view of a male *Loligo plei,* 175 mm ML, showing many of the same chromatic components in (H) towards a squid on its left (towards the bottom in the photograph). Note that LATERAL FLAME is expressed unilaterally only on the side towards the other squid. DORSAL MANTLE SPLOTCHES are prominent on the mantle, and the posterior portion of the conspicuous white testis is very lightly shaded by the chromatic component SHADED TESTIS. DORSAL ARM IRIDOPHORES is weakly expressed, and ARM SPOTS and STITCHWORK FINS are shown bilaterally.

J) Female *Loligo plei,* 121 mm ML, showing the chromatic components RING and DORSAL MANTLE SPLOTCHES. RING is produced by the selective nervous excitation of Standard Discoid Units on the mantle and fins. Note the silver-green appearance of the Reflector Cell units on the sclera of the eyeball.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but they are nevertheless recognizable due mainly to the typed array of adjoining units. This "standard part" arrangement (Riedl, 1978) is theorized to be a result of the processes of morphogenesis that insure the regular spacing of dermal elements during ontogeny (Packard, 1982). The morphological units described herein must be distinguished from the physiological units described by Packard (1982); the physiological units represent the concept of neurophysiological motor units that reflect nerve or muscle connection, not the anatomical placement of elements. This paper emphasizes the arrangement of the chromatic units, because the static morphological array of elements provides the framework for both the morphological and physiological unit structures, and these are the very bases of color and pattern change.

In the initial stages of this study it appeared to me that observed differences in components were due mainly to selective nervous excitation and that the chromatophores were generally evenly distributed over the entire body surface. However, this could not completely account for the wide variations of appearance in different chromatic components, and upon closer observation it became apparent that in certain body areas the arrangement of chromatophores and iridophores was organized to produce specific visual effects. Accordingly I have grouped the two types of elements into seven chromatic units to facilitate the description of sixteen chromatic components. This has inevitably involved some arbitrary decisions, but as a guide I have always chosen the most obvious recurring arrangement of elements into which chromatic components can be most easily divided, and those that can be readily identified on a macroscopic level.

Standard Discoid Unit

This unit is characterized by a large brown chromatophore surrounded by a circlet of red chromatophores, with small yellow chromatophores interspersed throughout (Fig. 1A). The term "discoid" describes the generalized dimensions of the morphological unit, i.e. it is circular in the horizontal plane (1–4 mm in diameter) and flat in the vertical plane (50–150 μm). The static morphological array of retracted chromatophores in this unit is shown in Figs. 2A and 4. The Standard Discoid Unit is the most common unit on the body surface (Fig. 3). Adjoining units are most easily recognized by the large, central brown chromatophore, but some red chromatophores in the circlet may be shared by the adjoining unit. On the dorsal mantle, most of the larger units have a large iridophore splotch centered beneath the center brown chromatophore (Figs. 10, 11). In this type of unit brown chromatophores are larger than reds, and reds are larger than yellows; this holds true both at full retraction and full expansion (Table 1). Their vertical distribution in the dermis, as shown by frozen sections, is: browns deepest, reds intermediate and yellows shallowest (Fig. 1C). When retracted, each color class is generally identifiable by its minimal chromatophore diameter; e.g. all yellows are smallest and of the same approximate size. A great deal of overlap occurs when all chromatophores in the array are at full expansion. Yellows and reds overlap with all colors, while browns overlap only with reds and yellows. Browns do not overlap with other browns because they are so widely and evenly spaced that it precludes overlap. In adult squids the average Standard Discoid Unit comprises one brown chromatophore, approximately 7 to 13 reds, and 15 to 30 yellows; it may or may not have one underlying iridophore splotch.

Modified Discoid Unit

This unit is similar in most respects to the Standard Discoid Unit, except that some of the red chromatophores in the circlet are replaced by brown chromatophores similar in size to the reds (Fig. 2B). The larger proportion of brown chromatophores per unit produces an overall darker shade when full expansion is achieved. The sizes of the chromatophores and their vertical distribution are similar to the Standard Discoid Unit. These units are found in two areas (Fig. 3): (1) over bright underlying organs, such as the highly reflective sclera on the back of the eye (Fig. 5) or the white testis of males, and (2) where very dark spots are produced, as on the second and third arms of males (Fig. 1B).

Yellow-Brown Discoid Unit

This unit is characterized by the absence of red chromatophores. Yellows are the same size as in all other types of units, while the browns are slightly larger than yellows, though not as large as browns in Standard and Modified Discoid Units. Overlap occurs commonly and yellows always lie above the

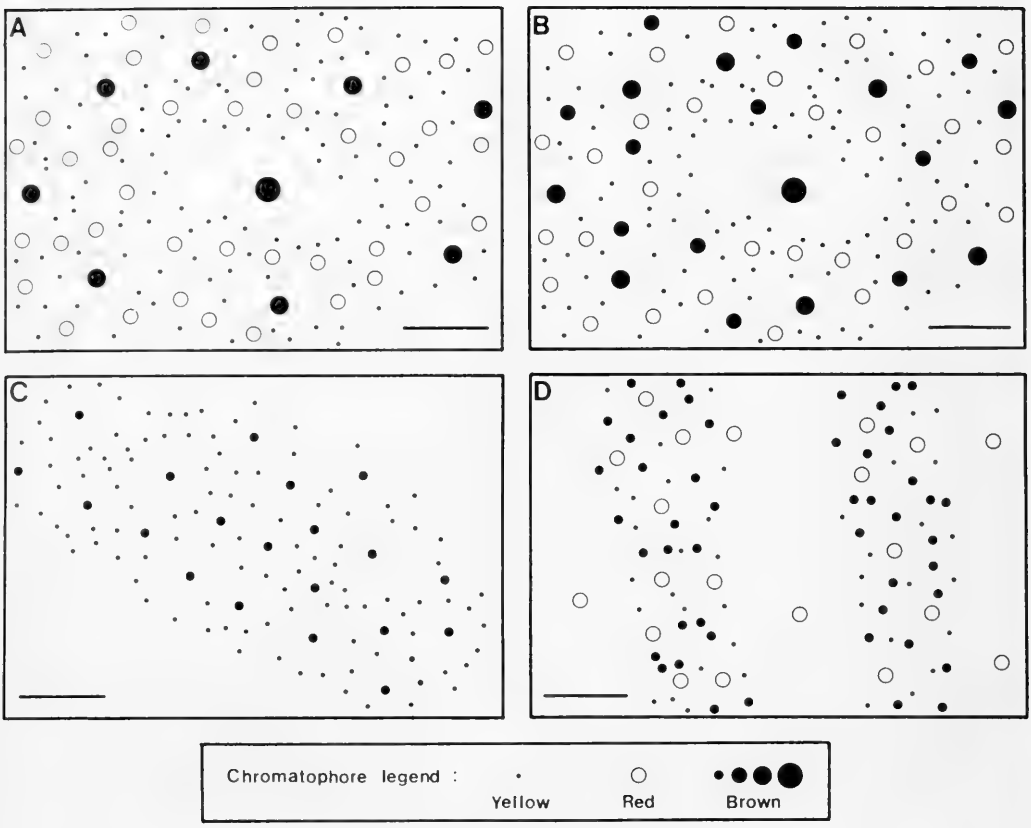


FIG. 2. Static morphological arrays of the four types of chromatophore units, each drawn to the same scale (line equals 1 mm) to accurately depict the distance between retracted chromatophores. All illustrations were traced from video tape recordings of live squids. More detailed descriptions are given in the text. A) Standard Discoid Units. Each brown chromatophore represents the center of one morphological unit. The central unit is the largest and presumably oldest one, and the brown chromatophore at its center is larger than surrounding browns both in the retracted and expanded states. Drawn from the dorsal mantle of a male, 170 mm ML. B) Modified Discoid Units. Identical to (A) except that some brown chromatophores have replaced reds in the circlet around the larger center brown. C) Yellow-Brown Discoid Units, distinguished by the absence of red chromatophores. The brown chromatophores are smaller than browns in Standard and Modified Discoid Units. This illustration was traced from one group of adjacent units that made up one "dash" of the chromatic component STITCHWORK FINS around the periphery of the fins of a male, 185 mm ML. D) Lateral Flame Units, characterized by longitudinally oriented rows of yellow and brown chromatophores. The browns in these units are nearly as small as the yellows. Drawn from the lateral mantle of a male, 150 mm ML. NOTE: The diameters of the dots and circles are not all drawn accurately to the scale bar. In (A) and (B), the yellow chromatophore dots must be increased in diameter by 1.5× to give an accurate scale size of chromatophores in the retracted state (see Table 1). In (C) and (D), the sizes are approximately accurate as shown.

browns. The static morphological array of retracted chromatophores is shown in Fig. 2C. The brown chromatophores are evenly dispersed relative to one another and form the centers of the units, which are 1–2 mm in diameter. Smaller yellows are evenly interspersed amid the remaining areas. There are generally 5–10 yellows and one brown per unit.

Distribution of this unit is confined to two areas (Fig. 3): (1) on the tentacular club and stalk of juvenile and adult squids, and (2) around the periphery of the fins of males larger than approximately 105 mm ML. On the inside surface of the tentacular stalk, the units are grouped into seven or eight distinct "spots," while the adjacent areas are without chromatophores (Fig. 1B). On the fins, the

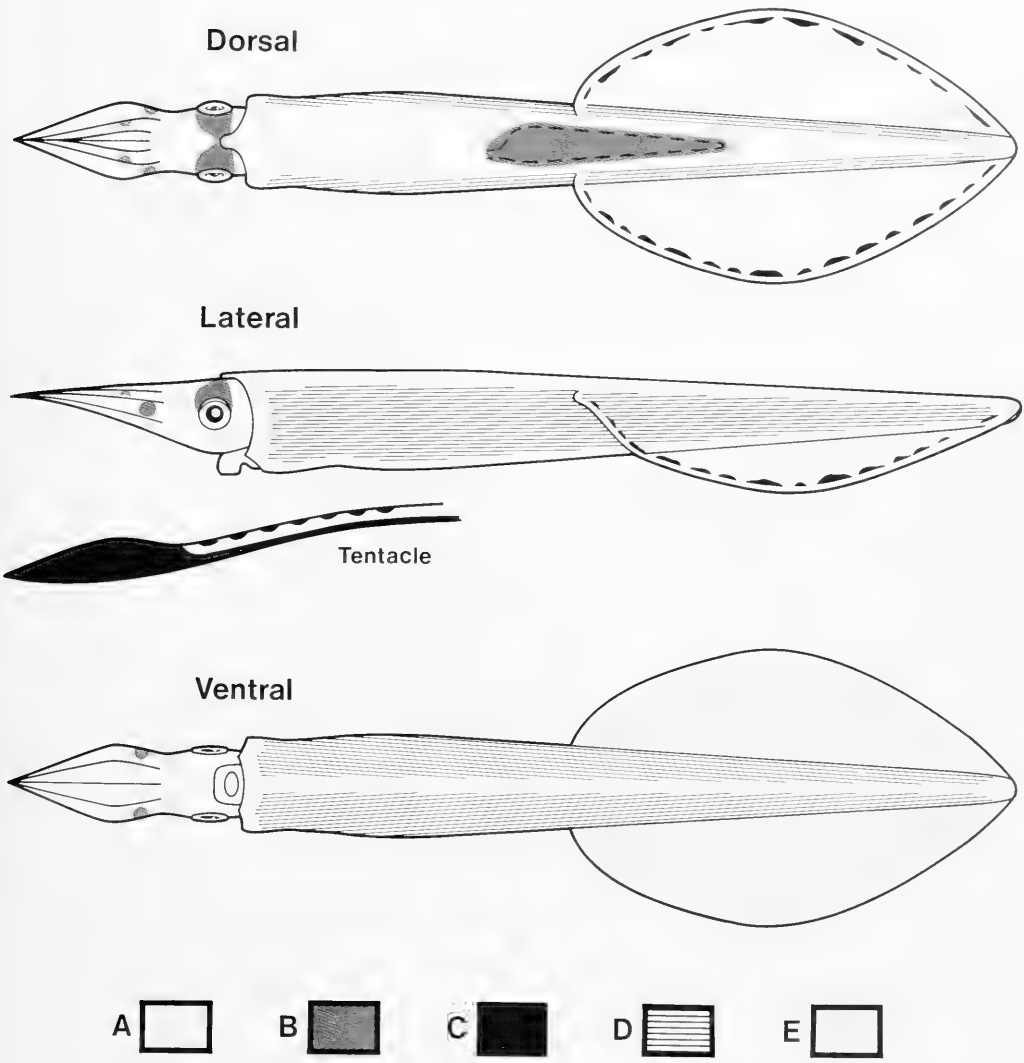


FIG. 3. Distribution across the skin surface of the four types of chromatophore units in male *Loligo plei*. Iridescent cell unit types are not shown. In the top figure the dashed line represents the position of the white testis. (A) Standard Discoid Units. B) Modified Discoid Units. C) Yellow-Brown Discoid Units. D) Lateral Flame Units. E) Areas devoid of chromatophores. The drawing of the outside portion of the left tentacle is slightly enlarged. Females have Standard Discoid Units all over the body surface except for Modified Discoid Units over the top of the eyes and no chromatophores on the underside of the fins. Illustration by Charles Moen.

units are grouped 2–3 units wide into a continuous line, 2–5 mm inside the margin of the fin, that is present around the periphery of the fins.

Lateral Flame Unit

This unit, found only in mature males larger than approximately 70 mm ML, is markedly different in nearly every respect from the three types of discoid units. Adjoining units are organized into long, longitudinally oriented rows of yellow, red and brown chromatophores separated by areas relatively free of chromatophores except for widely spaced large red chromatophores (Figs. 2D and 6). The rows are 1.5–3.0 mm wide. Yellow chromatophores are smallest, browns are the same size or

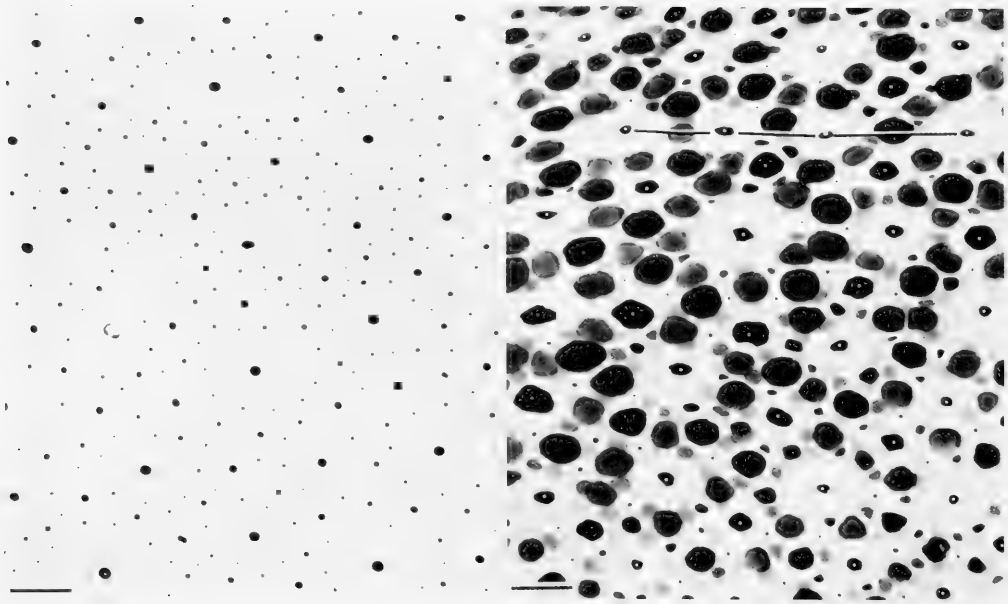


FIG. 4. Photographs of Standard Discoid Units on the mid-dorsal mantle of live squids. Left: Retracted units from a male squid, 190 mm ML. Compare unit structure with the diagrammatic sketch in Fig. 2A. Line represents 1 mm. Right: Expanding units on a male squid, 147 mm ML. Brown chromatophores at the center of each morphological unit are identified by a small white dot at their centers. This photograph represents one transient appearance of very rapidly expanding chromatophores. There are many examples of units in different states of expression. Browns in similar states of expansion are members of the same physiological units (e.g. the four browns in the upper right hand corner connected by line). Note the many retracted chromatophores (smallest dark dots) that are members of physiological units different from those that are expanded. Line represents 1 mm.

TABLE 1. Sample chromatophore diameters (in mm) of *Loligo plei*. Measurements taken from Standard Discoid Units on the dorsal mantle of a freshly dead male, 88 mm mantle length. Measurements were made with a micrometer eyepiece at 25×.

		Yellow n = 20	Red n = 20	Brown n = 20
Retracted	mean	0.07	0.14	0.24
	range	(0.04–0.12)	(0.08–0.24)	(0.12–0.48)
Expanded	mean	0.35	0.68	1.07
	range	(0.12–0.60)	(0.36–1.12)	(0.80–1.52)
Expansion factor	mean	5.00×	4.86×	4.46×
	max.	(11.00×)	(14.00×)	(10.67×)

slightly larger than yellows, and reds are very large. Their vertical distribution in the dermis differs from the three discoid units. It is: yellows shallowest, browns intermediate and reds deepest (Fig. 1D). When fully expanded, yellows always overlap browns and browns always overlap reds. When retracted, yellows and browns are nearly identical in size and color and are generally indistinguishable; reds are larger and identifiable. At full expansion, the yellow and brown chromatophores form long distinct rows that are accentuated by the adjacent clear areas that have relatively few large red chromatophores. The distribu-

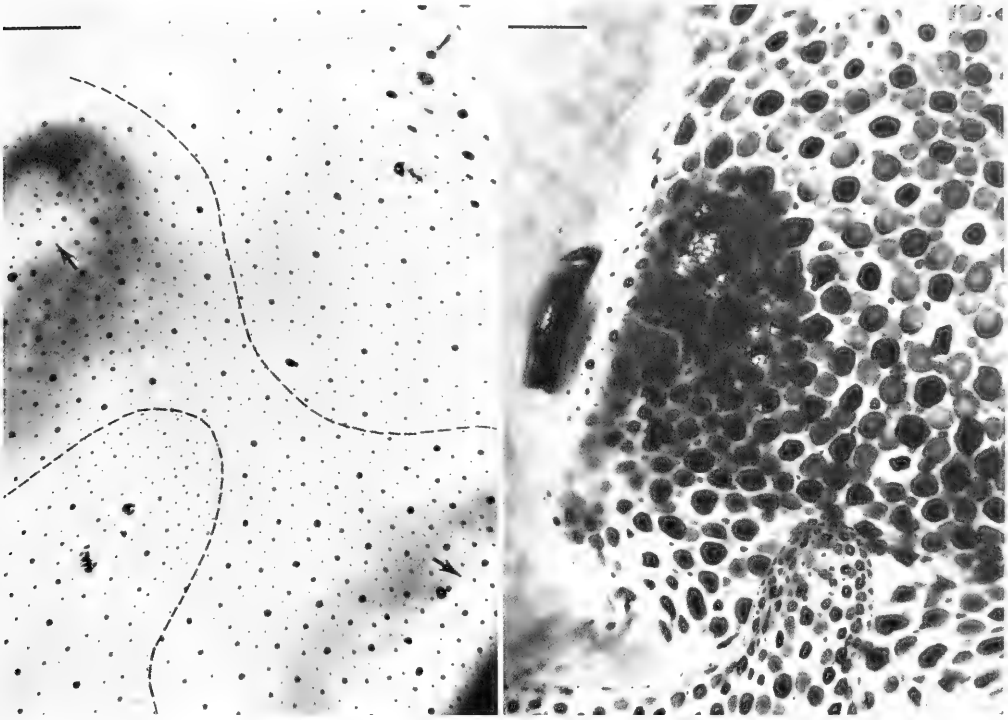


FIG. 5. Photographs of Modified Discoid Units on the dorsal head region over the eyes (arrows) of live squids. The rounded tip in the bottom of each photograph is the anterior portion of the mantle. Left: Retracted units on a male, 190 mm ML. The area within the dashed lines represents the approximate distribution of the Modified Discoid Units. Compare with the diagrammatic sketch in Fig. 2B. Line represents 2 mm. Right: Expanded Modified Discoid Units over the top of the left eye of a male squid, 147 mm ML. The expansion of these units results in the chromatic component SHADED EYE. The units are not yet fully expanded, but it can be seen that they are effective in blocking the reflection from the silvery Reflector Cells on the sclera of the back of the eyeball. Line represents 2 mm.

tion of these units is outlined in Fig. 3 and may be noted in photographs in Figs. 1H, 23 and 27. The long, flame-like streaks of yellow and brown chromatophores bifurcate and later merge as they progress down the mantle. The exact placement of the rows differs slightly from animal to animal and may be as distinctive as a fingerprint, but on a macroscopic scale the visual effect is the same. The most startling visual expression results when the yellows, browns and reds in the rows are maximally expanded while the reds between the rows in the clear areas remain retracted.

It is noteworthy that the rows are delineated by the distribution of the small yellow and brown chromatophores, and not in any degree by the large red chromatophores that are fairly evenly distributed throughout the entire area. This becomes obvious if one concentrates on observing only red chromatophores in photographs or if red chromatophores are selectively traced from a photograph (Fig. 7). The sequence in Figs. 1E, 1F and 1G shows the progressive neural recruitment of yellows and browns that result in the flames. The delineation of rows is further illustrated in the transition zone between the Standard Discoid Units and the Lateral Flame Units (Fig. 8). The transition is accomplished by (1) the disappearance of the large, deeply placed brown chromatophores that are at the center of Standard Discoid Units, (2) the appearance of a continuous, longitudinal row of small conspicuous brown chromatophores shallower in the dermis, just below the yellows, (3) the reorganization of the small, evenly distributed yellow chromatophores of the Standard Discoid Units into distinct rows and (4) the

FIG. 6. Photograph of retracted Lateral Flame Units that make up the chromatic component LATERAL FLAME. From the lateral mantle of a male squid, 190 mm ML. Compare with diagrammatic sketch in Fig. 2D and photographs of expanded units in Figs. 1E, 1F, 1G, 8 and 23. Line represents 1 mm.

gradual reorganization of red chromatophores from circlets in the Standard Discoid Unit into an evenly dispersed array of red chromatophores that are approximately the same size range as reds found in Standard Discoid Units.

Reflector Cells

This term refers collectively to the reflective layers on the ink sac and around the eye—the eyelids, iris and the sclera. These cells reflect light constantly and are visually conspicuous except when the overlying chromatophores are maximally expanded. The iris, eyelids and ink sac reflect silver, while the sclera on the top and back of the eyeball reflects silver-green (Fig. 1J).

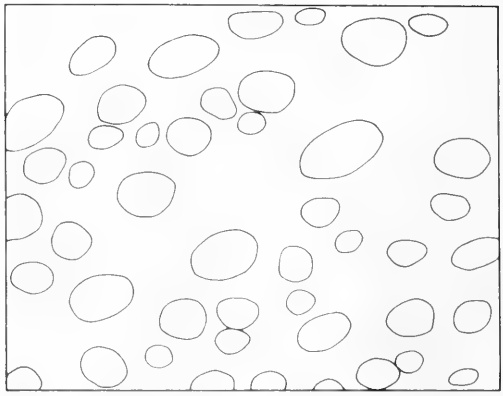


FIG. 7. Distribution of red chromatophores traced from a mirror image of Fig. 1F. Red chromatophores are fairly evenly distributed among Lateral Flame Units and are not aligned into distinct rows as are yellow and brown chromatophores.

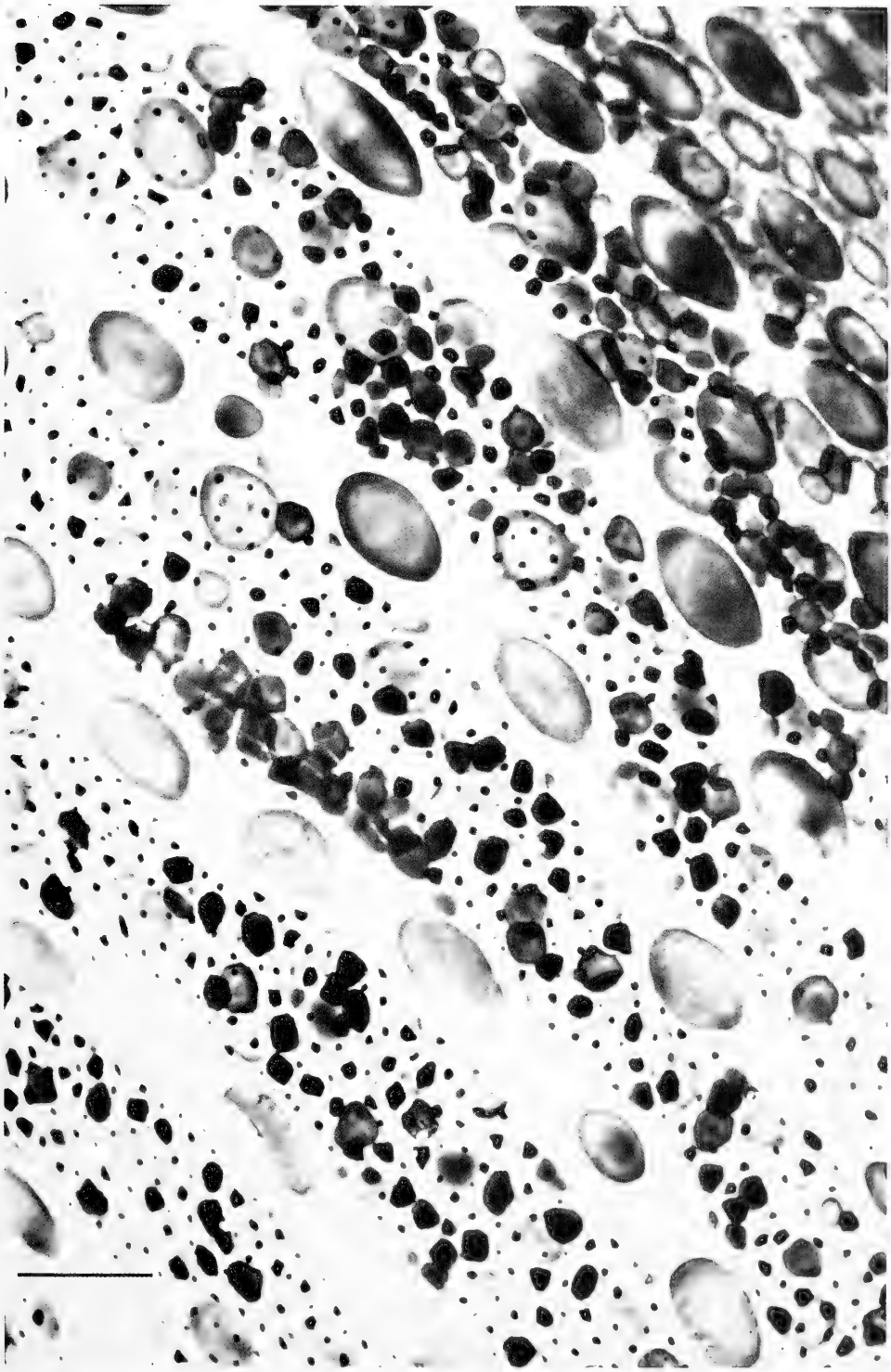
Iridophore Splotches

These splotches are distributed somewhat evenly over the dorsal mantle and fins and they act as a unit by showing the same degree of prominence. The splotches are distinct, irregulary shaped, 1–3 mm in diameter, and they appear either pink, green or yellowish-white (Fig. 1I). They are not always visible, and they show varying degrees of prominence even when the overlying chromatophores are retracted. The splotches are generally centered beneath a large brown chromatophore of the Standard Discoid Unit (Figs. 10, 11).

Iridophore Sheets

These iridophores appear as a continuous sheet of pink, green or yellowish white iridescence that has a visual effect similar to that of the splotches. Like the splotches, they are not visible at all times. These units are distributed over the dorsal head region of both sexes. They are particularly evident on the first pair of arms in adult males, and they periodically render the entire arm brightly iridescent (Fig. 1H). Adult females have fewer and less conspicuous iridophores on those arms by com-

FIG. 8. Transition zone between Standard Discoid Units of the dorsal mantle (extreme upper right hand corner) and Lateral Flame Units of a male squid, 220 mm ML. See text for explanation. Line represents 1 mm.



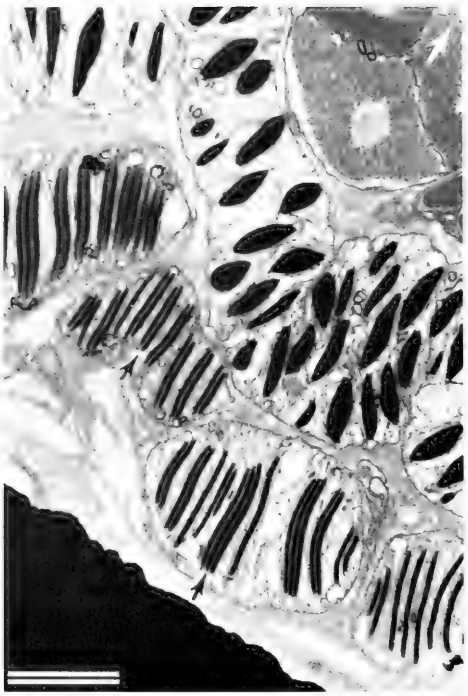


FIG. 9. Electron micrograph of iridophores from the dermis of the lateral aspect of the mantle of a male, 120 mm ML. Iridosomal platelets (black arrows) within iridophore cells are clearly evident. The ovoid inclusions in the cells lying between the iridophores and the muscle cells in the upper right hand corner are unknown structures that may be light-scattering organelles similar to leucophores (S. Brocco, personal communication, 1980). White arrow points toward the epidermis. Black area on bottom left is the grid border. Line represents 2 μm. Micrograph courtesy of M. R. Villoch.

parison. On the mantle, iridophores appear to be densely clustered around the anteriormost margin of the mantle, and when expressed they appear as a collar of pink or green (Fig. 12).

CHROMATIC COMPONENTS

Components are, by definition, the parts that make up a pattern. Therefore, a body pattern can be described in terms of the relative position and intensities of the components regularly present (Packard & Sanders, 1971). Chromatic components come and go, leaving no trace in the static morphological array (Packard & Hochberg, 1977). Thus emerges

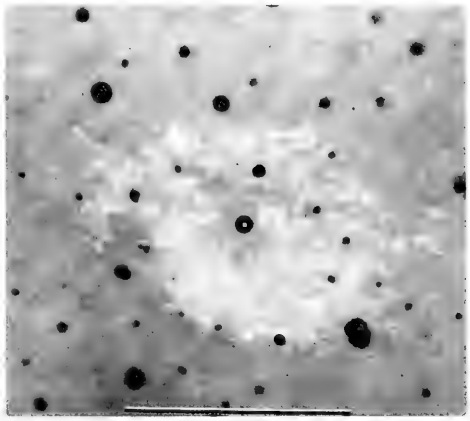


FIG. 10. Dorsal iridophore splotch on the fin of a male squid, 190 mm ML. The shingled appearance is characteristic of the splotches. The overlying chromatophores are mostly retracted, and the white dot marks the center brown chromatophore of a Standard Discoid Unit under which the splotch is centered. The striated fin muscles appear as faint, oblique lines oriented top right to bottom left. Line represents 1 mm.

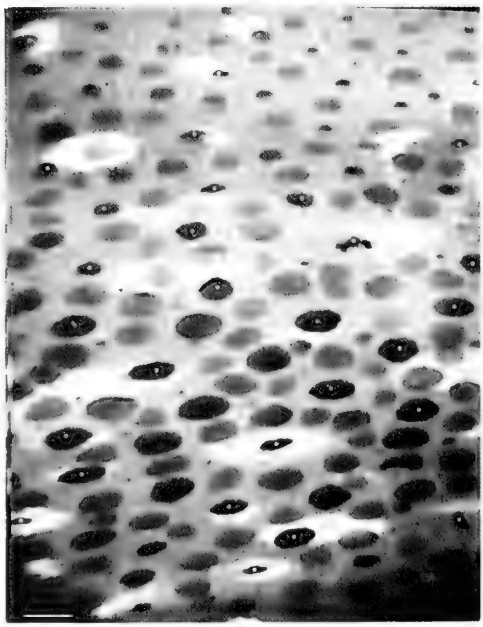


FIG. 11. Video tape recording from the dorsal mantle of a live male, 150 mm ML, showing the DORSAL MANTLE SPLOTCHES generally positioned beneath the brown chromatophores (marked with small white dots) of Standard Discoid Units. Line represents 1 mm.

FIG. 12. Male squid, 201 mm ML, showing the chromatic component DORSAL ARM IRIDOPHORES. In this case the chromatophores of the first pair of arms are retracted while the remainder on the head and arms is expanded, resulting in the boldest expression of the component. The Iridophore Sheets on the head can be seen, as well as DORSAL MANTLE COLLAR (arrow), DORSAL MANTLE SPLOTCHES and the outline of Lateral Flame Units on the mantle.

one of the general principles of patterning in cephalopods: that components vary in the extent of their expression from barely perceptible to fully expressed (Packard & Hochberg, 1977). The changes in expression reflect neural control and, because of the "fine tuning" that nervous control provides, the components may change abruptly (in tenths of seconds according to Hill & Solandt, 1934) or they may grade one into another. For this reason it is difficult to make definitive classifications of patterns, but by describing the discrete chromatic components that are commonly repeated, one can obtain a basis for describing body patterns.

The 16 chromatic components described here are based upon a wide size range of animals, and future work may reveal additional components. Some components are specific to a certain size range of squids, some to a specific sex, and some are common to all members of the species. When possible the components are described in terms of the types of units that constitute them.

CLEAR

This component (Fig. 13) is characterized by the complete retraction of all the chromatophores, which renders the squid translucent. The chromatic units of Reflector Cells on the eye parts are very prominent, and Iridophore Splotches may be present. Many of the internal organs are visible through the translucent skin. When viewed from above,

FIG. 13. Mating pair of *Loligo plei*. The female (top, 93 mm ML) shows the CLEAR chromatic component with DORSAL MANTLE COLLAR and DORSAL MANTLE SPLOTCHES. The white spot in mid-mantle is the ovary and the small dark spot below it (arrow) is the red accessory nidamental gland. The male (175 mm ML) shows ARM SPOTS, DORSAL ARM IRIDOPHORES, DORSAL MANTLE COLLAR and DORSAL MANTLE SPLOTCHES. The large white organ is the well-developed testis.

the testis of mature males and the ovary of mature females are clearly visible, as are the esophagus, stomach and mid-gut gland during feeding. The buccal mass and systemic heart are also visible. Laterally, the ovary, egg mass and red accessory nidamental gland (Figs. 13, 20) are clearly visible in sexually mature females. This component has been observed in males and females throughout the entire size range, from hatching (3 mm ML) to adult (348 mm ML).

ALL DARK

In this component all of the chromatophores are expanded and the squids appear uniformly dark over the entire body surface (Fig. 14). The overall hue is a deep red-brown. ALL DARK has been observed on squids of both sexes throughout the entire size range, including hatchlings.

Three variations have been noted. First, small, young squids (less than 70 mm ML) produce a lighter red color than adults because: (1) they have fewer chromatophores per unit area, (2) red and brown chromatophores of young squids are very similar in size and (3) there appear to be more pink-colored chromatophores than in the adult. Second, a dark mottled variation was observed on three occasions in an adult male (140 mm ML) sitting motionless on the bottom. The mottling was produced when small, irregular splotches of chromatophores were retracted, while the units of Iridophore Splotches became very distinct. Third, a female (93 mm ML) once produced a pulsating, rippling wave of chromatophores over the entire dorsal surface for 15 mins, ranging from light to dark red.

RING

Considerable variation appears in this component, but it is always distinguished by three or four transverse rings around the mantle. The most common form consists of four mantle rings (Figs. 1J, 15), the second anteriormost ring being normally the most prominent and well developed. This second ring is located anterior to the fin insertion, approximately ⅓ of the way to the anterior margin. The first ring is next most prominent and lies midway between the anterior mantle margin and the second ring. These first two rings completely encircle the mantle and in most cases are best developed dorsally. The third ring bisects the anterior fin insertion and generally extends transversely over the dorsal ½ to ⅔ of the mantle. The fourth ring is actually a transverse line of expanded Standard Discoid Units across the middle of the fin, but it gives the visual impression of a ring extending around the narrow posterior mantle. Regions between the rings and over the head and arms are normally clear, and Iridophore Splotches are usually prominent. This component has been observed on females of 34–136 mm ML and males of 85–252 mm ML.

The most common variation is the three-ring component, which is found in certain individual squids (mostly females) and is characterized by a single ring that is situated between where the second and third rings would be in a squid with the four-ring component. Another variation occurs when the Modified Discoid Units between the eyes become dark while the remainder of the head and arms remain clear; when viewed from above this produced the visual effect of an extra ring. In still

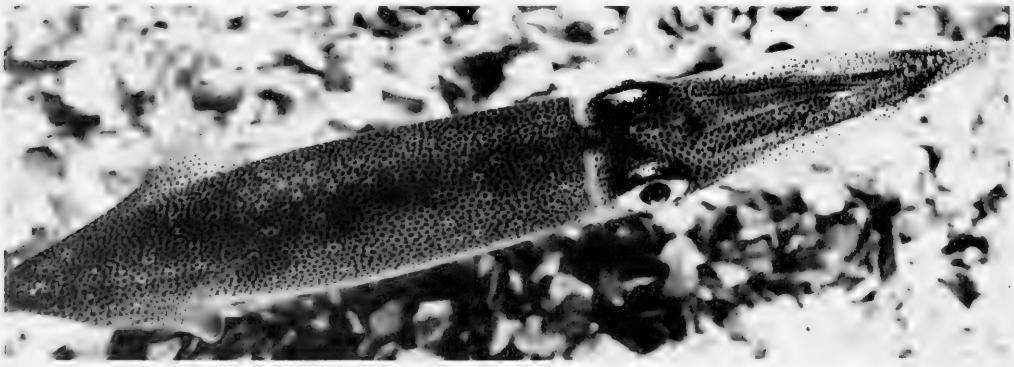


FIG. 14. A small female (67 mm ML) in ALL DARK. Note that the center brown chromatophores of some Standard Discoid Units on the mantle are only partially expanded, and the underlying iridophores of DORSAL MANTLE SPLOTCHES can be seen.

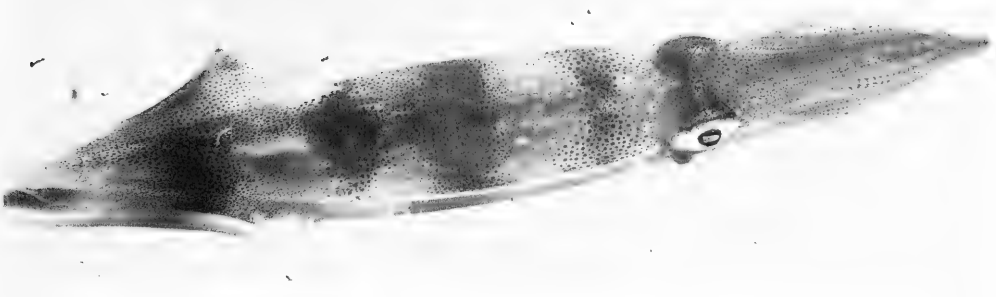


FIG. 15. Male (115 mm ML) swimming near the bottom in the RING chromatic component, with DORSAL MANTLE SPLOTCHES prominent. The head and arms are often clear when RING is shown.

FIG. 16. Same female as in Fig. 13 (93 mm ML) in the three-ring variation of RING, with SHADED EYE and DORSAL MANTLE SPLOTCHES. The first and third mantle rings are poorly developed in this photograph.

another variation, females in the three-ring component sometimes appear to have only one ring when the first and last are weakly developed due to the graded retraction of certain elements in Standard Discoid Units on the periphery of the ring (Fig. 16). Any of the aforementioned variations may be seen with uniformly dark head and arms (Figs. 1J, 15).

ACCENTUATED TESTIS

The squid is uniformly dark, as in ALL DARK, except for a completely clear area of retracted chromatophores around the conspicuous, white testis of males (Fig. 17). In most cases the clear area extends well beyond the outline of the testis. This component has been observed in sexually mature males of 39–285 mm ML.

Two variables regulate the apparent size and conspicuousness of the testis: (1) the size of the clear area and (2) the degree of expression of the surrounding Standard Discoid Units. Fig. 18 shows the clear area at its greatest dimension. In contrast, Fig. 17 shows a small clear area over the testis with maximally expanded surrounding units. The combination that produces the most conspicuous component is produced when the clear area over the testis is largest and the chromatophores of adjacent Standard Discoid Units are completely expanded.

SHADED TESTIS

In this component a male squid is clear except for the area over the testis, which is

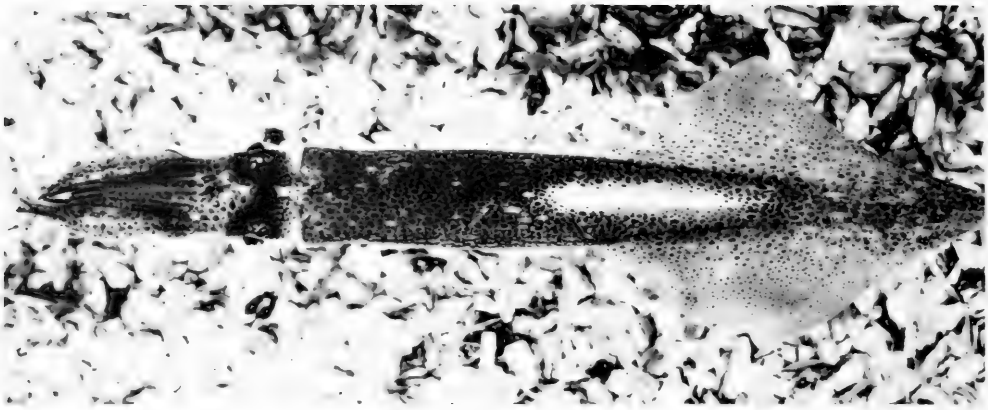


FIG. 17. A male (133 mm ML) in ACCENTUATED TESTIS, ARM SPOTS and SHADED EYE. The testis of this male is not well developed and the clear area over the testis is not at its largest dimension.

FIG. 18. A male (205 mm ML) guarding recently laid eggs and showing ACCENTUATED TESTIS, SHADED EYE, ARM SPOTS (third arms), TENTACULAR STRIPE and weakly developed DORSAL ARM IRIDOPHORES. The clear area over the testis is at its greatest dimension.

shaded by a latticework of brown chromatophores (Figs. 1J, 19).

The latticework is presumably produced by the selective nervous excitation of brown chromatophores in the Modified Discoid Units that cover the testis. Usually the center browns are only ¼ expanded while the browns in the circlet are near maximal expression; a few yellow chromatophores of each unit may also appear. These units produce a stippled effect that obliterates the highly conspicuous white testis by reducing its luminance. The interspersed yellows reduce the luminance of the bright testis so that it approximates that of adjacent areas on the dorsal mantle. SHADED TESTIS has been observed in sexually mature males of 20–285 mm ML.

Only minor variations have been noted. In smaller squids (approximately 29–50 mm ML) the shaded area is more diffuse and covers a greater area than the testis. In squids this small the units are not as well defined and do not selectively delineate the testis. The shaded area may vary from the testis proper to a larger area such as that included in ACCENTUATED TESTIS.

It is noteworthy that on a few rare occasions females (50–90 mm ML) have shown a component, somewhat similar to SHADED TESTIS, that generally covers the posterior ⅓ of the mantle including the area of the ovary. I do not consider it a distinct component because (1) the ovary is not generally very conspicuous through the mantle, (2) the area covered by chromatophores is not well defined and looks more like DORSAL STRIPE (see below), and (3) it has only rarely been seen. In fact, females appear to have no counterpart component homologous to ACCENTUATED TESTIS in males.

SHADED EYE

The highly reflective sclera of the back of the eye is shaded by Modified Discoid Units

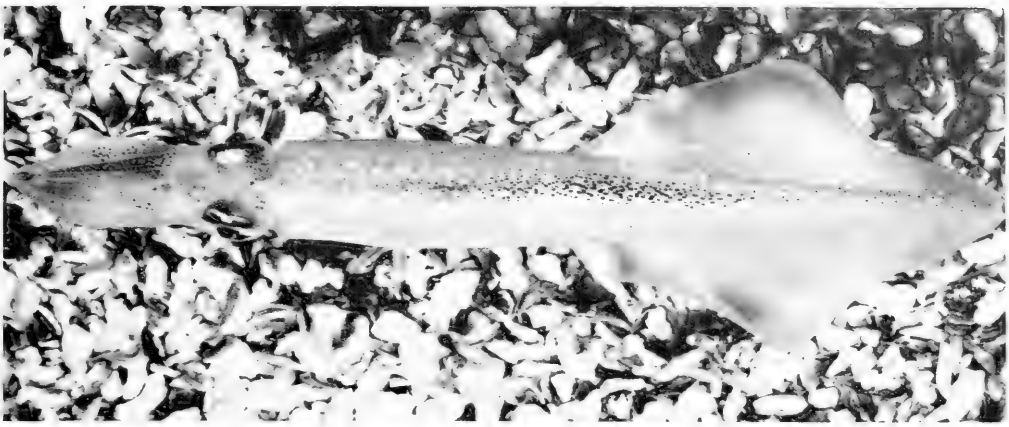


FIG. 19. Same male as in Fig. 17 (133 mm ML) shown minutes later in SHADED TESTIS and DORSAL MANTLE SPLOTCHES.

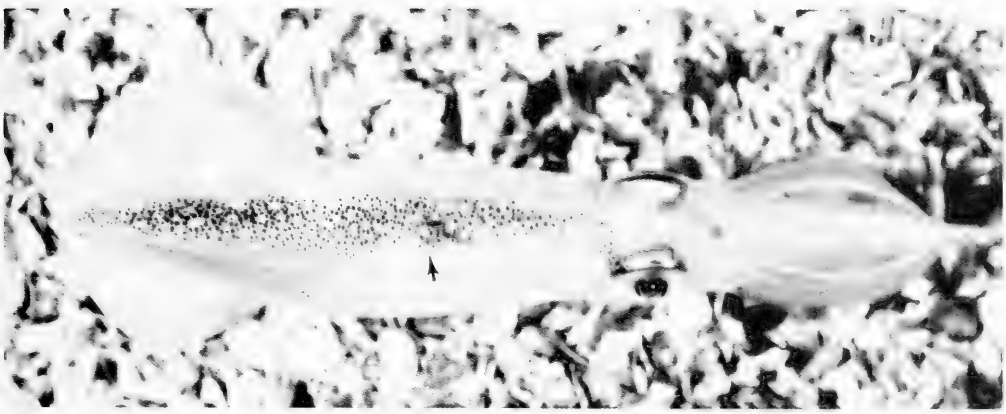


FIG. 20. Female (89 mm ML) in DORSAL STRIPE and DORSAL MANTLE SPLOTCHES. Arrow indicates the bright red accessory nidamental gland. Note the brightness of the sclera of the eye when the overlying chromatophores are retracted.

that are situated on the dorsal head region directly above them (Figs. 5, 14, 16, 17, 18, 27). When these units are fully expanded the browns overlap and provide complete shading of the eyeballs. This component may be expressed in varying degrees that shade the eyes correspondingly. It has been seen on females and males as small as 40 mm ML.

DORSAL STRIPE

The squid is basically translucent with a diffuse longitudinal stripe of expanded chromatophores extending the entire length of the dorsal mantle (Fig. 20). The stripe is centrally aligned on the dorsal mantle and extends laterally ⅓ to ⅔ of the mantle width. The stripe is characterized by Standard and (in males) Modified Discoid Units in which the center browns are only partially expanded while the yellows, reds and browns are usually ½ to fully expanded. In most cases the Iridophore Splotches on the dorsal mantle are simultaneously prominent. Occasionally the Modified Discoid Units between the eyes appear dark also. DORSAL STRIPE has been observed on females of 74–136 mm ML and males of 128–226 mm ML.

ARM SPOTS

There are two expressions of ARM SPOTS. The first is a compact, well-defined dark

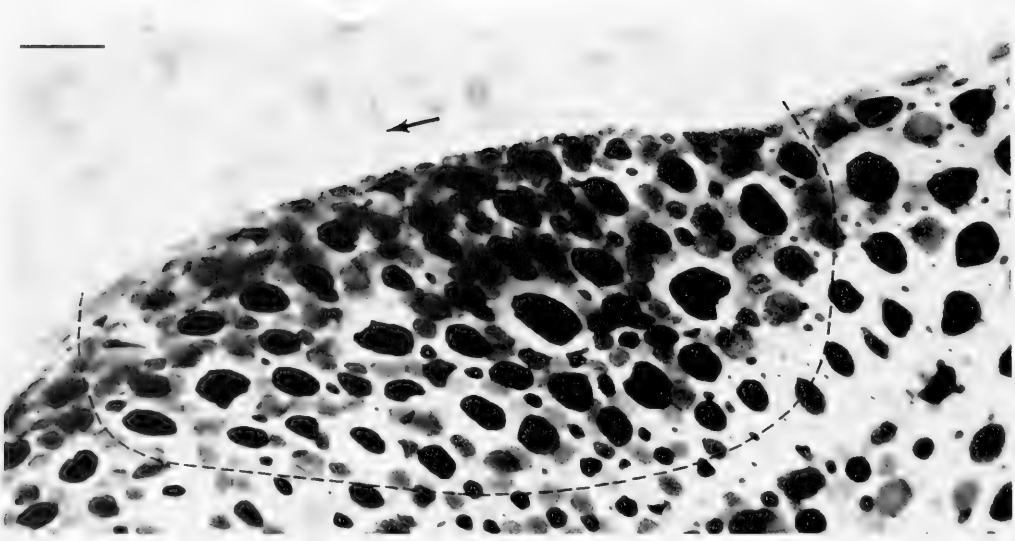


FIG. 21. Dorsal base of the third right arm from a male (147 mm ML) showing the approximate area of Modified Discoid Units (within dashed line) that produces the chromatic component ARM SPOTS. The units are shown only partially expanded. The distribution of the Modified Discoid Units continues onto the ventral portion of the arm and covers the same approximate area as on the dorsal side. The skin is transparent, and when all these units are expanded simultaneously, the spot appears darker and more distinct than if only one surface had Modified Discoid Units. See Fig. 23 for the appearance of this distinct spot. Arrow indicates anterior direction. Line represents 1 mm.

brown spot that occurs near the bases of the second and third arms (Figs. 1B, 21, 23, 27). These distinct spots are produced by a cluster of Modified Discoid Units in which all chromatophores are maximally expressed. The browns in this unit are morphologically arranged in such a manner that they overlap one another when fully expressed to provide a very dark brown base for the spot. The yellows and reds contribute to the darkening as well. On the second pair of arms the Modified Discoid Units occur only on the dorsal surface of the arm, but on the third arms (Fig. 21), which are spread laterally as swimming keels, the units occur both on the dorsal and ventral surfaces of the outside edge of the arm, making the spot even more conspicuous.

The second expression of ARM SPOTS is a diffuse area of expanded chromatophores that is centered at the base of each third arm, and there is considerable variation in its size and shape. It may appear as a diffuse reddish-brown splotch covering up to ⅓ to ½ of each third arm (Fig. 13) or a large reddish-brown darkening of most or all of the second and third arms (Fig. 1I). Any of the aforementioned expressions may occur independently or in combination and they are all nearly always exhibited bilaterally. The ARM SPOTS component has been seen on males of 41–285 mm ML.

Only twice were females observed showing similar markings. An egg-laying female (89 mm ML) once briefly showed spots at the bases of the second arms, and three females (100–114 mm ML) once showed uniformly dark arms for 30 secs, with the remainder of the body clear.

STITCHWORK FINS

This component appears as a series of dark dashes, or stitches, around the periphery of the fins (Figs. 1I, 22, 23, 27). The stitchwork appearance results from the full expression of the Yellow-Brown Discoid Units that are grouped 2–3 units wide into a continuous line, 2–5 mm inside the margin of the fin. The fins are generally clear but in some cases the component appears when the adjacent fin areas are ALL DARK. Since the Standard Discoid Units on the outer ⅓ of the fin periphery are somewhat dispersed relative to those on the mantle, STITCHWORK FINS is still con-

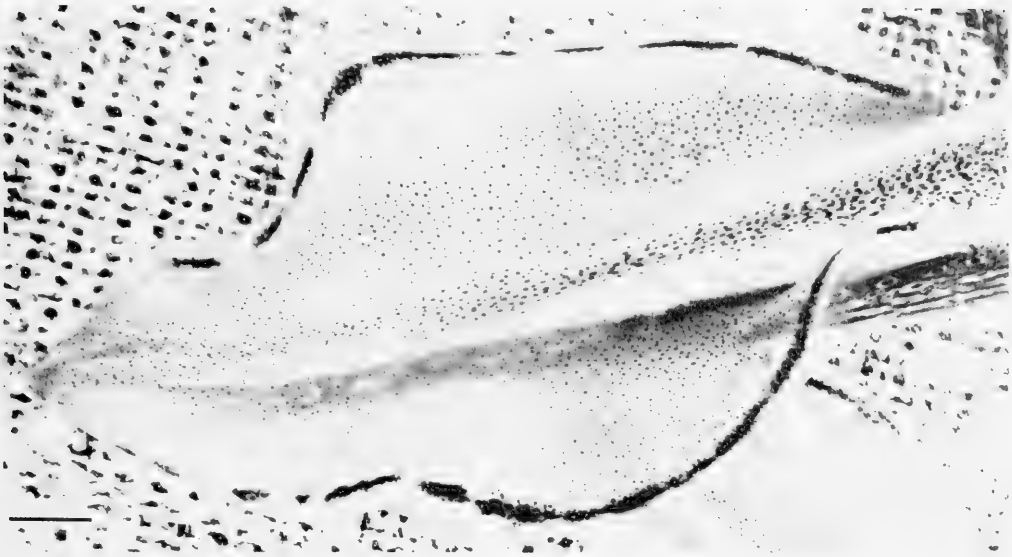


FIG. 22. Fin of a large male (220 mm ML) showing the distribution of Yellow-Brown Discoid Units that produces STITCHWORK FINS. Line represents 10 mm.

FIG. 23. Lateral view of a male squid (174 mm ML) showing the chromatic components STITCHWORK FINS, LATERAL FLAME, MID-VENTRAL RIDGE, ARM SPOTS, TENTACULAR STRIPE AND SPOTS and DORSAL ARM IRIDOPHORES towards a squid on its right. The tentacles are not often extended in this manner.

spicuous when ALL DARK is shown. On some squids the stitchwork arrangement is not so conspicuous and the component appears as a somewhat continuous line around the fin periphery; it is nearly always bilateral although in rare cases it appears unilaterally. Only males of 105–285 mm ML have been seen to exhibit it.

MID-VENTRAL RIDGE

Viewed laterally, this component appears as a distinct, dark red solid line extending the entire length of the midline of the ventral mantle (Figs. 1H, 23). It is a protrusible flap of skin that is muscularly extended downward 1–3 mm while the chromatophores are fully ex-

panded. The chromatophores along the midline are arranged into Standard Discoid Units (Fig. 24); beyond this line on each side are the Lateral Flame Units. When the skin is protruded downward the chromatophore units on either side are compressed "back to back," which results in a double layer of units that result in a solid dark appearance. It has been seen in males of 41–285 mm ML.

Females of 50–90 mm ML have on rare occasions shown a component similar to MID-VENTRAL RIDGE. It appeared as a ventral stripe of expanded chromatophores (similar to DORSAL STRIPE) in the same position as MID-VENTRAL RIDGE, but without the ridge.

LATERAL FLAME

This component appears as longitudinal, flame-like streaks of expanded Lateral Flame Units (Figs. 1H, 8, 23, 27). It covers the entire lateral aspect of the mantle and is usually deployed unilaterally (Figs. 1I, 23). It has been seen only in males from 70–285 mm ML.

LATERAL BLUSH

This component has two forms. Most commonly it appears as a diffuse, dark, lateral stripe 4–5 mm below the fin insertion, extending longitudinally from the arm tips to the mantle tip, with the remainder of the squid clear (Fig. 25). The component arises from the expansion of Standard Discoid Units, and the width of the stripe is usually 2–3 units wide. It is generally shown unilaterally although occasionally it is seen bilaterally. It has been observed on small males of 41–86 mm ML and females of 82–107 mm ML. As young male squids grow older, this component persists

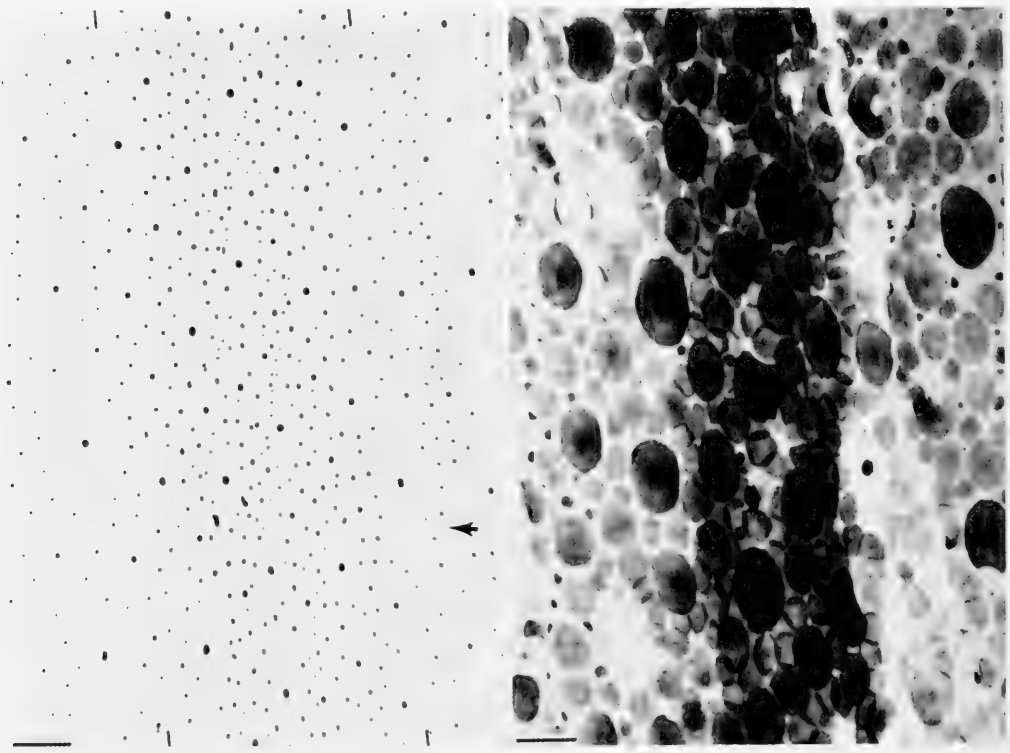


FIG. 24. Portions of the ventral midline of males showing the Standard Discoid Units that constitute the chromatic component MID-VENTRAL RIDGE. Left: Retracted units (within area between hash marks) from a live male, 190 mm ML. Right: Expanded units from a live male, 147 mm ML. Note the transition into Lateral Flame Units on the left and right of each photograph, and the waves of muscular contractions (arrow) produced by the same muscles that protrude this skin downward when the component is expressed. Complete expression of this component may be seen in Figs. 1H and 23. Lines represent 1 mm.

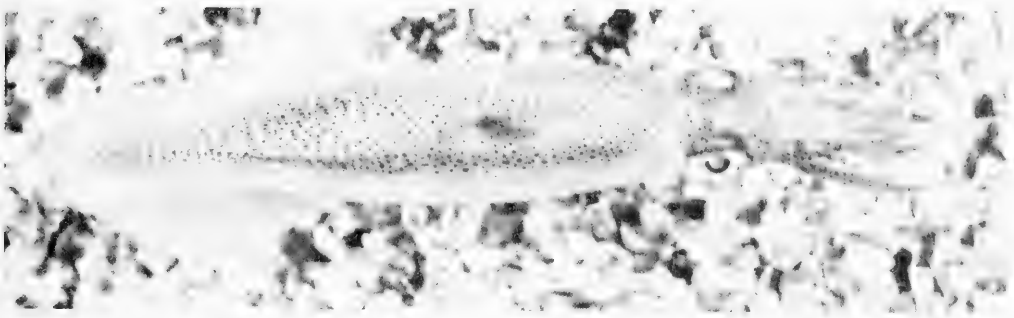


FIG. 25. One form of LATERAL BLUSH in a small male (86 mm ML). The component ARM SPOTS is very weakly developed.

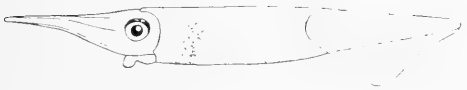


FIG. 26. Another form of LATERAL BLUSH that is seen only in sexually mature females. Drawing by D. A. McConathy.

and becomes the uppermost line of expanded chromatophores in LATERAL FLAME.

In another form it appears as a diffuse splotch of expanded chromatophores on the anterior, lateral portion of the mantle (Fig. 26). This has been observed only on sexually mature females of 83–114 mm ML.

TENTACULAR STRIPE AND SPOTS

The stripe consists of a single, thin brown line of expanded chromatophores along the lateral margin of the entire length of each tentacle (Figs. 1H, 18, 23, 27). At the distal end of each tentacular club this line ends in a darkened tip. The chromatophores along the stripe and tip are mostly arranged into Yellow-Brown Discoid Units, except that isolated reds are found intermittently along the tentacular length. There are approximatlely seven or eight distinct brown spots placed along the middle third of the inside of each tentacular stalk, posterior to the tentacular club (Figs. 1B, 2, 23, 27). These spots are composed exclusively of Yellow-Brown Discoid Units, the smaller isolated spots comprising approximately 10–15 brown chromatophores and 6–10 yellows. This component is most readily seen when the tentacles are extended; however, it can also be seen amidst or through the other arms when the tentacles are held at arm's length. It has been seen on males of 90–285 mm ML. In one rare instance it was seen on a female of 50 mm ML.

DORSAL MANTLE SPLOTCHES

This component is characterized by the frequent visual appearance of all units of Iridophore Splotches (Figs. 1I, 1J, 10, 11, 13, 14, 15, 16, 19, 20, 27). The splotches are evident in both sexes in sizes as small as 21 mm ML.

DORSAL ARM IRIDOPHORES

The component is produced by the bright, distinct Iridophore Sheets that extend the length of the first pair of arms of males (Figs. 1H, 1I, 12, 13, 18, 23, 27). The chromatophores of the Standard Discoid Units on these arms are completely or mostly retracted and the Iridophore Sheets reflect pink, bright white or whitish-yellow. It has been seen on males of 74–285 mm ML. Although this bilateral component involves no chromatophores, it is not present at all times and it appears that the squids have some control over its appearance (see Discussion).

DORSAL MANTLE COLLAR

The Iridophore Sheets that are clustered around the anteriormost margin of the mantle periodically appear as a pink or green collar (Figs. 12, 13, 27). It appears in young squids of both sexes from 21 mm ML.

DISCUSSION

Previous workers have rightly suggested that the body patterns of *Loligo* are of simpler construction than those of *Octopus* and *Sepia*

(Boycott, 1953, 1965; Holmes, 1955; Wells, 1962; Messenger, 1974). Nevertheless, *Loligo plei* exhibits a diverse range of chromatic components that can be used in intraspecific signalling and camouflage. What is significant is that this diversity can be largely accounted for by the morphological arrangement of the elements.

When the body patterns of *Loligo plei* are analyzed and described in terms of their constituent parts (i.e. components, units, elements), it becomes manifest that, like *Octopus,* it is at the unit level that patterning is first developed, both in the morphological and physiological senses. In the following paragraphs I emphasize that the units are functionally organized to produce differently shaped and colored components that are unique not only to the species, but in many cases to each sex within the species. In *L. plei,* the expression of chromatic components is a result of (1) the particular static morphological array of elements within differently constructed units, and (2) the selective nervous excitation of those morphological units.

Functional Morphology of the Units of Patterning

The 16 chromatic components seen thus far in *Loligo plei,* when combined with postural components and behavioral movements, allow for a wide range of body patterns, each of which coincides with a particular aspect of behavior (Hanlon, 1981; in prep.). While this discussion is limited to the functional morphology of the constituent parts of body patterns, the following brief and very simplified explanations of the interrelationships between groups of various chromatic components and associated behavior are given to provide some perspective concerning the circumstances in which they are expressed. Eight components are seen on calmly swimming squids and presumably aid in camouflage: CLEAR, RING, SHADED TESTIS, SHADED EYE, DORSAL STRIPE, DORSAL MANTLE SPLOTCHES and DORSAL MANTLE COLLAR. Two components are exhibited by squids that are closely approached by a predator or large object: ALL DARK and RING. Eight components are seen almost exclusively during intraspecific aggression among males: ACCENTUATED TESTIS, ARM SPOTS, STITCHWORK FINS, MID-VENTRAL RIDGE, LATERAL FLAME, LATERAL BLUSH, TENTACULAR STRIPE AND SPOTS and DORSAL ARM IRIDOPHORES.

The Standard Discoid Unit is most widely dispersed over the body surface in both sexes and is responsible for producing the many variations of the components ALL DARK, RING, ACCENTUATED TESTIS, DORSAL STRIPE and LATERAL BLUSH. The Modified Discoid Units have a greater number of browns that provide an important function: a greater capability to darken an area of the skin, either to shade underlying reflection for camouflage (SHADED EYE, SHADED TESTIS), or to produce a distinctly dark spot for visual communication (ARM SPOTS). Yellow-Brown Discoid Units are distributed in such a manner that they produce distinctly dark brownish dashes (STITCHWORK FINS), stripes or spots (TENTACULAR STRIPE AND SPOTS). For example, the spots of TENTACULAR STRIPES AND SPOTS are a result of the distribution of units into circular spots that are bordered by areas devoid of chromatophores or iridophores; they are not a result of selective nervous excitation. In summary, discoid chromatophore units are best suited for (1) uniformly darkening large skin areas, (2) forming loosely defined, large dark areas such as transverse rings and longitudinal stripes, (3) shading or highlighting organs, and (4) creating dark spots and splotches.

In contrast, the Lateral Flame Unit is not circularly arranged but is functionally organized into longitudinally oriented dark streaks highlighted by adjacent light areas. In addition to the different spatial distribution of elements within the unit, the depth distribution changes as well, with brown chromatophores now lying above reds (Fig. 1D). The small size of the yellow and brown chromatophores, and their concentrated distribution into well-defined rows, both enhance the distinct appearance of LATERAL FLAME. This arrangement of elements is not suited for uniform darkening as are the circularly arranged units. This is illustrated when males are in the ALL DARK chromatic component, with all chromatophores maximally expanded, and the lateral flame streaks are still conspicuous (Figs. 12, 27).

The organization of iridescent cells is notably different from that of chromatophores. Some difficulty arises when attempting to organize them into morphological units, since they are deeper in the dermis and are not visible at all times; in contrast, chromatophores are visible both in the retracted and expanded states. The only way to verify the distribution and morphology of all iridescent cells would be to make a histological survey of

the entire dermis of the squid. Consequently, they have been described from their visual appearance. The Reflector Cells are structured to reflect all light constantly, whereas the iridophores probably are not. The Iridophore Splotches on the mantle are not seen at all times, but mostly in combination with components that are used for camouflage, such as CLEAR, SHADED TESTIS and DORSAL STRIPE. The slightly irregular spacing of the splotches and their reflection of pink, green and yellow light promote concealment when viewed from above against a variegated substrate (Cott, 1940). Many of the splotches are positioned beneath the central brown chromatophore of the largest Standard Discoid Units (Figs. 1J, 10, 11) and the regularity with which this occurs is reminiscent of the positioning of some black chromatophores over gaps between underlying leucophores in the center of chromatic units in *Octopus vulgaris* (Froesch & Messenger, 1978). It is unclear whether the placement of these elements is related in morphogenesis, and whether there is coordination between the chromatophores and iridophores of each morphological unit during patterning. The iridophore splotch has not been included in the description of the Standard Discoid Unit for these reasons, and also because all units do not have an underlying iridophore splotch.

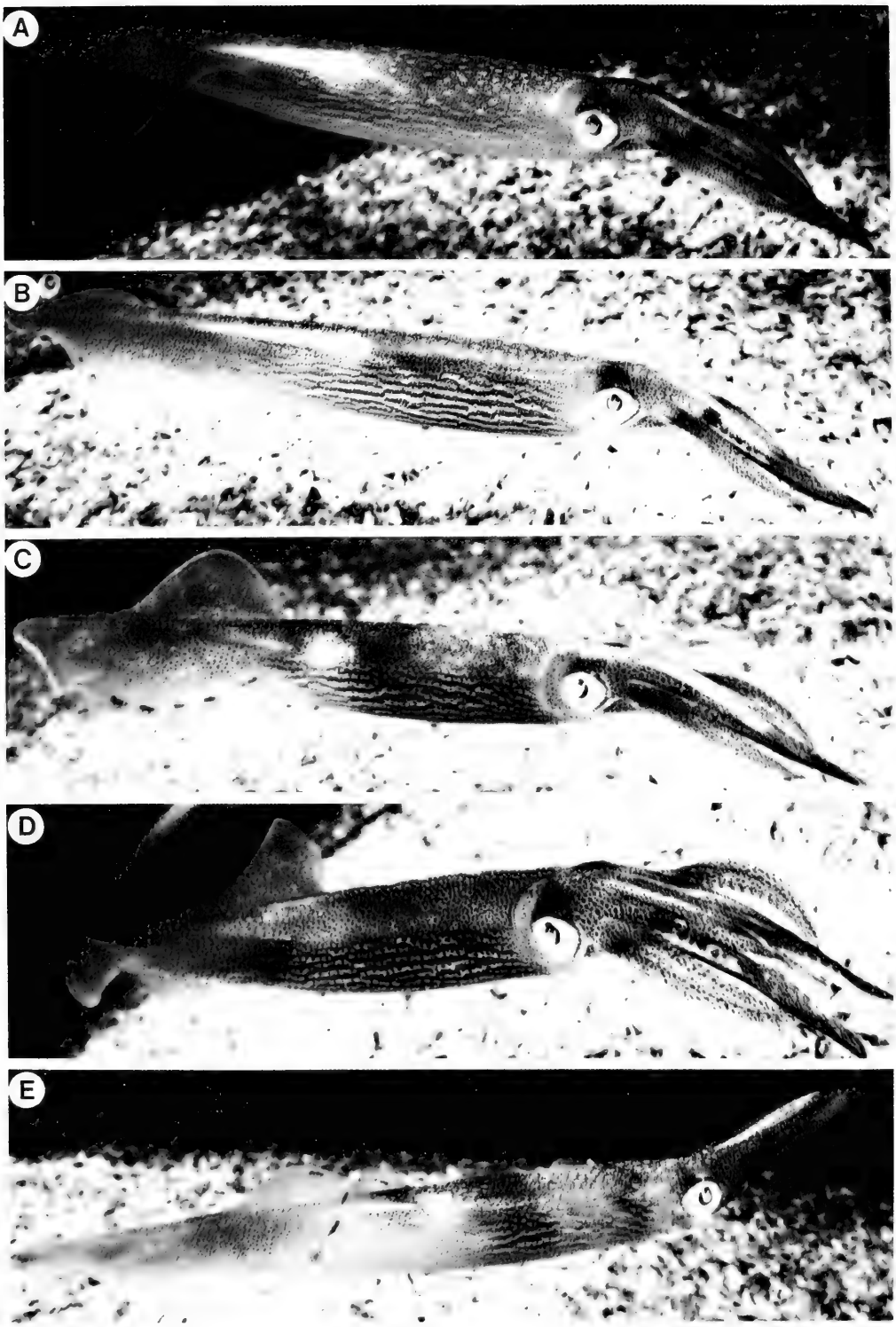
The Iridophore Sheets have a visual quality similar to that of the Iridophore Splotches, and histological investigation may determine that they are morphologically identical; the colors that they reflect are similar. These cells are organized to produce a continuous sheet of iridescence that is used for camouflage, obliterative counter-shading and, among males, intraspecific signalling. The iridescence in the first pair of arms (DORSAL ARM IRIDOPHORES) provides a particularly conspicuous signalling device that is far brighter than any chromatophore expression. This iridescence is evident even when the overlying chromatophores are expanded, but to promote its conspicuousness, the first pair of arms usually has the chromatophores retracted (Figs. 1I, 12, 13, 23, 27), and the arms are often arched upward during intense agonistic displays (Fig. 1H).

An important question arises at this point: is the appearance of iridophores controlled by the squid? Based upon my observation the answer is probably affirmative. Video tapes from a stereomicroscope clearly show iridophores appearing then disappearing under identical lighting and viewing conditions. Series of photographs taken from a single port of the holding tank show the same squid expressing different iridophore units under unchanging light conditions. Additional evidence is that males show DORSAL ARM IRIDOPHORES particularly during intraspecific aggressive bouts with other males, indicating that this component is controlled. Furthermore, when neurotransmitter substances such as dopamine, acetylcholine and 5-hydroxytryptamine are applied to the skin of live, narcotized or freshly dead squids, both chromatophores and iridophores are often expressed. This phenomenon has also been observed in *Loligo vulgaris* in Naples (A. Packard, personal communication, 1979). This opens up the interesting possibility of whether the iridophores are under muscular control. Further investigation will be rewarding, because at present the iridophores are thought to be static cells whose appearance is regulated only by the structure of the cell and the quality and angle of light falling upon them.

Selective Nervous Excitation

The neural organization of chromatophores is poorly understood because of the difficulties in tracing nervous interconnections. A further complication is evidence that chromatophores may be also linked by their muscles, which means that the innervation of one chromatophore may have a muscular effect on neighboring chromatophores (Froesch-Gaetzi & Froesch, 1977). In *Loligo plei,* the yellow, red and brown chromatophores of a single morphological unit are innervated in different combinations. Proof of this may be seen when examining video tapes of close-up recordings of individual chromatophore units of live squids. Within a single Standard Discoid Unit, groups of reds are separately innervated from other groups of reds, as well as from the brown and yellow chromatophores. The same is true of the yellows. The single brown chromatophore of each unit fires synchronously with nearby browns of a similar size (Fig. 4).

The appearance of a squid can change within a matter of seconds, as shown in Fig. 27. In these photographs the same squid progresses from a relatively light, translucent body pattern to a dark body pattern by selective nervous excitation. It is critical to note that it is not only the *number* of units expressed


A
B
C
D
E

that results in darkening, but the *degree* to which they are expressed. For example, in Fig. 27 (A–D), the dorsal mantle becomes progressively darker not because more Standard Discoid Units are expressed, but because the chromatophore elements within each unit are expanded to greater degrees. Within a single Standard Discoid Unit, a typical representation of the progression from light to dark would be as follows: (1) all chromatophores are retracted, (2) reds are expanded to ½ full size, (3) browns are then concurrently expanded to ¼ full size, (4) yellows are expanded to ½ full size, (5) reds are expanded from ½ to full size, and (6) browns and yellows are also expanded to full size (i.e. all chromatophores are now maximally expanded). In other words, not only are more chromatophores of more colors being expanded, but each chromatophore is expanded to a progressively larger size until all are maximally expanded.

Figs. 1E, 1F and 1G indicate how complex the neural connections in LATERAL FLAME may be. In Fig. 1E, the reds between rows are expanded, as are a few within rows. A few yellows are fully expanded, and some browns and yellows are partially expanded; in general the rows are weakly developed. In Fig. 1F, several more reds within the rows have expanded, and groups of yellows are expanded, especially in the bottom row, and the rows are clearly distinguishable. Note, however, that certain groups of yellows, browns and reds within the rows still remain unexpanded.

From a functional standpoint, the complex morphological and physiological organization of Lateral Flame Units provides the capability of expressing LATERAL FLAME in varying degrees of intensity. This is important because during intraspecific aggression, males use the unilateral expression of this component (Figs. 1H, 1I) along with other components during agonistic displays with other males, and the degree of intensity of the LATERAL FLAME component is directly related to the degree of aggression during the display (Hanlon, in prep.). Another function of the complex organization is to allow certain Lateral Flame Units to be used in the expression of other chromatic components. For example, when large males are in RING, selected Lateral Flame Units are expressed in combination with selected Standard or Modified Discoid Units to produce a transverse band of expanded chromatophores around the mantle (Fig. 27E).

Significance of Color

Cephalopods are almost certainly color blind (Messenger et al., 1973; Messenger, 1977, 1979) and therefore it is highly unlikely that color is of significance in intraspecific communication. Pattern and contrast are the information transmitters among conspecifics, as postulated by others (Packard, 1972; Messenger et al., 1973; Messenger, 1974) and reinforced by my observations of *Loligo plei*. The components used in intraspecific encounters (e.g. ACCENTUATED TESTIS, ARM SPOTS, etc.), especially LATERAL FLAME, are most conspicuous by their configuration, and are most effective as they become brighter.

Color would be most effective in camouflage patterns used to avoid predators with color vision, such as fishes. An important consideration in this regard is the water depth at which these colors are viewed. Only at the shallowest depths would the long-wavelength colors (especially red) retain their distinct hue because the longer wavelengths of light are more attenuated in sea water than the shorter wavelengths. *Loligo plei* is found occasionally on and around shallow coral reefs throughout the Caribbean and in these circumstances the warm-toned chromatophores, in conjunction with the iridophores that reflect all wavelengths of light, are probably effective in achieving color as well as tone matching when the squids are sitting on or swimming near substrates that are colored brown, red, green or yellow. During mid-water swimming or schooling in the day, the iridophores may help achieve obliterative counter-shading (Cott, 1940). *L. plei* actively forages and feeds in the water column at night and the chromatic components are probably not effective in very low light levels. During the day, when the squids school near the bottom, the various

FIG. 27. Example of selective nervous excitation changing the appearance of the same male squid (201 mm ML) within a few seconds. From A to E note the transient appearance of the conspicuous white testis and the chromatic components LATERAL FLAME, ARM SPOTS, TENTACULAR STRIPE AND SPOTS, STITCHWORK FINS, RING, DORSAL ARM IRIDOPHORES, DORSAL MANTLE SPLOTCHES and DORSAL MANTLE COLLAR. See Discussion for further explanation.

body patterns may be used for predator escape, camouflage and intraspecific communication.

The vertical distribution of differently colored chromatophores in *Loligo* is exactly opposite to that of *Octopus vulgaris* (Froesch & Packard, 1979). In *Loligo,* it is yellow over red over brown (in Standard Discoid Units), whereas in *Octopus* it is brown over red over orange over yellow. The significance of this is unknown. From a functional standpoint, the chromatophores of *Loligo* act somewhat like a neutral density filter in a manner similar to *Octopus*, the principal differences being the slightly narrower color range and the vastly greater size of chromatophores in *Loligo.* With fewer and less dense chromatophores, *Loligo* is unable to produce chromatic components as complex and varied as *Octopus vulgaris.* However, the fine pattern reticulation and skin sculpture required of a benthic octopus are not needed by a schooling squid that spends most of its time in the water column. In contrast to *Octopus,* whose finely differentiated skin is constructed to produce subtle, refined patterns mainly for camouflage and predator escape, *Loligo* uses most of its body patterns for intraspecific communication in which bold, coarse patterns are sufficient to visually communicate with a nearby squid. Hence, large chromatophores may be regarded as an efficacious means of producing a chromatic component with the simplest skin differentiation.

Sexual Dimorphism

Three aspects of sexual dimorphism related to chromatic behavior may be demonstrated in *Loligo plei*: (1) the distribution of chromatic units in the dermis, (2) the range of chromatic components, and (3) the general behavior of each sex. Table 2 shows that most of the chromatic units and components of *L. plei* are shared by both sexes, but that males have acquired one unit and seven components that are unique to that sex alone. In contrast, females do not have any unique units or components. Among the shared units, males have a greater number and distribution of certain types. For example, both sexes have Modified Discoid Units over the eyes (SHADED EYE), but only males have them over the testis (SHADED TESTIS) and on the second and third arms (ARM SPOTS). In addition to having Yellow-Brown Discoid Units on the tentacles, males have them on the periphery of the fins (STITCHWORK FINS). Males have prominent Iridophore Sheets on the first pair of arms (DORSAL ARM IRIDOPHORES).

The overall behavior of each sex is markedly different. Females are relatively passive

TABLE 2. Breakdown of the chromatic units and components of *Loligo plei* that are found (1) only in males, (2) only in females, or (3) shared by both sexes.

CHROMATIC UNITS		
Male only	Female only	Shared
Lateral Flame Unit		Standard Discoid Unit
		Modified Discoid Unit
		Yellow-Brown Discoid Unit
		Reflector Cells
		Iridophore Splotches
		Iridophore Sheets
CHROMATIC COMPONENTS		
Male only	Female only	Shared
ACCENTUATED TESTIS		CLEAR
SHADED TESTIS		ALL DARK
ARM SPOTS		RING
STITCHWORK FINS		SHADED EYE
MID-VENTRAL RIDGE		DORSAL STRIPE
LATERAL FLAME		LATERAL BLUSH
DORSAL ARM IRIDOPHORES		TENTACULAR STRIPE AND SPOTS
		DORSAL MANTLE SPLOTCHES
		DORSAL MANTLE COLLAR

and docile laboratory animals, whereas males are strongly aggressive and spend a great deal of time fighting among themselves and courting females (Hanlon, in prep.). Of the male-only components, all but SHADED TESTIS are used during intraspecific aggressive encounters. Another dimorphic character is the mean and maximal size of adults; males are substantially larger. With these morphological and behavioral differences it is quite easy to distinguish the sexes of living squids, even in certain cases where very young squids as small as 35 mm ML attain precocious sexual maturation in the laboratory.

Comparisons Within the Genus Loligo

Although this study represents the first detailed description of the organization of chromatic elements in *Loligo*, several comparisons may be made with other members of the genus. Table 3 is a tabulation of incidental reports of various chromatic components that have been mentioned in the literature. The fact that all five of the species when observed alive showed CLEAR, ALL DARK and DORSAL MANTLE SPLOTCHES indicates that these are common components. MID-VENTRAL RIDGE, which is often clearly evident in preserved specimens, has been seen in four species besides *Loligo plei,* and evidence of LATERAL FLAME has been seen in two other species. The chromatic components ACCENTUATED TESTIS, SHADED TESTIS, SHADED EYE, TENTACULAR STRIPE AND SPOTS, DORSAL STRIPE, LATERAL BLUSH and DORSAL ARM IRIDOPHORES have not yet been reported in other species, but this undoubtedly is partly a result of the general difficulties encountered in keeping squids alive in captivity for long periods or in making long-term observations in the field. It is likely that many of these components, as well as new ones, will be seen in other species when detailed observations are made. It is presently impossible to determine whether *L. plei* can show more than 16 components, or if it possesses a wider repertoire of chromatic components than other members of the genus, although it seems that *L. plei* has more chromatic expression than *L. pealei* or *L. opalescens.* More information is urgently needed for other species, for this will be invaluable in understanding the role and ontogeny of behavior, reproductive strategies and phylogenetic relationships among cephalopods.

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TABLE 3. Incidental reports of chromatic components seen in live or preserved squids of the genus *Loligo*. M = male; F = female; J = juvenile.

Species/Author Year	Chromatic Components											
	CLEAR	DARK	RING	ACCENTUATED TESTIS	SHADED TESTIS	DORSAL STRIPE	ARM SPOTS	MID-VENTRAL RIDGE	LATERAL FLAME	DORSAL IRIDOPHORE SPLOTCHES	DORSAL MANTLE COLLAR	Other Components
Loligo plei												
Rees, 1950									dead M			
Boycott, 1965			live	live	live							
LaRoe, 1967	live	live	live					dead M,F	dead			
LaRoe, 1970		live	live	live	live		dead	dead	live	live		
Moynihan & Rodaniche, 1977		live		live								
Loligo pealei												
Verrill, 1881								live M				
Williams, 1909	live	live	live				live			live		Dark arms, head, anterior mantle; live
Stevenson, 1934		live	live									
Arnold, 1962							live M					Dark spots along fin margins; live
LaRoe, 1967								dead M				
Mirow, 1972b										live		
Loligo vulgaris												
Jatta, 1896										live	live	
Naef, 1923	live J	live						dead	dead M			Fin spots, stripes on fin base; live

Tardent, 1962	live	live					Fin spots, red fin saddle, spot below eye; live
Wells, 1962	live	live					White flecks, ventral line; live
Neill, 1971		live	live				Fin spots, fin saddle; live
Loligo opalescens							
McGowan, 1954	live						
Fields, 1965	live						Dark arms and head; live
Mirow, 1972a		live					
Mirow, 1972b						live	
Hurley, 1977		live		live			Dark arms and head; live M
Loligo forbesi							
Jatta, 1896					live M,F	live	
Naef, 1923					dead M,F		
Holmes, 1940	live	live					
Rees, 1950					dead		
Voss, 1952					dead M		
Holme, 1974					dead		
Loligo edulis							
Sasaki, 1929				dead M			
Loligo bleekeri							
Sasaki, 1929				dead M			

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THE "KIDNEYS" OF CEPHALOPODS: A UNIQUE HABITAT FOR PARASITES

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ABSTRACT

The fluid-filled renal and pancreatic coela ("kidneys") of cephalopods are an ideal environment for the establishment and maintenance of parasites. Periodic, but incomplete, elimination of urine from the renal sacs provides an abundant source of nutrients rich in nitrogen and carbohydrate compounds. The epithelium of the convoluted renal appendages provides an excellent surface for attachment and the renal pores provide a simple exit to the exterior. As such the "kidneys" have been exploited by a number of phylogenetically distinct symbiotic organisms. These include: a virus, a fungus, three distinct groups of ciliates, dicyemid mesozoans, a digenetic trematode, larval cestodes, and larval nematodes. Of these forms, the dicyemids and the apostome ciliate, *Chromidina*, are known to occur only in cephalopods and only in the "kidneys." Benthic cephalopods are infected exclusively with dicyemids whereas pelagic cephalopods harbor only chromidinids. An overlap of hosts occasionally occurs when ciliates are acquired by planktonic stages of otherwise bottom-dwelling octopods and sepioids. The remarkable similarities in morphology and diphasic life cycle exhibited by the dicyemids and chromidinids indicate a high degree of convergence in response to selective pressures associated with life in the renal habitat.

Key words: cephalopods; "kidneys"; excretory organs; parasites; dicyemids; ciliates; *Chromidina*; convergence.

INTRODUCTION

The kidneys of any organism are an unusual place to find parasites, and in fact in the entire animal kingdom this organ has rarely been exploited. It is then with some interest that we view the rather remarkable situation in cephalopods where a majority of species and individuals of squids and octopuses harbor a diversity of parasites, several of which are found exclusively in the excretory organs.

This paper reviews the morphology of the cephalopod excretory system and relates it to the parasites which occur there. The discussion primarily considers similarities between the chromidinid ciliates and the dicyemid mesozoans, but other renal parasites are briefly treated.

MATERIAL AND METHODS

Over 2,300 cephalopods representing 91 species in 56 genera and 22 families have been examined for parasites. In all cases the excretory organs were examined and any parasites encountered were removed. Parasites were smeared and fixed on coverslips or prepared as whole mounts for later identification (see Hochberg, 1971 for details). Species descriptions have been published or are being prepared.

A review of the literature revealed another 56 species of cephalopods in 29 genera for which parasite information has been additionally recorded. However, in only 6 of these genera has the presence or absence of "kidney" parasites been specifically indicated. All genera considered are listed in Table 1.

TABLE 1. Genera of cephalopod hosts and their "kidney" parasites.

Hosts	Parasites	
	Chromidinid	Dicyemid
Nautiloidea		
Nautilus	–	–
Coleoidea		
Sepioidea		
Spirula	+	–
Euprymna	–	–
Heteroteuthis	+	–
Rondeletiola	–	+
Rossia	–	+
Sepietta	–	+
Sepiola	+	+
Sepia	+	+
Teuthoidea		
Alloteuthis	–	–
Loligo	+	–
Loliolopsis	–	–

TABLE 1 (Continued)

Hosts	Parasites	
	Chromidinid	Dicyemid
Lolliguncula	–	–
Sepioteuthis	–	+
Abralia	+	–
Abraliopsis	+	–
Enoploteuthis	–	–
Pterygioteuthis	+	–
Pyroteuthis	–	–
Thelidioteuthis	–	–
Octopoteuthis	–	–
Moroteuthis	–	–
Onykia	–	–
Berryteuthis	–	–
Gonatopsis	–	–
Gonatus	–	–
Bathyteuthis	–	–
Histioteuthis	+	–
Ctenopteryx	+	–
Dosidicus	+	–
Illex	+	–
Ommastrephes	+	–
Symplectoteuthis	+	–
Todarodes	+	–
Chiroteuthis	–	–
Mastigoteuthis	+	–
Bathothauma	–	–
Cranchia	–	–
Galiteuthis	–	–
Helicocranchia	–	–
Leachia	–	–
Liocranchia	–	–
Megalocranchia	–	–
Phasmatopsis	–	–
Sandalops	–	–
Octopoda		
Grimpoteuthis	–	+
Opisthoteuthis	–	+
Japetella	+	–
Bathypolypus	–	+
Bentheledone	–	+
Benthoctopus	–	+
Eledone	+	+
Graneledone	–	+
Octopus	+	+
Pareledone	–	+
Pteroctopus	–	–
Robsonella	–	+
Scaeurgus	+	+
Thaumeledone	–	+
Ocythoe	–	–
Argonauta	–	–
Vampyromorpha		
Vampyroteuthis	–	–

CEPHALOPOD "KIDNEY" PARASITES: RÉSUMÉ

Historically, the first known reference to a cephalopod parasite is found in a brief note by Cavolini (1787). In the kidneys of an octopus (probably *O. vulgaris*) he discovered "little infusorial organisms, shaped-like eels, having a muzzle with a trembling beard, darting, dividing themselves into many portions." This description could easily apply to either the chromidinid ciliates or the dicyemid mesozoans. Some 50 years following Cavolini's report, Krohn (1839) first documented the dicyemids and this well known group has been extensively detailed by numerous investigators. In 1881, Foettinger described the ciliate genus now referred to as *Chromidina*. This unusual parasite is known from only a few studies. Both these groups of ciliated, vermiform parasites are known only from cephalopods and characteristically occur only in the "kidneys." I will present additional details on these two groups farther on in this paper.

Several other parasites have been described which are either rare in cephalopods or rare in their excretory organs.

Dobell (1909) reported on a small ovoid infusorian, *Opalinopsis,* from the "kidneys" of *Sepia officinalis*. This ciliate is known from a number of cephalopods but is normally restricted to the digestive gland (see review in Hochberg, 1971). Its presence in the "kidneys" is presumed to be an error which may have resulted from accidentally cutting the digestive gland while removing the renal organs.

Raabe (1934) noted a fungus in the "kidneys" of several specimens of *Sepia officinalis* and *Octopus vulgaris* in the Mediterranean. The filamentous thalli of this fungus invade the renal appendages and the blood spaces causing considerable damage to the host tissue. Tentatively identified as, *"Aspergillus,"* the fungus is a highly destructive pathogen. This parasite must be quite rare, since it has never been reported or mentioned again, in spite of the numerous cephalopods examined in the Mediterranean and elsewhere.

A number of larval and adult helminths have been reported in cephalopods, but only one is known to regularly inhabit the "kidneys." Allison (1966), and later Short & Powell (1968), described the digenetic trematode, *Plagioporus maorum,* and reported its

presence in *Octopus maorum* and *Robsonella australis* in New Zealand. The worms typically are found crawling on the renal appendages within the renal sacs, or are located beneath membranes of nearby organs. This is the only sexually mature (not progenetic) digenean known from a marine invertebrate.

Larval stages of digeneans (didymozoans), cestodes, and nematodes have all, on occasion, been observed in the "kidneys" of squids. This phenomenon is rare, and should not be confused with the natural condition. It occurs only when massive numbers of larval helminths, which normally inhabit the digestive tract, invade a cephalopod host, and penetrate all the organs of the viscera (Hochberg, 1971).

What appear to be virus particles have been observed in the nuclei of the epithelial cells of the renal appendages of several *Octopus* species from New Zealand, Florida, and California (Short & Hochberg, unpublished). This viral infection is found not only in the nuclei of the epithelial cells of the host but in the nuclei of the somatic cells of the dicyemid parasites as well (Short & Hochberg, 1969).

More recently, representatives of two additional families of ciliates have been recovered from the excretory organs of pelagic squids off Hawaii and California. Though distinct from *Chromidina*, they have not been identified or described and will not be treated here (Hochberg, unpublished).

DICYEMID MESOZOA

The dicyemids are a puzzling group without definite affinities in the animal kingdom. They exhibit an impressive array of truly unique characters which hold a special curiosity for zoologists. Along with the orthonectids they have long been considered a class within the phylum Mesozoa (see Hyman, 1940; Grassé, 1961). In light of their dissimilar internal morphologies and the lack of homologies in stages of their life cycles, it is best to treat these two assemblages as separate phyla and to use the term "Mesozoa" to refer to their grade of organization only.

The dicyemids are the most common and characteristic parasites of the excretory organs of cephalopod molluscs. These minute, vermiform organisms attach principally to the renal appendages while the remainder of their worm-like bodies float in the fluid-filled renal coelom. Occasionally they are found in decapods in the pericardium attached to the branchial heart appendages and also in the reno-pancreatic coelom attached to the digestive duct appendages. They live and reproduce in these organs, doing no apparent harm to the host.

A total of 50 species of cephalopods representing 18 genera are currently known to host dicyemids (Table 1). These parasites occur in sepioids, especially cuttlefishes and sepiolids. In the octopods, both cirrate and incirrate groups are parasitized. In the teuthoids, only *Sepioteuthis,* an epibenthic loliginid, is infected. Each host species usually harbors a specific dicyemid species or a complex of species.

Dicyemids exclusively parasitize benthic or epibenthic cephalopods. In temperate and polar waters, adult, benthic cephalopods are 100% infected, whereas in the tropics and off oceanic islands no cephalopods have been reported to be infected. In subtropical waters the incidence of infection varies but is always less than 100%. In other words, the distribution of dicyemids in benthic hosts is by no means universal.

Initial infection normally occurs in very young animals, either immediately following hatching, in cephalopods with demersal juveniles, or following settlement to the bottom, in those host species with planktonic larval stages. In all the animals I have examined I have never encountered dicyemids in neritic or oceanic cephalopods. However, McConnaughey (1959) recorded a species of *Dicyemennea* as occurring in *Loligo opalescens* and a single dicyemid was reported in a single specimen of *Illex illecebrosus* by Aldrich (1964). These reports are probably in error considering that thousands of *Illex* and *Loligo* have been examined by many other investigators and all have been uninfected. Both *Loligo* and *Illex* are neritic genera which come inshore as adults only to mate, spawn and die. In essence, they are pelagic, and would predictably be free from dicyemids.

To date, 67 species of dicyemids have been described. On the basis of the undescribed species in my collections and considering the number of potential host species still to be examined it is possible to project a total of about 200 species in the phylum. Seven genera are currently recognized and placed in two families—DICYEMIDAE: *Dicyema, Dicyemennea, Dicyemodeca, Pleodicyema,* and *Pseudicyema*; CONOCYEMIDAE: *Conocyema* and *Microcyema*.

The number and orientation of cells in each tier of the calotte, the presence or absence of abortive axial cells and the presence or absence of syncytial stages determines the genus. The size of the adult stages, the number of cells comprising the body, the shape of the calotte, the anterior extension of the axial cell, the presence or absence of verruciform cells and the structure of the infusoriform larva are characteristic for each species.

When the dicyemids are examined closely, a simple structure is revealed. A single internal, axial cell runs almost the entire length of the body in the nematogens and rhombogens. In total length these adult vermiform stages range from 500 to 10,000 μm depending on the species. Reproductive products are relegated to the interior of the axial cell of the parent. These cells function as nurse or follicular cells providing both protection and nourishment for the germ cells and developing embryos. The axial cell is surrounded by a jacket of 20 to 40 large somatic or peripheral cells which are entirely ciliated externally. The head or anterior end is modified into a calotte, by means of which the parasite attaches to the host renal tissue. The actual shape of the calotte varies a great deal depending on the species. There is no trace of a differentiated digestive, circulatory, nervous, respiratory, glandular, or excretory system. No muscles, sensory receptors, or skeletal elements are present. In fact, nothing comparable to organs, tissues or glands are observed.

The life cycle (Fig. 1) has been a controversial subject since Erdl (1843) and von Kölliker (1849) first observed the presence of two stages in the renal organs of the cephalopod host. Despite comprehensive study, the life cycle is still incompletely known (see reviews in Hyman, 1940; Nouvel, 1947; McConnaughey, 1951, 1968; Stunkard, 1954; Czihak, 1958; Grassé, 1961; Lapan & Morowitz, 1975). In its simplest expression it is composed of an alternation of essentially isomorphic, parent generations. The embryos of all known stages develop intracellularly until released through rupture of the parent's body wall. Cleavage is determinant. A definite cell number is attained early in development and subsequent growth is by cell enlargement.

The mode of entry and the initiation of the infection is not known or has not been demonstrated experimentally. The earliest known stage observed in juvenile cephalopods is termed a stem nematogen. This stage differs from the typical adult vermiform stages principally in having three axial cells instead of the usual one. However, all vermiform embryos produced by stem nematogens have only one axial cell.

Immature hosts harbor populations of nematogens all of which contain elongate vermiform embryos in their axial cells. The embryos develop asexually from agametes (axoblasts) and resemble the parents when released. Constant proliferation of daughter nematogens eventually results in an enormous population of dicyemids which fills the renal organs of the cephalopod host.

In older hosts the vermiform embryos are replaced by gamete producing infusorigens. The parent is now called a rhombogen. The resulting zygotes develop into ovoid embryos, which, when full grown are termed infusoriform larvae. The infusoriform larvae are anatomically the most complex of any stage in the life cycle. After breaking out of the parent's body the infusoriforms escape from the renal environment with passage of the urine. The fate of this dispersal stage and the phase(s) of the cycle which occur(s) outside the cephalopod host are still a mystery. Several authors have suggested that the infusoriform larvae or their released germinal cells must infect a secondary benthic host since they are not attracted to young cephalopods (see Nouvel, 1947; McConnaughey, 1951; Stunkard, 1954). On the other hand, Lapan & Morowitz (1975) recovered dicyemids in the renal organs of *Sepia* reared from eggs in isolated aquaria and exposed only to infusoriform larvae. This indicates that an intermediate host may not be necessary.

Twice during the course of an infection a change of phase takes place. The initial infective phase is brief and when the stem nematogens are spent they disappear and are replaced by nematogens. As the cycle progresses all nematogens are eventually transformed into rhombogens during which stage gametic reproduction takes place. In octopods, the transition from nematogens to rhombogens is prolonged and a mixture of stages is often found (Hochberg, 1971) whereas in cuttlefish a rapid metamorphosis is completed at the time of sexual maturation of the host (Nouvel, 1933). Because the shift in phase is particularly evident in adult cephalopods, most authors have suggested that the hormonal flux associated with host maturation acts as the trigger. However, at the time of transition the renal organs are maximally crowded with parasites. Hochberg

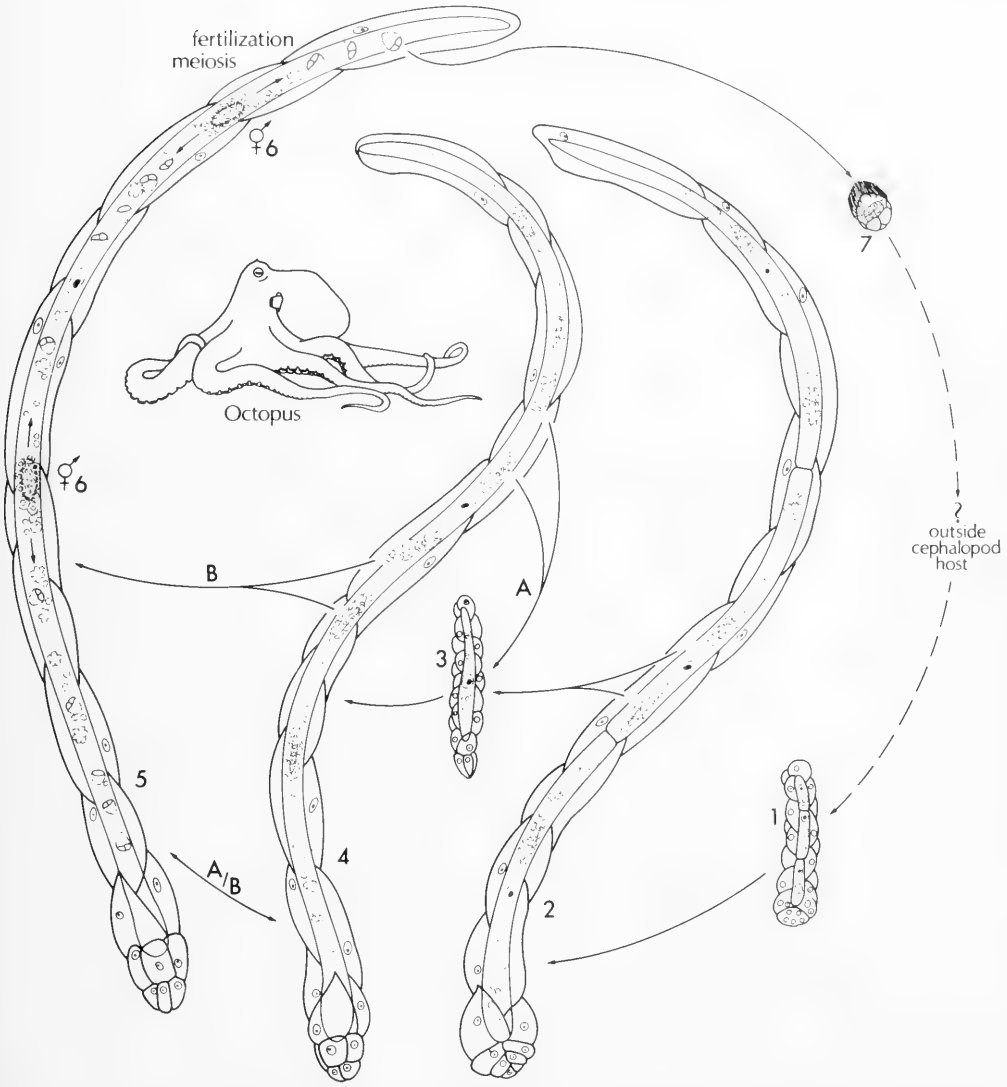

FIG. 1. Life cycle of the dicyemid mesozoan, *Dicyemennea,* in octopus host. 1. larval stem nematogen; 2. stem nematogen; 3. vermiform embryo; 4. nematogen; 5. rhombogen; 6. infusorigen; 7. infusoriform larva released from parent. Density of parasites: A, low; B, high.

(1971) postulated and Lapan & Morowitz (1975) later demonstrated that population pressure or crowding may actually be the key factor which initiates the shift from the nematogen to the rhombogen phase.

CHROMIDINID CILIATES

Next to the dicyemids, ciliates are the most frequently encountered parasites of cephalopods. However, only a few published studies deal with the many unusual forms that occur in the excretory organs, digestive glands and on the gills of squids and octopods.

The genus *Chromidina* is restricted to a small group of vermiform ciliates which infect the renal organs of cephalopods. In the past, *Chromidina* has often been lumped with *Opalinopsis,* a genus of ovoid ciliates which infects the digestive glands of a number of cephalopods. The taxonomic position of these

two genera has been the subject of considerable debate. An affinity between these two highly specialized cephalopod parasites and the apostomes, which typically occur on crustaceans as epibionts, was first postulated by Chatton & Lwoff (1926) who two years later proposed this more definitely (Chatton & Lwoff, 1928). Their ideas with regards to this relationship, were expanded in 1930 and finally in 1931 they demonstrated morphological stages in the life cycle of *Chromidina* that were very similar to stages in the life cycle of the apostomes, especially the foettingeriids (Chatton & Lwoff, 1930, 1931). The extensive monograph by Chatton & Lwoff (1935) provides the only definitive study of *Chromidina* and the apostomes as a whole.

As part of my doctoral research I reviewed the systematic literature and together with new material examined I reaffirmed placement of *Chromidina* in the order Apostomea but relegated the genus to its own family, the Chromidinidae, as separate from the Opalinopsidae (Hochberg, 1971). Though widely accepted as a well characterized and relatively homogeneous group, the apostomes are still an enigmatic assemblage without definite affinities in the accepted scheme of ciliate evolution (Corliss, 1979).

A total of 23 species of cephalopods representing 20 genera are currently known to harbor chromidinid ciliates (Table 1). These ciliates characteristically infect epi- and mesopelagic squids and octopods. Infection of benthic or epibenthic hosts has been occasionally reported but in all cases the ciliates are found only in octopods which have planktonic larvae (i.e. *Octopus salutii, O. vulgaris, Scaeurgus unicirrhus,* and *Eledone cirrhosa*) or in sepioids whose young feed in surface waters (i.e. *Sepia elegans, S. orbigniana* and *Sepiola rondeleti*). The ciliates, which are contracted through association with crustaceans living in the water column, are brought to the bottom at the time of settlement. Once dicyemid infections are established, the ciliates are slowly eliminated as the dicyemids multiply and fill the entire renal habitat (Hochberg, 1971; Nouvel, 1945).

Only three species of *Chromidina* are described in the literature, though most earlier work has not been critically evaluated. On the basis of recent work at least ten different species are recognized and considering the potential hosts not yet examined, a total of perhaps twenty species may eventually be referred to the genus. Two basic body shapes are observed. *Chromidina coronata* has an inflated anterior end and a conspicuous crown of elongate cilia, whereas in *C. elegans* the anterior end is not swollen and the ciliary crown is lacking. In other ways the species are almost identical.

Like the better known foettingeriids, *Chromidina* undergoes a complex polymorphic life cycle which involves an ordered sequence of distinct phases. Hochberg (1971) elucidated the two-host cycle as seen in Fig. 2. Young squids pick up the ciliates when they associate with or feed on swarms of pelagic crustaceans, such as euphausiids. At present the method of entry is not known. Within the cephalopod, the stages of the cycle show considerable modification and condensation as compared with the small, ovoid and less specialized foettingeriids (see especially Bradbury, 1966; and Chatton & Lwoff, 1935). In *Chromidina*, the vegetative and divisional phases are combined into long, thin trophotomonts. These vermiform individuals attach to the renal appendages or digestive duct appendages by means of stiff thigmotactic cilia covering the anterior end. The posterior end which is actively involved in nutrient uptake and division, hangs free in the fluid-filled coelomic sac. Reproduction takes place by unequal, transverse fission or budding at the posterior end of the body. *Chromidina*, unlike the foettingeriids, does not encyst prior to division. Two distinct budding patterns are observed, monotomy and palintomy.

In young hosts, the ciliates all produce large, single buds, termed apotomites, which resemble the parents. When detached they are transformed directly into daughter trophotomonts. By means of this initial budding process the number of ciliates within the renal sac is greatly increased.

Eventually, the renal habitat is saturated with ciliates. Chemical factors related to the density of the parasites or to host maturation probably trigger the second divisional phase as occurs in the dicyemids. In older hosts, the ciliates undergo palintomy, a multiple fission process which results in long chains of 8, 12 or 24 small buds. Eventually, tiny, ovoid dispersal stages, termed tomites, are produced which bear little resemblance to the parents. The tomites conjugate immediately after detachment from the parent tropho-tomonts and then exit through the renal pores with the passage of urine.

Once in the sea the ciliates swim about searching for a new host. Upon contact with a

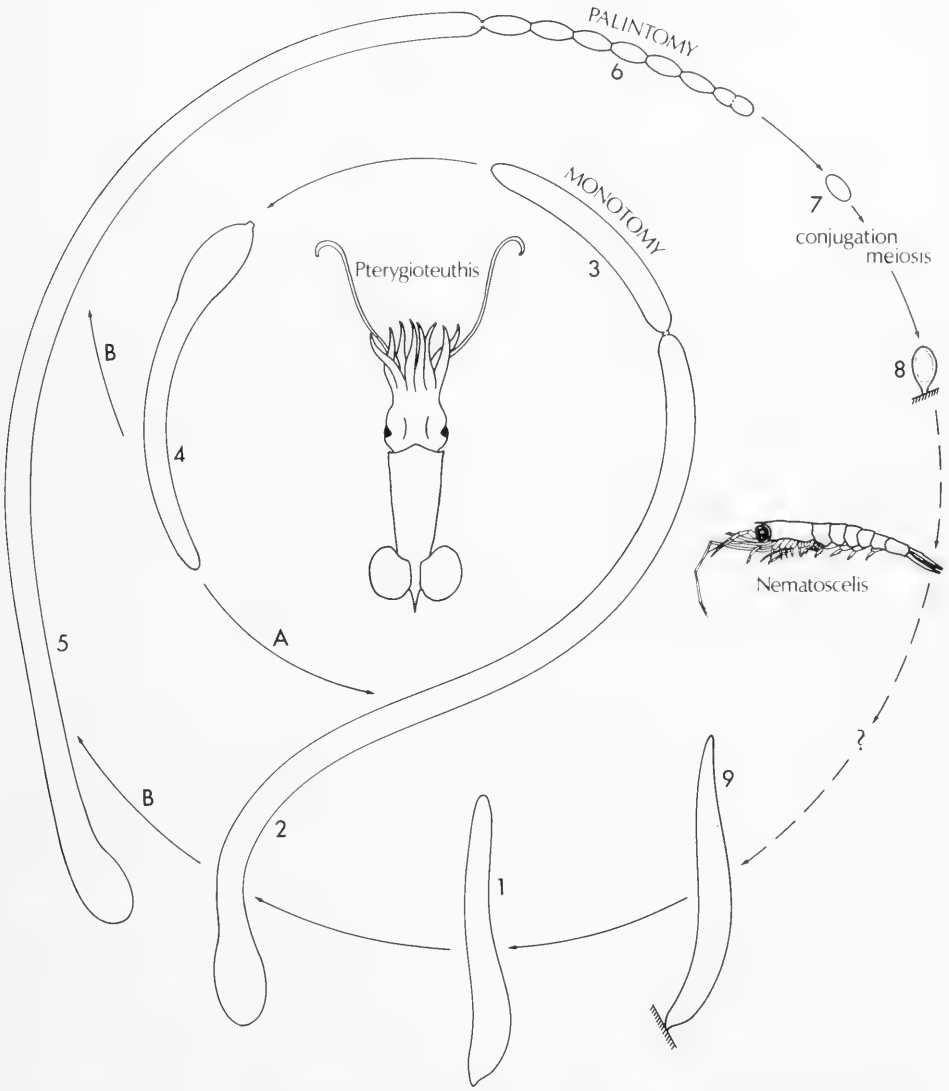

FIG. 2. Life cycle of the apostome ciliate, *Chromidina* in squid (1–7) and on euphausiid hosts (8–9): 1. protropho-tomont; 2. 1° tropho-tomont; 3. production of apotomite via monotomy (single fission); 4. apotomite; 5. 2° tropho-tomont; 6. production of tomites via palintomy (multiple fission); 7. tomite detached from parent; 8. 1° phoront; 9. 2° phoront. Density of parasites: A, low; B, high.

euphausiid or other appropriate crustacean host the tomites encyst on the mouthparts and setaceous appendages of the second host. Phoronts, or encysted resting stages of several sizes have been found indicating that the ciliates undergo a series of growth phases. Euphausiids are known to moult every few days. It is presumed that with each moult the ciliates excyst, feed on exuvial fluids in the cast off moult, grow and then reencyst on another crustacean. Eventually a size is attained which is infective to the cephalopod and the cycle begins again.

The maximum length of vermiform stages in the cephalopod renal organs range from 400 to 2,000 μm depending on the species. The infraciliature of the tropho-tomonts consists of a tight dextral helix, continuous without breaks from the anterior to the posterior pole. Typically 12–14 kineties are present.

The macronucleus is an open network of chromatin which ramifies throughout the entire body. A tiny, spindle-shaped micronucleus is located in the posterior of the body in the region of the future fission plane. Mouth, rosette and contractile vacuole, typically found in other apostomes, are absent in the vermiform stages of *Chromidina*.

Occasionally hypertrophonts are found. Described by Collin (1914, 1915), they may measure up to 5,000 μm. These apparently are individuals that have penetrated the epithelium of the reno-pancreatic appendages and entered the blood spaces within. Here they increase rapidly in size, probably because of the high osmotic pressures. The ciliates which are thus imprisoned and immobilized, appear degenerative due to attack by phagocytes from the blood of the host.

With each successive division during palintomy the kinities are shortened and straightened. Fully developed tomites range in size from 15 to 30 μm. They are pyriform in shape with a convex dorsal surface and a flat or slightly concave ventral surface. The oral field and contractile vacuole develop following detachment from the parent. The tomites of *Chromidina* closely resemble the tomites of many of the crustacean epibionts and it is at this stage that affinities between the apostome families, the foettingeriids and the chromidinids, are most evident. This affinity allows comparison of *Chromidina* with the foettingeriids which are considered to be the most primitive of the apostomes. This in turn provides insight into the adaptations and modifications which have occurred as a result of an endoparasitic existence.

CEPHALOPOD EXCRETORY ORGANS: MORPHOLOGY AND FUNCTION

Physical and chemical environments encountered by parasites within hosts influence the selection of their form and life cycles. Similarities in form and reproductive strategies between the dicyemid mesozoans and the chromidinid ciliates, two phylogenetically unrelated parasites, suggest that the excretory organs are responsible for directing the course of their evolution. It is with this in mind that I will briefly review the main features of the excretory system of cephalopods which appear to be so ideally suited for maintaining parasites.

The first detailed examination of the excretory system of cephalopods was by Cuvier (1817) . The history of morphological investigations and many structural details are provided by Turchini (1923) and more recently by Marthy (1968) and Schipp & Boletzky (1975). The secretory and reabsorptive functions of the excretory organs are reviewed by Harrison & Martin (1965), Potts (1965), and Potts & Todd (1965) among others. Chemical composition of the excretory fluids are reviewed by Lapan (1975). Additional details and a review of the literature is provided in Hochberg (1971).

The term excretion refers to the sum of all activities involved in the formation and elimination of wastes. Through this process an animal regulates the fluid and chemical balance of its internal environment. As compared with other molluscs, cephalopods are very efficient in maintaining a rather constant internal medium. The elaboration of the excretory fluid or urine is actually the result of three separate physiological processes: a) ultrafiltration, b) secretion, and c) reabsorption. Wastes which are produced and eliminated by the cephalopod are only those compounds and molecules from which no further energy can be derived. These waste products, however, are not beyond the use of other organisms, namely parasites.

In cephalopods, excretion occurs specifically in areas where the blood comes into intimate contact with the transport-active epithelium. The main bulk of excretion is carried out by the renal complex (renal appendages and renal sac) in conjunction with the branchial heart complex (branchial heart appendages and pericardium). In the decapods (squids, cuttlefishes and sepiolids) the process is aided by the digestive gland complex (digestive duct appendages and reno-pancreatic sac). Parasites infest all three sites. Since the excretory system of cephalopods is actually a complex of organs and since the renal component may not function in filtration it is best to avoid referring to these organs as a kidney. However, since the word is so commonly applied to the excretory organs, I have continued to use it in quotation marks throughout this paper.

When the ventral mantle of a cephalopod is cut open and the mantle walls laid back, a pair of conspicuous fluid-filled sacs is seen lying on the surface of the visceral mass. The renal organs are the principal excretory organs in cephalopods and the principal site for attachment of parasites. They are derived from

mesodermal masses found in the coelomic complex of the developing embryos. As development proceeds one wall of the expanding renal coelom forms the thin wall of the urine-filled renal sac while the other wraps around the walls of the large veins which traverse the renal cavity. In the region of contact the walls of the vena cava become highly convoluted and eventually develop into the glandular renal appendages. In the appendages a single-cell thick excretory epithelium separates the blood from the urine in the renal sac. On the lumen side, the renal appendages are covered by a microvillar brush border.

Peristaltic activity of the circulatory system creates a flow of blood from the veins into the interior spaces of the renal appendages. Upon contraction, the muscular tissues of the appendages pump some blood back into the main venous vessels and compress the rest within the blind sacs of the highly folded appendages. This action may provide pressure sufficient to effect some filtration although this is debatable. The glandular nature of the renal epithelium and its intimate contact with the blood suggests that the main function is secretory. Harrison, Martin, Potts and others have demonstrated active transport of a number of inorganic and organic compounds through the epithelium of the renal appendages. Ionic resorption, important in the concentration of urine, is presumed to occur in the renal sac. Water resorption probably does not occur in the area of the renal organ.

Rhythmic contractions or pulsations of the renal appendages, as a whole, serve to constantly mix the fluids in the renal sac. Urine is periodically voided through tiny renal pores and then flushed from the mantle cavity during respiratory or locomotory contractions. Due to the dead spaces surrounding the renal appendages emptying is never complete. As a result fluid is always present. Accumulations of mucus, proteins and nitrogenous wastes may escape elimination for some time. The constant presence of a nutrient-rich fluid environment is considered essential for the maintenance of parasites.

The branchial hearts form a complex with the branchial heart appendages (or pericardial glands) in both octopods and decapods. The dark, muscular branchial hearts, located at the base of the gills, are accessory pumping structures which help to irrigate the gills. The lumen of these organs continues into the branchial heart appendages where it forms a system of blood sinuses. Each appendage is surrounded by a pericardium which empties via the reno-pericardial canal into the renal sacs. Similar to the renal appendages, the branchial heart appendages of sepioids and teuthoids are convoluted and are lined with a transport-active epithelium covered with a microvillar brush border. The high systolic pressure in the branchial hearts provides ample pressure for ultrafiltration to occur and in fact this region and not the renal appendages is the principal site for blood filtration. Cilia, present on the cells of the reno-pericardial canal, beat in the direction of the renal organs and are thought to additionally aid filtration by lowering the pressure inside the pericardial cavity. The resultant reno-pericardial filtrate or "primary urine" serves as the aqueous vehicle for excretory products formed by the renal appendages. Resorption of certain constituents such as glucose occurs in the pericardium and in the reno-pericardial canal.

A single species of dicyemid, *Dicyemennea brevicephaloides,* has been recovered from the branchial heart coela of *Rossia pacifica,* a small west American sepiolid (Hoffman, 1965). Other dicyemid and chromidinid parasites probably occur in this site in sepioids and teuthoids but simply have been missed in routine autopsies. In octopods, the coelomic spaces surrounding the branchial heart appendages are greatly reduced and the peritoneum of the appendages is smooth (Witmer & Martin, 1973), hence parasites are unlikely to be present.

In decapods, a third organ is present which serves an excretory function. These are the grape-like "pancreatic" or digestive duct appendages (see Bidder, 1976) which cover the paired ducts connecting the "liver" or digestive gland and the caecum. The elaborations of these ducts are located in a single median sac which lies dorsal to and opens into the renal sac. Taken together the renal and the digestive duct appendages are contained within the reno-pancreatic coelom.

The digestive duct appendages are composed of two layers of epithelial cells separated by a layer of blood vessels and sinuses. The low, outer epithelium is derived from the reno-pancreatic sac during development and is similar to the transport-active epithelium of the renal appendages, complete with microvillar brush border. In function, the digestive duct appendages are thought to secrete inorganic and organic compounds and to concentrate the urine by reabsorbing ions and water.

In decapods, the two organs are contained within a common coelomic cavity, hence, when parasites invade this region they are found attached to both the renal and the digestive duct appendages. In octopods, this organ is embedded within the capsule of the digestive gland and, hence, not subject to infection by "kidney" parasites.

In summary, the "kidneys" of cephalopods are ideally suited for the establishment and maintenance of parasites. As such, these organs meet the parasite's requirements by providing: a) a substrate for attachment; b) a constant fluid bath; c) a source of nutrients; and d) a simple exit for release of dispersal stages.

Vermiform stages of chromidinid ciliates and dicyemid mesozoans live and reproduce within the excretory organs of cephalopods. Specifically they are located on the renal appendages, digestive duct appendages and occasionally on the branchial heart appendages. In all cases the appendages to which the parasites are attached are convoluted and the epithelial substrate is transport-active and covered with a microvillar brush border. Dense thigmotactic cilia which interfinger with the microvillar surface of the appendages help to hold the parasites in place. Undulations of the body generated by ciliary beat create additional forces directed toward the surface of the excretory appendages. In many cases the anterior ends of both the dicyemids and chromidinids are imbedded between the convolution of the excretory appendages or they may even occupy individual depressions or "crypts" in the renal surface (Ridley, 1968).

All the appendages mentioned above are surrounded by fluid-filled coelomic spaces into which the parasites hang. Periodic and incomplete emptying of these coelomic sacs ensure that the parasites are constantly surrounded by fluids. The parasites derive all their metabolic requirements from the dissolved nutrients within the excretory fluids. A great deal of study has gone into unraveling the physiology of urine formation and the chemical composition of the urine, but little is known about the actual products utilized by the parasites. Since urine from uninfected cephalopods has never been analyzed we do not know what effects the parasites have on the excretory processes nor do we know how they modify the composition of the urine through discharge of their own metabolic wastes.

Finally, in order to complete their life cycles, the parasites must find their way to the exterior of their host. When dispersal stages are produced within the renal organs of the cephalopod, they are easily voided through the renal pores along with the urine and then flushed out of the mantle cavity of the host.

DISCUSSION

Possession of similar characters, by phylogenetically unrelated organisms, consequent upon a similar mode of living is termed convergence. The similarity in: a) site of infection; b) sedentary habits; c) general shape; d) body proportions; and e) diphasic life cycle, of both the dicyemids and the chromidinid ciliates is an example of such a phenomenon. Evidence suggests that the adaptive response of these two ciliated parasites to the excretory organs of cephalopods has resulted in the convergence of both their form and reproductive strategies. In essence, evolutionary processes have been restricted by environmental factors which operated to select for development in specific directions.

An elongate, vermiform shape; terminal (anterior) holdfast organ; and terminal (posterior) budding are not uncommon traits in the animal kingdom. These features are variously expressed in groups of hymenostome and astome ciliates, catenulate turbellarians, cestodes, and annelids among others. However, as displayed by *Chromidina*, these features are unique within the apostome ciliates.

Several additional features are displayed by the chromidinids which are not present in simpler, more primitive apostomes such as *Gymnodinioides* or *Hyalophysa.* These include: their location in the excretory organs of cephalopods; their greatly increased size, polarized along the anterior-posterior axis; the absence of a rosette and functional cytostome; and unencysted divisional stages. Unlike the more typical foettingeriids, single buds or apotomites, produced by monotomy, are a constant feature of the life cycle. In *Chromidina*, protomites, produced by palintomy, are attached posteriorly to the parent in long, linear, multiple bud chains.

Comparison of *Chromidina* with primitive or less complex apostomes, as mentioned above, provides insight into the modifications of morphology and life cycle which are correlated with their endoparasitic specialization. Using these modifications as a guide, it is possible to postulate that similar changes

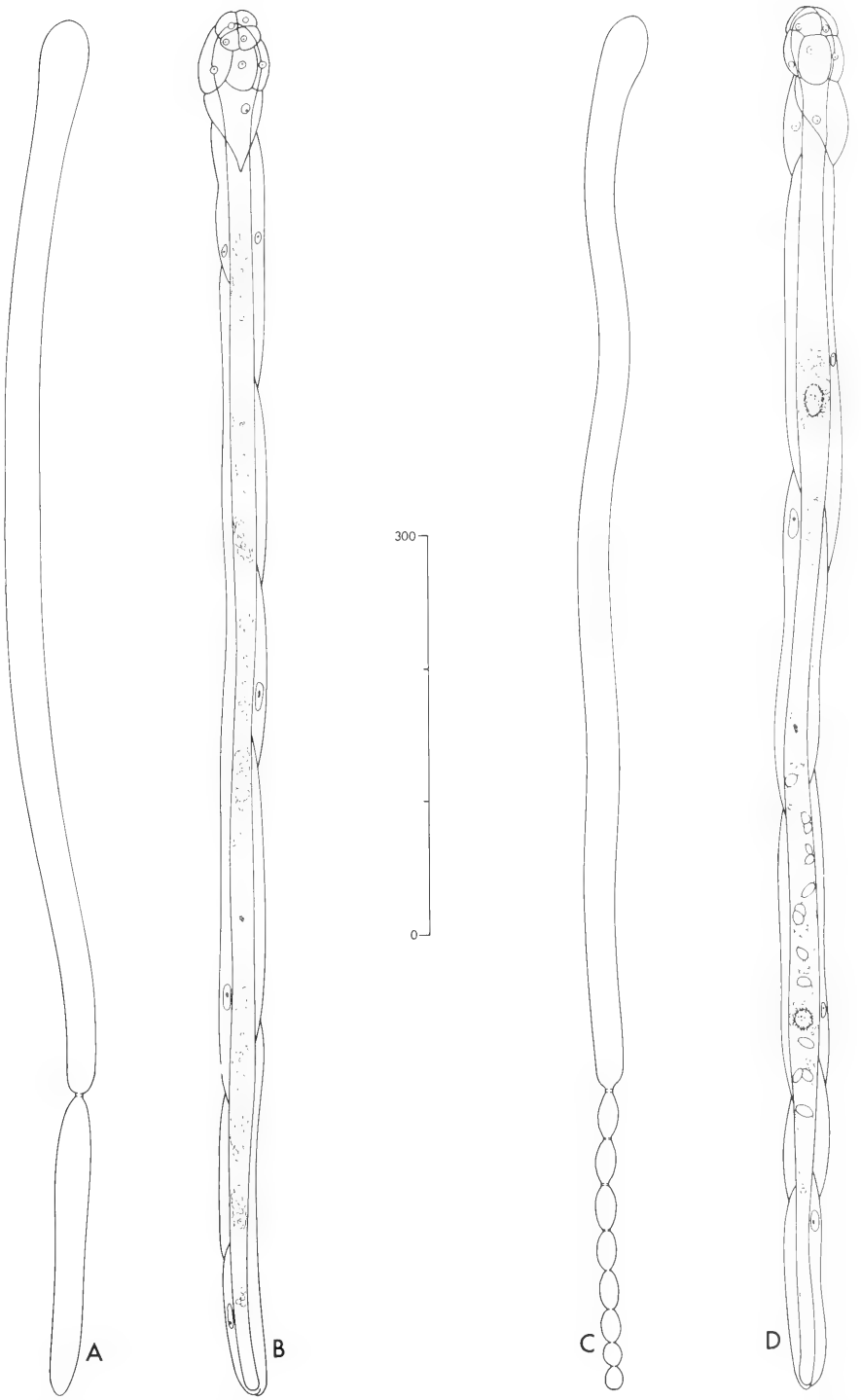

FIG. 3. Convergence of chromidinid ciliates and dicyemid mesozoans. Parasites in young cephalopods (A & B); parasites in mature hosts (C & D). *Chromidina* from *Pterygioteuthis* (A & C); *Dicyema* from *Octopus* (B & D).

must have occurred in the evolution of the dicyemid mesozoans, where no relatives are available for comparison.

The diphasic life cycle, of both groups, is beautifully adapted to the requirements of their endoparasitic environment. Two distinct types of embryos (dicyemids) or buds (chromidinids) are produced within or attached to essentially isomorphic parents (Fig. 3). In young hosts, where there may be only a few initial parasite individuals, elongate daughter individuals (apotomites and vermiform embryos), resembling the parents, remain in the coelom of the renal organs when released. Endogenous proliferation of this sort functions to greatly increase the numbers of vegetative stages which eventually spread throughout the excretory organs. In older hosts, a change of phase triggers the production of small dispersal forms (tomites and infusoriforms) which are discharged from the host with elimination of urine. Widely broadcast in the watery environment, these motile forms function to disseminate the parasites to new hosts.

A convergence in form is also evident. In both groups of parasites the anterior end is often inflated and is always covered with short, stiff thigmotactic cilia. The parasites adhere, by this modified anterior end, to the brush-bordered, transport-active epithelium of the excretory appendages. The gradient, established when the parasites attach at one end while the other end hangs free in a fluid-filled coelomic cavity, results in the differentiation of distinct anterior and posterior regions. Growth, which occurs along this antero-posterior axis, imparts a vermiform shape to both organisms. This is most obvious in *Chromidina* when contrasted with other more typical apostomes. Almost without exception, the foettingeriids are small and compact ciliates. Only in *Polyspira* is an elongate, linear form observed, and then only during palintomy when chains of protomites are being formed. Definite anterior and posterior regions are not distinguishable in this case (Chatton & Lwoff, 1935).

The relative body proportions of adult vermiform stages of both dicyemids and chromidinids are almost identical. Nematogens, rhombogens, and tropho-tomonts generally range in length from 1,000 to 5,000 μm. *Chromidina*, in comparison with other apostomes, has undergone a many-fold increase in length. It can be assumed that the same holds true for the dicyemids, and that any resemblance to recent invertebrate forms is coincidental.

The size of daughter individuals is equally similar. Vermiform embryos, produced within dicyemids, range from 100 to 300 μm in length. Apotomites, produced by chromidinids, tend to be slightly larger. The similarity, in size and shape, of daughter stages is best witnessed by comparison of the tiny, ovoid dispersal forms. Both the tomites and the infusoriform larvae range in length from 20 to 40 μm. Since these stages leave the host via the renal pores, it is assumed that their size is a function of the diameter of the openings of these tiny pores. In essence, they must be small enough to be expelled through the pores along with the urine.

Concurrent infections rarely occur in nature. Cephalopods hosting the two parasites are normally spatially isolated. *Chromidina* typically infects oceanic cephalopods which never contact the bottom, whereas dicyemids are known only from exclusively benthic or epibenthic hosts. The exploitation of the "kidneys" of cephalopods by these unusual vermiform parasites thus is facilitated and maintained by the habits of the hosts and the spatial separation of the infective stages. In the absence of competition, adaptation to the selective pressures within the excretory environment has favored convergence or the development of similar morphological and reproductive characteristics in these two unrelated groups of parasites.

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THE FUNCTIONAL MORPHOLOGY OF A VENTRAL PHOTOPHORE FROM THE MESOPELAGIC SQUID, *ABRALIA TRIGONURA*

R. E. Young[1] and J. M. Arnold[2]

ABSTRACT

The vertically migrating squid, *Abralia trigonura,* has at least two types of photophores involved in counterillumination. The most complex of these is described.

We suggest that this photophore functions in the following manner. Innervated photocytes which contain crystalloids extract a component of the luminous reaction, presumably luciferin, from blood vessels via numerous finger-like processes. Energy for the reaction is supplied by banks of mitochondrial cells. Light is emitted by the crystalloids which are stacked to form a photogenic cone. The photogenic cone lies at the focus of a spherical proximal reflector. This reflector is an interference structure that selectively reflects light outward and contributes to color regulation by the alteration of its reflectance characteristics through changes in the diameter of its collagen rods. An interference filter, the axial stack, selectively transmits light and contributes to color regulation by altering the thickness of the fluid-filled spaces between its platelets. The torus and distal cap are "thick film" reflectors that slightly diffuse the highly directional emission beam. Another interference structure, the distal reflector, reflects outwardly light emitted between the distal cap and the proximal reflector. Numerous chromatophores can conceal the photophore or aid in adjusting the radiance pattern of emitted light. The intensity of emitted light can be regulated over at least a 325-fold range, and the spectral emission maximum can vary between 480 and 536 nm. The angular distribution of emitted light can be regulated as well, but measurements have not been attempted. The photophore can rock on a fluid cradle which enables proper orientation regardless of the squid's attitude in the water.

The complexity and the alterability of the light emission properties of this photophore, in combination with one or two other types of photophores, indicate that this squid can match many of the varying patterns of intensity, color and radiance of downwelling light encountered in its oceanic habitat and, thereby, conceal itself by eliminating its silhouette from potential visual predators.

Key words: photophore; squid; ultrastructure; bioluminescence; color.

INTRODUCTION

Abralia trigonura Berry, 1913 is a small mesopelagic squid endemic to the region of the Hawaiian Islands. This squid usually occupies depths between 450 and 550 m during the day and the surface and 110 m at night (Young, 1978). At these depths the squid is often exposed to downwelling light that could silhouette it and, thereby, make it visible to potential predators. This squid, however, has the capability of eliminating its silhouette by counterilluminating with downward-directed bioluminescence at least under some laboratory conditions (Young & Roper, 1977). Effective counterillumination in the sea requires that the emitted light match the color, intensity and angular distribution of the downwelling light. During the day at depth only light intensity varies but at night, in near-surface waters, all three parameters can vary depending on the exact depth, moon phase and declination, and cloud cover. Photogenic organs capable of providing counterillumination under these variable conditions must be highly sophisticated.

The photophores of related species of *Abralia* have been briefly examined by Hoyle (1894) and Joubin (1895). Mortara (1922) described in more detail the general features of the photophores of *Abralia veranyi.* However, without the aid of the electron microscope little about the functional morphology of the organs could be determined.

MATERIALS AND METHODS

Photophores fixed for electron microscopy came from live squid collected during a series

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of cruises aboard the University of Hawaii's research ship, R. V. KANA KEOKI off leeward Oahu, between 1975 and 1980. Fixation and embedding were carried out aboard ship. Photophores were generally fixed for 1 hr in 2.8% glutaraldehyde in filtered seawater buffered with 0.01 M collidine at pH 7.5. Following a seawater rinse, the organs were post-fixed for 1 hr in 1% OSO_4 in seawater buffered with 0.01 M collodine and adjusted to pH 7.5 with HCl. The photophores were then dehydrated through an ethanol series and embedded in Epon for thin sectioning. Sections were stained with uranyl acetate followed by lead citrate. Thin sections were viewed with a Philips 201 electron microscope.

Measurements of the luminescent emission spectrum were made according to procedures outlined in Young & Mencher (1980). Live squid maintained in thin vinyl tubing were stimulated to luminesce by artificial illumination above the animal. A fiberoptic probe beneath the squid transmitted the luminescence to a double monochrometer connected to a photomultiplier tube (PMT). The output of the PMT was fed into a photon-counting system connected to a desktop computer. Since water continuously flowed through the vinyl tube, the water temperature could be easily controlled.

ABBREVIATIONS

A	Type A photophores
ac	apical chromatophore
ax	axial stack
axc	axial stack cell
axcl	axial complex
B	Type B photophores
bv	blood vessel
C	Type C photophores
c	photogenic cone
cac	(distal) cap chromatophore
cc	(proximal) cup chromatophore
co	core
coc	core cells
cr	crystalloid
crc	crystalloid cell
dc	distal cap
dm	dense cytoplasm
dr	distal reflector
ds	dense substance
es	extracellular space
gr	girdle
ic	inner region of cap
icc	immature crystalloid cell
lm	lamella
m	muscle
mi	mitochrondria
mt	microtubules
n	nucleus
nc	nucleus of core cell
ne	nerve
oc	outer region of cap
os	orbital space
pc	proximal cap
php	photogenic plexus
pr	proximal reflector
r	root
ri	ribbon
s	(collagen) sheath
sc	sheath cell
sur	surface
to	torus
v	vesicles
vc	vesicle cell

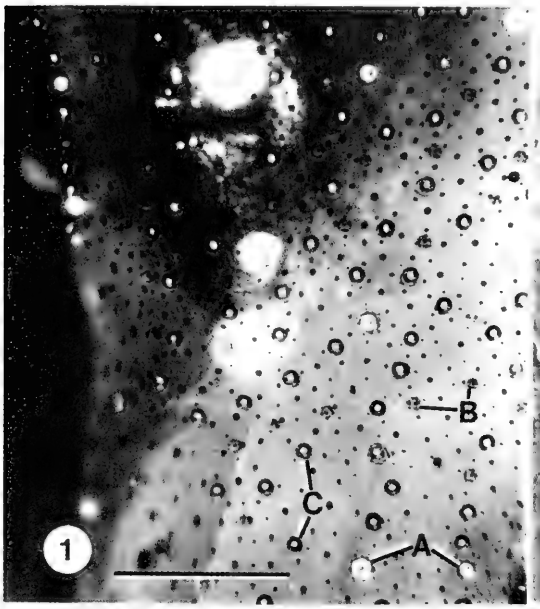
RESULTS

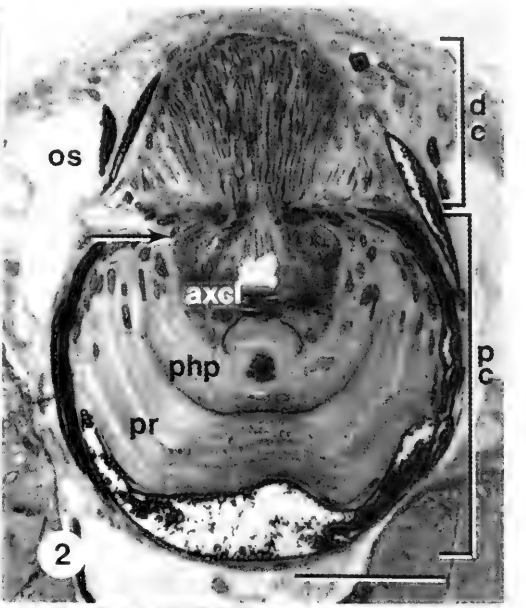
Abralia trigonura possesses five types of photophores. Two types lie in a series on the ventral surface of each eyeball and are not involved in counterillumination. The most abundant photophores (termed the Type A,

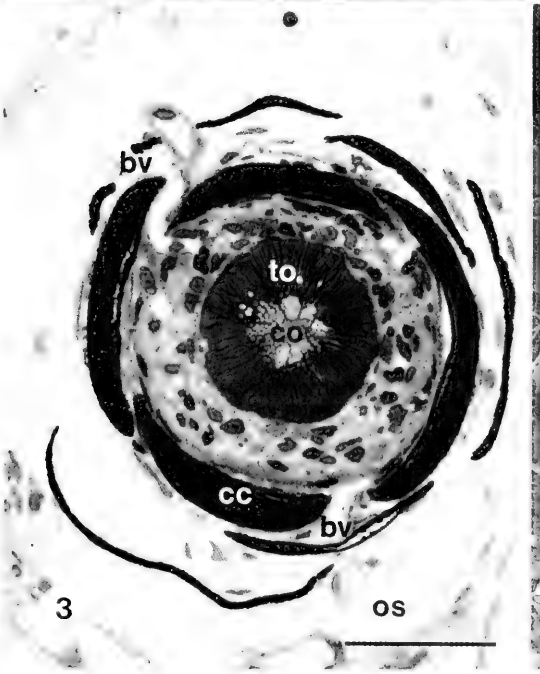
FIG. 1. Ventral head region of an adult *Abralia trigonura* showing the distribution of the three types (A, B, C) of integumentary photophores. The large white areas are ocular photophores. Scale bar is 4 mm. FIG. 2. Longitudinal section of the Type C photophore showing the general morphology. Compare with Fig. 5. Surface of the animal would lie above the top edge of the figure. The irregular outer convex surface of the posterior reflector is probably a fixation artifact. The large chromatophores surrounding the posterior reflector absorb light that is not reflected by this color-selective reflector. Arrow—see Fig. 3. Scale bar is 50 μm. FIG. 3. Cross-section of the Type C photophore at the level of the torus. Level of cut indicated by arrow in Fig. 2. Note the extensive orbital space and associated blood vessels. Scale bar is 50 μm. FIG. 4. Longitudinal section nearly through the optical axis of the axial stack and photogenic cone showing the general relationships of the photogenic cone, the proximal and distal region of the axial stack and the zone of vesicles. Variations in spacing of the platelets of the axial stack is probably a fixation artifact. Scale bar is 5 μm.

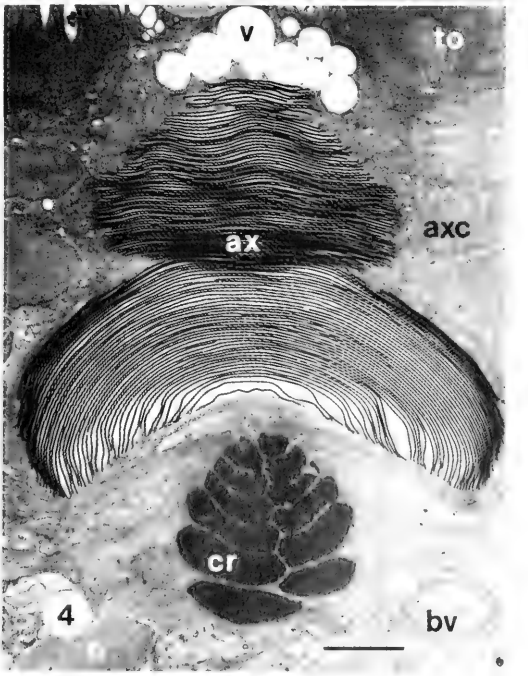
Type B, and Type C) comprise the remaining three types. These types occur approximately in the ratio of 1(A):7(B):10(C) and they are intermingled and scattered over the ventral surface of the head, arms, funnel and mantle (Fig. 1). We describe here the structure of the Type C photophore which is not only the most abundant type but the most complex as well.

Each Type C photophore is a small organ (140–180 μm in diameter) that lies just beneath the epidermis in a cup-like depression of the body musculature. For descriptive purposes the photophore can be subdivided into several components: 1) PROXIMAL CUP—a hemispherical region that forms much of the proximal half of the photophore; 2) DISTAL









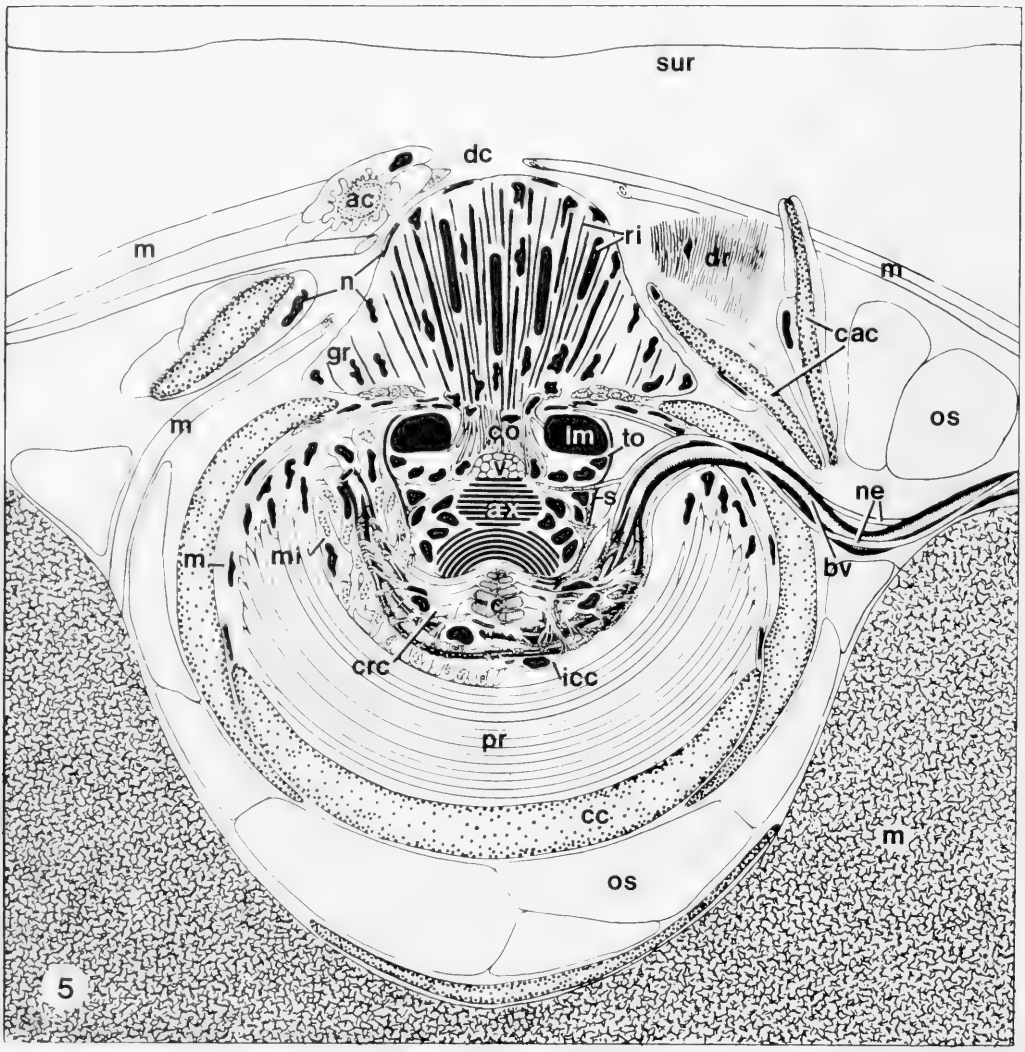
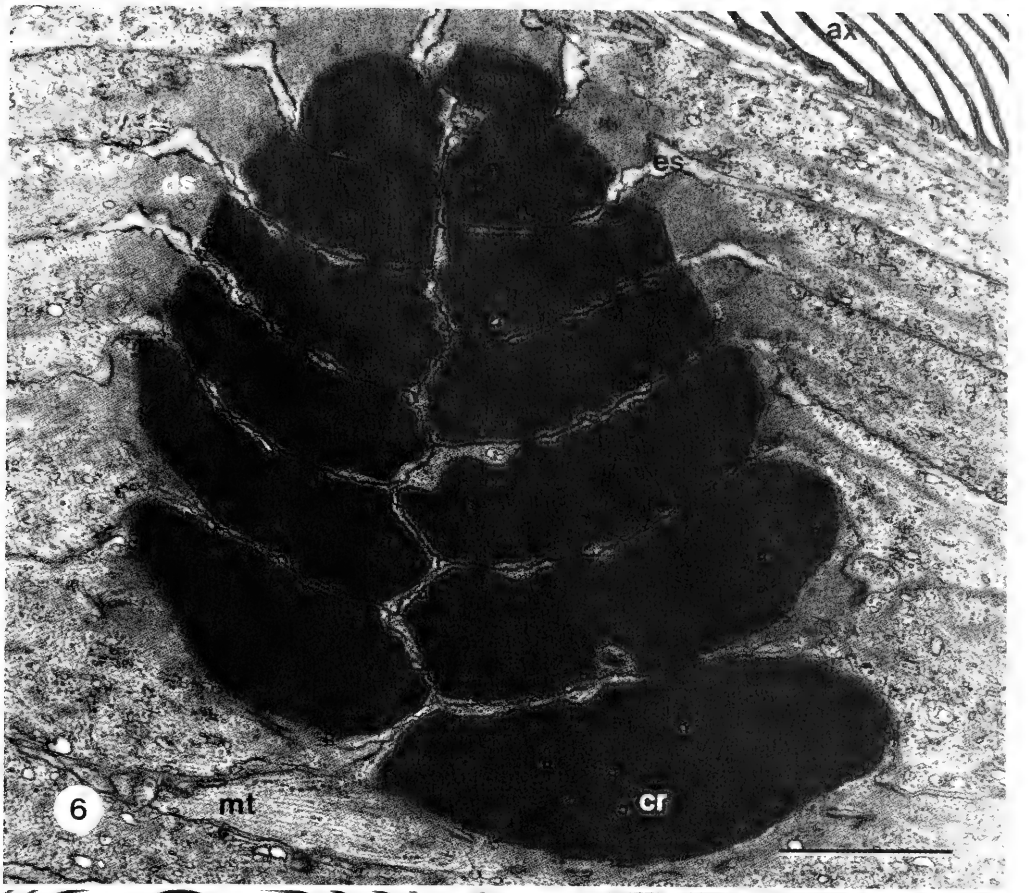


FIG. 5. Schematic diagram of the Type C photophore. For clarity, nerves and mitochondrial cells are drawn only on opposite sides of the photogenic plexus.

FIG. 6. Longitudinal section of the photogenic cone showing the stacked crystalloids each in an individual cell. Note the close apposition of the crystalloids, and the dense material where the cytoplasm of the cell joins the crystalloid. Numerous microtubules can be seen in the crystalloid cells. Note the increase in size of the extracellular spaces toward the apex of the cone. Scale bar is 2 μm. FIG. 7. Glancing section at the edges of the crystalloids showing the interconnecting extracellular spaces. Scale bar is 1 μm. FIG. 8. High magnification of crystalloids showing the lamellar organization with similar alignment in adjacent crystalloids. Membranes bordering the extracellular spaces are associated with regions of dense cytoplasm (dm). Scale bar is 0.2 μm.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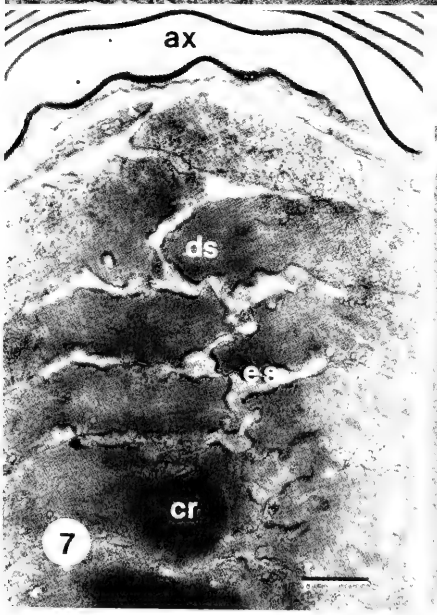
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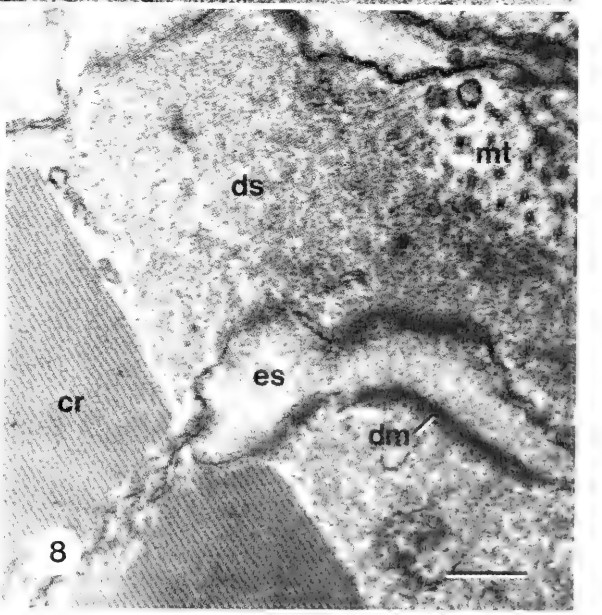
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CAP—a bell-shaped structure that sits atop the proximal cup; 3) DISTAL REFLECTOR—a lamellar organ that surrounds much of the dome of the distal cap and whose broad surfaces tilt slightly away from the optical axis of the photophore; 4) CHROMATOPHORES—several sets surround the photophore; 5) ORBITAL SPACE—large fluid-filled lacunae that surround the proximal cup. (See Figs. 2, 3, 5 for general morphology.)

1) PROXIMAL CUP—This portion of the photophore is composed of three primary regions: A. the photogenic plexus, B. the proximal reflector, C. the axial complex (Fig. 2).

A. *Photogenic Plexus.* This plexus is a highly vascularized region lying between the axial complex and the posterior reflector. The plexus is composed of several different cell types and tissues.

Crystalloid cells. The fully differentiated crystalloid cells lie in the center of the photogenic plexus. Each cell contains a single large crystalloid that lies freely within its cytoplasm (Figs. 4, 6, 9). Each crystalloid cell has many branches. The crystalloid is located at the center of the branching and the nucleus lies in one branch (Fig. 9). The crystalloid within each cell lies adjacent to those of neighboring cells. Together the crystalloids form a conical stack (conoid) which we term the photogenic cone (Figs. 4, 6). Individual crystalloids usually have the shape of a thick half-disc (Figs. 6, 9). The two adjacent stacks of these half-discs form a conoid. For the most part, crystalloid cells branch only to one side of the photogenic cone forming a "half-star" (Fig. 9). Nuclei of these cells are clustered in two small regions that lie on opposite sides of the cone (Fig. 9). The branches extend outward from the crystalloids, and subdivide into slender, finger-like processes that encircle blood vessels of the plexus, often forming a dense mat around the vessels (Fig. 10).

The crystalloid cells are packed with dense arrays of microtubules that predominantly extend between the crystalloids and the tips of the branches (Figs. 6, 9, 11). Near the crystalloids radiating microtubules are intermingled with microtubules that tend to parallel the surface of the crystalloid (Figs. 6, 8). Adjacent to the crystalloids most microtubules blend into an electron dense, amorphous substance (Figs. 6, 7, 8). This substance is more extensive near the apical end of the cone. Occasionally structures resembling ciliary rootlets extend from this amorphous substance into the region of microtubules (Fig. 11). The crystalloid cells also contain scattered tubular, branching, smooth endoplasmic reticulum which is most abundant near the crystalloids. Numerous vesicles, about the size of synaptic vesicles are observed in Glutaraldehyde-Osmium fixations (Figs. 6, 11). Mitochondria, however, are infrequently observed.

The crystalloid is a highly organized crystal-like array of electron dense lamellae (Fig. 8). In section at right angles to the lamellae, the lamellae exhibit minor Z-shaped undulations, the result of alternating ridges or knobs. Adjacent crystalloids often exhibit parallel alignment of their lamellae (Fig. 8). At their edges, the crystalloids blend abruptly into the cytoplasm; they are not membrane-bound although they lie adjacent to the plasmalemma (Figs. 6, 8). The shape of the crystalloids near the apex of the cone is often more irregular than those at the base (Fig. 4).

Near the crystalloids is a complex system of interconnecting extracellular spaces (Figs. 6, 7, 8). These spaces are large near the lateral edges of the crystalloids and are continuous with broad concavities or sometimes channels that extend well into the adjacent cells. The spaces become progressively more extensive toward the apex of the cone. Cell membranes bordering the channels are frequently distinguished by narrow, electron dense regions of cytoplasm (Fig. 8). The material within the extracellular spaces is not homogeneous. Electron light and dense areas intermingle often giving an indistinct striated appearance. The system of extracellular spaces is continuous with a broad, plate-like space that immediately underlies the axial stack (Fig. 7). The extracellular spaces of the axial stack are completely homogeneous electron light areas in contrast to spaces associated with the crystalloids. Places can be found where the extracellular areas of these two regions join; however, they maintain their distinctiveness.

Developing crystalloid cells which lack the

FIG. 9. Cross-section through the crystalloids and the photogenic plexus. The crystalloid cells branch only to one side of the plexus. Their nuclei are grouped into two regions which lie on opposite sides of the plexus. Blood vessels and nerves are numerous in this section. Scale bar is 5 μm.


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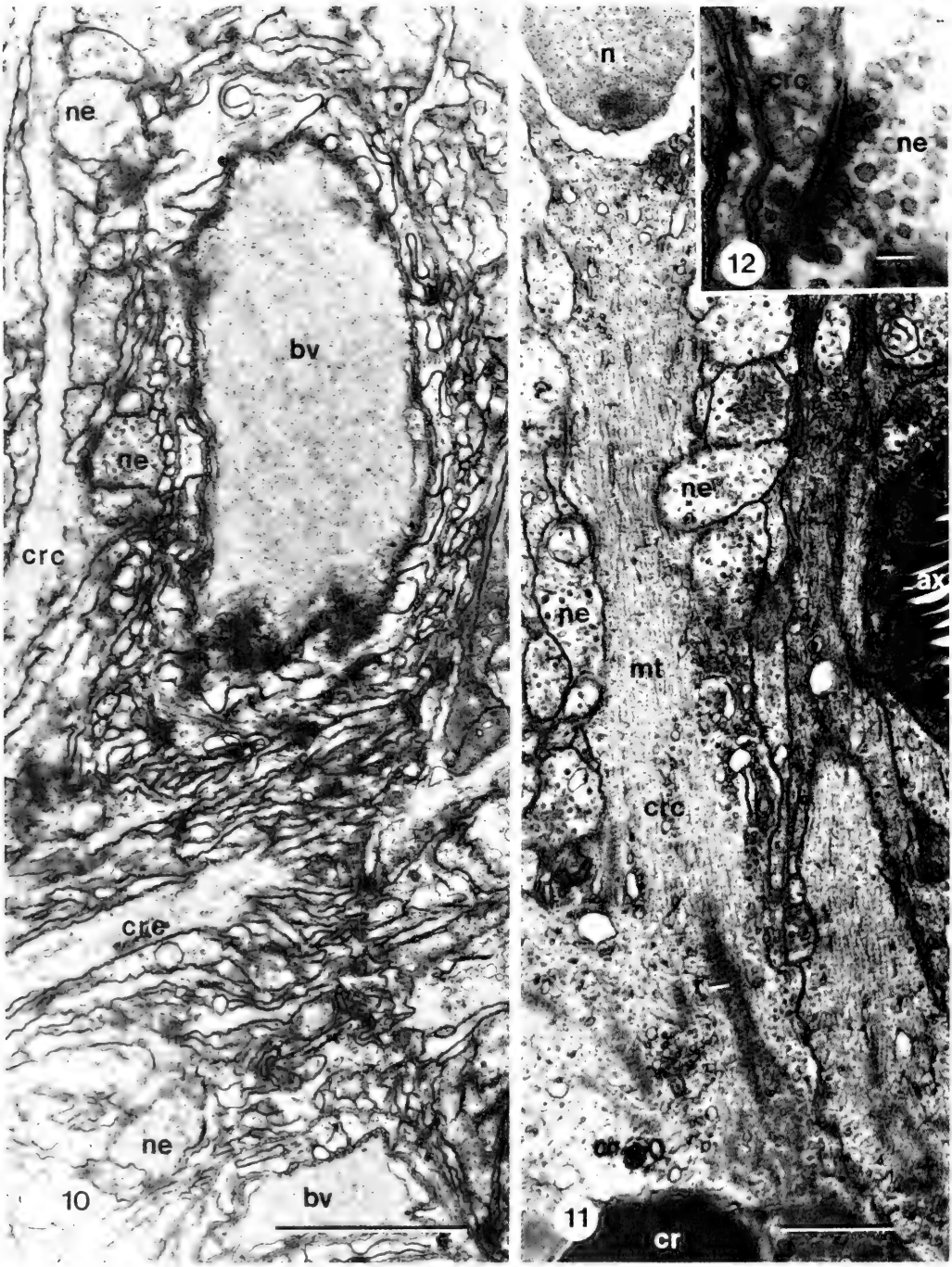


FIG. 10. A crystalloid cell exhibiting multiple branching near the surface of a blood vessel. Note the numerous slender extensions of the crystalloid cell that cover the blood vessel. Scale bar is 2 μm. FIG. 11. A crystalloid cell showing numerous adjacent nerves. Abundant microtubules can be seen within the crystalloid cell. Scale bar is 2 μm. FIG. 12. Synapse between a nerve and a crystalloid cell. Scale bar is 0.1 μm.

dense arrays of microtubules and which frequently contain very small crystalloids are found in the fully differentiated photophore between the photogenic cone and the posterior reflector (Fig. 5). Like the mature crystalloid cells these branched cells are in close contact with blood vessels. The branches that contact the blood vessels, however, are not as small or as numerous as are those of the mature cells. Mitochondria and Golgi bodies are common in these cells and tubular, smooth endoplasmic reticulum extends throughout the cytoplasm. The crystalloid cells of the developing photogenic cone from an immature Type C photophore exhibited these same characteristics.

Mitochondrial cells. The mitochondrial cells are highly branched structures that possess numerous large mitochondria (Fig. 15) The branches ramify throughout much of the photogenic plexus. Nuclei of these cells are located in the peripheral region of the plexus adjacent to the cells that form the proximal reflector. Because of the extreme tangle of the branches, individual cells cannot be traced very far from their nuclei. The branches are easily identified deep within the plexus by the lack of microtubules, the presence of numerous large mitochondria, and often, by a more electron dense cytoplasm. Cytoplasm in the region of the nucleus contains abundant smooth endoplasmic reticulum and occasional Golgi bodies. Within the proximal region of the plexus the mitochondrial cell processes are concentrated near the surface of the proximal reflector and

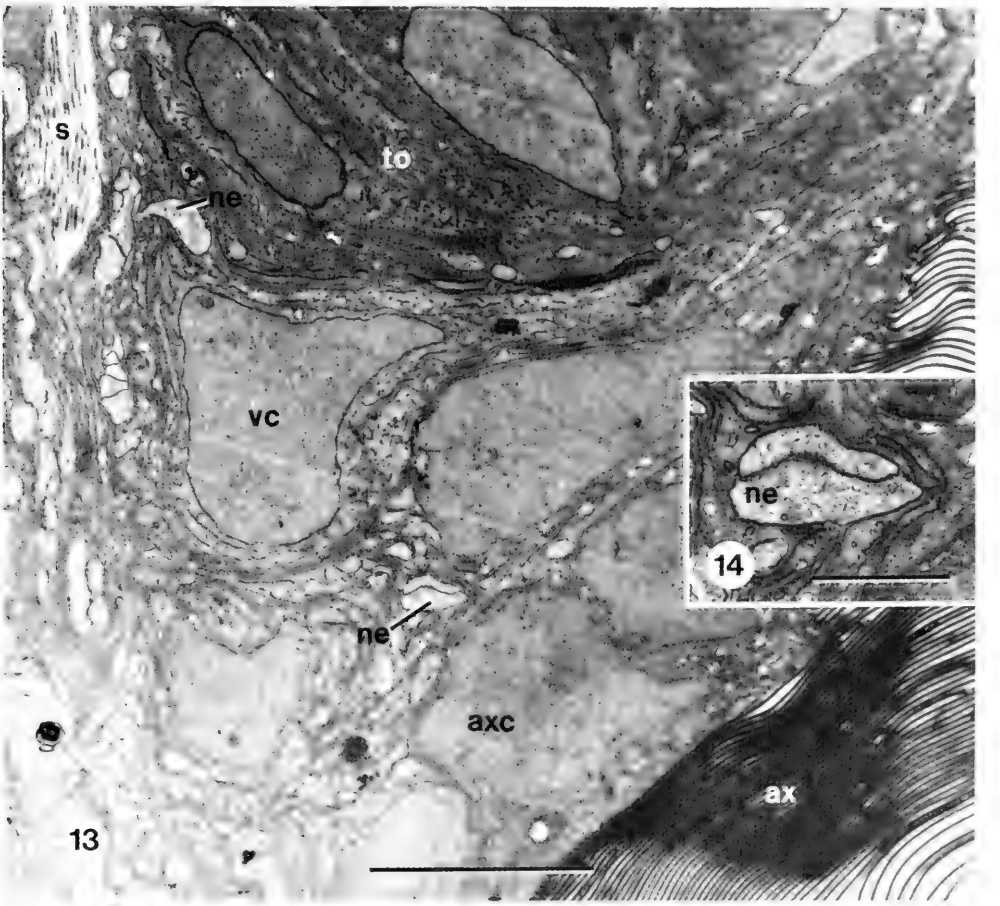


FIG. 13. Nerves in the region of the cells that form the axial stack. Two nuclei of the vesicle cells are evident. Scale bar is 5 μm. FIG. 14. Higher magnification of the nerves seen in Fig. 13 showing presynaptic vesicles. Scale bar is 1 μm.

extend inward between blood vessels, photocytes and nerves. They do not penetrate, however, to the region surrounding the crystalloids which is occupied only by crystalloid cells and occasional nerves.

Blood vessels. Blood vessels are observed only in the region of the photogenic plexus and enroute to and from this tissue (Fig. 15). Relatively large vessels penetrate the photophore from the periphery at various points. They pass between the chromatophores that surround the proximal cup, over the reflector-secreting cells and into the photogenic plexus (Figs. 2, 3). There the vessels subdivide forming about 30–35 small (~2.5 μm) vessels that pass beneath the photogenic cone (Fig. 9). The morphology of these vessels is typical of exchange vessels (i.e. capillaries, see Browning, 1979) (Fig. 10). A thick basal lamina is always present. The endothelial layer is incomplete and a single layer of deeply interdigitating pericytes composes the vessel wall. Myofilaments, microtubules, smooth tubular ER, and mitochondria are common in the pericytes. No extensive extracellular matrix surrounds the vessels.

Nerves. Numerous nerves packed with synaptic vesicles penetrate the photogenic plexus, presumably entering with the blood vessels above the margins of the proximal reflector. A few small nerves pass into the region of the axial stack (Figs. 13, 14). Most penetrate and entwine the branches of the crystalloid cells (Fig. 11). Rarely, however, do nerves penetrate to the immediate region of the crystalloids. Occasionally synapses are seen between the nerves and photocytes (Fig. 12). Other peripheral nerves are present that probably innervate the numerous chromatophore and other muscles of the photophore.

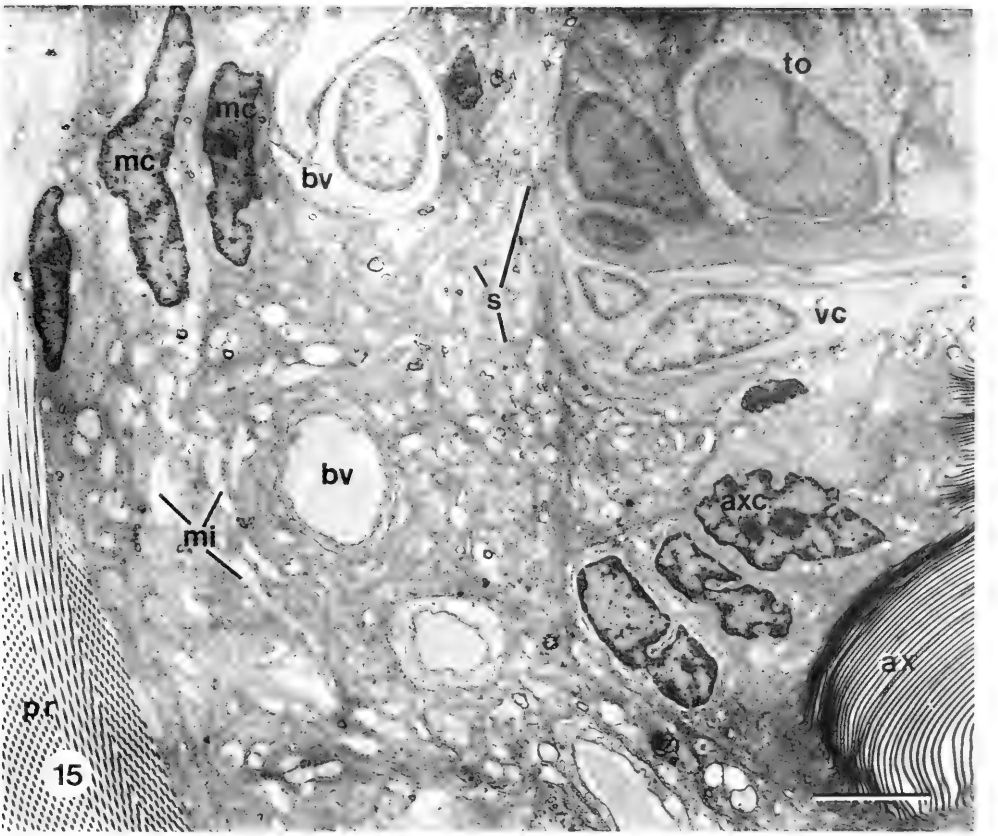
B. *Proximal Reflector.* The proximal reflector is cup-shaped with surfaces that appear to be portions of a sphere (Fig. 2). The reflector is an extracellular structure that consists of concentric arrays of collagenous rods arranged into primary and secondary layers (Figs. 16, 17). There are about 12 primary layers, each composed of 15–20 secondary layers. Each secondary layer consists of one layer of parallel rods. The parallel alignment of rods also exists between all secondary layers within a primary layer but usually they are not parallel to those of adjacent primary layers. The difference in axial orientation of the rods between primary layers varies randomly from near 0° to approximately 90°. A section at right angles to the rods reveals that each rod, excepting those of the marginal layers, is surrounded by six equally spaced neighbors (Fig. 16). The rods lie in an extracellular medium and often appear to have a thin coating and a network of interconnecting filaments. The rods exhibit 53 nm periodicity in cross-banding which lies within the range of invertebrate collagen. The rods are circular in cross section and uniform in diameter (117 nm; SD = 6) in the central portion of the reflector, but at the periphery they are slightly larger in diameter (130 nm, SD = 7). The distance between rods is generally affected by the fixation and embedding process. The rods, in sections showing the greatest spacing, are separated by about 120 nm.

Cells that secrete the collagen rods are located primarily adjacent to the periphery of the reflector (Fig. 17) although a few secretory cells can be found along the outer (convex) surface of the mirror. The secretory cells are characterized by an extensive granular endoplasmic reticulum which frequently contains large cisternae filled with moderately electron dense material.

C. *Axial Complex.* This complex occupies the center of the photophore between the photogenic plexus and the distal cap (Fig. 2). The complex is formed of four distinct structures: the axial stack and the cells that form it, a zone of vesicles situated atop the stack, a core above the vesicles and a surrounding toroidal structure (Fig. 5).

Axial stack. The axial stack consists of two adjacent multilayered structures, a proximal concave stack and a distal flat stack (Figs. 2, 4). Each stack contains approximately 50–60 electron dense layers (platelets) alternating

FIG. 15. Distal region of the photogenic plexus. The mitochondrial cells dominate this region, although blood vessels are also common. The mitochondrial cells tangle between the blood vessels, crystalloid cells and nerves. The proximal reflector is on the left and the axial complex on the right. Scale bar is 5 μm. FIG. 16. High magnification of the proximal reflector showing the banding pattern and spacing of the collagen rods. The consistent orientation of all rods within a primary layer is evident. Scale bar is 2 μm.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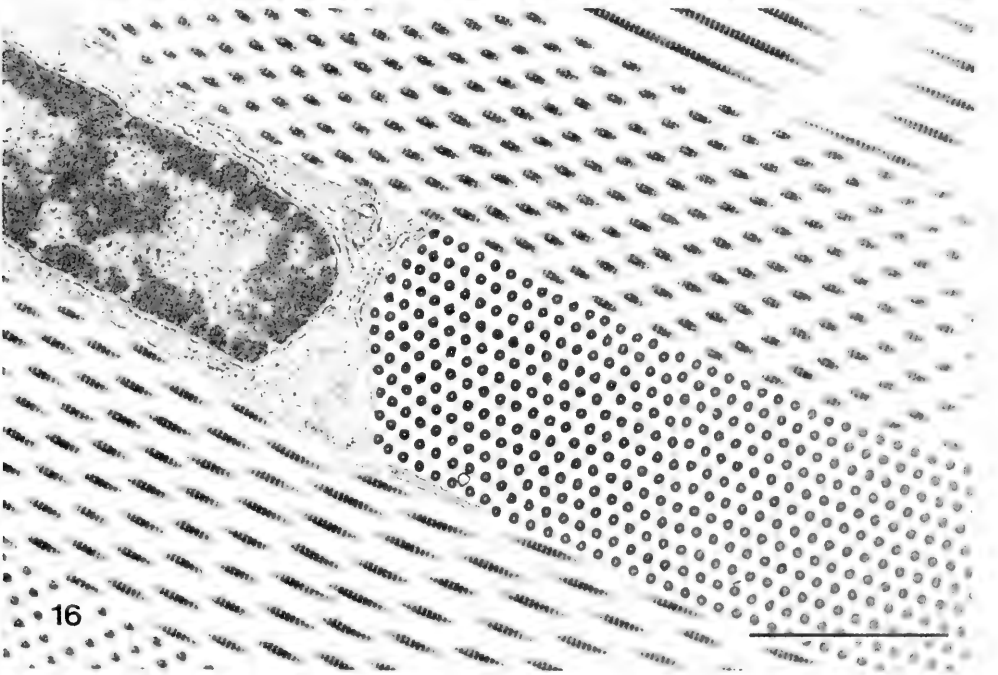
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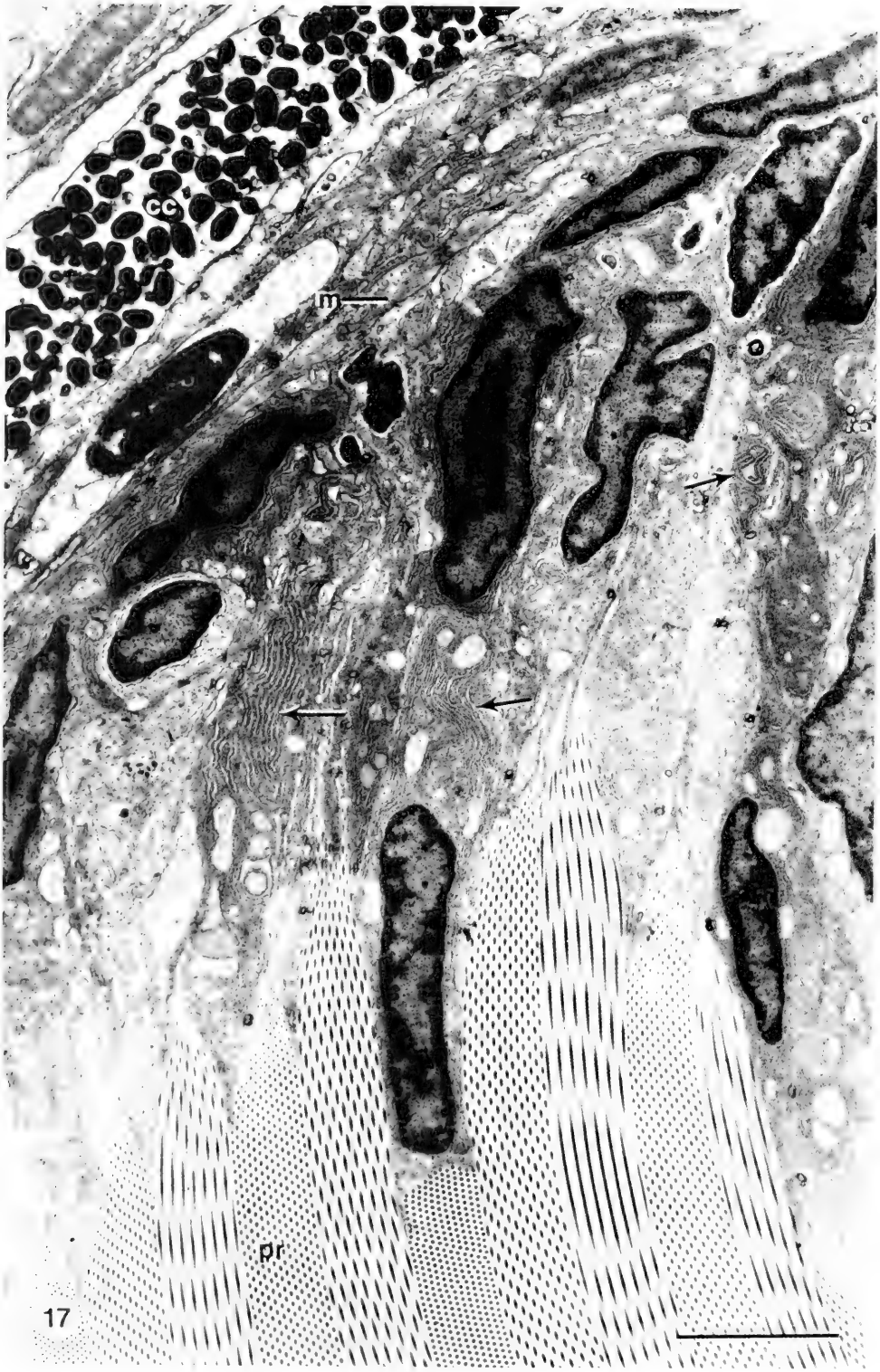

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with electron transparent layers (spaces). The exact shape of the proximal stack is difficult to determine with certainty. However, the concentric layers apparently are portions of a sphere whose center lies at the center of the photogenic cone. Each electron dense layer consists of eight closely applied plasma membranes while each electron transparent layer is extracellular space (Figs. 18, 19). The axial stack is formed by approximately four stacked rings of surrounding cells (the axial cells) with a maximum of about 10–12 cells in each ring. Each platelet is formed by lamellar interdigitations of the plasmalemma of at least several cells (Fig. 20). A single cell contributes lamellae to many platelets. Each platelet is circular but is divided into segments. The membranes of each segment are separate from those of adjacent segments and, therefore, do not extend the full width of the platelet (Fig. 19). Small knobs occur on the adjacent edges of the membranes from different cells and apparently contribute to some type of specialized junction although the details have not been resolved. The average thickneess of the electron dense layers is 79 nm (SD = 5, range = 72–91 nm). The thickness of the electron transparent layers is affected by fixation and embedding and measurements are not meaningful.

Vesicles. The vesicles, which occupy a zone between the axial stack and the core (Figs. 2, 4, 23) appear to be completely separate from any cells. They are large, closely

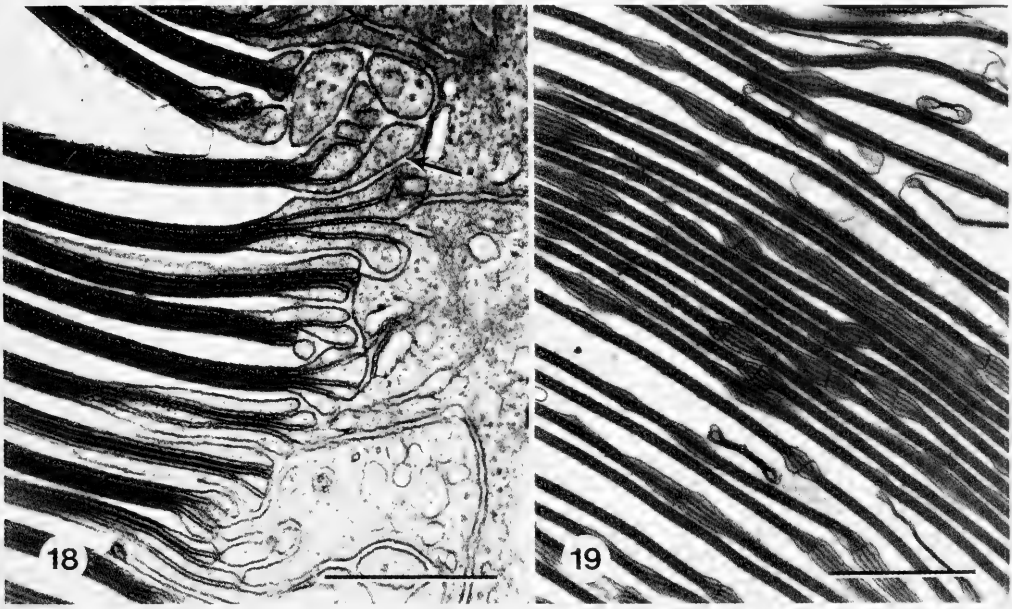


FIG. 18. Edge of the distal region of the axial stack showing the typical membrane looping and the apparent continuity between extracellular spaces separating platelets (arrow). Each platelet can be seen to be formed of eight plasma membranes. Scale bar is 0.5 μm. FIG. 19. Platelets in the axial stack of a developing photophore. The platelets are made of segments that abut against one another. The enlarged joints with easily visible membranes shrink in the mature photophore to approximately the same thickness as the platelets. Scale bar is 1 μm.

FIG. 17. Section through the periphery of the proximal reflector showing the cells that secrete the collagenous rods. These cells are rich in rough endoplasmic reticulum (arrows). The collagen appears to be lain down parallel to the surface of the cells. Note the different orientation of collagen rods in different primary layers of the reflector. The muscle above the collagen-forming cells is one of the radial muscles that attaches to the torus. In the upper corner is one of the chromatophores that surrounds the posterior cup. Scale bar is 5 μm.

FIG. 20. Schematic drawing of how two cells might interdigitate to form segments of platelets, and how they produce the characteristic membrane looping at the edges of the platelets.

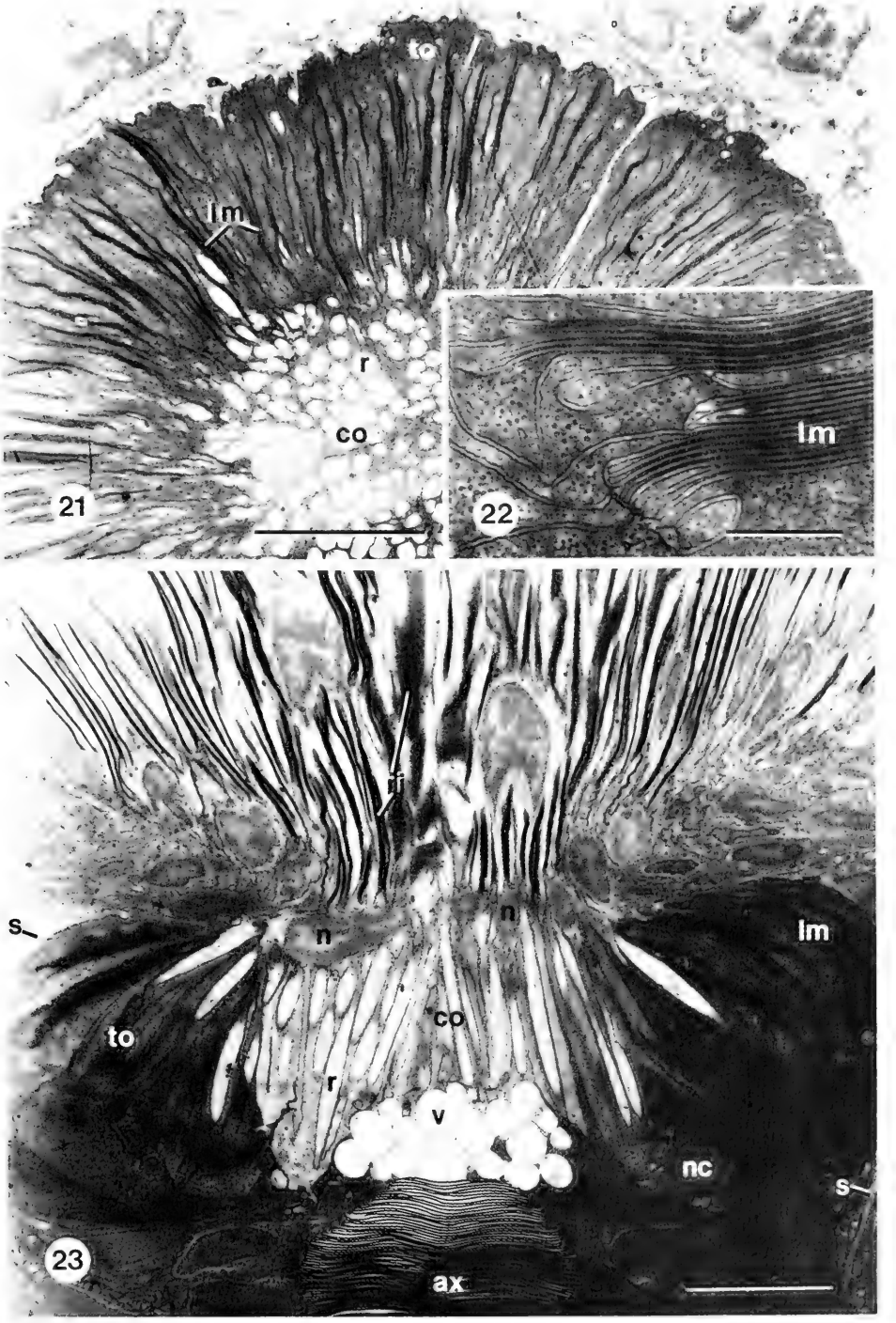
packed, and sub-spherical in shape. The lumina of the vesicles are homogeneous and electron transparent. Lateral to the vesicles and situated between the axial cells and torus is a wedge-shaped ring of cells (Fig. 13). These cells extend to the vesicles but we have not found continuity between the two. However, since these cells may be responsible for the formation of the vesicles, we tentatively name them the vesicle cells. A discontinuous sheath of collagen fibers covers the lateral surface of the torus and extends proximally over the lateral boundaries of the vesicle cells (Figs. 13, 15, 23).

Core. The core is a junctional area between the distal cap and the proximal cup (Fig. 23). Three cell types are present. A circlet of cells, the core cells, occupy much of the core and have their nuclei arranged in a ring at the base of the core where the latter surrounds the vesicles (Fig. 23). The lateral margins of the core cells intermesh in an irregular fashion with the cells of the torus. The second cell type, the junctional cells, have nuclei lying in a narrow, circular region (approximately one-fourth the diameter of the cap in the region of the girdle) that marks the separation between the core and the cap. Numerous cylindrical extensions from these cells (the roots) penetrate the core cells (Fig. 23). Similar "roots" extend from cells in the distal cap between the junctional cells and into the core. The relationship of the junctional cells to the cap cells is uncertain. We have been unable to confirm the presence of extensions of the former into the cap. In the distal portion of the core, the roots occupy over 60% of the cross-sectional area (Figs. 21, 24). Many of the roots penetrate past the nuclei of the core cells to the lateral base of the core; others penetrate to and abut against the vesicles, and a few roots extend at oblique angles into the torus (Fig. 23). The roots carry a uniform array of rather evenly spaced microtubules although the density of microtubule packing sometimes differs in different roots (Figs. 24, 25). Numerous fibrils extend between the microtubules. The roots have little else in internal structure and they appear quite electron transparent. The core cells also contain numerous microtubules and generally appear more electron dense than the roots (Figs. 24, 25).

Torus. A torus containing membranous lamellae surrounds the core and the vesicles of the axial complex (Figs. 3, 21, 23). These lamellae are radially arranged around the optical axis of the photophore (Figs. 3, 21). The plane of each lamella intersects a hypothetical central axis. The lamellae are similar in orientation to the radially arranged septa in a sea anemone. The lamellae are composed of electron dense layers of plasma membranes apparently formed by interdigitating membranes of adjacent cells as was found in the axial stack (Fig. 22). Large intracellular spaces, however, are lacking. The lamellae may be composed of just two membranes or as many as 40 to 50, and the spacing between lamellae is highly variable. The lamellae are not completely flat or precisely oriented. The edges of the lamellae are irregular since the component membranes have different lengths. Nuclei of the cells that form the lamellae lie proximal and lateral to the lamellae.

Muscle cells, presumably emanating from the large chromatophore at the base of the proximal cup, course radially over the surface of the cup to insert on the distal edge of the

FIG. 21. Cross-section through the torus and core showing irregular alignment of the lamellae. Scale bar is 10 μm. FIG. 22. High magnification of the medial end of a lamella from the torus showing its multimembranous structure and the characteristic membrane looping. Scale bar is 0.5 μm. FIG. 23. Longitudinal section through the torus and core showing their relationship to the zone of vesicles and the distal cap. Nuclei of the junctional cells (n) are seen at the top of the core and one nucleus of a core cell (nc) is seen at the right of the vesicles. Scale bar is 10 μm.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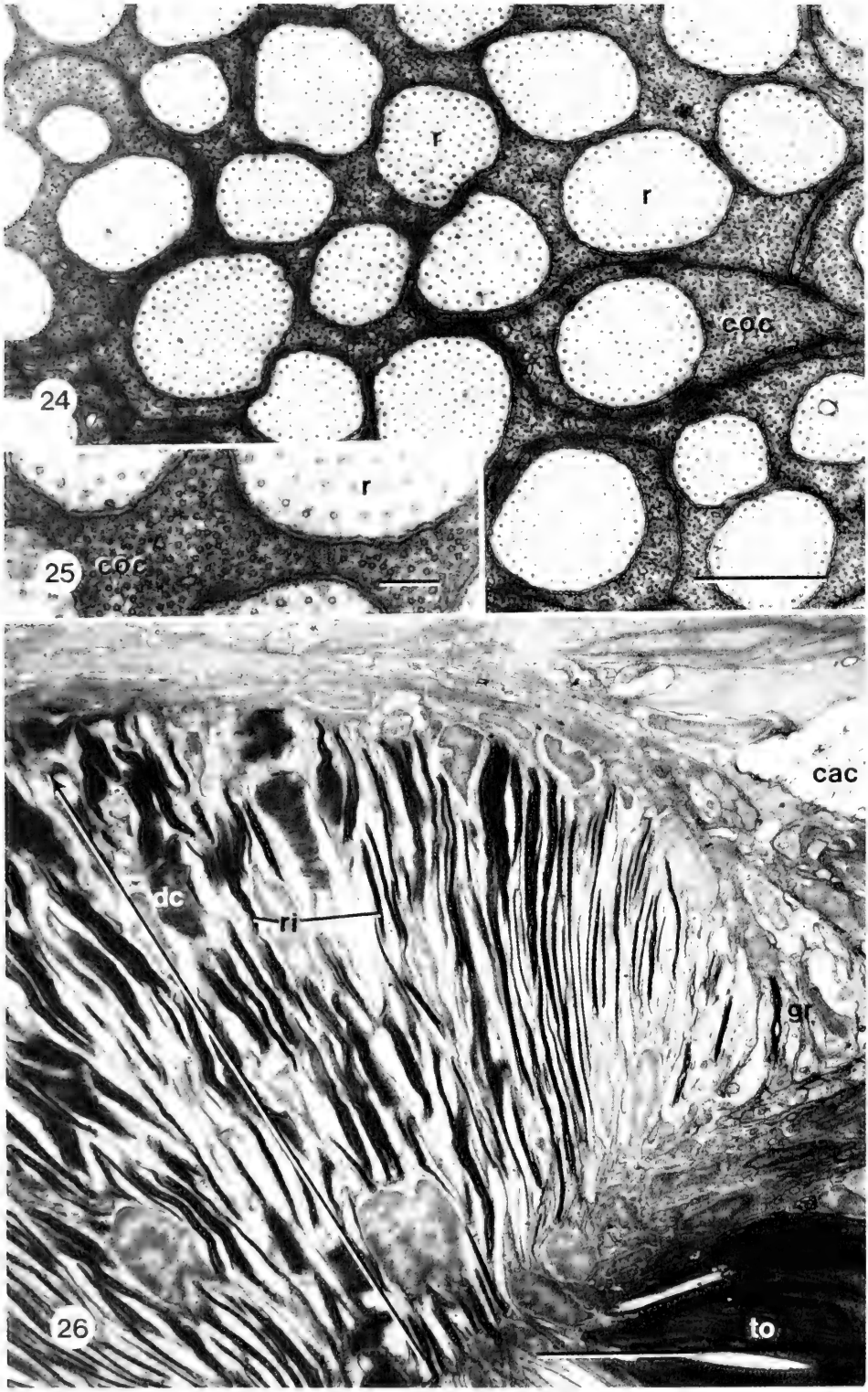
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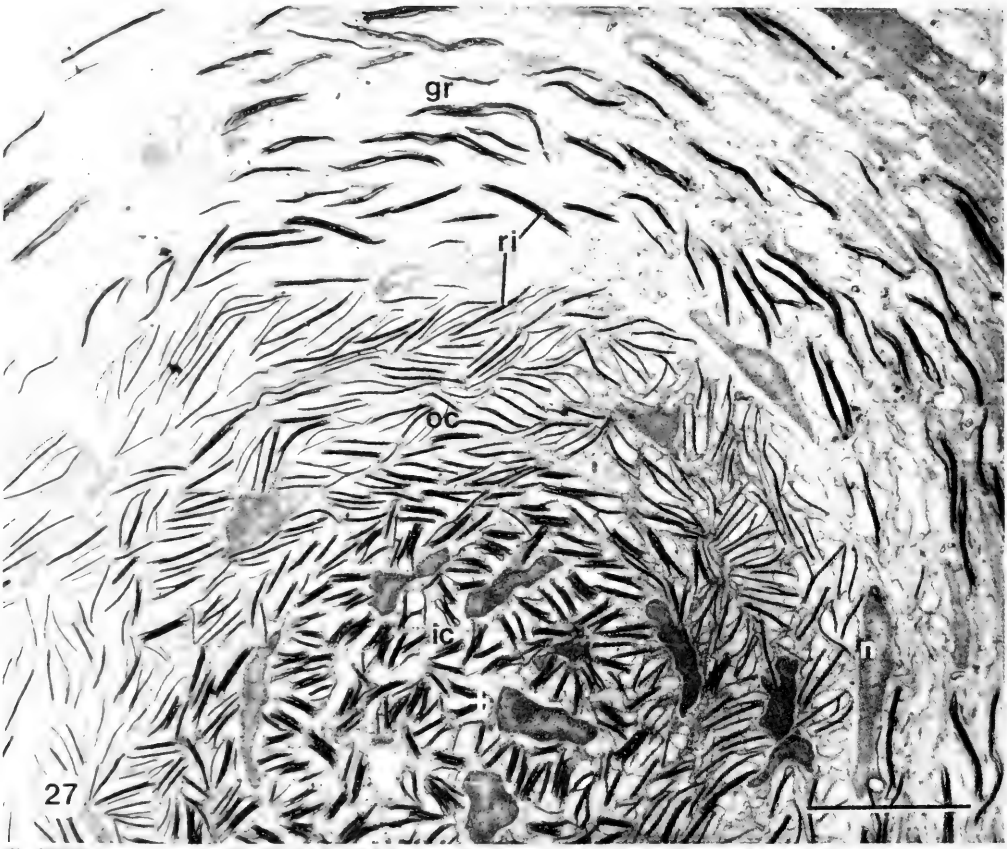
torus (Figs. 5, 17). Unlike most cephalopod muscle cells which have a solid core of mitochondria (Fig. 30), these contain scattered mitochondria exhibiting no consistent positional relationship to the myofilaments.

2) DISTAL CAP. The distal cap is a bell-shaped structure that can be divided into a central oval region and a surrounding low bulbous girdle (Figs. 2, 26, 27). The size and shape of the cap varies somewhat between photophores, but the basic structure is constant. Nuclei are scattered throughout the cap and no lining membrane is present (Fig. 26). The most distinctive feature of the cap is the numerous long ribbons that appear to extend from its distal to its proximal margins (Fig. 26). The ribbons are formed of layers of membranes. The characteristic membrane looping at the edges of the ribbons indicates that they are formed, as in the case of the membrane platelets of the axial stack and the lamellae of the torus, by the interdigitating of plasmalemma from adjacent cells (Figs. 28, 29). Intracellular spaces between groups of membrane layers are virtually absent. In the central portion of the cap, the ribbons are narrow and thick, involving in some cases more than 100 membranes. In cross-section the ribbons in the central-most region of the cap have a somewhat random orientation and occupy about one-third of the area of the region (Figs. 23, 28). Peripheral to this region, the ribbons are broader and thinner and their orientation is more regular (Figs. 27, 29). In these ribbons there is a dominant trend toward an orientation with the broad, flat surface parallel to the edge of the cap, and in cross-section they occupy about one-fifth of the total area of the region. In the girdle the ribbons are fewer and thicker but very broad, and more regular still in paralleling the circumference of the cap (Fig. 27). They occupy 10–15% of the cross-sectional area of this region. The ribbons of the cap tilt toward the optical axis of the photophore with the angle of tilt increasing from the center to the periphery (Figs. 2, 26). The maximum angle of tilt is about 25–30° to the optical axis. Although the tilt is not precise, the projected proximal ends of the ribbons in the central region would seem to converge at a point in the center of the axial stack. Nuclei are scattered throughout the cap. In the central region, the cytoplasm of the cap cells is packed with regularly arranged microtubules that run parallel to the long axis of the ribbons (Figs. 28, 29). In the girdle the microtubules are less regularly arranged and, while some have the same orientation as those of the central region, most lie at right angles to this direction and parallel to the circumference of the cap.

Many muscles that originate on the body musculature surrounding the photophore course toward the distal cap and apparently spread over and attach to or near the surface of the cap (Fig. 31). However, we have not located specialized cell junctions that would define the exact areas of insertion.

3) DISTAL REFLECTOR. Almost completely surrounding the sides of the distal cap is an extensive lamellar structure similar in overall appearance to a series of typical cephalopod iridophores but differing in details of structure (Figs. 30, 31). Each iridophore bears numerous platelets separated by extra-cellular spaces. The plasmalemma of the iridophore forms both surfaces of a platelet which extends outward from the nuclear region. The extracellular spaces lack structure and are electron transparent. The cytoplasm within a platelet is granular in appearance and is somewhat more electron dense near the center (Fig. 31). The thickness of the platelets varies by as much as 20–25% within a single platelet. The average thickness of the platelets is 136 nm (SD = 11). Each platelet is nearly formed into two layers by a single collapsed cisterna that extends almost the full length and width of the platelet (Fig. 31). Occasionally the cisterna is aligned with flattened vesicles that lie at a uniform distance from the plasmalemma in the region of the cell nucleus suggesting a stage in the formation of the cisterna. The platelets are imprecisely ar-

FIG. 24. Cross-section through the core showing roots of the junctional cells and the axial-cap cells embedded within core cells. Numerous microtubules are present in all cell types. Scale bar is 1 μm. FIG. 25. High magification of core cross-section. The relationship of the cell membranes is evident. The numerous microtubules presumably have a structural function. Scale bar is 0.2 μm. FIG. 26. Longitudinal section through the distal cap. The arrow indicates the optical axis of the photophore. The ribbons exhibit considerable irregularity in alignment, and their tilt with respect to the optical axis increases laterally. Scale bar is 10 μm.



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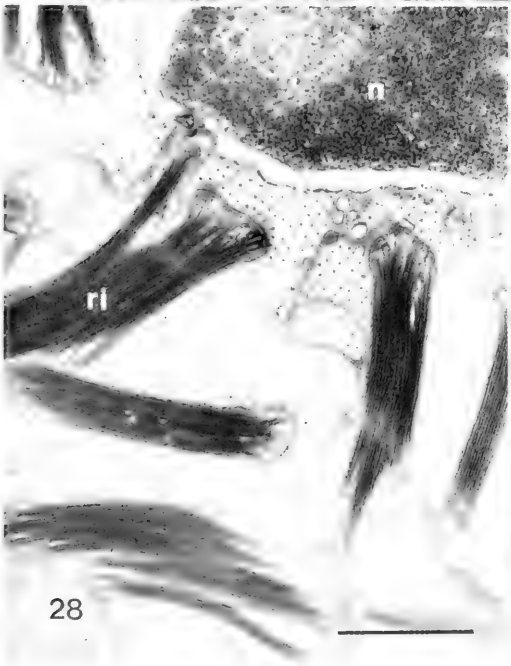

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ranged into concentric rings around the dome of the cap. The flat surfaces of the platelets face the optical axis of the photophore and are generally tilted so that the proximal end of a platelet is somewhat closer to the optical axis than is the distal part (Fig. 5). The angle of tilt, however, is not consistent. Within a single longitudinal section through the reflector, groups of platelets may vary in their angle of tilt from other groups by as much as 90°.

4) CHROMATOPHORES. Large chromatophores cover the proximal cup (Figs. 2, 3, 17). Generally, one large chromatophore covers the apex of the proximal reflector and four overlapping chromatophores cover the sides. A series of chromatophores surrounds the sides of the distal cap. These chromatophores, like those of the proximal cup, have a structure typical of cephalopod chromatophores and are accompanied by large sheath cells (Fig. 30). A separate group of 4 or 5 small chromatophores, the apical chromatophores, surround the distal tip of the cap (Fig. 5). The pigment granules of these chromatophores are about ½ to ⅔ the size of the granules in other chromatophores. Numerous muscles associated with the chromatophores encircle the photophore and some pass beneath the girdle of the distal cap.

5) ORBITAL SPACE. Surrounding the proximal cup is a series of large apparently fluid-filled lacunae (Figs. 2, 3). These lacunae are all bound by thin membranes and lack association with muscle fibers. This space provides a thick cushion between the photophore and the body musculature.

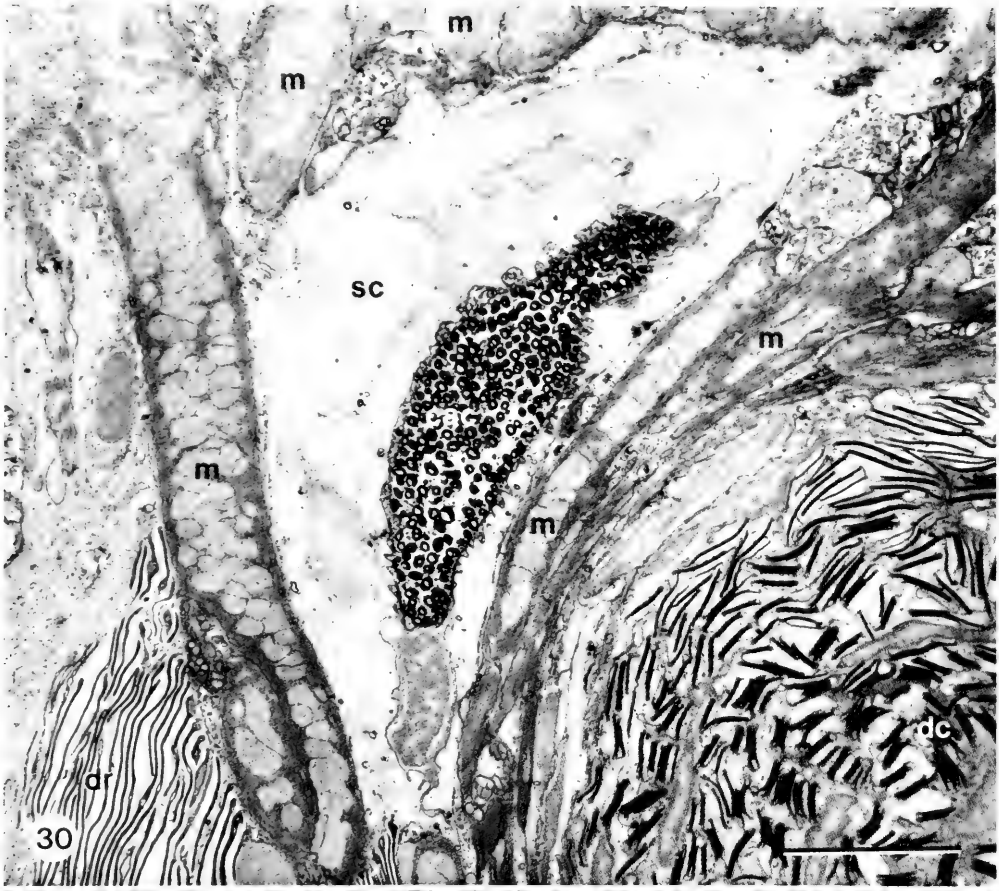
Observations on Fresh Photophores

Observations have been made, with the aid of a dissecting microscope and lamp (artificial light), on the photophores of recently dead or dying *A. trigonura*. When a photophore is mechanically stimulated its muscles will frequently contract. Repeated stimulation demonstrates that the photophore is capable of rocking in any direction. The maximum tilt of the optical axis was difficult to assess but appeared to be at least 45°. The photophore also could be rotated slightly on its optical axis. All chromatophores exhibited considerable activity. At maximum expansion the cup chromatophores conceal the posterior cup except for a distal opening (i.e. the cap-core junction). This aperture is less than a third the diameter of the proximal portion of the photophore. The apical chromatophores were yellow-brown in color which contrasted with the brown color of the other chromatophores. The former could converge (i.e. shift position) over the center of the distal cap and expand. If all chromatophores expanded at once the photophore would be completely concealed.

Transmitted artificial light when viewed from the side of the rather firm but colorless distal cap, exhibits little distortion. Considerable scattering of the light, however, is apparent from a top view (i.e. down the optical axis of the photophore). The axial stack is easily seen through the microscope when the distal cap is dissected away. Artificial light incident on the distal end of the stack is generally reflected as a small brilliant yellow disc. This color could result from the reflection of all but the blue component of the incident light. This reflected light swamps any transmitted light that may have bounced off the posterior reflector. With the distal cap in place the reflection off the stack is diffused as it passes through the cap. The degree of diffusion (scattering) varies between photophores and occasionally little if any diffusion of the reflected light is seen.

The proximal reflector is difficult to observe directly. Because of the spherical geometry of the reflector, most artificial light directed into the photophore is not reflected directly back. In addition the optical inhomogeneities of the distal cap (and presumably the torus) make clear viewing of the reflector difficult. However, judging by the color, two sources of light

FIG. 27. Cross-section of the distal cap showing the inner and outer areas of the central region and the surrounding girdle. The cross indicates the optical axis of the photophore. The differences in alignment, thickness and width of the multimembranous ribbons in the three region are apparent. Scale bar is 10 μm. FIG. 28. Cross-section through inner area of the central region of the distal cap. Note thickness of multimembranous ribbons, and the membrane looping at their edges. The numerous microtubules presumably have a structural function. Scale bar is 1 μm. FIG. 29. Cross-section through outer area of the central region of the distal cap. The multimembranous ribbons are thinner and longer than those in Fig. 28; however, the pattern of membrane looping is similar. Scale bar is 1 μm.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emerging from the photophore can be attributed to reflection of artificial light off the posterior reflector: (1) a diffuse reflection generally is observed from the photophore peripheral to the reflection from the axial stack; (2) a series of brilliant tiny points of reflected artificial light arises lateral to the diffuse reflection. Light from these two regions gives the photophore its predominant color. With the distal cap dissected away bright points of light can be observed to be directly reflected off the posterior reflector. The color of this light is the same as that from the two previously mentioned sources. This color is either blue or green, depending on the particular photophore. Occasionally when the cup chromatophores are strongly retracted and the photophore is turned over, the outer (convex) surface of the posterior reflector is visible. As would be expected, artificial light reflected off this convex surface is the same color as that reflected off the concave surface.

Generally, most Type C photophores under artificial light appeared predominately green in freshly captured animals although sometimes a mixture of blue and green photophores was seen. In a few squid most Type C photophores were blue. On one occasion the color of the reflected artificial light was observed to change with time in a dead squid. After two days in 10°C seawater photophores that were initially green had become a dark blue, but, as the water warmed under microscope lights, the reflection became green again. The water was cooled to 0°C but the Type C photophores remained green. However, as the water warmed slightly the photophores first turned blue and then returned to green as the water warmed further. Not all the Type C photophores exhibited these dramatic color changes, and a repeat of the temperature changes had a negligible effect on the color. The predominant color of blue or green was due to light reflected off the proximal reflector. Changes in the reflection off the axial stack shifted from pale yellow (blue photophores) to yellow-orange (green photophores) or, in photophores that did not make the full change, from the blue type to the green type, to lavender. In spite of the length of time the animal had been dead, the skin had not turned opaque and chromatophore and photophore muscles were still responsive to mechanical stimulation.

The distal reflectors were almost impossible to see under the microscope when viewing directly into the photophore although occasionally some golden iridescence was observed. However, a small fibre-optic light guide (~60 μm diam.) with one end placed atop the distal cap directed light into the organ and produced a bright blue-green oblique reflection off the distal reflector. When the photophore was tilted and light struck the reflector at near normal angles (roughly 60–70°), a red-orange reflection was observed. The endowment of distal iridophores varied between individual photophores. In addition, the arrangement of these iridophores varied. In some photophores they closely surrounded the photophore and were covered laterally by large chromatophores. In other photophores, often adjacent to the first type, the iridophores were spread over a broad region half again the diameter of the proximal cup. Mechanical irritation failed to induce one type to transform into the other.

Optical effects of the torus could not be detected.

Observations on Bioluminescence

1. *Intensity regulation.* Young & Roper (1977) demonstrated that this squid could effectively counterilluminate over at least 20-fold change in light level. We have measured the emission spectrum of the luminescence as it increased in intensity by 325-fold in response to changes in the overhead light. Young et al. (1980) found that a closely related species, *Abraliopsis* sp. B, could adjust its bioluminescence during counterillumination over a range of at least 15,000-fold. We would expect to find the same capability in *A. trigonura* if tested in a similar manner.

2. *Color regulation.* Young & Mencher (1980) demonstrated that the color of light produced during counterillumination under simulated day conditions (i.e. cold water environment of 6–8°C) was a narrow unimodal

FIG. 30. Cross-section of the distal region of the photophore showing the central region of the distal cap, a chromatophore with sheath cells, numerous large muscles which presumably orient the photophore, and the distal reflector. Scale bar is 10 μm. FIG. 31. Platelets of the distal reflector. Collapsed cisternae form the distinctive double membrane in the midline of the platelet. The matrix of the platelet is heterogeneous. The platelets have a uniform thickness but imprecise alignment. Scale bar is 1 μm.

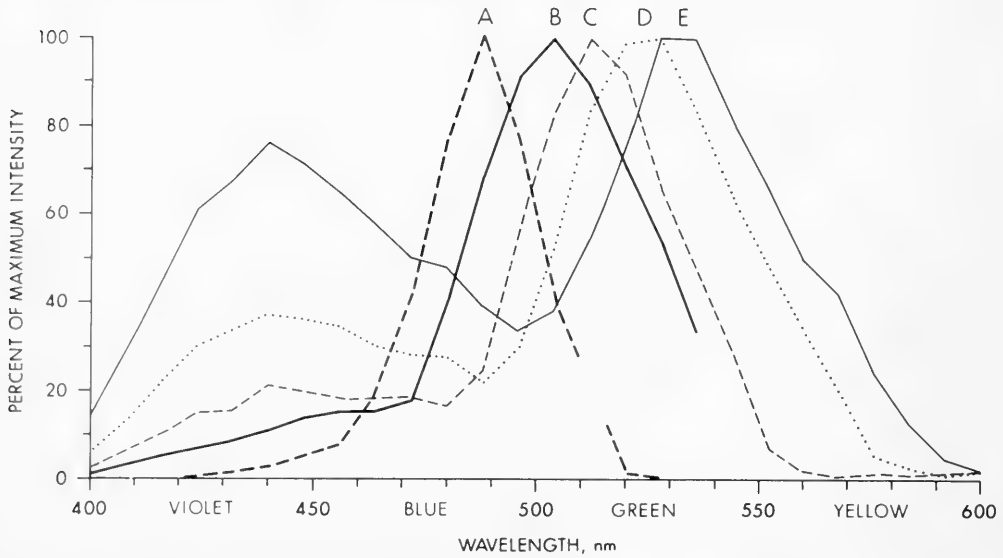

FIG. 32. Luminescent emission spectra taken during counterillumination from the ventral surface of the head of *A. trigonura*. The overhead light which stimulated counterillumination was held at a constant intensity throughout the experiment. The system for detecting bioluminscence covers a large region of the ventral surface of the head and, therefore, it can receive light from all three types of photophores. The temperature of the water surrounding the squid was adjusted to a different temperature for each measurement. Curve A: water temp. 6–8°C; Curve B: water temp. 11–12°C; Curve C: water temp. 14–15°C; Curve D: water temp. 19–20°C; Curve E: water temp. 23°C. Curve A presumably represents the luminescent spectrum the squid would produce in its day habitat and Curve E the spectrum in its night habitat. Each curve represents six measurements. The two peaks of the night curve form in different ways. The shift of the peak at A to the right indicates that one set of photophores is changing the color of light it emits. The peak on the left gradually increases indicating a new set of photophores is on and increasing in intensity.

band with peak emission at about 480 nm (full width at half maximum (FWHM) of 33 nm). Under simulated night condition (warm water environment of 23–25°C) a bimodal peak appeared with peaks at 440 nm (FWHM-55 nm) and 536 nm (FWHM-46 nm). Under night thermal-conditions direct visual observations on a brilliantly lit squid revealed a mix of photophores of different brilliance suggesting that more than one type of photophore was involved. However, under day thermal-conditions at the same intensity of overhead light all photophores had a uniform brilliance suggesting a single photophore type was involved. Examination of the emission spectra during a gradual change between day and night temperature conditions confirmed that two sets of photophores are involved in producing the night colors. During this changeover, one of the night color modes (peak at 440 nm) appeared and gradually increased in height while the other night color mode (peak at 536 nm) developed from a gradual shift of the day mode to longer wavelengths (Fig. 32). Apparently, the mode at 440 nm is produced by a set of photophores turning on for the first time, while the mode at 536 nm is produced by the same set of photophores that produces the day peak at 480 nm. That is, the latter photophores can change the color of light they produce. The large number of active photophores observed during counterillumination eliminates the relatively few Type A photophores as the source of the day color. Under the microscope unfixed Type B photophores reflected a blue-violet color indicating these were the source of the 440 nm peak. The Type C photophores remain as the source of the 480 nm and 536 nm peaks.

Additional color shifts have been measured under both day and night conditions at high light intensities (Young & Mencher, 1980). At the day temperatures the peak broadens slightly and at the night temperatures the 536 nm peak shifts back toward the blue region as light intensity increases and combines with the 440 nm peak to form a complex curve (Fig. 33)

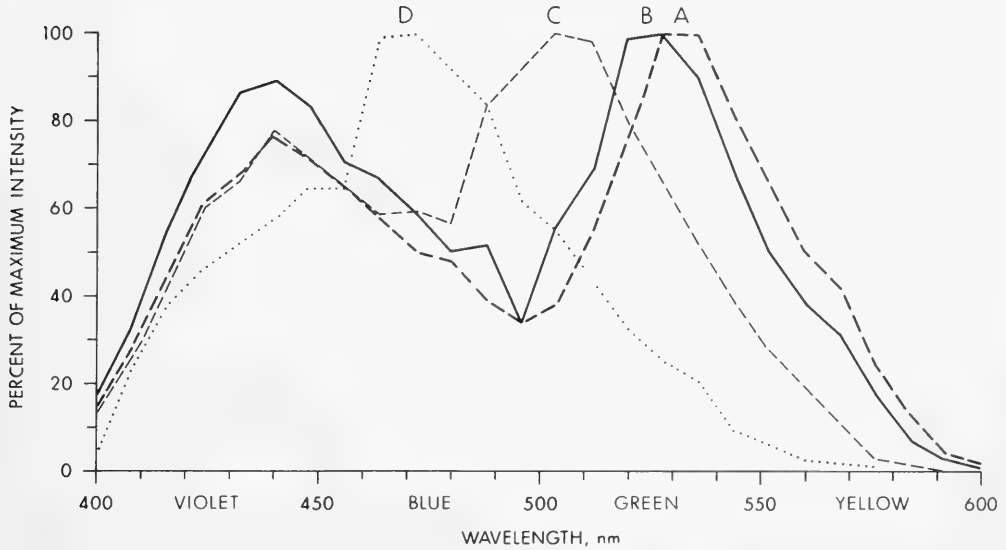

FIG. 33. Luminescent emission spectra taken during counterillumination from the ventral surface of the head of *A. trigonura*. Temperature was held constant at 23°C during the entire experiment. Beginning with curve A, which is curve E in Fig. 32, each subsequent curve was measured after increasing the overhead light. Each curve is the average of 1–6 measurements. Curve A: overhead light intensity approximately 2.5×10^{-3} $\mu W/cm^2$; Curve B: overhead light intensity increased by 6 times; Curve C: overhead light intensity further increased 3.5 times; Curve D: overhead light intensity further increased 4.4 times. Note that the peak of A shifts to shorter wavelengths as the light intensity increases.

3. *Angular distribution.* We have no precise data on the angular distribution of the light except for visual observations that indicate it is highly directional (directed ventrally) when the squid is in cold water and somewhat less so in warm water in our experimental apparatus.

DISCUSSION

The site of light production is, almost certainly, the crystalloids of the photogenic cone. Thus the crystalloid cells appear to be the photocytes. The cone's strategic optical location, its crystalloid nature and its close association with blood vessels strongly support this assumption: 1) The cone sits at the focus of the spherical proximal reflector, and is centrally located with respect to the axial stack and the optical axis of the photophore. 2) The presence of crystalloids has been reported in the presumed photogenic cells of three other cephalopods (*Bathothauma lyromma,* Dilly & Herring, 1974; *Watasenia scintillans,* Okada, 1966; *Enoploteuthis* sp., Young, 1977). 3) Intense vascularization is characteristic of all known photogenic tissue in cephalopods (e.g., Girsch et al., 1976; Arnold et al., 1974; Dilly & Herring, 1974; Okada, 1966).

We do not know the chemical composition of the crystalloids. Similar crystalloids in the photophores of *Watasenia scintillans* are thought to be proteinaceous (Okada et al., 1934). We suspect the crystalloids of being composed predominantly of luciferase while luciferin, in some form, is constantly supplied to the crystalloids via the circulatory system. Young et al. (1979) found evidence for the presence of luciferin in the blood of the squid *Symplectoteuthis oualaniensis.* The intimate association between photocytes and blood vessels suggests that a component of luminescent reaction, possibly luciferin, is supplied by the circulatory system. Browning (1979) suggests that in *Octopus* transport of molecules from the blood of less than 120Å in diameter occurs through pericyte junctions. Luciferin extracted from the squid *Watasenia* is a small molecule with a molecular weight of 572 (Inoue et al., 1976). In *Abralia* the finger-like extensions of the crystalloid cells that surround the blood vessels could provide a large surface area for acquiring such molecules.

We view the presence of dense arrays of microtubules within the dendritic photocytes as evidence for the intracellular transport of materials between the blood vessels and the crystalloids. While microtubules are generally considered to be cytoskeletal elements, they have also been implicated in the transport of cell structures.

The presence of immature crystalloid cells near the proximal end of the photogenic cone indicates that crystalloids are continuously added to the stack as the photophore grows. The extracellular spaces and channels and/or the nearby tubular ER may function as a reservoir for some component of the luminescent reaction.

The scarcity of mitochondria in the crystalloid cells seems inconsistent with their presumed high metabolic activity. Presumably ATP is transported to the photocytes from the intertwining mitochondrial cells. Arnold et al. (1964) suggest a similar mechanism in the ocular photophores of the squid *Pterygioteuthis microlampas*.

Two different types of iridophores are present in the Type C photophores: those of the axial stack and those of the distal reflector. Both are distinctly different in their structure from any previously described in cephalopods (i.e., Kawaguti & Ohgishi, 1962; Arnold, 1967; Mirow, 1972; Arnold et al., 1974; Young, 1977; Brocco & Cloney, 1980). A third interference structure, the proximal reflector, is present; however, it cannot be properly termed an iridophore since the reflecting apparatus is entirely extracellular.

To predict the reflectance and transmittance characteristics of iridophores, the refractive indices and thicknesses of the high (platelets) and low (spaces) refractive index layers must be known (Land, 1972). The refractive index of the spaces can be assumed to be that of tissue fluid, 1.33, as measured by Brocco & Cloney (1980) in *Octopus*. The refractive indices of the platelets in iridophores of *Abralia* are unknown. Brocco (see Brocco & Cloney, 1980), however, measured the refractive index of the proteinaceous platelets of *Octopus* iridophores as 1.42. Denton & Land (1971) found a value of 1.56 for platelets in squid and cuttlefish; however, Brocco & Cloney (1980) suggest the latter value may be high due to the method of measurement. The structure and composition of the platelets in *Abralia* differ from those measured. However, for lack of more accurate data, we assume a refractive index of 1.49 (i.e. midway between measured values) for the high refractive index structures in the iridophores.

The axial stack could function either as a filter or a mirror. Direct observations of the reflection of artificial light off the distal end of the axial stack in fresh photophores suggest transmission of blue to green light (i.e. yellow or yellow-orange reflection). If the stack were to function in reflecting all bioluminescent light, it would reflect blue-green light or, perhaps, a much broader band (i.e. silvery reflection). The axial stack therefore, selectively transmits light and functions as a filter. In *Abralia* the narrow transmission band measured under day conditions indicates that the filter reflects light to either side of the band pass back toward the proximal reflector and in this manner controls the transmission bandwidth.

The refractive index of the plates affects not only the wavelength of maximum reflection but also the reflection bandwidth (Land, 1972). For a refractive index of 1.49 and a large number of platelets, the bandwidth would be about 40 nm and at a lower refractive index the width would be even narrower. (Smoothed bandwidths can be computed following the procedure in Huxley, 1968: 241, except that for equation 28 the following is substituted: $|R|^2 = 1 - \sqrt{1 - 1/k^2}$. This equation is the counterpart of Huxley's equation 40 but applies to non-ideal stacks.) If the axial stack were formed of two uniform functional units, two reflection bandwidths of 40 nm each would be inadequate, judging from the measured emission spectra. Apparently, the slight variation in the thickness of the plates (and perhaps the spaces) functions to broaden the reflection bandwidth. The exact reflectance of the platelets is impossible to calculate since the widths of the spaces between plates are highly variable, apparently due to fixation effects. However, for an ideal $\frac{1}{4}\ \lambda$ stack the plate thickness predicts a reflectance maximum at 471 nm [i.e. $\lambda_{max} = 2(n_a d_a + n_b d_b)$, where $n_a n_b$ are the refractive indices of the light and dense layers in the stack respectively and $d_a d_b$ are the thicknesses of these layers (Land, 1972)], and a range of 429 to 542 nm based on the measured range of thicknesses. Although we have reason to suspect that the stack cannot be ideal (see below), the calculations suggest that the stack is close to ideal.

The iridophores of the distal reflector have thicker platelets with somewhat irregular spacings. If we consider this an ideal stack,

maximum reflectance at normal angles of incidence to the broad surface of the platelet would be 811 nm. This value would shift to longer wavelengths for a non-ideal stack (although a second order peak at $\frac{1}{2}\ \lambda_{max}$ would occur in the visible region in the latter case [Land, 1972]). The iridophores, however, seem to function in reflecting oblique light. We roughly estimate that light from extreme spherical aberration off the posterior reflector or light exiting between the posterior reflector and the distal cap will arrive in the region of this reflector at angles of 30° to 70° to the optical axis of the photophore. If we consider the tilt of the platelets to be 7° to 17° to the optical axis (angles which would cause reflected light to depart the region at half its original angle to the optical axis) then the angles of incidence would be about 52° to 65° and reflectance maxima would vary between about 410 and 535 nm. For a non-ideal stack in which the spaces are wider than the platelets, these values would shift to longer wavelengths. These values agree well with direct observations.

The third interference system (the proximal reflector) is based on arrays of rods rather than layers of platelets. Unfortunately, a mathematical treatment of the properties of rod-based systems, apparently, has not been published (Land, 1972). However, Land (1972) suggests that the reflectance maximum for normally incident light can be approximated by "$\lambda = 2$ nd, nd, $\frac{2}{3}$ nd etc., where n is the mean refractive index of the structure and d is the separation of the centers of the rods in one plane to those in the next." The latter measurement in our material, unfortunately, is unreliable due to fixation effects. Using the maximum distances measured, and assuming an ideal system, we calculate a primary maximum at 680 nm and a secondary maximum at 340. At greater rod separation the secondary maximum would move into the visible region.

A spherical interference reflector requires a complex construction as light of a given wavelength will strike the reflector at normal angles along the optical axis of the photophore but will strike at increasingly oblique angles laterally. Perhaps the slight lateral gradient of increasing rod diameter partially compensates for the different angles of incidence. Other than this lateral gradient, the rods are quite uniform in diameter. This uniformity indicates that the mirror acts as a narrow-band reflector. Direct observations with reflected artificial light confirm the narrow-band properties. In contrast, the rod-based reflectors of the cat tapetum and of certain bird feathers that are broad-band reflectors contain rods of various sizes (Land, 1972). The silvery reflector of certain cephalic photophores of the related squid *Abraliopsis* contain rods that also vary greatly in size (personal observation).

In order to understand the effects of other optical structures in the photophore we determined the approximate optical pathways within the photophore. Because of the large size of the photogenic cone relative to the posterior reflector, light cannot be considered to arise from a single point. Instead, we considered an arbitrary array of 46 points evenly distributed throughout the cone to represent the light source. Also one cannot consider reflection off the posterior reflector as arising at its front surface. Reflectance off an interference structure is exponentially related to the number of platelets within the reflector (Land, 1972). We divided the reflector into 15 equal layers which were considered to function as individual platelets in an ideal stack. The contribution of each layer was then determined according to the procedure in Land (1972: 82). In using this approach, we assumed that the entire thickness of the reflector was required to produce 100% reflectance. If we ignore the optical effects of other accessory structures, representative optical pathways would appear as in Fig. 34, and the calculated angular distribution of the light would appear as in Fig. 35.

The angular distribution of light from the photophore differs slightly from that of daylight in the habitat of the squid (Fig. 35). If the radiance pattern from a single photophore is to match that of daylight or nightlight (this is uncertain since it is the combined output of all photophores that is important), then accessory structures must compensate for the differences. The torus, the distal cap, the distal reflector and the chromatophores appear to be such structures.

The presumed optically functional components of both the torus and distal cap are similar in structure to those of the axial stack. However, the thickness of their layers is highly variable and is generally too thick and irregular for interference effects. These structures, which presumably act as "thick-film" rather than "thin-film" reflectors, should be effective if the incident light is strongly oblique and if multiple reflections are allowed.

The tilting ribbons of the distal cap will tend

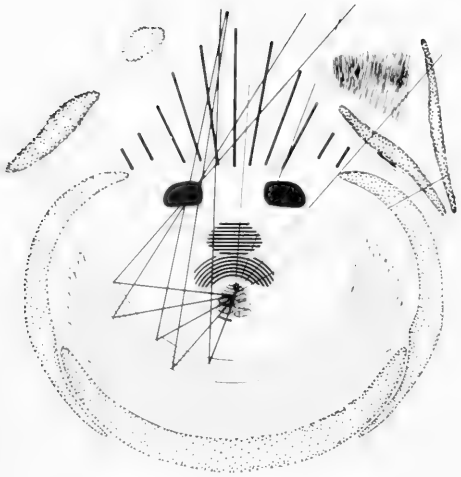

FIG. 34. Ray diagram showing representative light rays emerging from the center of the photogenic cone. Compare to Fig. 5. Heaviest straight lines represent ribbons of the distal cap. The effects of chromatophores, cap ribbons and distal iridophores on the ray paths are ignored. Note that most laterally reflected rays pass through the torus. Note angles of incidence of rays on cap ribbons and distal reflector, and the potential effect of chromatophore movement. The cup chromatophores are drawn in a partially expanded position.

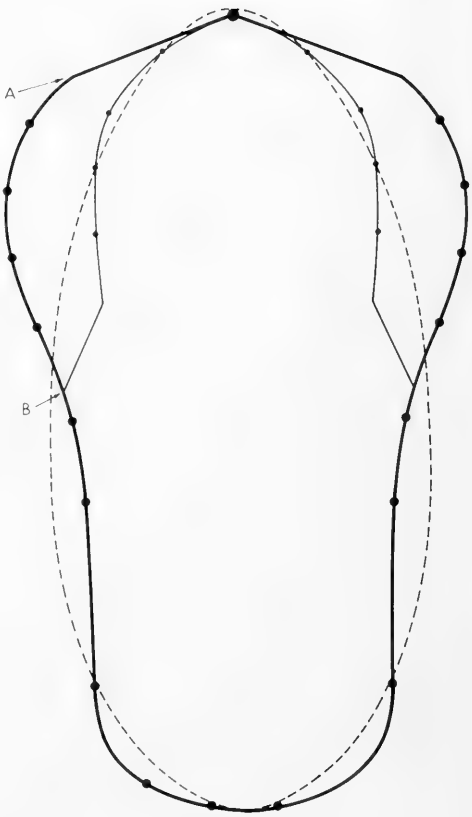

FIG. 35. Polar diagrams. Each diagram represents a vector diagram in which the tips of all the vectors are joined to form a curve. The length of each vector represents light intensity and the angle of the vector represents the direction of the light. The dashed curve represents the angular distribution of daylight in the sea based on Denton et al., 1972. The large dot represents the photophore. The heavy solid curve represents the angular distribution of light emitted from the photophore if the effects of the torus, distal cap, distal iridophores are ignored and if chromatophores are retracted. The thin curve represents the effect on the previous curve if the cup chromatophores are expanded. Point A represents the effect of the edge of the posterior reflector on the emission beam and point B represents the effect of the edge of the expanded cup chromatophores.

to deflect, away from the optical axis, rays emerging from the posterior cup at angles less than roughly 15° to the optical axis (according to Fresnel's equations for reflectance) (see Fig. 35). The result is to spread somewhat the directional beam arising from the photophore.

Nearly all aberrant rays reflected from the spherical posterior reflector pass through the torus where they may encounter the vertical membrane lamellae. The surfaces of the lamellae will scatter light at low angles of incidence but would appear to affect azimuth more than the declination of the resulting rays. This could in turn affect the angle of incidence on the more precisely aligned ribbons at the periphery of the cap. Unfortunately, the imprecise geometry of both the torus and the cap make it impossible to determine exactly what the effect of the torus is. At any rate we consider that the torus scatters light to increase, in some manner the proportion of rays striking the cap ribbons at angles sufficiently high to allow reflection. In addition, a considerable portion of the light must be intercepted by the ends of the lamellar stacks of the torus and cap. The fate of light guided within the stacks is uncertain due to their complicated terminations but the net effect must be to increase the scattering of the light. While we describe the functions of the torus and distal cap as those of a weak diffuser, the organization of these structures indicates that this is an oversimplification.

The distal reflector, due to its slope to the optical axis, will intercept and redirect outward some of the directly emitted light passing between the central portion of the cap and the proximal reflector when the posterior cup chromatophores are retracted (Fig. 35).

Thus far this discussion has considered a more or less static photophore. We know, however, that both intensity and color are capable of being regulated and circumstantial evidence indicates that the angular distribution of emitted light can also be varied. The enormous range over which light intensity can be regulated, combined with the requirements for adjusting intensity independent of the angular distribution of the light eliminates the chromatophores as the primary regulators of intensity. The presence of numerous nerves and occasional synapses associated with the photocytes argue for intensity control via direct nervous regulation.

The Type C photophores appear to be responsible for the measured shift in the "day" blue luminescence peak at 480 nm to the "night" green peak at 536 nm. This ability to change color is confirmed by visual observations which show that colors reflected from artificial light in these photophores can either be blue or green in fresh animals and can exhibit reversible post mortem shifts between these colors. The axial-stack filter and the posterior reflector probably control the measured color shift: the properties of both can be altered and all emitted light encounters one or both of these structures. Neither the axial stack nor the proximal reflector *alone* could account for the color shift. The effects of one cannot be physically separated from those of the other since much of the light that encounters one or the other of these structures is intermingled in the distal cap.

It is unlikely that changes at the site of the luminescent reaction contribute significantly to this shift. The inability of the squid to maintain the green color at high light intensity suggests that the filters and reflectors in the "green mode" are selecting light from the tail of a fixed chemical emission spectrum.

The simplest system for altering the transmission characteristics of the axial stack would involve a change in the spacing between platelets. A change in the thickness of the spaces by about 20% will shift the reflectance maximum by about 55 nm. This could be accomplished by moving fluid from one portion of the stack to another. Where fluid is withdrawn the reflectance maximum would shift to shorter wavelengths. Where it is added the opposite would occur and a new bandpass would arise in-between. Continuity between some spaces clearly exists (Fig. 13). The mechanism that would control fluid movement, however, is obscure. The presence of nerves adjacent to the axial cells (Fig. 8) indicates a potential means of regulation.

This mechanism is not applicable to the posterior reflector which is an array of collagen rods suspended in a single fluid-filled extracellular space. In this case the change must occur in the thickness of the high, rather than the low, refractive index layers (the other option, an alteration in refractive index, would have only a minor effect due to the low range of refractive indices found in biological materials). We suspect the mechanism involves changes in the hydration states of the collagen rods and/or *simple osmotic swelling.* Under severe conditions collagen can swell over threefold in diameter (Gustavson, 1956). In the photophore approximately a 20–30% increase in diameter presumably would be adequate. Temperature, salts and pH strongly affect one or both mechanisms. Resulting changes in refractive index and spacing would have an opposite but minor effect. Although reflectance changes were correlated with temperature changes in the dead animal, the color changes in life are not a passive response to temperature changes since in the living animal the color can shift from green to blue at constant high temperatures under the stress of high intensities of overhead light (Fig. 26). Apparently, the change is under behavioral control. Perhaps nervous or hormonal messages to nearby cells trigger alteration of pH, salts or some other factor in the fluid bath which in turn controls the hydration or swelling of the rods.

The angular distribution of the luminescence apparently can also be regulated. The expansion state of chromatophores surrounding the photophore must have strong influence on the angular distribution of the emitted light (see Figs. 35, 36). In addition, between individual Type C photophores considerable variation is seen in the straightness of the ribbons of the distal cap and some variation occurs in the shape of the cap. Large variations can be observed between adjacent Type C photophores in the spread of the distal reflector. Numerous muscles lie in the region of these structures. Muscles attach near the distal surface of the torus that presumably allow this structure to be altered. The nearby vesi-

cles which easily distort may facilitate this movement. Thus the chromatophores, the distal lens, the distal reflector and the torus all have the potential of being altered. Unfortunately, the demonstration of such effects will be difficult. In addition, as the animal's attitude in the water changes, so must the radiance pattern of the luminescence with respect to the body axis. Presumably the lacunae of the orbital space and the numerous muscles that tilt the photophore enable its proper orientation regardless of the squid's attitude.

Although we are only beginning to understand the complex functioning of this minute photophore, we can speculate on its method of operation. The general features of which are summarized as follows. Light is produced by the photogenic cone which is under neuronal control. Luciferin, or other key components of the luminescent reaction, is supplied through the blood and collected by numerous extensions of the photocytes. The proximal reflector and the axial stack are interference structures that determine the color of the emitted light. Complex interaction between chromatophores, proximal and distal reflectors and the torus and distal cap determine the angular distribution of the emitted light. The light intensity, color and probably the angular distribution of the light can be regulated. Because of this flexibility, we expect that these photophores, in combination with other types, allow the squid to conceal itself with bioluminescence under a variety of enviromental lighting conditions that are found in the squid's open ocean habitat.

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DEVELOPMENTAL ASPECTS OF THE MANTLE COMPLEX IN COLEOID CEPHALOPODS

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ABSTRACT

The embryonic development of the mantle complex in some decapod cephalopods is briefly described. Emphasis is placed on the relationship between the muscular fins and the shell sac. In the Decapoda (Sepioidea and Teuthoidea), the base of the fins tends to become detached from the actual shell complex by forming a pair of basal pouches that may completely separate from the shell sac. The embryonic development of the mantle-fin-shell complex in forms having an extremely reduced shell demonstrates the morphogenetic interdependence of the three components.

Key words: Cephalopoda; mantle; fins; shell sac; development; morphology.

INTRODUCTION

When considering the form and function of the mantle in cephalopods the question arises as to how and when the particular form is achieved that corresponds to a particular function. The cephalopod mantle together with the funnel and collar acts as a pump; water drawn into the mantle cavity through the peripheral slits between the funnel pouch (or collar) and the mantle margin is expelled through the funnel tube by muscular contraction of the mantle. This concentration and the resulting water jet can be more or less vigorous. The water is expelled gently during the regular water exchange in the respiratory cycle. More vigorous expulsion always has a locomotory effect; this is used for the typical jet propulsion, especially in attack and escape movements. The "dynamic lift" provided by rapid respiratory jets is characteristic of "hovering" in small animals.

In the evolution of the "modern" coleoid cephalopods, the muscular mantle has largely replaced the protective shell, which has become an internal, residual "backbone." Along with this replacement, the mantle has been equipped with a special device for stabilization and dynamic lift in hovering and slow locomotion, namely the muscular fins.

In this paper, I briefly describe and discuss the early embryonic development of the complex comprising the muscular mantle, the fins and the shell sac.

The cephalopods are characterized by an essentially direct development. The hatchlings already have the complete mantle complex of the adult type. During the later stages of embryonic development, mantle contractions become increasingly frequent and may serve respiration even before hatching, although to a limited extent.

The general process of shell sac formation in cephalopod embryos has been described by Koelliker (1844), Naef (1928), and Spiess (1971). In all the decapods, a circular fold forms around the central part of the mantle rudiment at about stage VIII of Naef. This fold grows over the central area that is surrounded by the ring- or clasp-shaped rudiment of the muscular mantle; during this process the rudiments of the fins appear on each side of the now closing pore of the shell sac. Their bases are attached to the outer wall of this sac.

In the octopods, shell sac formation begins earlier than in the decapods, at least in the Incirrata. The rudimentary shell sac is largely modified in its form, but not in its relation to the fin rudiments which appear even in the embryos of the finless Incirrata but do not continue to develop (Naef, 1928). A discussion of the octopodan relationships is presented in an earlier article (Boletzky, 1978). The present description is limited to the coordinated processes of the mantle-fin-shell sac formation in decapods, especially the process by which the fins may detach from the actual shell sac. This phenomenon has briefly been mentioned by Naef (1923, 1928), but has not yet been described in detail. In a recent study of shell sac formation (Spiess, 1971), it is entirely ignored.

MATERIALS AND METHODS

In addition to live observations of various embryonic stages, fixed embryos of different species were analysed. For scanning electron microscopy (SEM), embryos of *Loligo vulgaris* Lamarck, 1798 were fixed in buffered glutaraldehyde and post-fixed in OsO_4, critical point dried and coated with gold-palladium.

A series of ethanol-fixed embryos of *Sepioteuthis lessoniana* Lesson, 1830 from the Red Sea were imbedded in paraffin and sectioned. The sections were stained with Azan or Masson's trichrome.

Embryos of *Sepia officinalis* Linnaeus, 1758 and *Rossia macrosoma* (Delle Chiaje, 1829) were fixed in Bouin's and processed for histology as indicated above. Early organogenetic stages of *Rossia macrosoma* were studied after fixation by detaching the embryonic "cap" from the yolk mass and viewing it under the dissecting microscope in incident or transmitted light.

OBSERVATIONS

Loligo vulgaris

When viewed under the SEM, the mantle rudiment at stage IX shows the peripheral tissue concentration giving rise to the mantle muscle and two slight elevations on either side of the shell sac pore, the fin rudiments (Fig. 1). They rise from the mantle surface during the ensuing stages when the shell sac becomes completely sealed (Fig. 2). These stages are characterized by a general contraction of the embryo by which the organ rudiments are "assembled" into a more compact embryonic body.

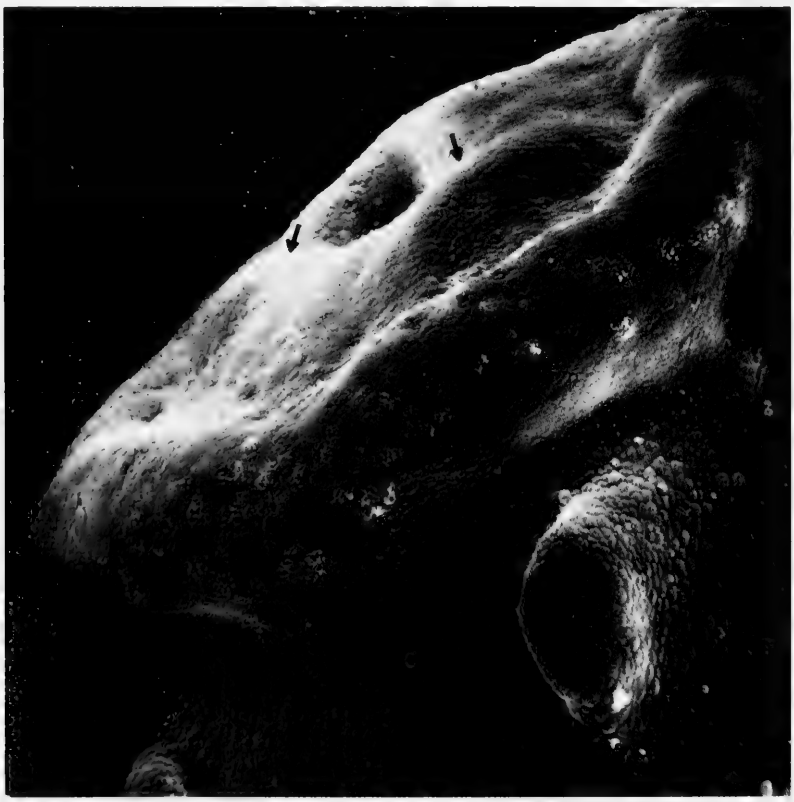

FIG. 1. Ventrolateral view of *Loligo vulgaris* embryo at stage IX of Naef (SEM photograph). Arrows mark the fin rudiments. The two gill rudiments are indicated by arrow heads. The light patches (x) on the mantle border are ciliary cells. Scale bar = 50 μm.

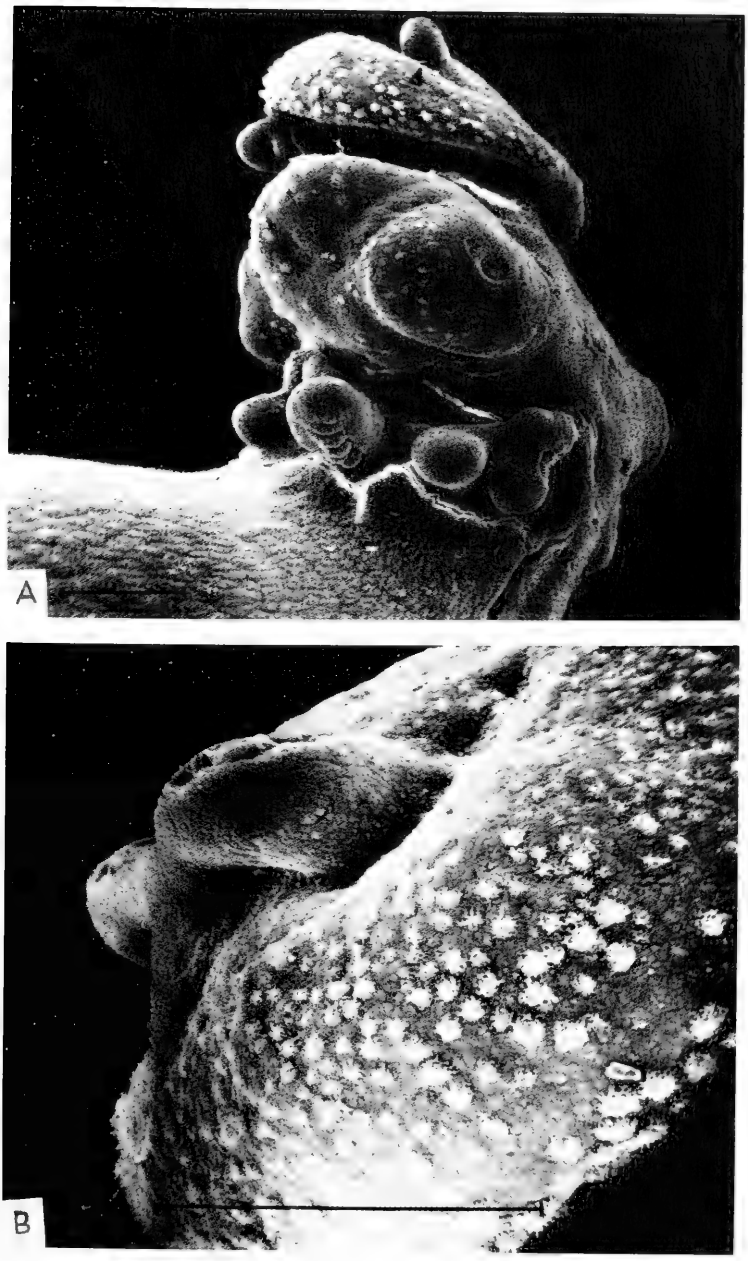

FIG. 2. A. Right-side dorsolateral view of *Loligo vulgaris* embryo at stage XI (SEM), with one fin showing on top of the mantle rudiment (arrow head). B. Ventrolateral view of mantle and fins (partly damaged during preparation) in *Loligo vulgaris* embryo at stage XII. Scale bars in A and B = 100 μm.

FIG. 3. Ventrolateral view of mantle and oblique fins in *Loligo vulgaris* embryo at stage XIII (SEM). Note that fins remain unciliated on most of their surface. Scale bar = 50 μm.

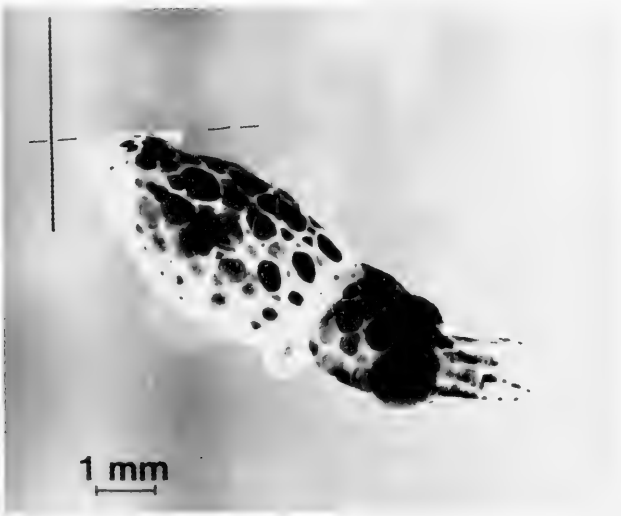

FIG. 4. Juvenile *Loligo vulgaris*, 18 days old, in normal "hovering" position. Solid line indicates vertical axis in space, broken line corresponds to the plane of the fins (shown during a down beat).

The fins lie in an oblique plane in relation to the longitudinal axis of the animal, closer to perpendicular than to parallel (Fig. 3). This is the position typical of the later embryonic and post-embryonic stages. When hovering, the hatchlings and early juveniles swim in an oblique position with the head down. The plane of the fins, oblique in relation to the body axis, is then roughly horizontal in space (Fig. 4). In rapid forward movements (e.g. attack), these terminal fins counteract the upward thrust of the reversed funnel tube and are thus essen-

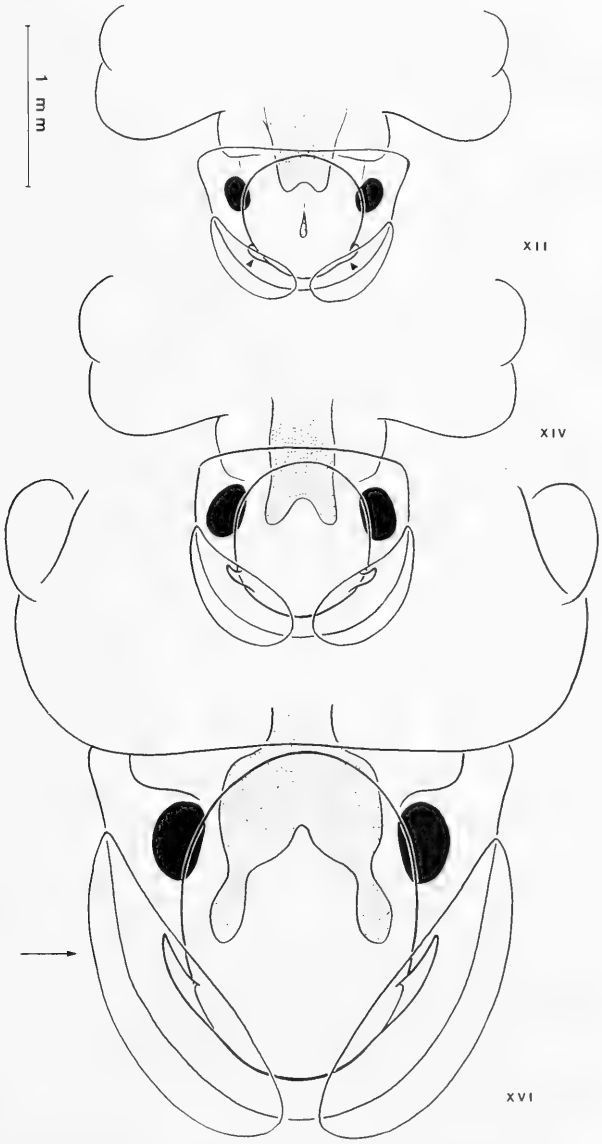

FIG. 5. *Sepia officinalis* embryos, reconstructed from histological cross sections, at stages XII, XIV and XVI. Arrow heads in the upper figure indicate the fin pouches forming under the fin rudiments. The stellate ganglia are marked black and the inner yolk sac is dotted (the posterior "appendix" of the yolk sac at stage XII is constricted by the closing mid-gut rudiment and will soon disappear). Arrow in lower figure indicates position of section shown in Fig. 6.

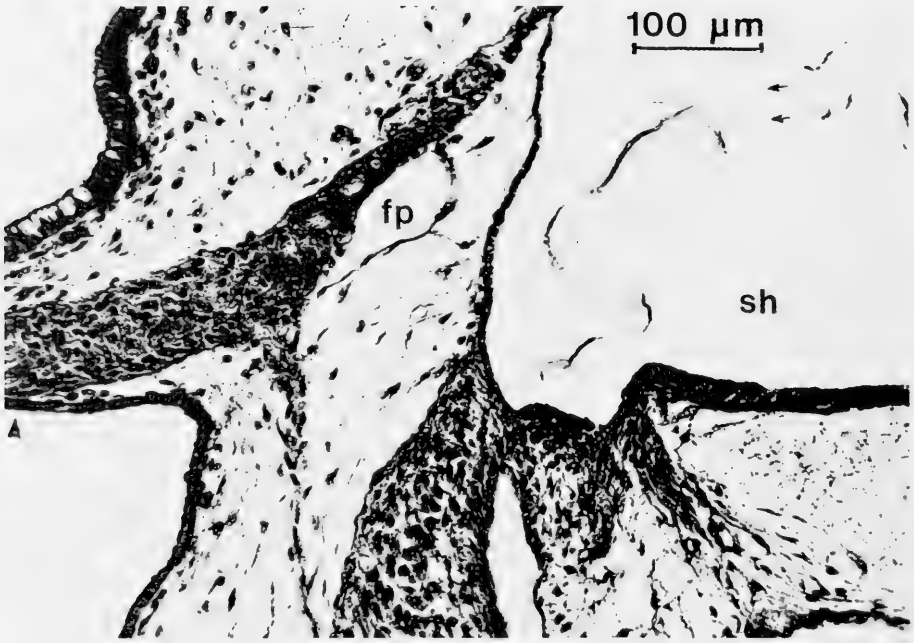

FIG. 6. Histological section (cf. Fig. 5) showing the basal part of the fin (arrow head), the fin pouch (fp) and the shell sac (sh), dilated by the fixation procedure, containing the chambered shell (arrows mark the two "chambers" formed at this and the preceding stage).

tial for the maintenance of the direction of forward movements. At hatching and early in juvenile life, the base of the fins lies above the broad posterior part ("vane") of the organic shell (or "pen"). Only later do they become separated from the actual shell sac by the musculature of the mantle, which grows dorsally over the shell.

Sepioteuthis lessoniana

The process occurring only after hatching in *Loligo* begins at the end of organogenesis (from stage XV onward) in *Sepioteuthis lessoniana*, a species characterized by very large eggs. The sections show that the process corresponds exactly to that described for *Sepia*.

Sepia officinalis

At stage XII, the closed shell sac still has a roughly circular outline and contains the early embryonic shell (Fig. 5). In the posterior part, the outer wall of the sac formed from the early ring-shaped fold begins to "invaginate" under each fin rudiment. By stage XIV, these re-

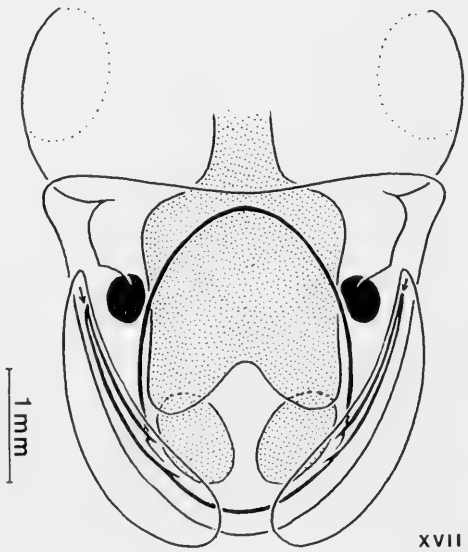

FIG. 7. Reconstruction of *Sepia officinalis* embryo at stage XVII, showing the large extent of the tube-shaped fin pouches (arrows) reaching to the foremost part of the fins (inner yolk sac has also undergone differentiation by forming two distinct posterior lobes).

cesses have become slightly curved tubes. At stage XVI, when the calcified shell is already well differentiated, the recesses or pouches are still comparatively short; their anterior ends lie far behind the stellate ganglia (see also Bandel & Boletzky, 1979). The basal part of the fins begins to differentiate into cartilaginous tissue overlying the thin outer wall of the tube-shaped pouches (Fig. 6). These pouches then grow rapidly in length and reach the anterior part of the fin, adjacent to the stellate ganglia, by stage XVII (Fig. 7). This corresponds closely to the adult situation in that the bases of the fins lie on the surface of the mantle muscle, in a plane parallel to the longitudinal axis of the animal (in lateral aspect).

Rossia macrosoma

The general aspect of the early embryo of *Rossia* is almost identical to that of *Sepia* (Fig. 8). The fin rudiments become distinct long before the shell sac is closed. However, after its closure, this sac takes on a different form, with an increasingly tapering anterior part. Fin pouches form at a similar stage, but they are much wider antero-posteriorly even though the fin rudiments are shorter than in *Sepia* (Fig. 9). The later development demonstrates the basic difference between *Rossia* and *Sepia*. In fact, the entire posterior half of the early shell sac differentiates into fin pouches. The remaining anterior part becomes the actual shell sac, in which a small

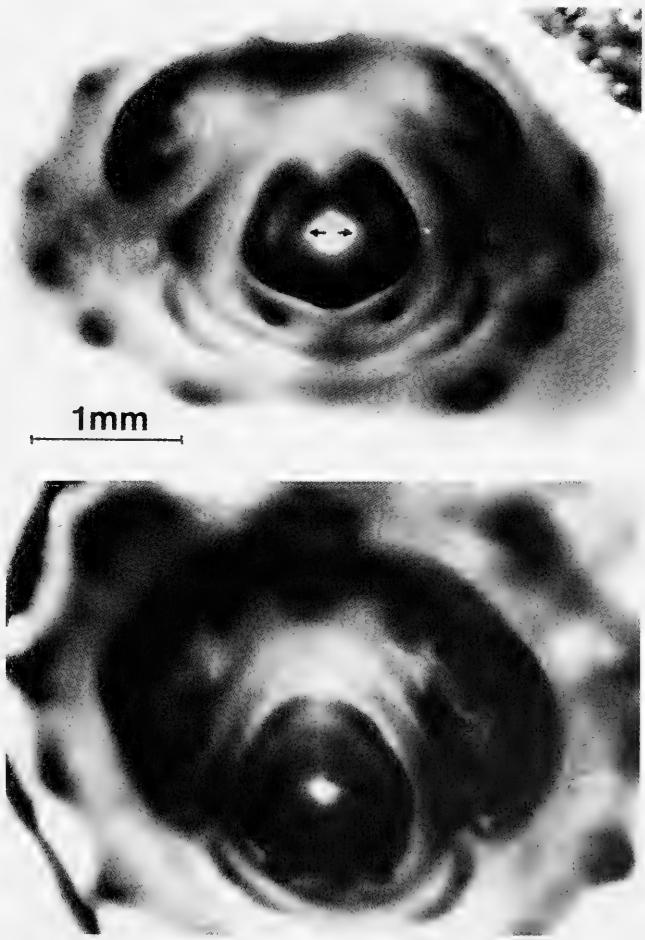

FIG. 8. Fixed embryos of *Rossia macrosoma* under transmitted light. Stage VIII (above) shows an early aspect of shell sac closure (arrows) in the central part of the mantle rudiment. At stage IX (below) the shell sac is half-closed and the fin rudiments (arrow heads) become distinct on each side of the pore.

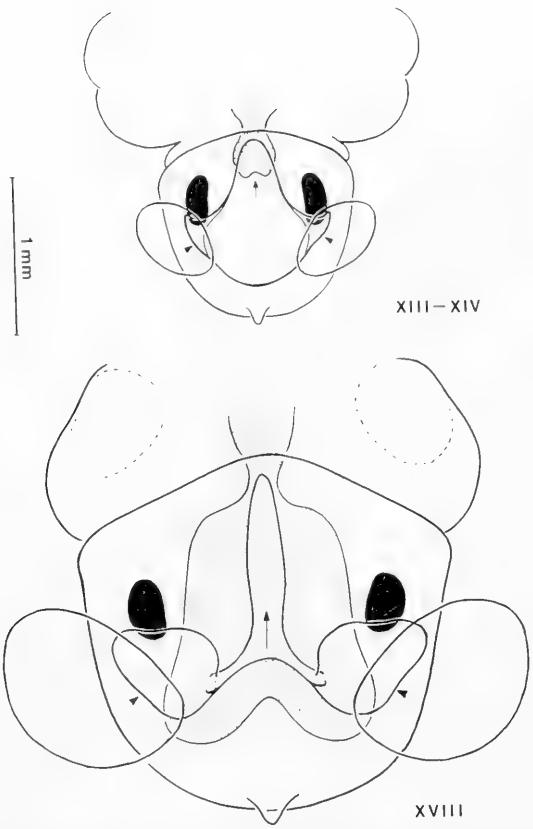

FIG. 9. Reconstruction of *Rossia macrosoma* embryos at stage XIII–XIV (above) and stage XVIII (below), showing the differentiation of the actual shell sac (arrow) in the mid-dorsal line of the mantle, and of the fin pouches (arrow heads). The stellate ganglia are marked black and the inner yolk sac is dotted.

but otherwise typical organic "pen" is formed. The positional relationships between the different components of this complex are, however, identical to those observed in *Sepia.* Thus the anterior part of the fin pouches lies close to the stellate ganglia, and the communication with the actual shell sac lies in the vane area of the pen. As in *Sepia,* the fins lie in a plane nearly parallel to the body axis (in lateral aspect). The base of the fin forms a cartilaginous attachment to the wall of the pouch, with muscular attachments joining the mantle muscle (Fig. 10). The basal fin cartilages become relatively large and form two dorso-lateral plates (Fig. 11) in a position reminiscent of the vane in a typical teuthoid pen.

The completely different aspect of the mantle of all sepiolids compared to *Sepia* is due to the "blowing up" of the posterior mantle end (with its peculiar tissue "spine"), on the one hand, and to the preservation of the rounded fin shape, on the other. This fin shape is typical of all decapod hatchlings except those of *Sepia* which show an extremely early deviation towards the adult condition as far as the proportions of the fins are concerned.

DISCUSSION

The formation of fin pouches which allow the fins to "detach" from the shell complex appears as a common ontogenetic character of all decapods, as Naef (1923, 1929) has already emphasized. However, this process of fin detachment may begin at very different ontogenetic stages, according to the species or genera considered.

This latter fact calls attention to the orienta-

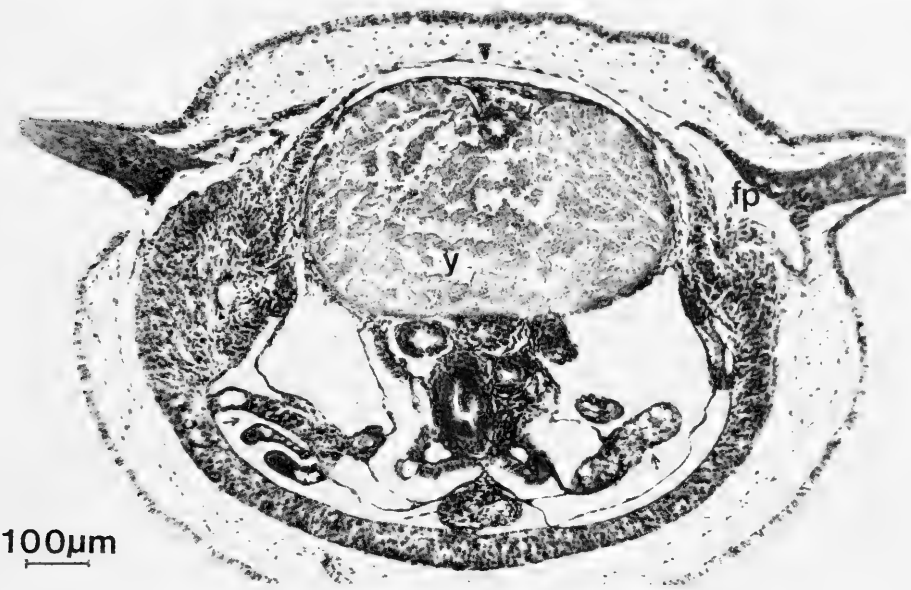

FIG. 10. A histological cross-section through the rear part of the mantle in a *Rossia macrosoma* embryo at stage XV–XVI. The inner yolk sac (y) has considerably increased in size (cf. Fig. 9, above). The dorsal mantle musculature (arrow head) is extremely thin in this area. The fin pouches (fp) are in the course of their separation from the actual shell sac not shown here. Below the yolk sac, the intestinal complex is visible; arrows indicate the gills and a broken line points at the ventral mantle adductor.

tion of the fins in relation to the body axis. Fin pouches apparently are formed in conjunction with obliquely orientated fins that are tilted into a plane roughly parallel to the body axis. The pouches are inconspicuous in many oegopsid squids in which the fin cartilages are fused in the dorsal midline. They are very conspicuous in the sepioids and in the loliginids where the fins remain separated, and this condition does not necessarily imply great fin lengths as demonstrated by the sepiolids (represented here by *Rossia*).

For the evolution of the coleoid cephalopods, one would expect that these peculiar pouches appeared only after the fins had been "invented." Indeed the most archaic recent coleoid, *Vampyroteuthis,* has fins set directly on the shell sac (Pickford, 1949). An intriguing peculiarity of this form is that two pairs of fins are formed successively. R. E. Young (personal communication to Jeletzky, 1966) notes that the first (so-called "larval") pair of fins structurally resembles the decapod fins, whereas the adult pair is much more similar to the fins of cirromorph octopods. Whatever the origin of the fins in the latter group may be, there can be no doubt that they are homologous to either one or the other pair of the *Vampyroteuthis* fins (they would be homologous to both only if fin development in the Vampyromorpha had secondarily been broken up into two phases, after the octopods had diverged from the common stock). There can also be no doubt that the shell sac in all octopods is homologous to the shell sac of Vampyromorpha and Decapoda.

Taking as a primitive condition the roughly circular early shell sac rudiment common to all coleoid embryos (and probably to all cephalopod embryos in general), one can make out two different lines or trends of shell reduction. One line leads from the vampyromorphs to the living decapods, where the shell can completely disappear without any parallel reduction of the fin apparatus (e.g. *Heteroteuthis*). This appears to imply an early separation of the fins from the actual shell sac.

The other line leads from a primitive situation, with the fin bases firmly attached to the actual shell sac, as in *Vampyroteuthis,* via a reduction of the parts of the sac not "used" as

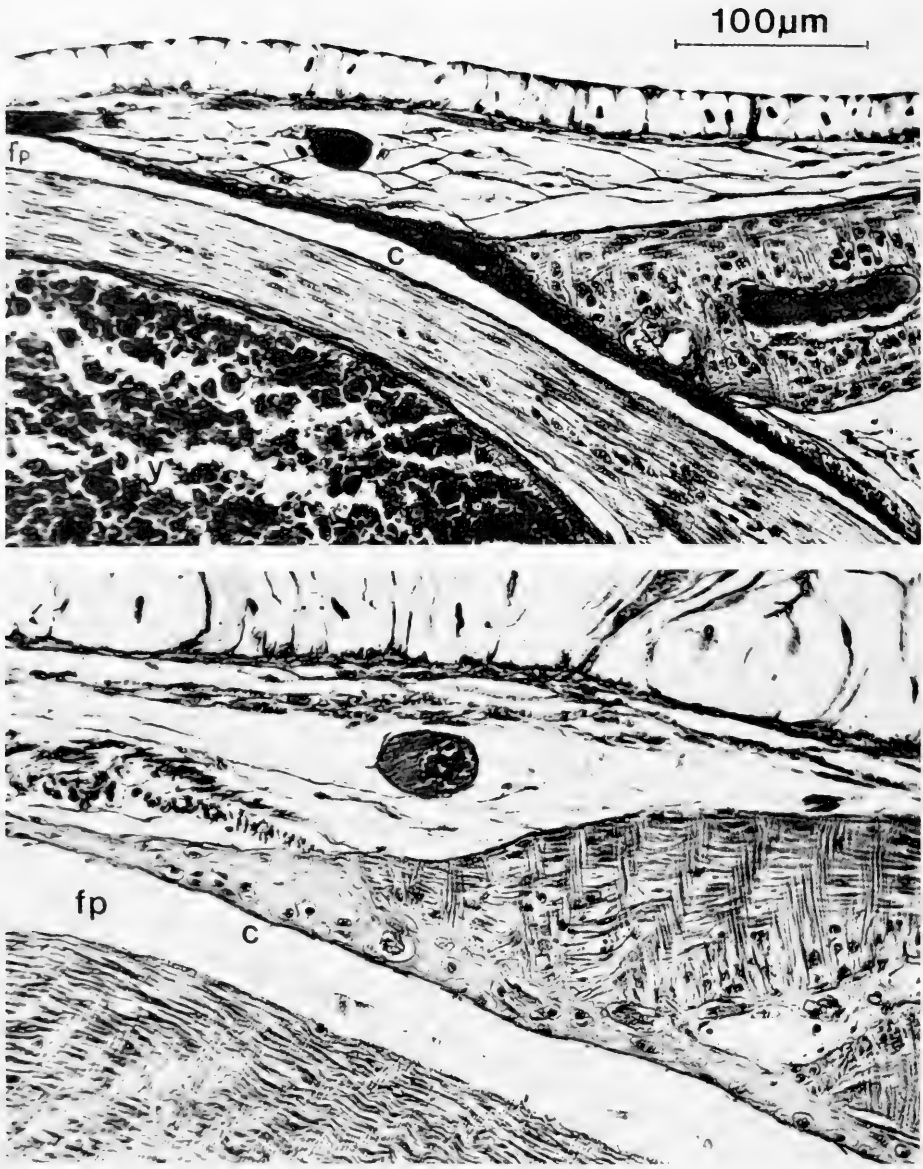

FIG. 11. Histological cross sections through the fin cartilage (c) and fin pouch (fp) in *Rossia macrosoma*, at late embryonic stage (XIX–XX, above) and in early juvenile, 20 days after hatching (below) at the same magnification.

fin support (Cirrata) to the further reduction of both shell and fin (Incirrata). This "trend" does not of course explain what actually happened in the ancestor of the Incirrata. Other arguments will be necessary in formulating a coherent hypothesis for this crucial event. These arguments again will have to encompass all aspects of developmental coordination, which is crucial in any constructional "deviation."

In the mantle complex of cephalopod embryos, such a deviation is not limited to gross morphology of muscular components in relation to the shell; it includes also finer details in

the structure of the integument. Indeed the absence of fins in incirrate hatchlings appears to be correlated with the presence of special integumental structures (the Koelliker organs) that act as auxiliary hatching equipment adjusted to the particular egg structure of incirrate octopods (Boletzky, 1978).

A similar correlation between the morphology of the mantle complex and the differentiation of a particular hatching equipment exists in the decapods. In contrast to the incirrate octopods, all decapods have transitory ciliation that covers large parts of the embryonic integument. This ciliation, especially the bands of short cilia on the dorsal and ventral mantle surface of squid hatchlings, provides locomotion when the animal crosses the egg jellies (Boletzky, 1979). These specialized ciliary bands are absent on the mantle integument of sepiolid hatchlings (Boletzky, in press); they are "replaced" by an integumental organ apparently involved in breaking the outer shell, which is rigid in *Rossia* eggs. The differentiation of the "terminal spine" on the mantle end is clearly correlated with the marked expansion of the rear part of the mantle and the corresponding reduction of the shell size described here.

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MALACOLOGIA, 1982, 23(1): 177–192

HISTOCHEMISTRY AND FINE STRUCTURE OF THE ECTODERMAL EPITHELIUM OF THE SEPIOLID SQUID *EUPRYMNA SCOLOPES*

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ABSTRACT

Histochemical analysis and electron microscopy reveal the presence of three distinct cell types comprising the ectoderm of *Euprymna scolopes*. The most prominent types were ovate- and goblet-shaped secretory cells. The secretory product of ovate cells was unreactive to periodate oxidation, alcian blue, azure A, colloidal iron and all iron diamine reactions, but was reactive with beibrich scarlet, bromphenyl blue, and the ninhydrine-Schiff test indicating the presence of basic proteins. In contrast, the product of ovate cells fixed in the process of active secretion was strongly metachromatic with azure A, alcianophilic at both pH 2.5 and pH 1.0, strongly basophilic, and was vigorouly reactive with all diamine sequences and colloidal iron. Furthermore, the ovate cell product remained periodate unreactive during secretion, but reactivity with stains for basic proteins was lost. The ovate cell secretion product was judged by these criteria to be a strongly acidic protein-polysaccharide complex. The goblet cell secretion product was judged by these same histochemical tests to be a neutral polysaccharide having little if any protein. The third cell type was not obviously secretory and was found toward the outer surface of the epithelium. The mucous layer covering the epithelial surface stained as did the product of goblet cells.

Electron microscopy revealed that the 30 μm to 50 μm thick epithelium was underlain by a 0.2 μm thick basement membrane and below this a layer of orthogonally arranged striated collagen fibrils. The apical surfaces of all cells possessed microvilli covered with a thin layer of mucous material. Ovate cells had a single large vesicle filled with a fine granular material. In contrast, the secretory product of goblet cells consisted of large, electron dense membrane-bound granules. Goblet cells contained an extensive smooth ER-Golgi complex, whereas ovate cells possessed only small amounts of rough ER. Ovate cells had a meshwork of 5 nm to 8 nm microfilaments surrounding the large vesicle. Goblet cells contained small numbers of longitudinally oriented microtubules extending to the apical surface which were closely associated with secretory granules. The histochemically unreactive microvillous cells were revealed by TEM to contain very small membrane-bound vesicles containing a discontinuously electron dense material.

The distribution of cell types was non-random. Ovate cells occurred in greatest numbers and on both dorsal and ventral surfaces. Goblet cells were found only in the dorsal epithelium and in greatest abundance on the head and back of the animal. Microvillous interstitial cells were found in both dorsal and ventral epithelia, but in greater numbers dorsally.

The differential distribution and histochemical nature of the secretory products of the two glandular cells suggest that they play opposing roles in *Euprymna*'s camouflaging behavior.

Key words: Cephalopoda; Sepiolidae; *Euprymna scolopes*; integument; histochemistry; behavior.

INTRODUCTION

The sepiolid squid *Euprymna scolopes* Berry, 1919, like most members of the Sepiolidae (see Boletzky & Boletzky, 1970; Boletzky et al., 1971), is primarily nocturnal and burrows into the sandy ocean bottom during the day. In addition to burrowing in the sand, *Euprymna* is able to cause sand and other materials of the substratum to adhere to its dorsal surface. This behavior creates an effective camouflage while the animal is hunting during the daylight or when it is disturbed from its hiding place. When the camouflage is no longer required the squid is able to release the adherent materials instantaneously. This sort of camouflaging behavior has not previously been reported in a cephalopod.

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The character of *Euprymna*'s camouflaging behavior suggests a mechanism involving the epidermis and its secretory activity. However, the nature and functions of cephalopod epithelial secretory products (indeed those of molluscs in general) have received little attention regarding either their histochemical or specific biochemical characteristics (Hunt, 1970). The only available information concerning squid skin mucopolysaccharides (MPS) is provided by two brief studies. Anno et al. (1964) found that the MPS from the skin of *Ommastrephes sloani pacificus* is approximately 70% unsulfated chondroitin. The skin of *Loligo opalescens* was found to contain MPS consisting of 70% highly sulfated chondroitin sulfate and 25% chondroitin (Srinivasan et al., 1969). There appears to be no information on the biophysical properties of such secretions.

This paper describes the results of an investigation of the possible mechanism of sand adhesion and release by *Euprymna scolopes*. The nature of *Euprymna*'s epidermal secretions was studied using standard histochemical techniques. The histochemical characteristics of these secretions suggest that they may be involved in sand adhesion and release. The structure of the epidermis was studied in further detail by transmission electron microscopy. Examination of the fine structure of the epidermis provides additional insight into the possible mechanisms involved in *Euprymna*'s camouflaging behavior.

MATERIALS AND METHODS

Adult *Euprymna scolopes* were collected in shallow intertidal areas at the island of Oahu, Hawaii and were maintained in the laboratory as previously described (Arnold et al., 1972).

Histology and Histochemistry

Animals were anesthetized by cooling to near 4°C, after which they were decapitated and rapidly immersed in one of the following: 2% calcium acetate-10% formalin (Ca-F) (Lillie, 1965), for 24 hr at 4°C; 0.5% cetylpyridinium chloride-10% formalin (CPC-F), for 24 hr at 20°C (Williams & Jackson, 1956); 0.5% cetylpyridinium chloride in Carnoy's fixative (CPC-C), for 24 hr at 20°C to precipitate polyanionic polysaccharides; Lillie's (1949) acetic-alcohol-formalin (AAF), for 24 hr at 4°C; or 10% formalin in sea water, for 24 hr at 4°C. Samples of both dorsal and ventral epidermis were excised from the mantle while in the fixative. Samples of fins and arms were also removed and processed for histological comparison.

To ensure the identical treatment of tissues subsequent to fixation, samples of epidermis fixed by each of the different methods were processed simultaneously. Tissues were dehydrated through ethanol, cleared in xylene, and infiltrated with paraffin. Samples from each fixative were then embedded in the same block and sectioned at 5 μm. This method of processing made it possible to evaluate the effectiveness of each fixative in preserving the secretory products of the skin and the effects of each fixative on the various staining procedures.

Lillie's (1949) AAF provided the best cytological detail and most vivid staining. All staining reactions are described from material preserved by this method. No major deviations from these results were observed with the other fixatives. Neutral calcium acetate-formalin and formalin-sea water provided good preservation, but less intense staining. The CPC-containing fixatives produced extensive cytological disruption, the loss of the epidermal surface and generally weak staining.

Various histochemical procedures were employed to elucidate the nature of the epithelial secretions. The procedures used were, except as noted, those outlined in Pearse (1968).

The periodic acid-Schiff (PAS) technique alone (McManus & Mowry's [1960] modification, with prior acetylation, acetylation-deacetylation, or analine blockage of −OH groups) was used to demonstrate mucosubstances having vicinal hydroxyls. Identification of mucosubstances presumably containing hexoses or deoxyhexose units, other than glucose, was accomplished using the periodic acid-*para*-diamine (pH 4.0) method (PAD) (Spicer, 1965). In addition, specific localization of periodate reactivity was confirmed at the ultrastructural level using the periodic acid-thiocarbohydrazide-silver proteinate method (Thiéry, 1967).

To differentiate between neutral and acidic mucosubstances, the following metachromatic and basic dyes were used: the mixed diamines (pH 4.0) technique (MD); 0.1% toluidine blue (TB) in 30% ethanol for 20 min (Kramer & Windrum, 1954); azure A (AA) in

various buffers at graded pH levels for 30 min; alcian blue G8X (AB) at graded pH levels for 2 hr and in combination with the PAS technique (Mowry, 1963); Mowry's (1963) modification of Hale's colloidal iron (CI) for 2 hr; aldehyde fuchsin (AF) for 10 min (Halmi & Davies, 1953); the low iron (LID) and high iron diamine (HID) reactions for 18 hr (Spicer, 1965); and 1% alcoholic thionin (pH 1.0) as a general metachromatic stain for acidic mucins.

Most of the preceding techniques were effected concomitantly with supplementary procedures and confirmatory tests which involved the chemical blockage, introduction or enzymatic removal of specific reactive groups in the mucosubstances. These procedures included both "mild" (4 hr at 37°C) and "active" (6 hr at 60°C) methylation, using methanol acidified to 0.1 N HCl, followed in control sections by saponification for 20 min with 1.0% KOH in 70% ethanol (Spicer & Lillie, 1959). These methylation and methylation-saponification procedures were effected in conjunction with AA, AB-PAS, AF-AB and HID-AB staining for selective blockage and restoration of various acid groups.

Sulfate esters were introduced using a 1:1 mixture of sulfuric acid and acetic anhydride for 3 min or a 1:10 mixture of chlorosulfonic acid and pyridine at 70°C for 5 min (Kramer & Windrum, 1954; Pearse, 1968) prior to staining with AA.

The Bial reaction was performed as a test for the presence of sialic acid (Ravetto, 1964). In addition, digestion with *Clostridium perfringens* neuraminidase (Sigma, Type VI) (Sialidase) was effected in conjunction with AA, AB, AF-AB and HID-AB staining for sialic acid containing mucosubstances. Sections were incubated at 37°C for 12 to 24 hr in 0.2 ml of 10 NFU/ml neuraminidase in citrate buffer at pH 5.0. Control sections were treated with buffer alone.

Basic proteins were visualized with Beibrich scarlet (BS). Sections were stained for 1 hr at 20°C in 0.04% BS in phosphate buffer at pH 6.0 or Laskey's glycine buffer at pH 8.0, pH 9.5 and pH 10.5 (Spicer & Lillie, 1951). In addition, comparative staining of proteins was effected using bromphenol blue (BPB) (for 2 hr at 20°C in 0.05% BPB, 1% $HgCl_4$ and 2% acetic acid), acid solochrome cyanine (ASC) (for 10 min at 20°C in 1% ASC in 0.1 M citric acid, pH 2.1), and the ninhydrin-Schiff method (NS) (16–20 hr at 37°C in 0.5% ninhydrin and Schiff's reagent for 30 min).

Electron Microscopy

Adult squid were anesthetized in 0.5% urethane in sea water. Immobilized animals were immersed in 2.5% glutaraldehyde in Millonig's phosphate buffer, pH 7.4, plus 0.4 M sucrose for 15 min at room temperature, after which samples of dorsal and ventral epidermis were excised from the mantle while submerged in the fixative solution. Primary fixation was continued for an additional hour at 4°C. Samples of epidermis were then rinsed in buffer for 20 min, post-fixed in 1% OsO_4 in bicarbonate buffer (pH 7.4) for 1 hr at 20°C, dehydrated through a graded ethanol series and propylene oxide and embedded in EPON 812 (Luft, 1961). Sections, 60 nm to 70 nm thick were cut with a diamond knife using a Reichert OM-U2 or a Sorvall MT-2B ultramicrotome and mounted on uncoated mesh grids. Section were stained with uranyl acetate (Stempak & Ward, 1964) and/or lead citrate (Venable & Coggeshall, 1965). Observations were made using a Philips EM 201 or EM 300 transmission electron microscope (TEM).

KEY TO ABBREVIATIONS

Stains		Colors	
AA	Azure A	B	blue
AB	Alcian Blue 8GX	C	grey
ASC	Acid Solochrom Cyanine	G	orange
BPB	Bromphenol Blue	K	black
BS	Beibrich Scarlet	N	brown
CI	Colloidal Iron	P	purple
D	p-Diamine	pk	pink
HID	High Iron Diamine	R	red
LID	Low Iron Diamine	Y	yellow
MD	Mixed Diamines		
NS	Ninhydrin-Schiff		
PAD	Periodic acid-p-Diamine		
PAS	Periodic acid-Schiff		
TB	Toluidine Blue O		

RESULTS

Histological Observations

The epidermis of *E. scolopes* was observed to be a pseudostratified columnar epithelium composed of three morphologically distinct cell types and underlain by a thick basement membrane. The interstitial cell type was

polymorphic, appeared restricted to the distal surface of the epithelium and was distinguished by a rounded nucleus. The ovate cell type was always the largest, ovate in shape, and contained a single, large secretory vesicle which flattened the nucleus against the basal surface. The goblet cell type contained darkly staining granules distributed from the region of the nucleus to the apical surface, producing the cells' distinctive shape. Goblet cells typically had elliptical nuclei located near their basal ends and generally extended from the basement membrane to the surface of the skin.

Interstitial cells were the most abundant cell type in the dorsal epidermis, but were observed less frequently in the ventral epidermis where the ovate type was predominant. Ovate cells were observed in all regions of the epidermis with the exception of the distal surfaces of the fins and arms. In contrast, goblet cells were found only in the dorsal epidermis and were especially abundant in the head and dorsal mantle. Goblet cells were observed less frequently in the dorsal skin of the arms and fins, and were completely absent in the dorsal-lateral surfaces of the fins and distal three-fourths of the dorsal arms where the epithelium was comprised of small, non-secretory cuboidal cells.

Histochemical Observations

Both the ovate and goblet cells secrete carbohydrate-rich substances. In addition, a distinct carbohydrate-rich layer covered the outer surface of the epithelium. The reactions of these cell types and the surface layer to specific histochemical staining procedures is summarized in Table 1–3 in which the figures

TABLE 1. Staining reactions dependent on periodate oxidation of vicinal hydroxyls, and tests for basic protein.

Histochemical method	Cell type or tissue region			
	Surface layer	Goblet cells	Ovate cells	Evacuating ovate cells
PAS	4PR	3–4PR	0	0
PAS–control	½pk	0–1pk	0	0
Acetylation (2 hr.)–PAS	0	1–2pk	0–1pk	0
Acetylation (9 hr.)–PAS	0	1pk	0	0
Acetylation (1 hr.)–Deacetylation–PAS	4PR	4PR	1–2pk	2pk
Acetylation (9 hr.)–Deacetylation–PAS	4PR	4PR	1–2pk	2pk
Analine–PAS	0	0	0	0
PAD (7 hr)	0	2YN	1C	4PK
(24 hr)	1–½C	2YN	1C	4PK
(48 hr)	2–3C	3–4N	2C	4PK
D (24 hr) without AP-oxidation	0	0	0	4PK
MD	0–1N	2N	1N	4P
Histochemical interpretation	Neutral mucosubstance with demonstrable vicinal-hydroxyls; suggests surface MPS are highly crosslinked.		No demonstrable vicinal hydroxyls; acidic groups present.	
BS (pH 6.0)	0	2G	2–3R	0
(pH 8.0)	0	1G	2–3R	0
(pH 9.5)	0	2–3G	2–3R	2R
(pH 10.5)	0	0–.5R	½–1R	0
BPB	2B	2B	4B	4B
BPB-NR	1B	0–1B	3B	2G
ASC (pH 2.1) (* biphasic)	1G	4B/G (*)	4G	4G/B (*)
NS	0	0–1PB	2pk	1PB
Histochemical interpretation	Some protein detectable in goblet cells, but absent or masked in surface layer.		Basic proteins present.	

TABLE 2. Alcianophilia at various pH levels and colloidal iron for acidic groups.

Histochemical method	Cell type or tissue region			
	Surface layer	Goblet cells	Ovate cells	Evacuating ovate cells
AB (pH 2.5)	0	0–1B	0	3B
AB (pH 1.0)	0	0–1B	0	3B
AB (pH 2.6)–after Sialidase Digestion	0	0–½B	0	3B
AB (pH 2.6)–after Active Methylation	0	0	0	2–3pk
AB–PAS	4PR	4PR	0	3B
AB (pH 2.5)–PAS–after Active Methylation	4PR	3R	0	3B
AB (pH 2.5)–PAS–after Active Methylation Saponification	4PR	3R	0	3B
CI	0–½B	½B	0	2–3B
CI–PAS	4PB	3–4PB	0	2–3B
CI–PAS–after Active Methylation	0	0	0	0
Histochemical interpretation	Periodate reactive with some orthochromatic alcianophilia; slight CI reactivity suggests some free –COOH groups.		Periodate unreactive; highly sulfated mucosubstance.	

TABLE 3. Azurophilia at various pH levels and tests for Sialic acid.

Histochemical method	Cell type or tissue region			
	Surface layer	Goblet cells	Ovate cells	Evacuating ovate cells
TB	0	0–3B	2B	4PR
TB after Active Methylation	0	0–2B	0–1B	2–3B
AA (pH 0.5)	0	0	0	4PR
(pH 1.0)	0	0	0	4PR
(pH 3.2)	0	0	0	4PR
AA (pH 1.0)–after Sulfation (alc)	0–1B	0–1B	0	3PR
(pH 3.2)–after Sulfation (alc)	2B	1B	0	3B
AA (pH 3.2)–after Sialidase Digestion	0	0	0	4P
Bial	0	0	0	0
Histochemical interpretation	Lack of metachromatic azurophilia confirms lack of acidic sulfate groups; no sialic acid.		Metachromatic azurophilia attributable to sulfate groups; acid groups appear masked in ovate cells.	
Observations of basophilia with various individual and combination dyes				
Thinonin	0	0	0	4R
LID	0	0	0	4P
LID–AB	0	0	0	4P
PA–LID	0	0	0	4P
HID	0	0	0	4P
HID–AB	0	0	0	4P
HID–AB after Salidase Digestion	0	0	0	4P
PA–HID	0	0	0	4P
AF	0	0	0	4P
AF–AB (pH 2.5)	0	0–1B	0	4P
AF–AB (pH 2.5)–after Sialidase Digestion	0	0–1B	0	4P
Histochemical interpretation	Neutral mucosubstance.		Highly sulfated, but acidic groups appear masked in ovate cells.	

represent visual estimates of the relative intensity of staining. Ovate cells which were fixed while in the process of secretion stain differently than non-secreting ovate cells so are treated as a separate cell type.

Ovate Cells. The secretory vesicles of these cells were unstained by the PAS reaction as well as most other stains used in conjunction with prior periodate oxidation (Tables 1, 3). However, a light grey coloration was produced by the PAD reaction and the acetylation-deacetylation-PAS sequence produced staining not observed with PAS alone. No staining was observed with AA, AB, CI or any other reaction to demonstrate acid groups.

The secretory vesicle of ovate cells stained dark red with Beibrich scarlet at pH 6.0 to pH 9.5, but stained only light red at pH 10.5 (Table 1). Positive reactions for protein were also produced with BPB, ASC (pH 2.1) and the ninhydrin-Schiff reaction.

Evacuating Ovate Cells. These, like ordinary ovate cells, were periodate unreactive. Staining with BS was observed only at pH 9.5 suggesting the presence of basic protein (Table 1). The partially secreted cell product also stained strongly with BPB and NS. ASC (pH 2.1) staining in these cells was biphasic. That portion of the product remaining within the cells was orange whereas the portion extending beyond the epithelial surface was blue. These cells exhibited strong orthochromatic alcianophilia and were dark blue with Hale's colloidal iron. In addition, they displayed strong basophilia with LID, HID and AF. Staining was not reduced with prior neuraminidase treatment. The secretory product of these cells displayed strong γ-metachromasia with TB and AA.

Goblet Cells. The granular secretory product of goblet cells was strongly periodate-reactive as evidenced by the dark magenta coloration with PAS (Table 1). These cells reacted only moderately with BS and minimal reaction was observed with BPB. A strong reaction was obtained with ASC (pH 2.1) and, like the product of evacuating ovate cells, the staining was biphasic. The granular substance stained blue whereas the intergranular substance stained orange (Table 1). These secretory granules displayed weak alcianophilia, however, a strong reaction was observed after active methylation (Table 2). Reactivity with CI was weak and no staining was observed with TB or AA. In addition, there was no observable basophilia.

Surface Layer. Staining reactions of the thin surface layer were essentially identical to those of the goblet cells with the exception that staining for basic proteins was weak or absent.

Electron Microscopic Observations

The dorsal epidermis ranges from 35 μm to 50 μm in thickness including the 0.2 μm basement membrane (Fig. 1). The three major cell types described from light microscopic observation were readily distinguishable with the TEM. The PAS-positive surface layer is comprised in part of a dense pile of microvilli (Figs. 2, 3). These microvilli are typically 0.5 μm to 0.6 μm in length and 80 nm to 12 nm in diameter, are electron dense at their tips, and contain few oriented filaments (Fig. 3). Associated with the outer surface of the microvilli is a thin layer (100 to 300 nm) of fibrous mucous material. Between the microvilli are seen small (35 nm to 45 nm) electron dense granules (Fig. 3).

Both the interstitial and goblet cells possess microvilli on their apical surfaces, whereas ovate cells lack microvilli. Interstitial cells constitute the largest proportion of the surface area of the skin by virtue of their greater numbers and their broad apical surfaces (Fig. 2). The relative numbers and distribution of these cells is best appreciated by viewing sections near and tangential to the apical surface (Fig. 4).

A complex system of nerve and glial cell processes is observed between the basement membrane and the secretory cells (Fig. 5) where they are most frequently associated with the bases of ovate type cells. Lying beneath the basement membrane is a layer of orthogonally oriented collagen fibrils (Fig. 6), below which are the variously oriented layers of dermal muscle (Figs. 5, 6).

Ovate Cells. Ovate cells of the dorsal epidermis are either roundly ovate or have the appearance of a drawstring purse with the nucleus flattened at the basal end (Fig. 1). Varying amounts of rough endoplasmic reticulum are observed within the cytoplasm surrounding the nucleus, but few Golgi bodies. The secretory material of these cells has a fine granular appearance (Fig. 7). Within the peripheral cytoplasm is a close reticulum of

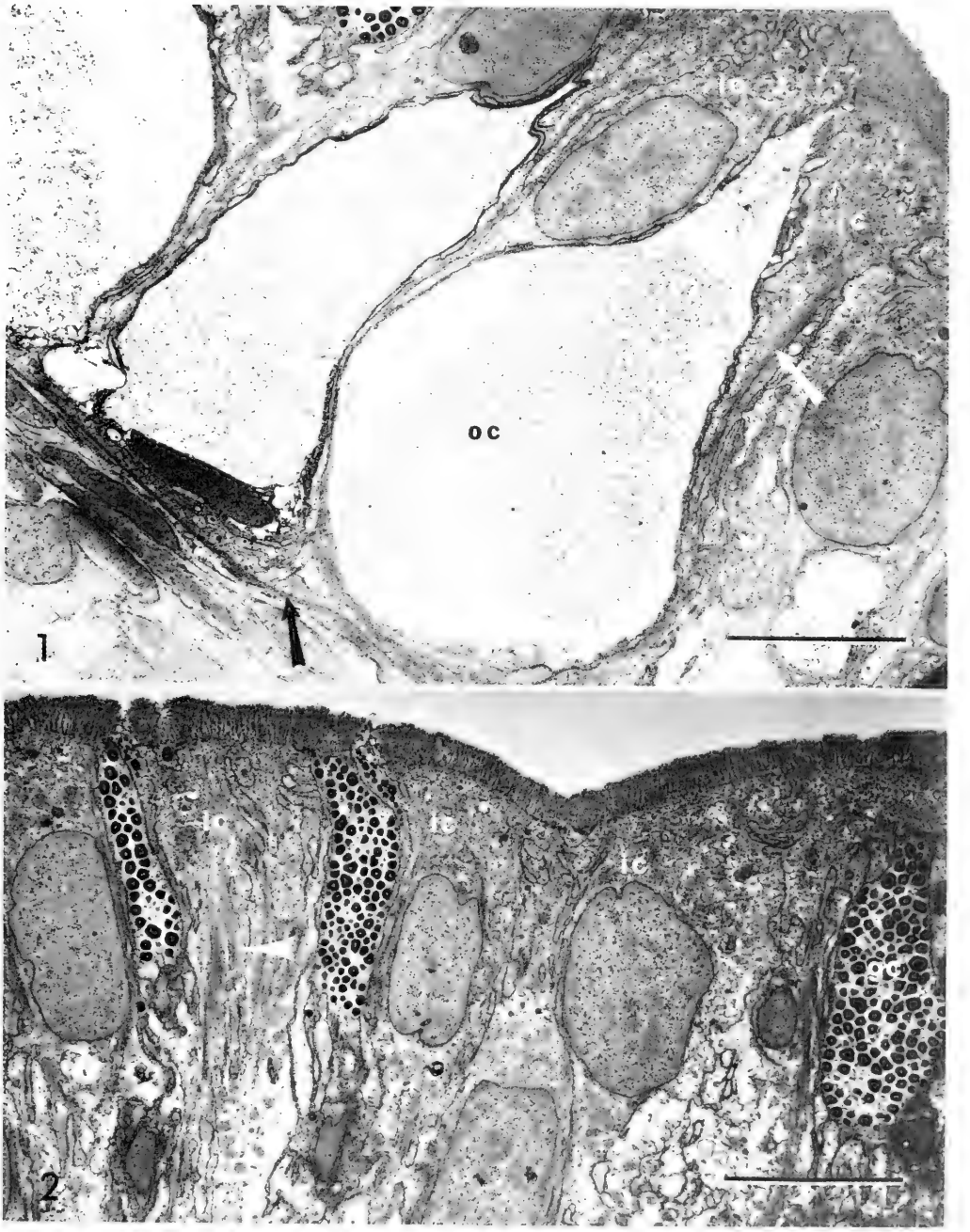

FIG. 1. Electron micrograph of a cross section of dorsal epithelium. Sac-like ovate cells (oc) lie between interstitial cells (ic) which appear to deflect the lateral surfaces of the ovate cells. Interstitial cells extend microfilament-filled process toward the basement membrane (arrow). Bar = 10 μm. FIG. 2. Goblet cells (gc) often appear grouped, but are always interspersed among interstitial cells (ic). Note intracellular filaments in ic's (pointer). Bar = 10 μm.

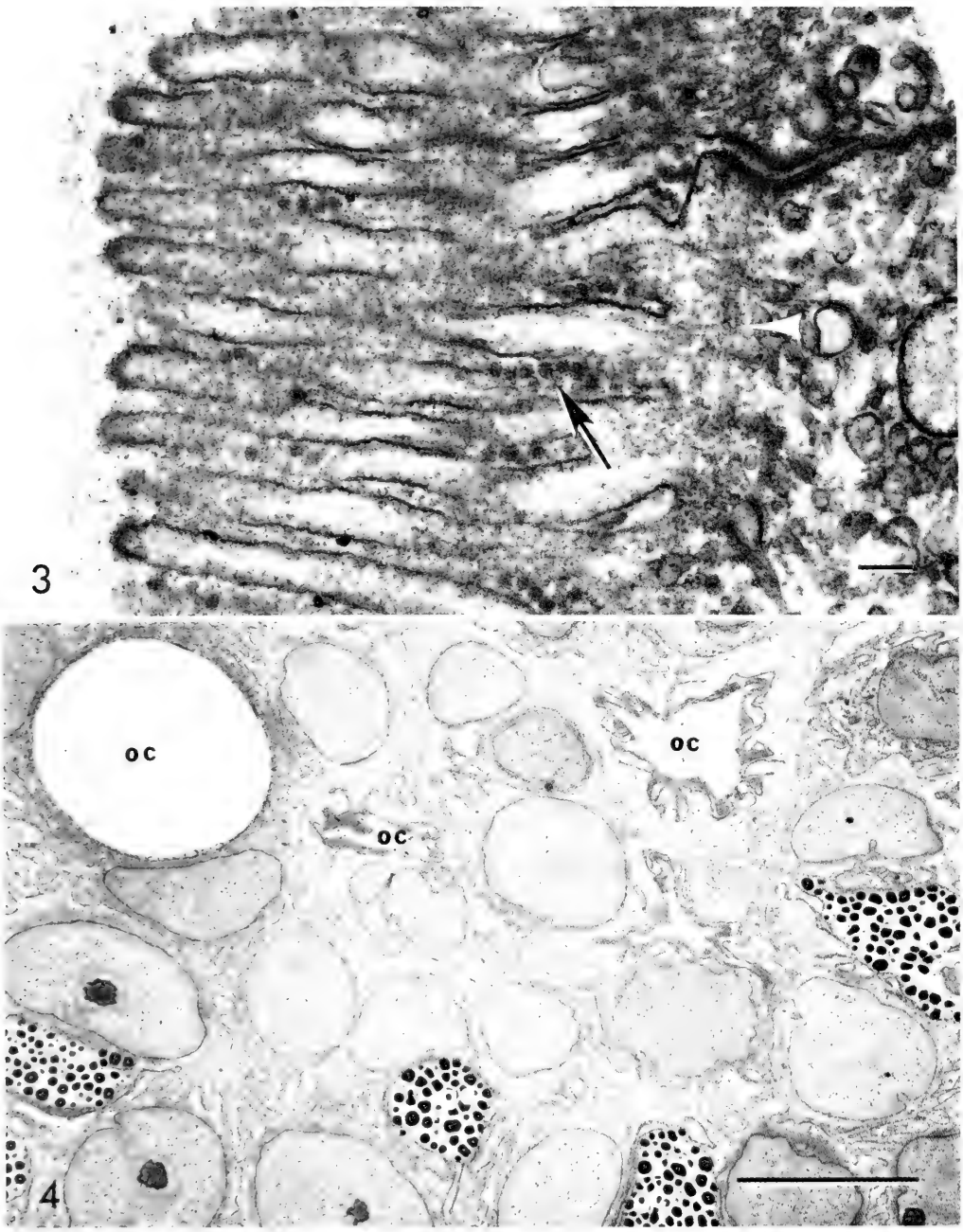

FIG. 3. Microvillous surface at high magnification. The surface mucous layer is fibrous and contains no large granular component. Electron dense granules lie between the base of microvilli even in ic's (arrow). Note the microfilamentous networks in the cell cortex (pointer). Bar = 200 nm. FIG. 4. This tangential section illustrates the relative abundance of interstitial cells. All nuclei visible in this section are interstitial cell nuclei. This section is cut below the infolded apical ends of the goblet cells. Bar = 10 μm.

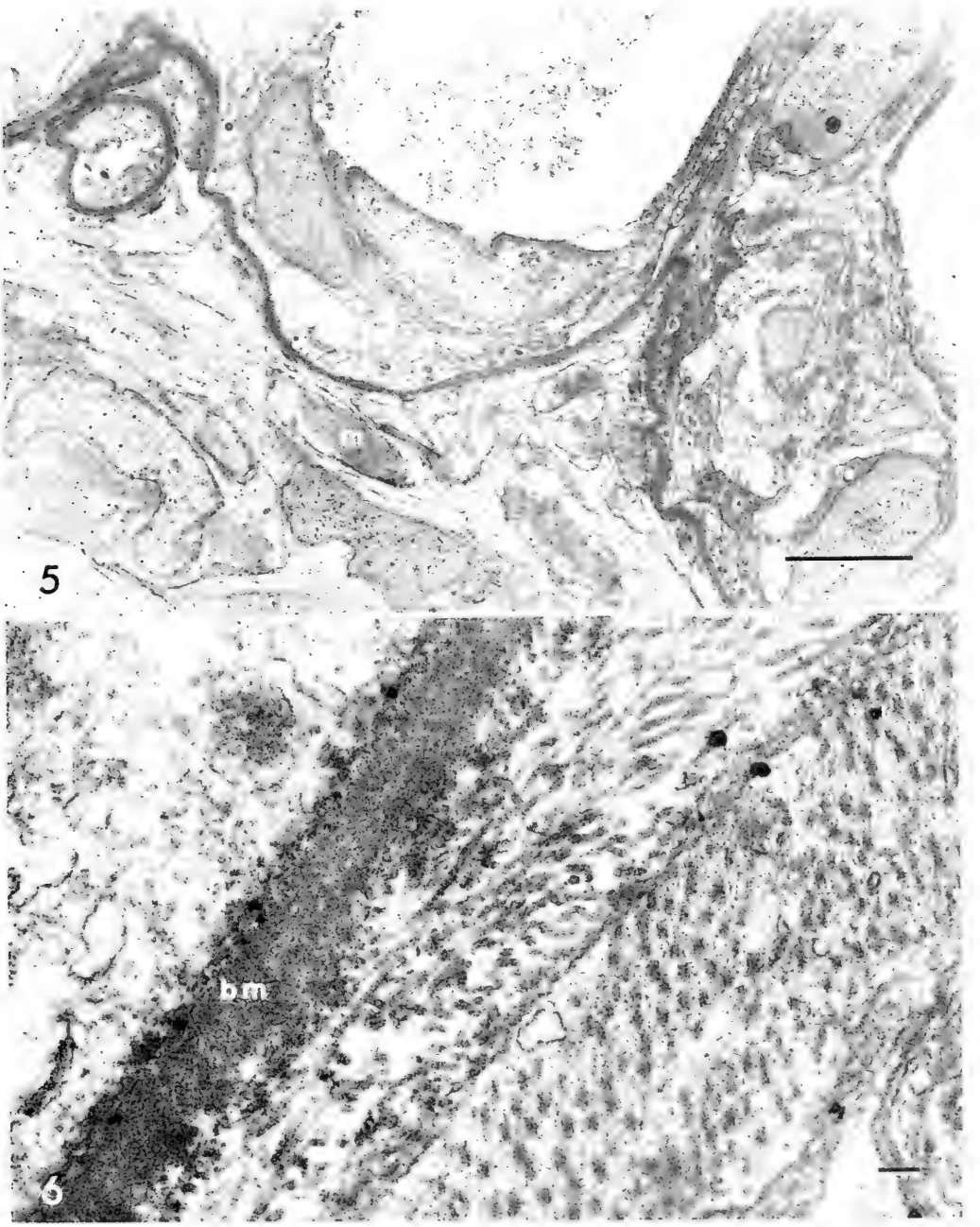

FIG. 5. The basal region of the epithelium is often folded as illustrated here. The electron dense granules of glial cells (gl) are prominent beneath the ovate cells. Mantle muscles (m) underlie the epithelium. Bar 5 μm. FIG. 6. High magnification micrograph of the basement membrane (bm) illustrating the relationship of underlying collagen fibrils (c) and dermal muscle (M). Bar = 100 nm.

5 nm to 8 nm filaments (Fig. 7). During secretory activity these filaments become rearranged and tightly packed. In addition, they appear to produce deep folds in the plasma membrane (Figs. 4, 8). Such folding begins near the apical end of the cells and progresses toward the basal end. The thickness of the peripheral cytoplasm in fully evacuated ovate cells is approximately twice that of presecretory ovate cells.

Goblet Cells. Goblet cells are rounded at their bases, possess roughly ovate nuclei, taper inward toward their apical ends (Fig. 2) and have rounded cross sectional profiles except at their apices (Figs. 4, 9). Toward their apices the cells are infolded laterally, producing a "pansy-like" profile in tangential section (Fig. 10). A branching profile is often seen in cells cut longitudinally (Fig. 11). The microvillate surface of these cells is much smaller in area compared with the interstitial cells and the microvilli are frequently much shorter (Figs. 9, 11).

Numerous microtubules (Mt) are observed oriented along the long axes of goblet cells (Fig. 9). These microtubules are always most numerous in the portion of the cells which appear to be actively secreting (Figs. 9, 10), and are frequently seen in close proximity to the secretory granules.

The highly electron-dense secretory granules are spherical and appear to vary considerably in size. The largest are typically 0.5 μm to 0.7 μm in diameter. The density of these granules is uniform and each is surrounded by a single unit membrane (Fig. 11). During active secretion the granules appear to break up into smaller units as they approach the apical surface (Figs. 9, 10).

The periodate reactivity of these granules and the surface mucous layer visualized by light microscopy is confirmed by the periodic acid-thiocarbohydrazide-silver proteinate (PATCSP) method of Thiéry (1967) (Fig. 12). The density of silver grains is uniformly greater over the large secretory granules than over the cytoplasm. The mucous layer at the surface also reacts strongly, with the grains found only over individual fibers. In contrast, the 35 nm to 45 nm granules lying between the microvilli are unreactive.

Near the bases of the goblet cells one typically observes a large rER-Golgi complex (Fig. 13). The Golgi is generally more massive than the rER and the Golgi saccules display a graded electron density.

Interstitial Cells. The TEM reveals that the interstitial cells span the full thickness of the epidermis (Fig. 2). In most interstitial cells the nucleus and the majority of the cytoplasm is concentrated near the epithelial surface (Figs. 2, 4). The remainder generally extends in a narrow process which contacts the basement membrane. The cytoplasm of these cells is typically filled with thick (10 nm to 15 nm) and thin (5 nm to 8 nm) filaments (Figs. 2, 11) which are especially prominent in the basal processes (Fig. 13).

Interstitial cells also possess small, membrane bound vesicles containing material with a non-uniform electron density (Figs. 8, 10). Although these granules may be for secretion, no conclusive evidence of their exocytosis has been observed.

During the preparation of tissues for the TEM, particles of anomalous material were often retained on the microvillous surface of the epithelium (Fig. 14). Such particles appear to be firmly attached to the microvilli by means of the fibrous surface mucus. No particles were observed adhering to the ventral skin.

DISCUSSION

Histochemistry provides some indication of the general characteristics of *Euprymna*'s epithelial secretions. The secretory products of the two glandular cells appear to be protein-polysaccharide complexes.

The secretory material produced by goblet cells appears to be a neutral mucopolysaccharide. This material is highly periodate-reactive indicating the presence of vicinal hydroxyl groups (Table 1). The reaction of this material to PAD and MD staining confirm its periodate reactivity and do not suggest the presence of hexose or deoxyhexose units other than glucose. The specific localization of periodate reactivity in both goblet cell granules and the surface layer is further confirmed by the PATCSP method (Fig. 12).

The lack of staining observed in goblet cell granules with AA, AF, LID and HID (Tables 2, 3) suggest the absence of strongly acidic groups. Whereas slight reactivity observed with TB and CI indicates the possibility that some carboxyl groups are present, is not confirmed by AF or LID staining. These results suggest that the observed periodate reactivity is due to vicinal hydroxyls rather than closely associated hydroxyl and carboxyl groups.

The goblet cell mucin displays moderate

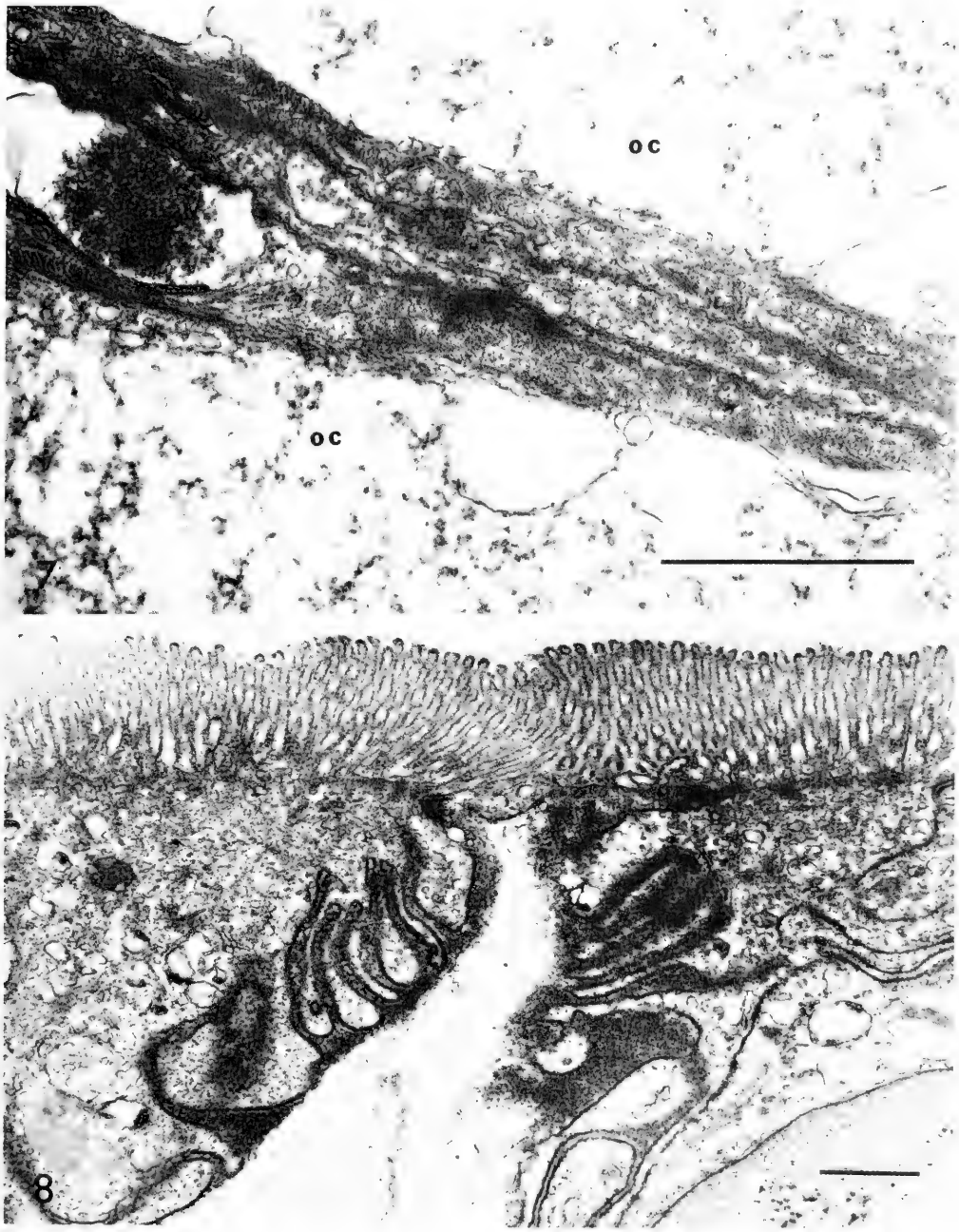

FIG. 7. Micrograph illustrating orientation of filaments within the peripheral cytoplasm of two adjacent ovate cells (oc). Bar = 1 μm. FIG. 8. Micrograph illustrating the folded aspect of the apical end of an ovate cell. Note the appearance of the peripheral filaments. Bar = 1 μm.

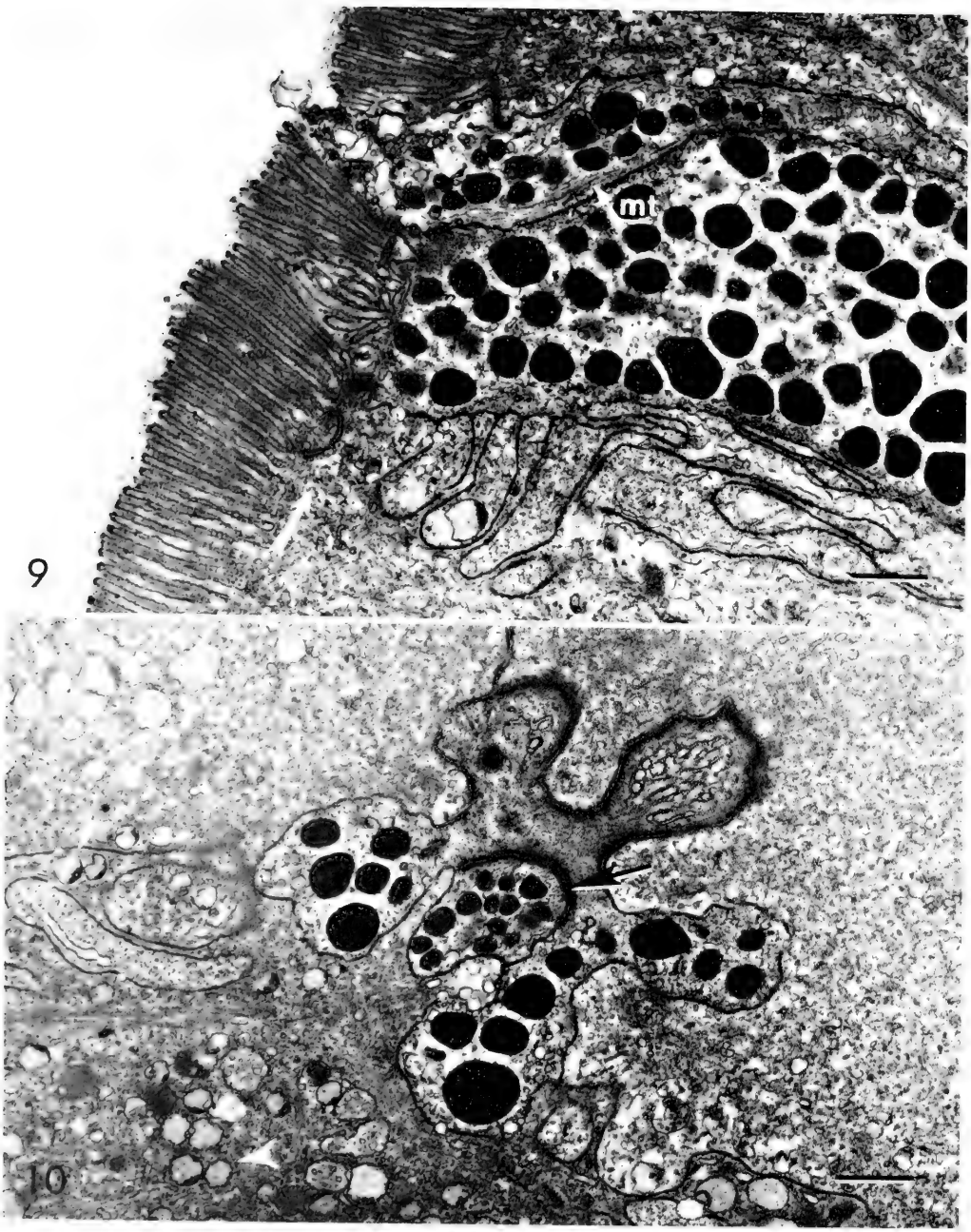

FIG. 9. A goblet cell illustrating apical narrowing and the presence of an apparently actively secreting segment. Note the orientation of microtubules (mt) within this area and the dispersion of the large secretory granules. Bar = 1 μm. FIG. 10. Illustration of the "pansy-like" profile of a goblet cell cut tangential to the surface at the level indicated by the large arrow in Fig. 9. Note the abundance of microtubules in the actively secreting branch (arrow). The non-uniformly electron dense granules of interstitial cells (arrowheads) are most numerous near the surface of the epithelium. Bar = 1 μm.

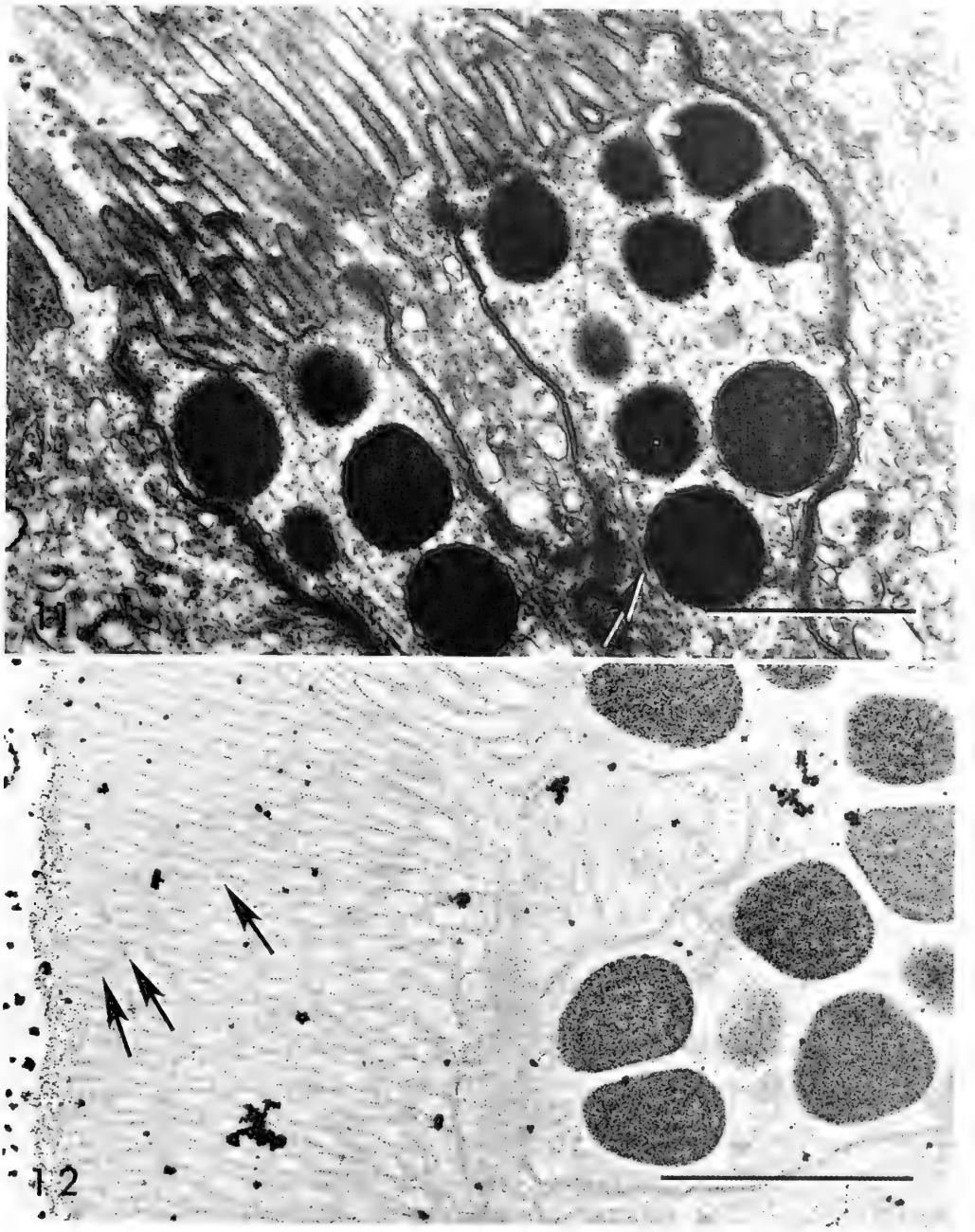

FIG. 11. Cross section at the apex of a goblet cell. The large membrane-bound granules are often seen in close proximity to microtubules (arrow). The apical ends of the cells typically form desmosomal junctions with neighboring cells. Bar = 1 μm. FIG. 12. This section stained by the PATCSP method illustrates the localization of periodate reactivity. Silver grains are prominent over goblet cell granules and the surface mucous layer whereas the small granules between microvilli are unreactive (arrows). Bar = 1 μm.

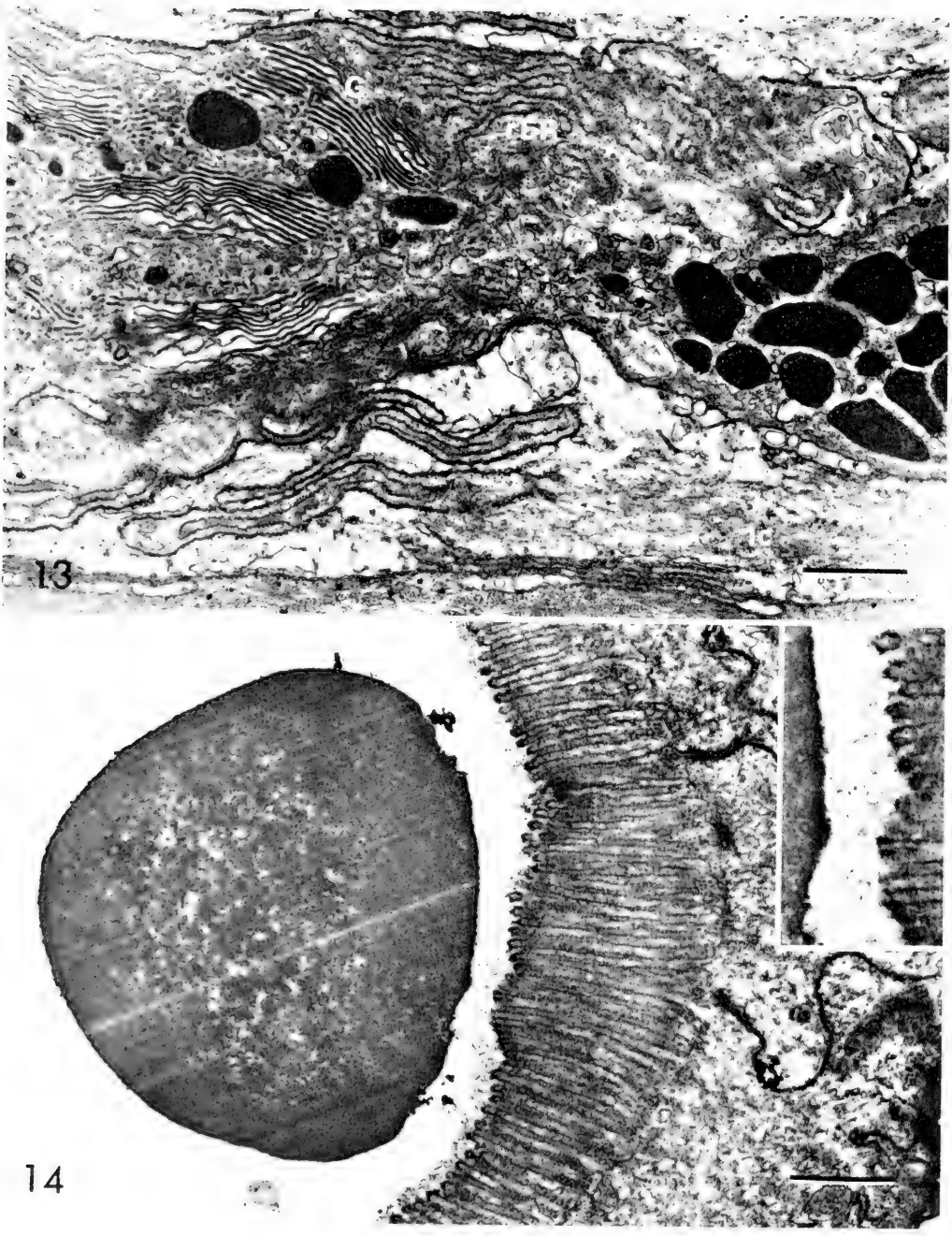

FIG. 13. Micrograph illustrating the rER-Golgi complex of goblet cells. In these cells the Golgi (G) was relatively more massive than the rough endoplasmic reticulum (rER). The adjacent interstitial cells (ic) contained large numbers of thin filaments which were often seen connected to highly folded membrane (arrow). Bar = 1 μm. FIG. 14. Micrograph of the microvillous epithelial surface with an associated particle of unknown composition. The fibrous surface mucus appears to be attached both to the microvilli and the particle (inset, ×30,000). Bar = 1 μm.

reactivity to all stains for protein (Table 1). The positive staining with BS at all pH levels below pH 10.5 suggests the presence of neutral or acidic proteins. The biphasic nature of staining with ASC is consistent with the results with BS. The blue coloration of the mucous granules suggests the presence of acidic protein, and is contrasted with the orange color produced by basic proteins in the cytoplasm.

The secretory material of the ovate cells appears to be a highly sulfated protein-polysaccharide complex. This material is periodate-unreactive (Table 1) and is strongly reactive with tests for basic protein. Their negative PAS reactivity and the lack of a positive reaction to tests for acidic groups by the majority of ovate cells, contrasted with the very strong staining of this material in evacuating ovate cells suggests that acidic groups are present but masked in the unsecreted material. It is possible that a protein component is interfering with the staining of the polysaccharide moiety (Pearse, 1968). If this is the case, then in order to produce the observed color differences there must be an alteration in the protein-polysaccharide association when this material comes into contact with sea water. In this regard, the biphasic staining of granules fixed after partial exocytosis is of considerable interest. With ASC at pH 2.1 (Table 1) the portion of the secretion product in contact with the exterior stained blue, indicating a shift from acidophilia to basophilia. Similar shifts are seen with thionin, LID, HID and AF staining (Table 3). In addition, the γ-metachromasia observed in evacuating ovate cells with AA and TB and comparison of the reactivity of ovate and evacuating ovate cells with AB and CI (Table 2) suggests an unmasking of strongly acidic groups. The marked reactivity of evacuating ovate cells suggests further that these are sulfate groups. The lack of a positive Bial reaction with this, as well as other materials in the epithelium, is consistent with previous results indicating a lack of sialic acids in invertebrates (Ravetto, 1964).

Inasmuch as the histochemical nature of the surface mucus parallels closely that of the goblet cell mucin, one might assume that this material consists largely of goblet cell secretions. The observed differences in their reactivity to stains for protein, however, argues that the secretory material is considerably modified. What these changes might consist of remains to be revealed by biochemical analysis. How such alterations might be involved in the functions of these materials during sand adhesion and release also remain to be studied.

The observation that goblet cells are found only in the dorsal epithelium, which is the only area of the surface where sand adhesion occurs, suggests that the secretory product of these cells may impart the adhesive characteristic of the surface mucus.

The presence of a massive, membrane-associated filamentous layer in the peripheral cytoplasm of ovate cells (Fig. 7) and the close proximity of gliointerstitial-nerve cell complexes (Nolte et al., 1976; Nicaise, 1973) to their bases (Fig. 5) suggests that the rate of secretion by ovate cells may be relatively rapid. By comparison, goblet cells contain no microfilamentous arrays and are seldom associated with glial cells (Figs. 2, 9, 10). This, in addition to the presence of oriented microtubules close to the secretory granules and the fact that only one segment of these cells appears to be actively secreting, suggests that goblet cells may secrete at a slower rate than ovate cells. If such differences in rates of secretion in fact exist, it would explain the observation (unpublished) that subsequent to the release of adherent sand, there is a period of several minutes during which squids are refractory to sand adhesion.

How then might sand release be accomplished? Since ovate cells secrete a highly acidic mucoprotein and are distributed over most of the squid's body, they would appear to be the most likely candidate for a means of accomplishing sand release (deadhesion). Highly sulfated mucins are generally "slimy" materials (Hunt, 1970), a character that fits the proposed role of these cells. Secretion of such a material by the ovate cells might cause the adhesive mucous layer and adherent sand to be lifted away from the microvillate surface. After release the adhesive characteristic of the surface would be restored.

The folded aspect of the epidermal basement membrane viewed in cross section (Fig. 5) suggests the possibility that active deformation of the skin might also be involved in deadhesion. Such deformations could be mediated by the layers of dermal as well as mantle musculature and/or possibly by the interstitial cells. The presence of large numbers of oriented thick and thin filaments within the basal extensions of interstitial cells (Fig. 2, 5, 13) suggests that these cells may possess contractile capabilities. Contraction of these

cells would not only result in a folding of the epithelial layer, but would also augment the secretory activity of the ovate cells.

Unfortunately, histochemistry and the TEM provide a static picture of the structure of *Euprymna*'s epithelial covering. A clear understanding of the mechanisms involved in *Euprymna*'s camouflaging behavior must await both the biochemical characterization of its epithelial secretions, including the putative secretory product of interstitial cells (Figs. 3, 10), as well as experimental evidence for the mechanisms of the control of secretory activity.

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MORPHOGENESIS OF CHROMATOPHORE PATTERNS IN CEPHALOPODS: ARE MORPHOLOGICAL AND PHYSIOLOGICAL 'UNITS' THE SAME?

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ABSTRACT

This paper discusses the differences between the two kinds of units in the skin of cephalopod molluscs that are recognized as entities of visible pattern generation—one anatomical and the other physiological—and points out that they are related in morphogenesis: i.e. to pattern generation in the developmental sense. The 'chromatic unit,' containing all the anatomically fixed elements (iridophores, leucophores, chromatophores) deployed in color change, should not be confused with the various transient physiological units (e.g. 'motor unit' neurophysiologically definable as a motoneurone and the particular elements it innervates). The anatomical unit develops through processes that parcel the skin up into compartments 1–4 mm across giving rise to the patches of *Octopus* and their equivalent (the large dark chromatophores and their satellites underlain by iridescent cells) in loliginid squids. Neurophysiological units too are potentially derivable as members of a developmental series; each is a *chronological unit* innervating a particular age-class of chromatophore elements identifiable by their size, color and relative positions in the morphological array but not confined to one particular anatomical unit. Examples are given of the different appearances produced by these units in the squids *Lolliguncula brevis* (Blainville, 1823) and *Loligo plei* Blainville, 1823 and in *Octopus vulgaris* (Lamarck, 1798) and *Octopus rubescens* (Berry, 1953). Nine rules are listed for the interpretation of chromatophore clusters based on *Octopus* studies but generalizable to other cephalopods.

Key words: octopus; squid; skin; pattern; morphogenesis; electrophysiology.

INTRODUCTION

The question of what it is that allows octopuses and squids to exhibit a range of patterns within the space of a few seconds is in one way easily answered. Distributed over the transparent body surface are chromatophores that expand or retract under nervous control: areas of expanded chromatophores turn dark or colored while the rest of the skin remains white or translucent. But in this relatively simple statement lurks a dilemma for the biologist, that has to do with the old dichotomy between form and function: anatomy and physiology. The arrangement of individual chromatophores is as much anatomically fixed in the skin as are the positions of light bulbs in an illustrated bill-board or the arrangement of muscles in the rest of the body, and it alters only with growth processes. But the patterns seen are transient phenomena that result from the various ways in which the chromatophore elements are switched on by electrical activities originating in the central nervous system. It would be foolish to try to account for the various displays that appear on an illuminated bill-board by giving a detailed description of the two-dimensional matrix of light bulbs making up the bill-board when such displays are a function of spatio-temporal connections encoded in a central programme. And yet a description of the matrix is necessary if one wants to account for the quality of the pictures displayed: for instance, the amount of detail or grain, and the colors available. In describing how patterns are generated, there is no easy way of resolving the dilemma of whether to adopt a morphological or a physiological approach, for both are needed.

This paper is intended a) to clear up a source of confusion that has already crept into the relatively young literature on body patterning in cephalopods, resulting from use of the word 'unit' both in physiological and in morphological senses; b) to show that the problem of choosing whether patterns are to be described in terms of the static morphological

array of chromatopores or in terms of the physiological responses (i.e. the *events* that play upon the array) can in part be resolved by recognizing that the two have common origins in *morphogenesis*. That is, by following the course of 'pattern generation' in the developmental sense.

The word 'unit'

(i) the term '*motor unit*' has been used by Maynard (1967) for the cuttlefish *Sepia*, Florey (1969) for the squid *Loligo* and by Packard (1974) and Packard & Hochberg (1977) for *Octopus*, on the basis of neurophysiological findings;
(ii) the term '*chromatic unit*' was coined by Packard & Sanders (1971) for the characteristic patch structure of the skin of octopuses and used by Hanlon (1982) for squids.

Motor units

Both Florey and Maynard used the term *motor unit*—in the classical sense of a motoneurone and the muscle fibers it innervates—to describe the responses obtained from electrical stimulation of nerve branches to the skin. They succeeded in isolating the all-or-nothing resonses of individual nerve fibres either by paring down the nerve branches or by adjusting the stimulus strength until it was clear that only single fibres were involved. The same rules hold for both the squid *Loligo* and the cuttlefish *Sepia*. One motoneurone innervates many chromatophores, and a single chromatophore can be innervated by more than one motoneurone. These two conditions are summarized in Florey's fig. 3.

Multiple innervation of chromatophores (i.e. the fact that the muscles radiating from a chromatophore are not necessarily innervated by branches of the same motoneurone) means that one and the same chromatophore can participate differently in different patterns. Maynard gave a dramatic example of this when he showed that some of the dark chromatophores (melanophores) in the units he was studying are used either to enhance a white spot—through contraction of the muscle fibres on the side of the melanophore away from the spot, giving the spot a dark edge—or

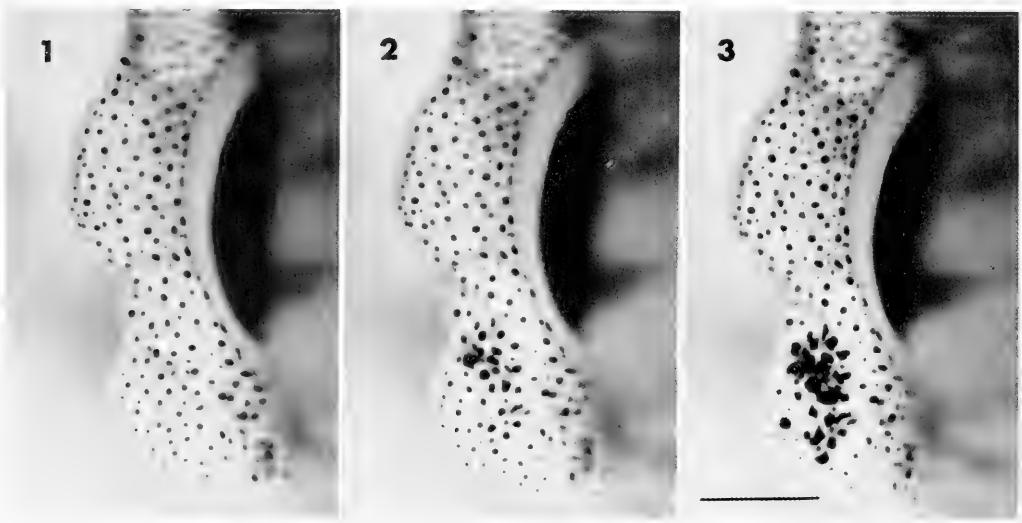

FIG. 1. Lower 'lid' of the left eye of a young *Octopus rubescens* (6 g body weight) showing a dark spot flashing on and off during partial alcohol anaesthesia. The constancy of this spot over several on/off cycles suggests it is part of a single motor unit (see text). Close inspection of the photographs reveals that the chromatophores involved in the response—seen in the resting, 'off' condition in 1, partly 'on' in 2 (all elements not yet invaded) and 'on' in 2—can be characterized by larger resting sizes than their immediate unresponsive neighbours. Most of the responding chromatophores occupy the slopes of a groove separating two patches.

Photographs taken with Wild 'Makro' optics and automatic camera using flash illumination and fibre optics light source. Scale bar 0.5 mm.

else to do the opposite, namely to help screen the white spot (through a second motoneurone that innervates the muscle fibres on the side over the spot). He cited this phenomenon as an illustration of what he called pattern-position separation. For our purposes of finding a suitable candidate to be classed as a unit of patterning, such "double agents," shifting one way or another depending on which motoneurone is firing, are clearly unsuitable. Single chromatophores are not units of physiological pattern.

The motor units of Florey and Maynard have the further characteristic that they play a recognizable part in generating whole body patterns. White spots on a dark ground are

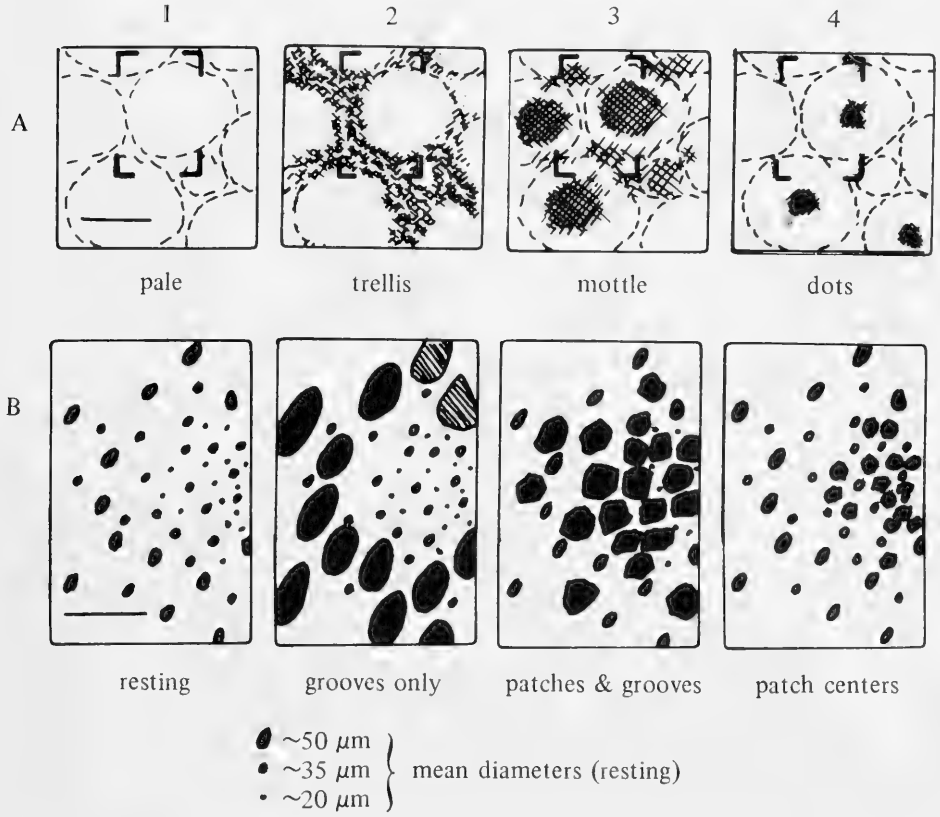

FIG. 2. Drawings of the dorsal skin of *Octopus vulgaris* (adult) showing 3 kinds of physiological unit occupying a region of patch and groove units, seen in the pale (resting) condition in 1. A) As they might appear during natural patterning (scale bar 1 mm). B) Details of the responses in the area indicated seen at the level of individual chromatophores (scale bar 0.5 mm).

Note that (a) in drawings 1–4 the same patches are present in A and the same chromatophores are present in B, (b) responses respect the patch and groove arrangement of the skin but are not confined to one patch and are not necessarily continuous with it, (c) responding chromatophores of the different physiological units, 2, 3 and 4 occupy complementary positions within a patch unit, (d) they belong to different resting size classes in descending order from the left.

Responses were obtained by electrical stimulation of the skin. In these units, only dark chromatophores (melanophores) are involved. The hatched melanophores in B2 are intended as an indication that more than one motor unit takes part in the groove response. N.B. Chromatophores not involved in any of the three kinds of response are not figured.

The drawing is a simplified version of the condition in life: e.g. there are fewer chromatopores than in life, and there are no shared chromatophores whereas in life 10–20% of the melanophores of neighboring size classes are shared by such complementary units. (After Packard, 1978). The area studied is just proximal to the large white spot on the second arm of *O. vulgaris*.

characteristic of some of the patterns of the cuttlefish; while they have other patterns in which the reverse is true: the white spots are overlain by dark screens. In other words, the motor units are also units of visible patterning.

A similar picture emerges in *Octopus* both during spontaneous patterning (Fig. 1) and following direct stimulation of the skin, evoking axon reflexes. Electrical stimulation produces darkening over definite areas that are recognizable as features of patterning displayed by the octopus in its natural environment (Fig. 2). A notable example is the eyespot of the two-spotted octopus *O. bimaculoides* (Packard & Hochberg, 1977). These features were originally referred to as chromatophore fields (Packard, 1974). The question of how many nerve fibres might be involved was unanswered.

Morphological units

Various details of the make-up of the patch and groove structure called—for better or worse—a chromatic unit have now been given, particularly for parts of the dorsal skin of *Octopus vulgaris* (Packard & Sanders, 1971; Messenger, 1974; Packard & Hochberg, 1977; Froesch & Messenger, 1978). Variations in the arrangement of the patches between one species of *Octopus* and another are tabled in Packard & Hochberg (1977).

The idea (Packard & Sanders, 1969; Packard, 1972) that the patches, with their combination of reflecting elements and dark screening chromatophores (melanophores), enable the octopus to achieve part of its camouflage by tone-matching (through neutral density filtering of the light striking the skin) has now been experimentally established by Messenger (1974), Froesch & Messenger (1978), and Messenger (1979) who have drawn particular attention to the arrangement of the leucophores. (See Discussion.)

The equivalent of the octopus patch and groove chromatic unit, which contains hundreds of chromatophores and many leucophores in an area little more than 1 mm², is not immediately apparent in other cephalopods. Squid chromatophores are almost an order of magnitude larger than octopus ones, and local densities are of the order of ten per square millimeter compared with 300 to 400 in *Octopus*. However, in loliginid squids (Figs. 3–5) the dark chromatophores (brown and red) on the dorsal mantle and elsewhere are arranged as a series of continuous and intersecting circles 1–2 mm across, each circle with a single large dark chromatophore at its

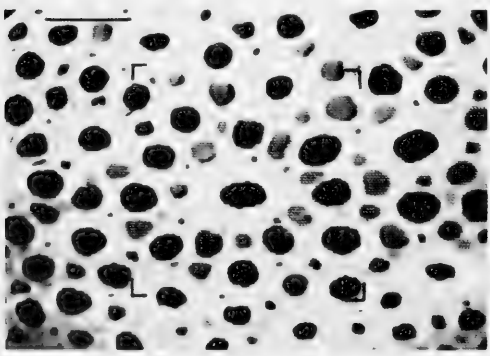

FIG. 3. Typical series of morphological units on dorsal mantle surface of loliginid squid *Loligo plei*, each centered on a single large dark chromatophore. Most chromatophore classes are physiologically expanded. Area framed in middle of photograph is analyzed in Fig. 4. Scale bar 2 mm.

The photograph is of the TV monitor screen taken during playback of a living squid exhibiting natural patterning recorded on videocasette by black and white TV camera through Wild dissecting microscope.

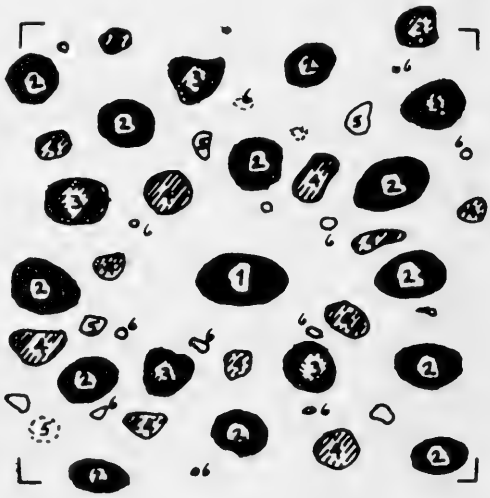

FIG. 4. Rough classification of individual chromatophores arrived at by inspection of the area indicated in Fig. 3 (*Loligo plei*). The classification is based on descending order of size and of pigment density. The numbers (2–5 etc.) give the putative succession in which chromatophores were recruited into the morphological unit centered on 1 (and into neighboring units) and define their membership in the various age-dependent physiological units (see Fig. 5 and text: Rules for the interpretation of chromatophore clusters).

centre and with pale (yellow) ones interspersed (Fioroni, 1965; Hanlon, 1982). The circles have similar dimensions to the patches of the octopus, and, like the patches, they are, in many parts of the body underlain by conspicuous accumulations of reflecting material (see Hanlon, 1982). Each circle is a morphological unit with the same status as the patch and surrounding groove of *Octopus. Each morphological unit contains all of the elements that contribute to patterning in that part of the skin.*

Morphogenetic Interpretation of Chromatophore Clusters

The rules for the development of the morphological array (morphogenesis) of chromatophore clusters have been worked out for *Octopus* but appear to be general. They are based on inspection of photographs taken in the plane of the skin surface with macro-optics at different stages of ontogeny and during different phases of activity of the different classes of chromatophore: at rest, spontaneously active and electrically stimulated. (The word recruitment is used in the sense of recruitment of new members into a population.)

1. *General recruitment.* Recruitment of chromatophores into the mantle, head and arm fields occurs from germinal cells spread throughout each expanding field.

2. *Field effects.* The rate of recruitment varies from one part of the field to another according to proximo-distal and dorso-ventral gradients established early in ontogeny (Fioroni, 1965) and to local conditions that alter with recruitment.

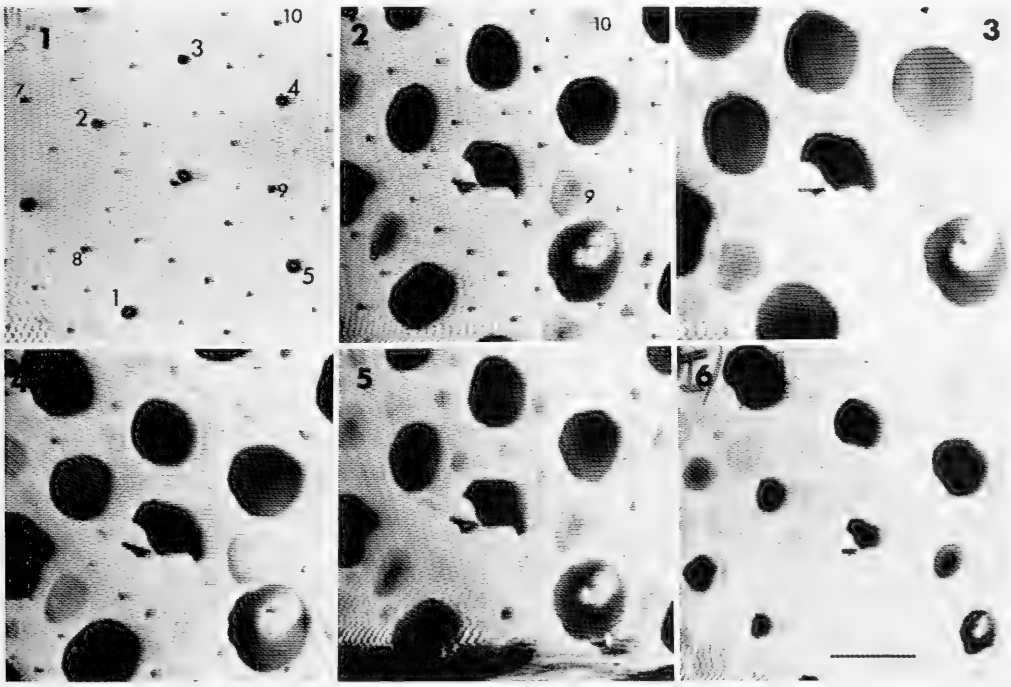

FIG. 5. Details of the responses of dorsal mantle chromatophores of the squid *Lolliguncula brevis* recorded with black-and-white video-technique (see caption to Fig. 3) during natural patterning. The same chromatophores are present in all six photographs; the central one appears torn and has been chosen as a marker.

1–3 serial expansion of different size classes. 1, all chromatophores at resting size (i.e. fully retracted); 2, responses confined to the larger resting-size classes (i.e. central chromatophore and chromatophores numbered 1–5 and 7–10 etc.); 3, all classes responding (N.B. in the expanded state yellow chromatophores are barely visible, but none remain in the resting state: cf. first photograph (1)).

4–6 partial responses of the array. 4 and 5 subdivision of the yellow chromatophores (i.e. smaller size classes) into complementary units whose boundary lies half way across the unit (compare photographs 4 and 5); 6 reduced responses of the large (dark) chromatophores (compare with 2) with near-maximal responses of the smaller size-classes (yellow).

3. *Field subdivision.* As each field expands, sub-fields arise within it.

4. *Position and size constancy.* Once established, a chromatophore does not alter its position in the field nor are there appreciable changes in resting size.

5. *Mutual relationship.* Established chromatophores influence the position, size and rate of differentiation of further chromatophores arising in their neighborhood through processes of *lateral inhibition* which result in chromatophores being spaced out relative to each other.

6. *Size hierarchy and age.* Chromatophores can be ranked according to size. The size-hierarchy (based on resting diameters) in a cluster reflects the order in which the chromatophores were born. The largest chromatophores are the earliest, the smallest are the latest. (N.B. The size hierarchy is maintained when all chromatophores in the cluster are uniformly expanded.)

7. *Age and color.* When first born, chromatophores are pale (usually yellow or orange in color) and become progressively darker with age as the density of the pigment increases.

8. *Color and degree of expansion.* The depth of a chromatophore's color is inversely proportional to its degree of physiological expansion.

9. *Separate responses.* Chromatophores of a given size and color class are able to expand or retract independently from other size and color classes (Figs. 2, 5), i.e. *each age class has its own innervation and muscle connections.*

These largely descriptive rules for understanding two-dimensional pictures in terms of the time dimension can be converted into *Rules for the Conduct of Young Chromatophores* such as Rule 4 "Stay put once you have arrived," Rules 5 and 6 "keep clear of neighbors already established and never grow larger than they."

Figure 4 is an example of how the rules can be applied for the interpretation of close-up photographs of the skin.

Figure 6 is a visual summary of the rules at work. *Every* structure is but a frozen stage of growth and the squid skin is not exceptional in this, but because comparatively few chromatophores are present and they are all size- and color-coded, the morphogenetic history of the skin can be followed with great clarity even in the adult. Here we find all four dimensions collapsed, as it were, into one plane.

Figure 6 also answers the question posed in the title of this paper:

Question: Are morphological and physiological units the same?

Answer: See how they grow.

DISCUSSION

This is not the place to go into details of how the rules for pattern generation were arrived at, but, as they have not been published elsewhere, some discussion is appropriate.

They are general rules that satisfy only part of the description. There are no values attached, and they say nothing about a number of interesting questions such as: 1) whether all yellow chromatopores eventually turn dark; 2) what is the influence of chromatophores upon the distribution of reflecting elements (leucophores and iridophores) which also contribute to a unit; 3) how do new units arise; 4) what accounts for the wide variety of units found in some species; 5) what are the details of innervation.

1) There are morphological units in which some of the yellow chromatophores are larger (in expanded diameter) than some of their darker neighbors. They may belong to an early age-class that is programmed to remain yellow/orange throughout ontogeny.

2) As Hanlon (1982) has shown, reflecting elements underly the central chromatophore of a morphological unit in loliginid squids much as the units are centered upon patches of reflecting elements in octopuses (see above: 'Morphological Units'). Presumably, both in octopuses and in squids, these reflecting elements (leucophores and iridophores) are exposed to the same sources of positional information that determines the differentiation of chromatophores. The empty spaces unoccupied by leucophores lying immediately below dark chromatophores in the small arm patches of *Octopus vulgaris* (Froesch & Messenger, 1978) can be interpreted as meaning that the leucophores are born later than the chromatophore they appear to surround and outside its sphere of inhibition.

3) New morphological units arise as the two-dimensional field expands. It appears that local centres of pattern generation concentric with the morphological units have a radius of influence limited to one or two millimeters, and that new centres will arise in areas of the expanding field that lie beyond this distance.

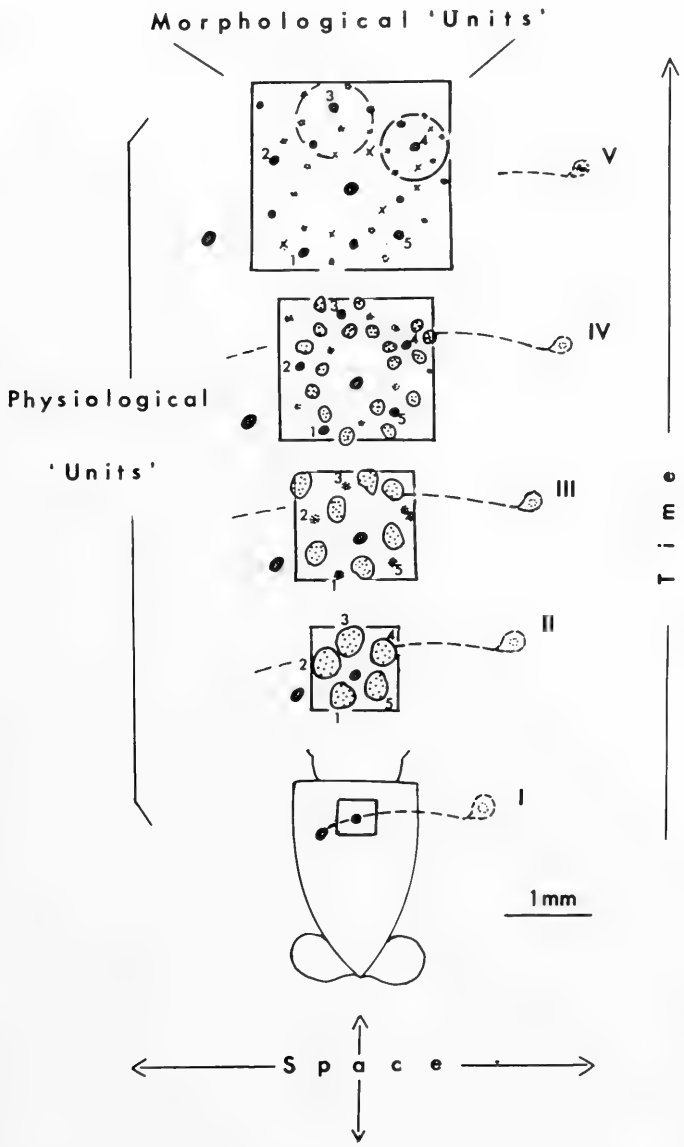

FIG. 6. Diagrammatic representation of the pattern of recruitment of chromatophores to form a single 'morphological unit' in the dorsal skin of a young squid during a 5-fold increase in linear size of the region indicated. Resting sizes are slightly exaggerated. Five successive age-classes of chromatophore (I–V) differing in size and colour at any one moment (see text) are figured each theoretically with its own nerve supply (right). At first appearance, age-classes II–IV are shown physiologically expanded, through contraction of their muscle fibers. Members of age-class V (x) are still being born and their nerve supply is in the process of growing in. Note that the members of a particular age-class have smaller resting diameters than their predecessors, and that chromatopores retain their relative positions during ontogeny but turn darker with time. In the last frame, two new 'morphological units,' centered on chromatophores 3 and 4 (age-class II), are seen in the process of formation.

Such a nascent unit is seen in the final state of the diagrammatic unit figured in Fig. 6.

This explanation of the patterns seen invokes the principle of lateral inhibition (Rule 5) not only within a unit, i.e. at the level of the spacing between individual chromatophore (and reflecting) elements, but also between units. A complete mathematical model for the role of lateral inhibition in morphogenesis was developed by Meinhardt & Gierer (1974).

4) The different appearance of the units from one part of the skin surface to another presumably reflects different local epigenetic conditions such as changes in the amount and distribution of inhibitor substance present or alterations in the rate of diffusion of a morphogen. Sometimes one gets a direct insight into the processes at work by inspection. The white spot on the mantle of octopuses which contains units that are separately innervated from the area immediately ahead of the white spot, starts as a forward-facing crescent of reflecting material behind which the local density of chromatophores is lower than in front and to the sides of the crescent. It is as if the crescent marked the edge of a partial barrier in the skin to morphogens (or activator substances) diffusing in a posterior direction, the polarity of the field being set with reference to the head (Rule 2). The area ahead of the white spot goes characteristically dark during patterning, the area over the white spot goes pale. The arm white spots have the same polarity (with respect to the head) as the mantle white spots (i.e. their polarity is rotated 180° with respect to the antero-posterior axis).

The straight pale areas that form part of the lateral flame of *Loligo plei* (Hanlon, 1982) where none but a few large dark chromatophores are present are strips of local inhibition where chromatophore genesis was completely suppressed after the early chromatophores became established. The strips of inhibition appear to be laid across the otherwise concentric arrangement of the units.

5) Because one cannot 'see' the nerves, the details of innervation of the living skin are hardest to know but potentially the most fascinating for a neurobiologist. The connections given in Fig. 6 are based on analysis of videotape pictures of natural patterning of loligind squids before and after alcohol anesthesia (see Fig. 5). They employ Rule 6 (relating chromatophore size and age) and Rule 9 (separate responses) and agree with the general findings for octopus chromatophores: namely, that each age-class of chromatophore has its own nerve supply and interconnected network of radial muscle fibers (Froesch-Gaetzi & Froesch, 1977). We know that brain cell numbers increase rapidly with growth (Packard & Albergoni, 1970) and it is assumed that the pattern of recruitment of chromatophores into the skin is paralleled by recruitment of motoneurones into the brain and outgrowth of their fibres into the skin. Thus the units shown are *chronological units* (or *chronomere* = new word), innervating particular age-classes of chromatophores and are not restricted spatially to one morphological (i.e. spatial) unit, but will pick up chromatophore muscle fibers (waiting to be innervated) wherever they find them, within the framework of competition from other ingrowing nerve fibres. As each chromatophore has many muscle fibres this explanation would allow for multiple innervation; but only by nerves of similar age-class (unless chromatophores acquire further muscle fibres as development proceeds).

Most important for the interpretation of pattern, the idea of the motoneurones as chronological units that successively intersect the morphological array as it develops accounts in rather simple terms for much of the seeming spatial complexity of the physiological units.

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MALACOLOGIA, 1982, 23(1): 203–208

COMMENTARY ON THE INTERNATIONAL SYMPOSIUM ON FUNCTIONAL MORPHOLOGY OF CEPHALOPODS

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Why do we study animals? Several months ago, while busily operating on a squid brain, Professor J. Z. Young posed this question to several of us onlookers and quickly gave a reply: "We study animals to learn principles." Principles that direct the course of living things. Functional morphology is at the beginning of the rainbow whose ultimate reward is an understanding of life processes. Indeed, most aspects of cephalopod biology are scarcely beyond the level of discovery and description. Exceptions, and there are several, the squid giant axon for one, have more often than not been related to some extremely unique aspect of anatomical structure. After an anatomical discovery has been described, the next step of investigation might be called a study of action mechanics. I once examined the ultrastructure of the Portuguese-man-of-war (*Physalia*) nematocyst and carefully described how the heavily armored thread is discharged from the capsule by turning inside out (Hulet et al., 1974). I was able to induce free nematocysts to discharge by adding household Clorox. The mechanics were clear, but to this day the mechanism of nematocyst

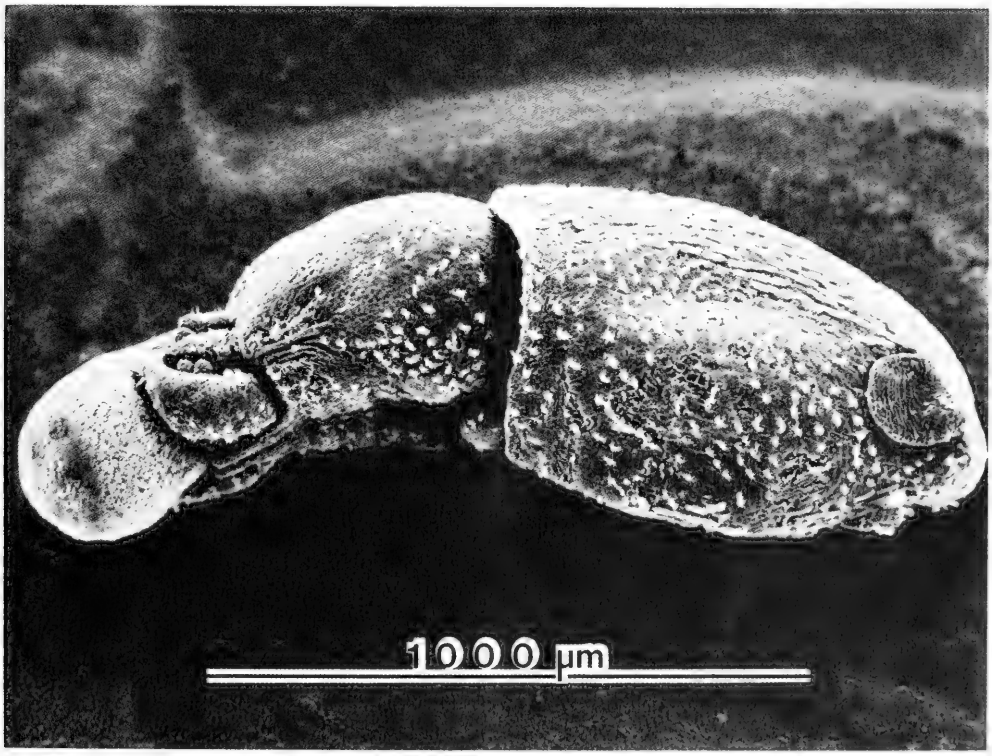

FIG. 1. A *Loligo pealei* hatchling minutes after emerging from the egg. Most of the dorsal and ventral ciliary bands are still intact. Between the fins the tip of the mantle is an actively protrusible organ.

FIG. 2. In *Loligo pealei* and other loliginid squids the anchor-shaped hatching gland sits between the two fins. The shaft of the anchor extends anteriorly over the dorsum of the mantle.

discharge in the living *Physalia* remains elusive.

Renal parasites, posterior salivary glands and ectodermal epithelium were the topics of three papers on descriptive morphology. The basic facts of structure presented in these papers elicited lively discussions and speculations on function. Many helpful recommendations for future study came from the symposium participants and it is worth noting that considerable attention was given to the future role of laboratory-reared cephalopods in unravelling many facts of cephalopod biology.

The two papers on cephalopod chromatophores clearly show that knowledge of chromatophore function is only beginning to be acquired. Before this symposium, I am sure that few squid specialists realized that the yellow, red and brown chromatophores of at least one species of squid, *Loligo plei,* were positioned by color at distinctly different depths in the dermis. Drs. Hanlon and Packard have reached beyond descriptive anatomy and have beautifully demonstrated that chromatophores, iridophores and leucophores are the instruments for expression for the highest level of communicative behavior in cephalopods.

A clear and minutely detailed account of a complex ventral photophore in the deep-sea squid *Abralia trigonura* highlighted the paper by Dick Young and John Arnold. Experiments on living animals aboard ship complemented the anatomical studies and brought to life many interesting aspects of function in such a complex organ. Without doubt, this work fulfills the symposium's ideal of a presentation on functional morphology.

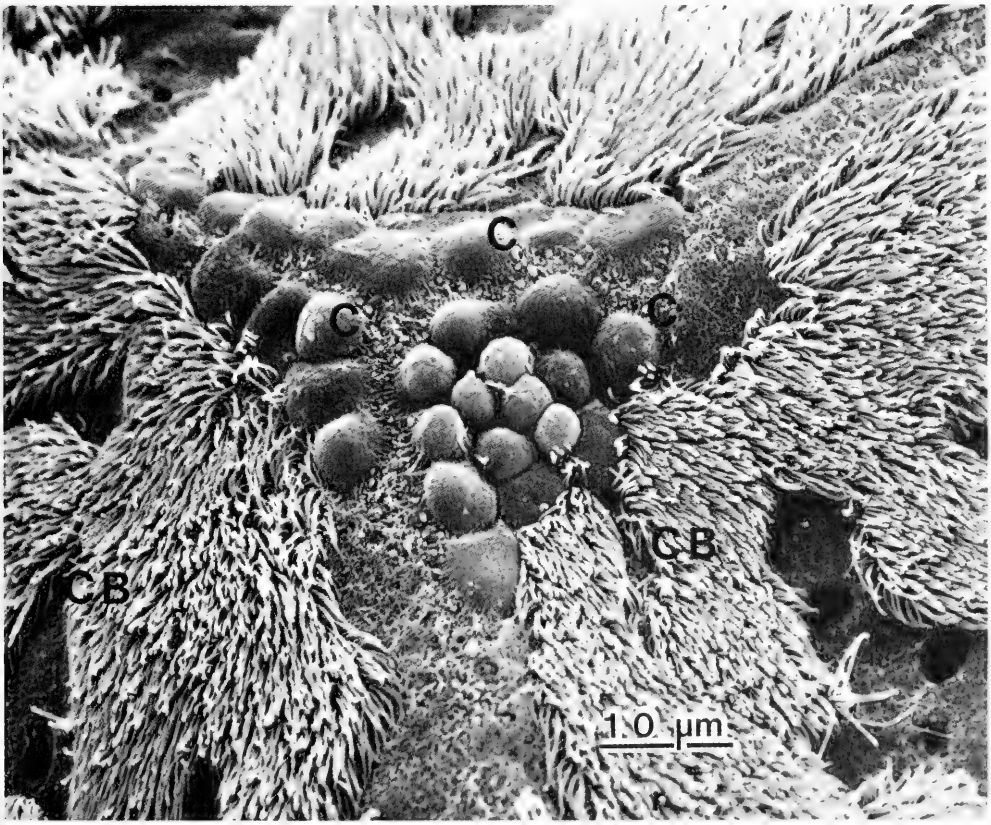

FIG. 3. Enzyme-laden cells (C) at the center of the hatching gland are ready to rupture and dissolve the chorion. The bands of cilia (CB) are in constant and rapid motion. *Loligo pealei* ready to hatch.

Dr. Boletzky has made significant progress in explaining the dissolution of the cephalopod mantle-fin-shell complex, and in his presentation he described the anatomical separation of the developing fins from the ontogenetically receding shell complex (Fig. 1). Concomitantly, the internalization of the shell complex in developing coleoid cephalopods has freed the mantle for almost limitless muscular growth and made way for the emergence of the giant fiber system of nerves. The closed pore of the submerged shell complex gives way and is covered over by Hoyle's organ or hatching gland. The anchor-shaped appearance of the gland is characteristic of decapods (Fig. 2). The cells of the hatching gland (Fig. 3) are swollen with proteolytic enzyme that digest the chorion and possibly liquefy the mucinous jelly that surrounds the egg and is the last barrier to freedom for the squid hatchling. Once free of the egg, effective passage through the jelly of the capsule is by means of numerous bands of motile cilia whose synchronous unidirectional beat propels the hatchling outward (Figs. 4, 5) (Boletzky, 1979). Free-swimming squid hatchlings shed these cilia-bearing bands of cells within hours after hatching (Arnold & Williams-Arnold, 1980).

Not all ciliated cells with the classical "9 + 2" arrangement of microtubules (Fig. 6), so abundant in the skin of loliginid hatchlings, are lost after the animal leaves the egg capsule. The hair cells of the statocyst are classical examples of functioning ciliated cells in adult cephalopods. Even in the skin, however, ciliated cells abound in mature animals (Fig. 7). Some are thought to be chemoreceptors (Emery, 1975) and others found on the mantle and fins might function as mechanoreceptors not too unlike the lateral line system of fishes.

The foregoing comments on structure and function of motile cilia are useful for a concluding remark to end this excellent symposium on functional morphology of cephalopods. My final thought is a reminder for all of

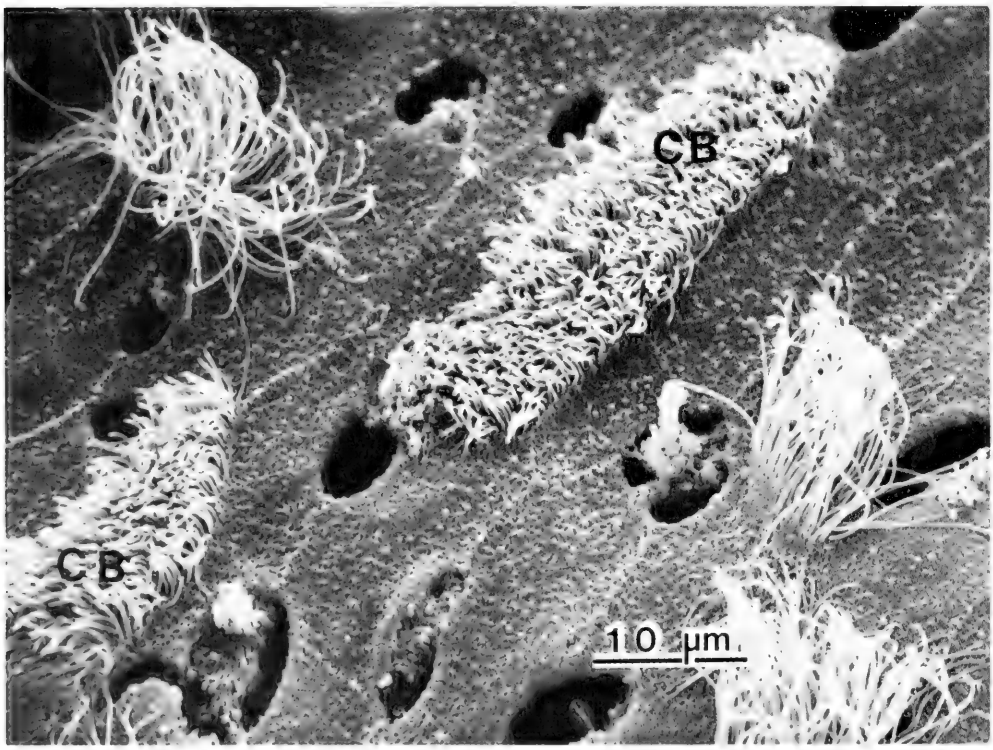

FIG. 4. The bands of moving cilia (CB) that nearly cover the mantle are shed within hours after hatching. Scanning electron micrograph. *Loligo pealei*.

us to exercise continuous vigilance for evidence of faulty repair in cells, tissues and organs. Cells can sustain a non-lethal injury, repair themselves and later express this faulty repair as a serious disturbance of function. So often the pursuit and study of an abnormality gives entry to knowledge of normal function. As illustrated in Fig. 6, the nine doublets of microtubules in all cilia are connected by arms containing the protein dynein which resembles the contractile protein myosin. Dynein acts as an adenosine triphosphatase (ATPase) and causes ciliary motion by forming temporary bridges between adjacent ciliary tubules. Although ciliary motion is far more complex than the bridging function of dynein, the absence of dynein arms destroys a coordinated ciliary beat and leaves the cilia immotile. One investigator interested in cilia was to discover the absence of dynein arms and unravel the mystery of why some children are born with the heart on the right side and suffer years of repiratory infections (Afzelius, 1976). Faulty cilia without dynein arms are to blame for situs inversus in the growing embryo and chronic bronchiectasis in later life. Kartagener described this debilitating illness nearly half a century ago (Kartagener, 1933). The discovery by Afzelius intensified work on the fine structure and chemistry of ciliary motion. There are now many known causes of the immotile-cilia syndrome. All were waiting to be discovered.

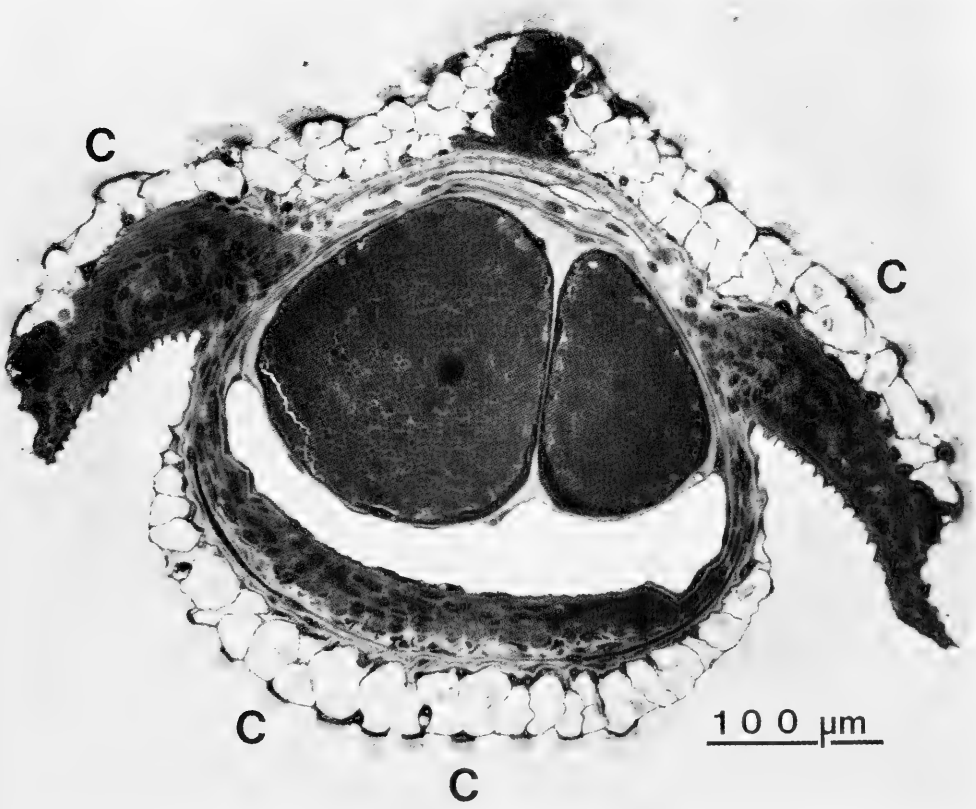

FIG. 5. A section through a whole hatchling near the fins shows the extent of the ciliation. After hatching most of these cells (C) can be found free in the seawater medium spinning around like free-living ciliates.

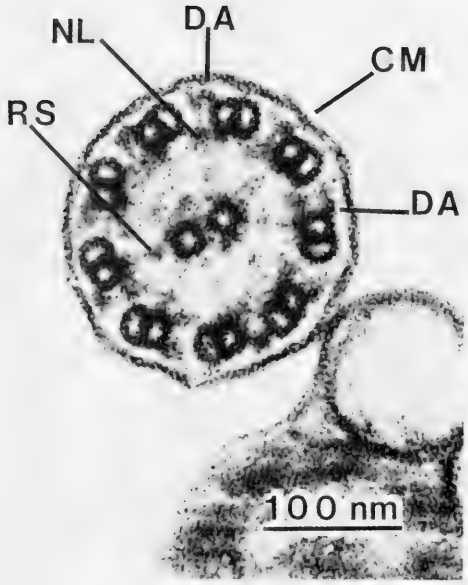

FIG. 6. A kinocilium from a *Loligo pealei* hatchling. The "9 + 2" doublets and two central microtubules are clearly visible. Dynein arms (DA), radial spokes (RS), nexin links (NL) are a few of the known structural faults that can produce immotile-cilia syndrome and its serious consequences. A transmission electron micrograph.

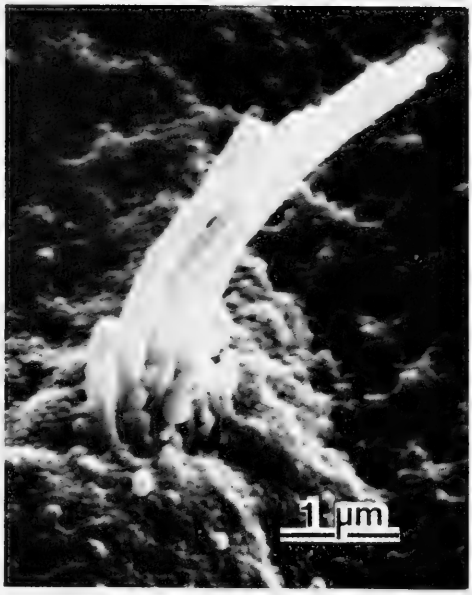

FIG. 7. This and many other similarly ciliated cells were found on the mantle of adult specimens of *Loligo plei.*

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BULIMULID LAND SNAILS FROM THE GALAPAGOS: 1. FACTOR ANALYSIS OF SANTA CRUZ ISLAND SPECIES[1]

Guy Coppois[2] and Claude Glowacki[3]

ABSTRACT

A biometrical comparison of Santa Cruz Island bulimulid land snails is made using factor analysis (7 variables, 140 specimens). The analysis points out two main factors, one representing the overall dimensions of the shell, the other its degree of slenderness or plumpness. In a practical way, these two factors can be represented by the height of the shell and its terminal apical angle. The analysis allows the separation of the specimens into 28 taxa, mostly representing different species, but it isolates some cases of infraspecific variations.

Key words: Bulimulidae; *Bulimulus; Naesiotus*; biometry; factor analysis; speciation; Galapagos.

INTRODUCTION

Long term research on the speciation of the bulimulid land snails in the Galapagos archipelago is undertaken. It started by a three years' stay in the islands where we were able to gather a large collection of these snails pertaining to most of the described species as well as undetermined taxa (species or variations, some obviously not described). Collections were made in most of the islands of the archipelago, but for this paper special attention is directed to the malacofauna of Santa Cruz (Indefatigable) Island. Many different species can be found on this island. Some are extinct or on the way to extinction, but others are still present in numerous and healthy populations. They occupy various complex habitats often closely related to well defined plant zonation (Wiggins & Porter, 1971; Smith, 1966). Thanks to this species richness (about sixty species in one genus in the whole of the archipelago—Dall & Ochsner, 1928; Smith, 1966), the Galapagos Bulimulidae constitute an exceptionally suitable group for the study of speciation in invertebrates.

Some species are easily identified by their obvious morphological characters; in other cases, less obvious morphological characters or wide intraspecific variations make it more difficult to separate specimens belonging to closely related species. Moreover, original descriptions are most of the time very brief and incomplete and almost never give any information on intraspecific variations (Dall & Ochsner, 1928; Smith, 1972; Vagvolgyi, 1977). This makes a certain degree of uncertainty in species identification unavoidable, noticed even in large reference collections like that of the California Academy of Sciences at San Francisco. For many species one must compare the specimens to the types (often empty shells, when available) to obtain a correct identification. We had the opportunity to compare our material with the collections of the California Academy of Sciences, the British Museum (Natural History) and the Naturhistoriska Riksmuseet of Stockholm. It has not always been possible to ascertain the correct name for all the taxa mentioned in this study. For clarity of the text, each taxon has been given a number. The identified taxa are listed with their corresponding numbers in the Appendix. The others, unnamed, will be subject to further investigations.

The frequent difficulty encountered in determining with precision the bulimulid species of the Galapagos led us to analyze very carefully the material gathered by using factor analysis, a method giving in this case objective criteria based on the biometry of the shells and allowing an easier comparison of the various species in this group—limited in a first step to the species inhabiting Santa Cruz Island. In the first stage, only the biometrical variables of the shells were studied (Table 1). Some quali-

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TABLE 1. Biometric measurements used in the factor analysis and their symbols in the following tables. Mean, standard deviation, minimum and maximum values observed for the repeated control measurements (n = 31) of one shell over a period of two years.

	Symbol	Mean mm	Standard deviation	Min. mm	Max. mm
Height of shell	H. shell	15.191	0.117	15.00	15.40
Maximum diameter of shell	D. max.	7.976	0.082	7.83	8.12
Height of aperture	H. apert.	5.608	0.104	5.42	5.77
Width of aperture	W. apert.	4.925	0.072	4.82	5.09
Height of last whorl	H. l. whorl	9.030	0.110	8.80	9.28
Terminal apical angle	T. ap. angle	44.097	0.396	43.00	45.00
Embryonic apical angle	E. ap. angle	72.484	0.926	71.00	74.00

tative factors, like colour, type of apex and umbilicus, superficial characteristics of shells, presence or absence of teeth in the aperture, or anatomical peculiarities, will be examined later on. This study, focused on shell characters alone, was necessary in order to obtain a correct identification of our specimens by comparing them with museum material and type-specimens which are almost only dry shells.

MATERIALS AND METHODS

SAMPLING AND MEASUREMENTS. The specimens used in this study were gathered between July 1973 and July 1976 by methodical sampling on the whole surface of Santa Cruz Island. These samplings were made by picking up the snails found in quadrats (size: 1 m^2 and 0.25 m^2) chosen at random in some areas, but also by local sampling in all the zones of the island or by quadrats regularly spaced along transects. One can safely assume that they represent all the bulimulid taxa (species or subspecies) that can be found on the island. The specimens belonging to each taxon were selected from lots corresponding to the various collecting stations. A detailed list of localities will be published later. For the purposes of factor analysis, only adult specimens were selected at random in restricted number (5 for every taxon) after having discarded juveniles and damaged specimens from the lots. Every shell was drawn using the Wild M5 stereoscopic microscope fitted with a camera lucida. Shells and the corresponding drawings are numbered individually, and the scale of every drawing was noted. This made possible, at any stage of the analysis, finding again individual specimens or drawings every time an observation had to be completed or verified. To facilitate drawings, every shell was placed in a small, flat-bottomed container filled with fine sand, and in a standard position, the three following points being put in a single horizontal plane (Fig. 1): 1) the apex (A), 2) the point marking the maximum advance of the parietal area on the columellar surface of the previous whorl (B), 3) the point (C) corresponding to (B) on the outer edge of the aperture, (B) and (C) being situated on the same plane perpendicular to the apparent axis of the shell. It should be noted that in this position the surface determined by the aperture is seldom also included in a horizontal plane. In practice the standard position was obtained by successively focusing on points A, B and C at maximum magnification (×50). At this magnification the depth of field is very narrow and the plane determined by these three points can be assimilated to the horizontal plane. The approximation was tested by repeated positioning of a shell and of a small object presenting a triangular plane surface on one of its sides (for the accuracy of the method see further on).

The three points considered could be located easily whatever the type of shell; shapes may of course vary considerably from one species to another. Once a shell has been drawn, the terminal apical angle, the sides of which are tangents to the first and last whorls of the spiral, was traced on the drawing. Then we added an imaginary axis, passing through the summit of the terminal apical angle and through the point (D) on the columellar edge which was nearest the actual axis of the shell. This imaginary axis is never identical with the actual axis. In optimal cases, the latter goes through the center of the columella. All measurements were made along straight lines either parallel or perpendicular to the imaginary axis, from the axis to the outermost points of the shell and of the aperture. These measurements appear on Table

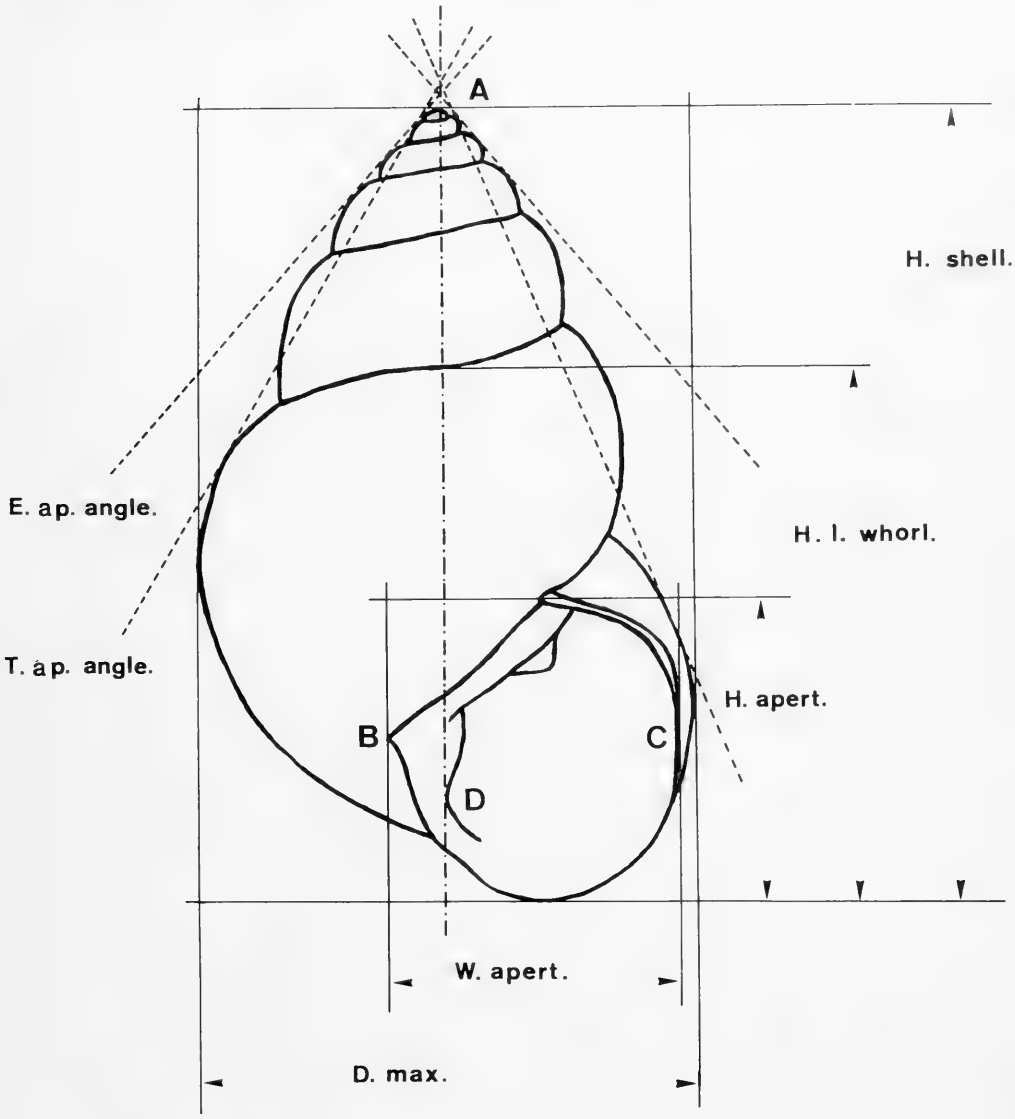

FIG. 1. Drawing of a shell in a standard position for measurements: *Bulimulus (Naesiotus) cavagnaroi* Smith, 1972, xanthic form (taxon 2, paratype n° GC.652 of *B. gilderoyi* Van Mol, 1972; H. shell: 22.14 mm, D. max.: 13.78 mm). Legend: see text and Table 1.

1. It should be noted that the height of the last whorl of the shell was always measured along the imaginary axis. An "embryonic apical angle" was also constructed on every drawing; its sides were tangents to the first whorls of the shell. In most cases, this was found to be identical to the "maximal aperture apical angle." As the sides of the embryonic apical angle could only be determined by closely spaced points on the drawing, its measurement was necessarily less accurate than that of other angles. The angles were measured with a protractor with an approximation of one degree. Length measurements were made on the drawings, using a sliding calliper (instrumental precision: ± 0.05 mm). To verify the accuracy of this method, measurements of the same shell (ref. GC 2, taxon 24) were taken 31 times at several months' intervals over a period of two years. The mean, stan-

dard deviation as well as the minimum and maximum value observed for each biometric measurement for these controls are presented in Table 1. The data thus collected were reproduced on cards to be analysed by a CDC 6000 computer.

FACTOR ANALYSIS. This type of analysis allows study of intercorrelations between variables. Sensu stricto, this term applies only to the treatment of aleatory variables which are supposedly known in terms of probability. This technique is essentially used to determine, among a group of variables, those most intercorrelated, in order to eliminate redundancies in explanatory variables, either by eliminating some of the variables, or by creating new variables which are linear combinations of the original variables. The sub-group of variables thus defined can be used for further analysis. In our case, the resulting subgroups of variables or factors enabled us to compare the taxa found on Santa Cruz Island on biometrical data alone. One hundred and forty shells were used for the analysis (five for each taxon, see Appendix); their mean and the general variance, shown in Table 2, were used to reduce the raw biometric variables in order to estimate the correlation matrix of these variables (Table 3).

TABLE 2. General mean, variance and standard deviation (140 shells).

Variable	Mean	Variance	Standard deviation
H. shell	13.8862	15.17258	3.8952
D. max.	7.8237	8.73202	2.9550
H. apert.	5.4159	4.03206	2.0080
W. apert.	4.8431	3.24144	1.8004
H. l. whorl	8.7746	10.05270	3.1706
T. ap. angle	48.2929	249.93712	15.8084
E. ap. angle	62.9143	289.10200	17.0030

TABLE 4. Percentage of total variance represented by each factor.

Factor	Percent of Variation	Cumulated percent
1	77.5	77.5
2	20.9	98.4
3	.7	99.1
4	.5	99.6
5	.2	99.8
6	.1	99.9
7	.1	100.0

The next step was the factor analysis proper (Lebart & Fenelon, 1971, Dagnelie, 1975; Nie et al., 1975). The whole of the observation realized by the reduced variables can be visualised as a cluster of points centered in a multidimensional space. In this space, factor analysis will determine a main axis corresponding to the maximal variance of the cluster (axis F_1). Other axes F_2, F_3, F_4 . . . corresponding to the next maximal variance can be determined perpendicularly to this axis F_1. These oriented axes or vectors are known as "factors." Table 4 shows the percentage of the total variance represented by each factor (shown in decreasing order).

It will be noticed that factor 1 explains 77.5%, and factor 2, 20.9% of the total variance. Together factors 1 and 2 explain 98.4% of this variance, the total of other factors accounting only for 1.6%. This means that in our multidimensional space, most of the observations are clustered nearly in a single plane. Thus the geometry of Santa Cruz Island bulimulid land snails is practically a bidimensional phenomenon, two axes being sufficient to characterize it almost completely. An important element of factor analysis lies in the interpretation of the selected factors or, more precisely, of the subspaces they determine. Generally the selected factors beyond the first two

TABLE 3. Matrix of correlations between variables.

	H. shell	D. max.	H. apert.	W. apert.	H. l. whorl	T. ap. angle	E. ap. angle
H. shell	1.00000						
D. max.	.85296	1.00000					
H. apert.	.92960	.94467	1.00000				
W. apert.	.87934	.98505	.96935	1.00000			
H. l. whorl	.92792	.97122	.98476	.97596	1.00000		
T. ap. angle	.06582	.56377	.35290	.50053	.40312	1.00000	
E. ap. angle	.28562	.70446	.53912	.66160	.58314	.93391	1.00000

TABLE 5. Correlations between initial factors and variables.

	Factor 1	Factor 2
H. shell	.85081	−.51395
D. max.	.99309	−.01170
H. apert.	.95995	−.23992
W. apert.	.99003	−.08080
H. l. whorl	.97700	−.19162
T. ap. angle	.57075	.81172
E. ap. angle	.72775	.66386

TABLE 6. Correlations between factors and variables after a Varimax rotation.

	Factor 1	Factor 2
H. shell	.99289	−.04691
D. max.	.87889	.46251
H. apert.	.95840	.24604
W. apert.	.90910	.40029
H. l. whorl	.95040	.29663
T. ap. angle	.11546	.98555
E. ap. angle	.32393	.93027

are not easy to interpret. Table 5 shows the existing correlations between factors 1 and 2 and the original variables. At this stage, it is clearly impossible to give a real meaning to the plane which has been defined.

To make it possible we shall rotate factors 1 and 2 around the origin while keeping them in the same plane, in order to correlate them as well as possible with a sub-group of reduced variables. This was, in the present case, effected by the well known VARIMAX method (Nie & al., 1975). Table 6 shows the correlations of the new factors with the observed variables. It will be clearly seen that factor 1 is closely correlated to: the height of the shell, the maximum diameter of the shell, the height of the aperture, the width of the aperture, the height of the last whorl; and factor 2 to: the terminal apical angle, the embryonic apical angle.

Thus factor 1 represents mainly a measurement of the overall dimensions of the animal; factor 2 shows its degree of slenderness or plumpness.

Since factor 1 is most closely related to the height of the shell, and factor 2 to the terminal apical angle, these two measurements can be used to describe accurately the geometry of the shell, on which they can easily be taken. The scores on factors 1 and 2 corresponding to each observation were calculated from the coefficients given in Table 7 using the method given by Nie et al. (1975: 489).

TABLE 7. Coefficients used for calculation of individual factor scores.

	i = 1	i = 2
$C^i_{H.shell}$	.30506	−.23386
$C^i_{D.max.}$	.16488	.08018
$C^i_{H.apert.}$	.23369	−.05975
$C^i_{W.apert.}$	.18685	.03843
$C^i_{H.l.whorl}$	.22075	−.02926
$C^i_{T.ap.angle}$	−.17127	.53747
$C^i_{E.ap.angle}$	.09774	.46248

RESULTS AND DISCUSSION

The distribution of the observations in the plane of factors 1 and 2 is shown on Fig. 2. On Fig. 3, the raw biometric data corresponding to each individual analyzed have been carried over into the plane of the variables "height of shell" and "terminal apical angle." For clarity, in Figs. 2 and 3, the five observations pertaining to each taxon are enclosed in a cluster delimited by a line, a cross indicates the mean position calculated from the corresponding scores or data. Sketches of the corresponding shell shapes have been added on Fig. 2. One will readily notice the similarity of these two graphs, which show a total of 28 taxa identified from Santa Cruz Island. Two extraneous taxa were added on Fig. 2 (indicated by arrows). They represent one species living on Isabela Island (*B. (N.) pallidus* Reibisch, 1892, taxon 51, from Alcedo volcano), and one found on Champion Island (*B. (N.) planospira* Ancey, 1887, taxon 49). The data pertaining to these taxa were not included in the global factor analysis to avoid any interference with the Santa Cruz data in calculations. Their position on Fig. 2, calculated from the scores of Table 7, are thus indicative. They are included to show that the variation in shapes of the bulimulids becomes even wider when taxa from other places in the archipelago are studied.

Factor analysis shows that in most cases these taxa can be identified even if only the biometric data of the shells are used. Obviously, an analysis including some qualitative characters of the snails will make possible an

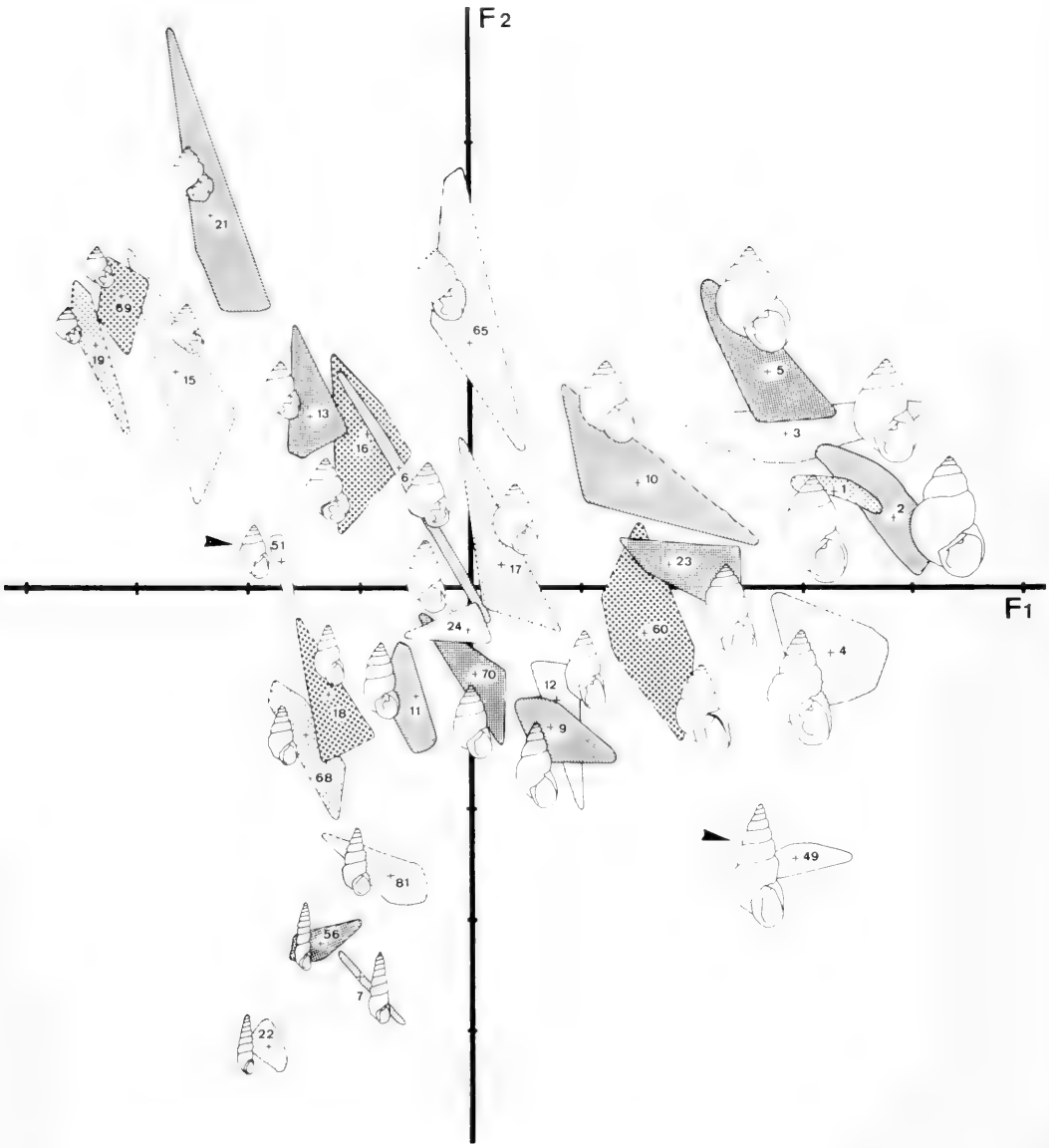

FIG. 2. Factor analysis: distribution of the observations in the plane of factor 1 and 2. The clusters of points enclose the five observations corresponding to each taxon, the mean of each cluster is marked by a cross and sketches of the corresponding shells are represented for the 28 taxa from Santa Cruz Island. Two extraneous taxa (n° 49 and 51) were added (see text, taxa listed in the Appendix).

even better separation between the different taxa (Coppois & Glowacki, in preparation). For example, taxa 9 and 12 are situated on largely overlapping areas of Fig. 2. But taxon 9 specimens, *B. (N.) eos* Odhner, 1950, have a light, smooth shell colored white or light brown, and no tooth in the aperture, or a very light parietal bump. Specimens of taxon 12, *B. (N.) scalesiana* Smith, 1972, have a thick, robust shell with an irregular bumpy surface, especially on the last whorl. The colour is white with a pink, or sometimes grayish apex; there is a tooth on the parietal wall in fully developed adults. So, in spite of their superposition on Fig. 2, these two taxa can be separated beyond any doubt. After examination of the

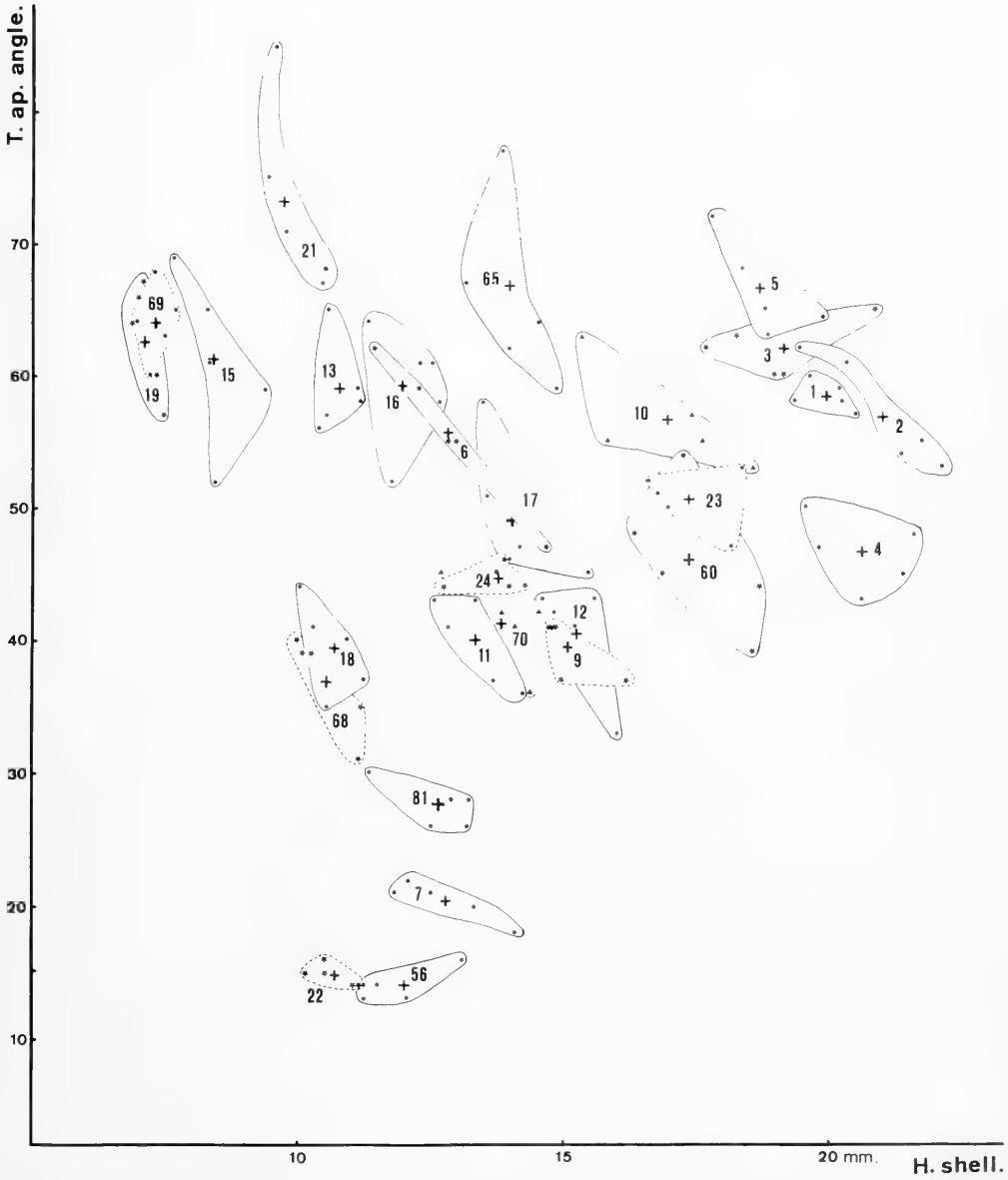

FIG. 3. Raw data, height of shell by terminal apical angle (see text). For clarity the five observations corresponding to each taxon are enclosed in a cluster of points delimited by a line. The mean of each cluster is marked by a cross. The identified taxa are listed in the Appendix.

type specimens of these two species and from our own conclusions there is good evidence that these two species are not synonymous as stated by A. G. Smith and A.S.H. Breure (Smith, 1974).

INTRASPECIFIC VARIATION. Although performed on a restricted number of snails (5 for each taxon) the factor analysis already provides interesting information on intraspecific variations. It is obvious that the clusters of points representing each taxon could be more extended if more cases were entered in the analysis, but the general pattern of Fig. 2 would only be slightly modified. Some preliminary tests have been made with samples of as many as 30 specimens, for taxa 1, 2 and 3;

the modifications are not significant. In taxon 6, *B. (N.) akamatus* Dall, 1917, smaller individuals are plump, while larger ones are more slender, although all specimens measured were adults. Other clusters similarly stretched on an upper left-lower right line can readily be observed.

Taxa 24 and 70 are clearly differentiated from their neighbours on Figs. 2 and 3 by their characteristic shell with a widely open umbilicus and the apex marked by straight axial riblets, the spaces between these riblets showing fine spiral striae (like taxa 10, 16, 18, 19, 22, 23, 49, 51, 56, 68, 81). Their neighbouring taxa: 6, 9, 11, 12, 17, and also taxa 1, 2, 3, 4, 5, 7, 13, 15, 21, 60, 65, 69 have another kind of apex with fine undulating, sometimes converging axial riblets. Although one can easily separate the shells of taxon 24 from those of taxon 70, these last ones being more slender and having a small bump on the columellar surface, both taxa are in fact representatives of one species *B. (N.) tanneri* Dall, 1895, which shows a considerable intraspecific variation when samples coming from different localities are studied. These shells can vary in size and shape (plump or more slender), having or not a small bump on the columellar surface. This can be observed in the field even within short distances: the localities where the specimens of taxa 24 and 70 were collected are distant by less than 1 km (Coppois & Glowacki, 1982).

Colour variations occur in two species, *B. (N.) eos* Odhner, 1950 (taxon 9), for instance, has mixed populations of two colour forms: light brown or white shells. For *B. (N.) cavagnaroi,* there are three main colour forms that are easily separated: normal brown (taxon 1), "xanthic" yellow (taxon 2) and "white unbanded" shells (taxon 3) (Smith, 1972).

RELATIONSHIPS BETWEEN SHELL SHAPES AND HABITAT. Although ecology is not the main purpose of this paper, it seems interesting to make some preliminary remarks on some striking correlations that seem to exist between shell shape and habitat (the ecological approach of our work is still in preparation). To give a better visualization of the variation of shell shapes over the whole group of bulimulid taxa found on Santa Cruz Island alone, we shall refer to Fig. 4. On this figure, the drawings of the shells are centered on the crosses which locate the mean position of each taxon in the factor analysis (similar to Fig. 2). We shall also refer to the observations of Smith (1966) and to the well known plant zonation (Wiggins & Porter, 1971; Van Der Werff, 1978).

Among the taxa found in the dryer zones (Arid and lower Transition zones), those living in the open, on tree trunks and vegetation are small (taxa 7, 22, 56 and to some extent 81), with slender shells, a small aperture (the latter characteristic being consistent with the necessity to minimize evaporation) and no teeth. Those living under rocks are bigger (taxa 6, 10, 11, 23, 24, 60, 65, 70, 81), with sturdier shells and a relatively wider aperture. In humid zones (upper Transition zone, *Scalesia* forest, summits zone), the variation in size is definitely wider. The biggest forms (taxa 1, 2, 3 and 5) are all found in the wettest habitats and live in the humus layer on the ground, as taxon 17 from the summits zone which is slightly smaller. The smaller forms (taxa 13, 15, 19, 21 and 69) may spread to less humid zones (e.g. moist forest of the upper Transition zone); they also live hidden in the humus layer. All these taxa are characterized by a very globular shape and a medium wide aperture which is always reduced by the presence of teeth. Taxa 18 and 68, which live in the humus layer like the above ones, but in relatively drier zones, are small, with a less plump shell shape and no teeth. This clearly places them in an intermediate category, which tallies with the intermediate situation of their habitat between dry and humid zones. A similar remark applies to taxa 4, 9, 12 and 16 which live exposed on the vegetation like taxa 7, 22 and 56 described above, but in definitively moister habitats.

CONCLUSION

This preliminary study shows that factor analysis can be usefully applied to the separation of the bulimulid land snail taxa of the Galapagos Islands and to the identification of species. Although focused on the shells' biometry alone, this method is a suitable tool for comparing our specimens with museum material and type-specimens which are mainly dry shells. Further studies will include other morphological characters of the shells, anatomical and ecological observations and, we hope, will help to clarify the taxonomy of this confusing group of snails.

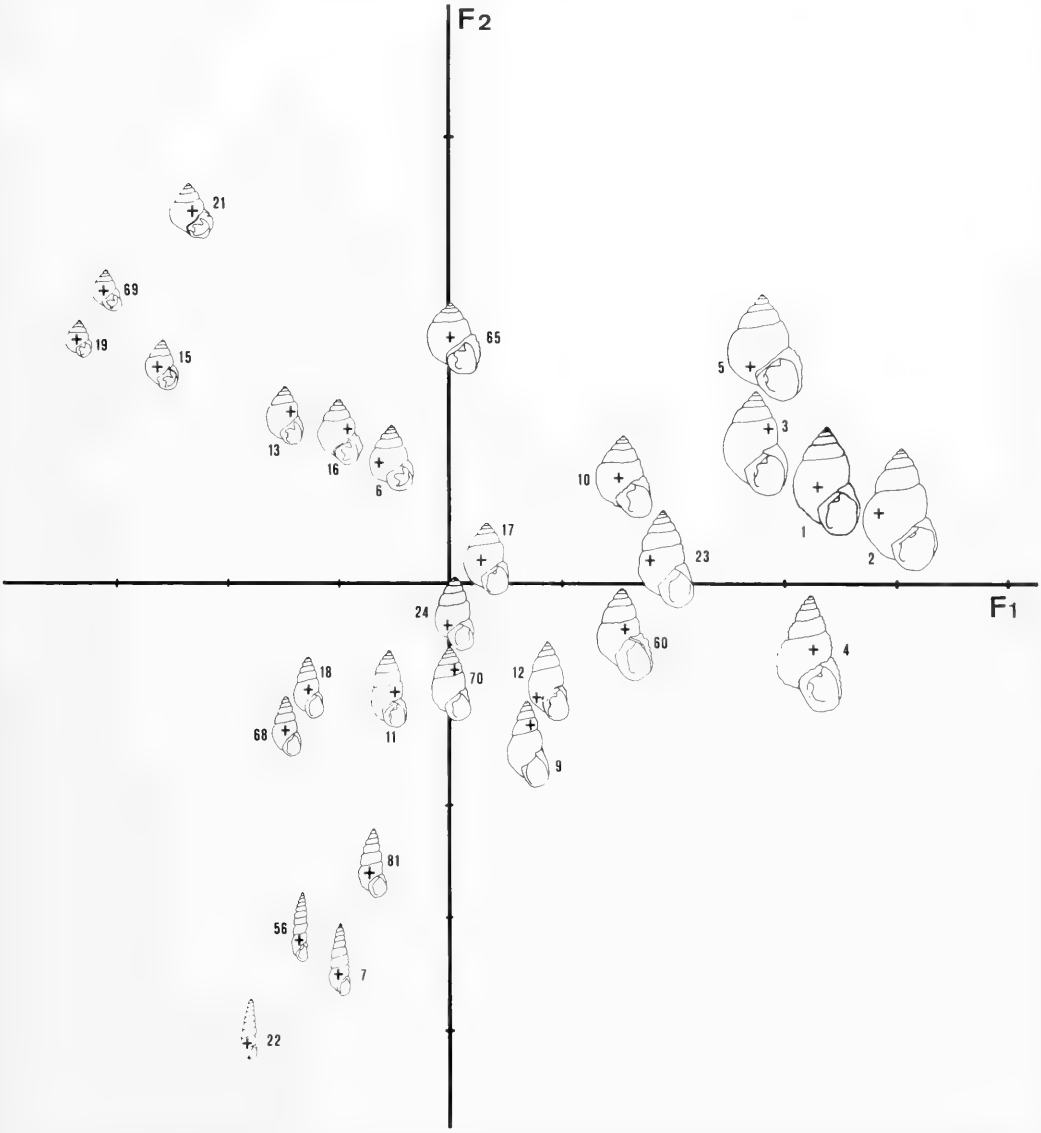

FIG. 4. Variation in shape of the shells in the bulimulid taxa found on Santa Cruz Island. Factor analysis (see text). Sketches of the shells are centered on the mean position of each cluster of points. The taxa are listed in the Appendix.

ACKNOWLEDGMENTS

We thank the Charles Darwin Foundation for the Galapagos Islands, the Parque Nacional Galapagos' authorities, the Belgian Ministère de l'Education Nationale and Professor Jean Bouillon who made the field work possible. We are grateful to Dr. Barry Roth for his kind welcome, his help and facilities at the California Academy of Sciences, San Francisco, and to Professors Guy Louchard and Jean-Jacques Van Mol for their advice and criticism during this work. We also thank Professor John F. Peake and Dr. Peter Mordan for access to the British Museum (Nat. Hist.) collections, and to Professor Å. Andersson for

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APPENDIX

The generic name for the Galapagos Bulimulidae is not accepted by everyone; two names were proposed, *Bulimulus* and *Naesiotus*. No really decisive arguments were presented by Breure & Coppois (1978) in their too short and tentative analysis of the genus *Naesiotus*. As this work leaves too many points unsolved, we decided on a more conservative approach and use *Bulimulus* as a generic name and *Naesiotus* as a subgenus. What is needed is a new analysis, including anatomical observations of all the Galapagos species as well as their continental relatives.

The taxa mentioned in this study are listed with the corresponding code number used on figures and in the text. Seven taxa were not identified (11, 15, 17, 21, 22, 56, 69) and will be subject to further investigations.

Code number	*Bulimulus (Naesiotus):* taxa from Santa Cruz Island
1	*cavagnaroi* Smith, 1972 (normal brown colour)
2	*cavagnaroi* Smith, 1972 ("xanthic" form)
3	*cavagnaroi* Smith, 1972 ("white unbanded")
4	*blomberghi* Odhner, 1950
5	*ochsneri* Dall, 1917a
6	*akamatus* Dall, 1917a
7	*reibischi* Dall, 1895
9	*eos* Odhner, 1950
10	*adelphus* Dall, 1917a
12	*scalesiana* Smith, 1972
13	*saeronius* Dall, 1917b
16	*lycodus* Dall, 1917a
18	*hirsutus* Vagvolgyi, 1977
19	*alethorhytidus* Dall, 1917a
23 cfr.	*duncanus* Dall, 1893 (no certitude)
24	*tanneri* Dall, 1895
60	*olla* Dall, 1893
65	*cymatias* Dall, 1917a
68	*jacobi* (Sowerby, 1883)
70	*tanneri* Dall, 1895 (modified form)
81	*nesioticus* Dall, 1896
	Taxa from other places in the archipelago
49	*planospira* Ancey, 1887; Champion Island
51	*pallidus* Reibisch, 1892; Alcedo volcano, Isabela

A detailed list of localities will be published later.

BULIMULIDAE (GASTÉROPODES, PULMONÉS) DES GALAPAGOS: 1. ANALYSE FACTORIELLE DES ESPÈCES DE L'ÎLE DE SANTA CRUZ

Guy Coppois et Claude Glowacki

RÉSUMÉ

Une comparaison biométrique des Bulimulidae (Pulmonés terrestres) de l'île de Santa Cruz est réalisée au moyen de l'analyse factorielle (7 variables, 140 spécimens). L'analyse met en évidence deux facteurs principaux, le premier représente une estimation des dimensions globales de la coquille, le second une estimation de sa corpulence. De manière pratique, ces deux facteurs peuvent être assimilés à hauteur totale de la coquille et à l'angle apical terminal. L'analyse permet la séparation des spécimens en 28 taxa, la plupart sont des espèces différentes mais quelques cas de variations infraspécifiques sont mis en évidence.

MALACOLOGIA, 1983, 23(2): 221–270

NEW ZEALAND SIDE-GILLED SEA SLUGS (OPISTHOBRANCHIA: NOTASPIDEA: PLEUROBRANCHIDAE)

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ABSTRACT

Five New Zealand species of the family Pleurobranchidae are recognized. *Berthella ornata* (Cheeseman) is endemic. *Bathyberthella zelandiae* is described as a new genus and species, and is also endemic. *Berthella aurantiaca* (Risso) is restricted to the Mediterranean Sea, but in New Zealand this name has been applied incorrectly and indiscriminately to two species differentiated here—*Berthellina citrina* (Rüppell & Leuckart) has a small shell, denticulate radular teeth, and possesses a prostate gland; *Berthella mediatas* Burn has a larger shell, smooth teeth and no prostate gland. *Pleurobranchaea maculata* (Quoy & Gaimard) is the sole species of its genus in New Zealand; *Pleurobranchaea novaezealandiae* Cheeseman and *Pleurobranchaea novaezelandiae* var. *granulosa* Bergh are synonyms of *P. maculata*.

Key words: Gastropoda; Opisthobranchia; Notaspidea; Pleurobranchidae; New Zealand; taxonomy; revision.

INTRODUCTION

Considerable advances have been made during the past decade in studies of nomenclature and relationships for New Zealand opisthobranchs. This paper reviews the order Notaspidea and in particular the family Pleurobranchidae. It arises from my investigations into the identity and ecology of shallow-water pleurobranchs from New Zealand (Willan, 1975) plus studies conducted since that time on additional material. These examinations extended the coverage to include deep-water species and thus this work now encompasses the entire New Zealand notaspidean fauna.

The Notaspidea have not been monographed before in New Zealand. Cheeseman (1878, 1879) was the only worker to describe pleurobranchs from New Zealand as new species. Others recorded side-gilled slugs from this country under the names of established species (Bergh, 1900; Odhner, 1924). These subsequent names were incorrect but inevitably became incorporated into important checklists of the New Zealand molluscan fauna (Suter, 1913; Powell, 1937, 1946, 1957, 1961, 1976, 1979) and so became entrenched. These incorrect taxa appeared in ecological works (Morton & Miller, 1968; Batham, 1969; Ottaway, 1977b). By noting that European names were being used incorrectly for New Zealand species, Burn (1962: 134) highlighted the taxonomic chaos that was the legacy of these early works.

Reviews and reappraisals of the genera *Pleurobranchella* Thiele (Willan, 1977), *Pleurobranchopsis* Verrill and *Gymnotoplax* Pilsbry (Willan, 1978) (none of which occurs in New Zealand) have already been published. This latter study was a prerequisite to this present paper in that it stabilized the taxonomy of *Berthellina* Gardiner. A review article on feeding within the Notaspidea that deals with two New Zealand species is currently in preparation.

MATERIALS AND METHODS

Material Studied

There are six species of the order Notaspidea in New Zealand; five belong to the Pleurobranchidae and one, *Umbraculum sinicum* (Gmelin, 1791), belongs to the Umbraculidae. *U. sinicum* is uncommon in this country and is found in northern waters only. As it has been described adequately from Australia by Burn (1959) and Thompson (1970), it is not treated further in this paper apart from its inclusion in the key.

All but one of the pleurobranchs occur in shallow water on the continental shelf. The taxonomic arrangement and sequence of

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TABLE 1. Classification of New Zealand pleurobranchs.

Family Pleurobranchidae
Subfamily Pleurobranchinae
Berthellina citrina (Rüppell & Leuckart, 1828)
Berthella ornata (Cheeseman, 1878)
Berthella mediatas Burn, 1962
Bathyberthella zelandiae n. gen. & n. sp.
Subfamily Pleurobranchaeinae
Pleurobranchaea maculata (Quoy & Gaimard, 1832)

presentation in this publication are given in Table 1. Because of the past cursory descriptions of New Zealand pleurobranchs and lack of comparative details derived from anatomical examinations of specimens from this country I have described each species fully. Such detailed descriptions are required for all pleurobranchs before there is critical reappraisal of taxa as biologically meaningful entities.

Locality data of material examined for each species are given in the Appendix.

Terminology

Terminology used for describing the Notaspidea throughout this paper is as follows (Figs. 1–4). The terms refer specifically to pleurobranchs.

The body has a dorsal mantle and ventral foot; posteriorly the foot sometimes has a pedal gland ventrally and/or a caudal spur on the dorsal face. Body length (living specimens) is the distance between the middle of the anterior border of the oral veil and the posterior extremity of either the mantle or the foot, depending on which extends farther to the rear in the actively crawling animal. The prebranchial aperture, nephroproct and reproductive apertures are situated in front of the gill on the right side; the mouth opens anteriorly beneath an expanded oral veil, which is derived from a forward-projecting flap of tissue that grows outwards from, and yet remains connected to, the oral tentacles during metamorphosis (Gohar & Abul-Ela, 1957). The rhinophores are scroll-like and located above the oral veil.

The terminology of the gill requires standardization (Fig. 4). The axis of the gill is the rachis, which is either smooth or tuberculate. From the rachis smaller side branches arise alternately; these pinnae (= primary lamellae or pinnules) consist of an axis and a series of minute, overlapping leaves down each side. These side leaves are here called pinnules (= secondary lamellae or plicae). A membrane attaches the ventral surface of the rachis, for the greater part of its length, to the side of the body. Morton (1972) felt there was not enough evidence to homologize the gill of the Notaspidea with that of other opisthobranchs. He established that opisthobranchs have a wide variety of gill structures (e.g. the plicatidium of the Cephalaspidea and Anaspidea, the plume-like gill of the Notaspidea, and the circum-anal gills of the Doridacea) that replace the ctenidium of prosobranch molluscs.

Labelling of parts of the radular tooth follows Bertsch (1977), since the teeth of pleurobranchs are homologous to those of chromodorid nudibranchs. I follow Burn (1962) for terminology relating to the jaws and my labelling of reproductive organs incorporates the terminology proposed by Ghiselin (1965) for an idealized, gonochoric opisthobranch system.

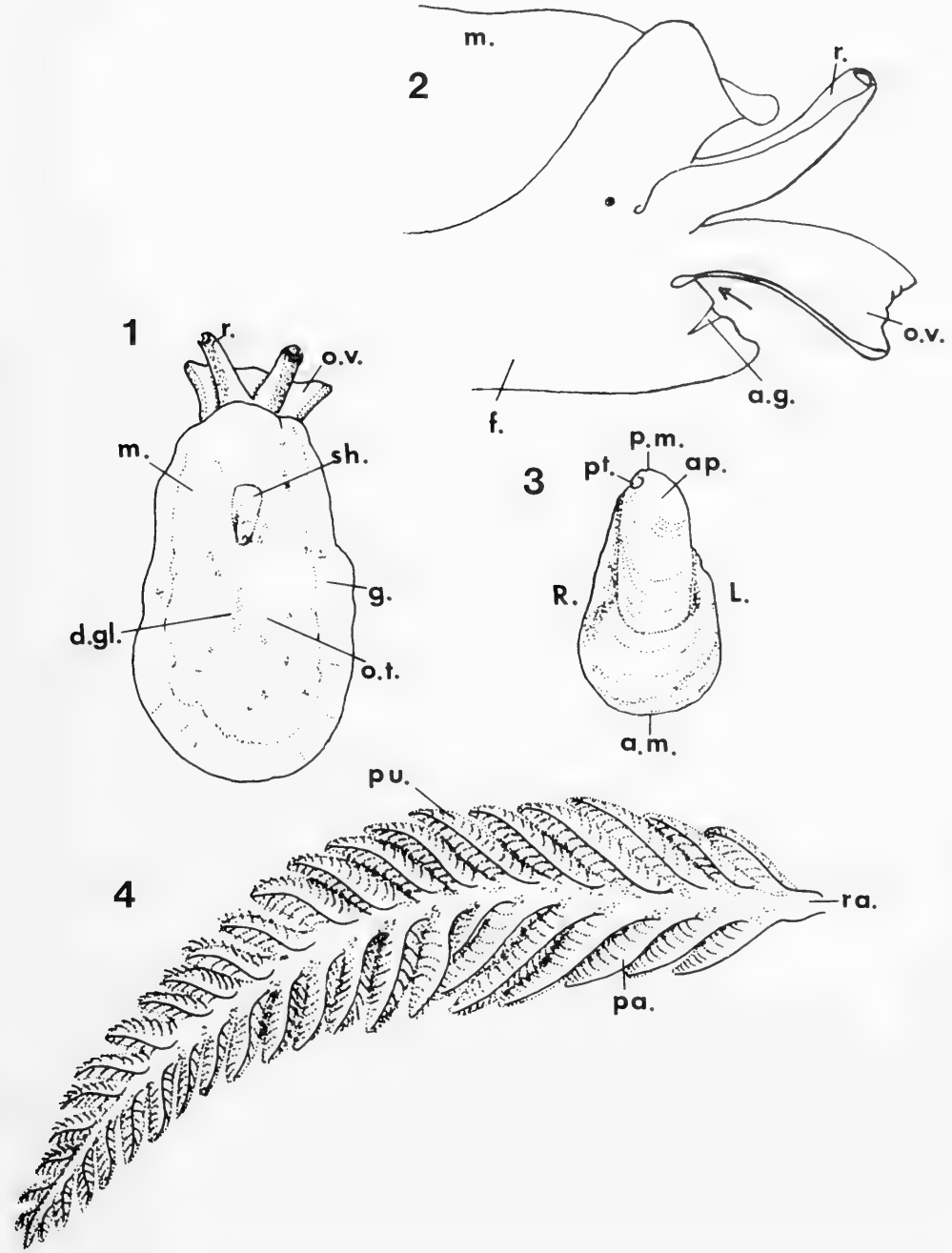
Many differences that have been used as generic characters are, in my view, only of use at the specific level. I list below those pleurobranch characters that have taxonomic value, and have attempted to assess their usefulness. Full information on all these characters, for both living and preserved animals where possible, is required in any future descriptions of pleurobranchs.

Mantle: Form, colour, extent and texture are distinctive and reasonably constant in living animals; these characters are most useful if there is some previous information on the degree of intraspecific variability.

Rhinophores: Very similar in construction throughout the group, all are scroll-like with a lateral groove and basal dilation. Data on size and position are of generic (rather than specific) value.

Oral veil: A most useful character to observe in living animals. Such features as relative width, strength of lateral extensions, degree of sinuosity and presence or absence of papillae on the anterior margin are good specific characters. All pleurobranchs have grooved edges to the lateral areas of the oral veil.

Foot and pedal structures: The foot offers the same types of characters as does the mantle. On the anterior dorsal surface a broad mucous gland and transverse slit are present. Some species possess a dorsal caudal spur and/or ventral gland towards the rear. The appearance of these structures probably indi-



FIGS. 1–4. Pleurobranch descriptive terminology. *Berthellina citrina* illustrated as a representative of the Pleurobranchidae. 1. Dorsal view of whole animal. 2. Lateral view of head; arrow indicates site of mouth. 3. Exterior of shell. 4. Lateral view of gill. Abbreviations. a.g. = mucous gland on anterior border of foot; a.m. = anterior margin of shell (i.e. edge facing anteriorly when shell is in place in living animal); ap. = apical region of teleoconch; d.gl. = digestive gland; f. = foot; g. = position of gill on right side beneath mantle; L. = left side of shell in place in living animal; m. = mantle; o.t. = ovotestis; o.v. = oral veil; pa. = pinna of gill; p.m. = posterior edge of shell (i.e. margin facing posteriorly when shell is in place in living animal); pt. = protoconch; pu. = pinnule of gill; R. = right side of shell in place in living animal; r. = rhinophore; ra. = rachis of gill; sh. = position of shell beneath mantle.

cates attainment of sexual maturity rather than similarity between species. The significance of these structures is discussed further at the end of this section.

Gill: The gill has been employed as an indicator of genus, depending whether the rachis is smooth or tuberculate (but I shall later query the importance of this feature). Generally the number of pinnae shows considerable intraspecific variation, particularly in relation to the size of the individual—juveniles have fewer pinnae and pinnules.

Shell: Presence or absence is important at the generic level; generally shape varies considerably within any one species. The shell is never wholly, or even partially, uncovered by the mantle in any pleurobranch when alive (Willan, 1978). In the literature are records of specimens of normally-shelled pleurobranchs being found without shells—*Pleurobranchus peroni* and *P. inhacae* (Macnae, 1962) and *Berthellina citrina* (Thompson, 1970; Edmunds & Thompson, 1972). So absence of a shell, by itself, should not be thought of as significant when an unknown specimen is being checked against a published description.

Gut: Pleurobranchs show little variation in the basic structure of the alimentary canal. Guiart (1901) used *Pleurobranchus membranaceus* (Montagu) in his description of the gut in the Pleurobranchidae, and Thompson & Slinn (1959) figured the alimentary canal of this species. Both the gut and nervous system are characteristic at the generic level.

Radula: Provides excellent diagnostic characteristics for delimiting genera (except *Pleurobranchus* and *Berthella*). Generally considerable intraspecific variation exists, particularly with respect to age of the individual (as is the case in doridacean nudibranchs—see Bertsch, 1976, 1977). Nevertheless, radular details are most useful at the species level when the extent of this variability is understood.

Jaws: As for the radula.

Reproductive system: A highly important source of differential characters at higher levels. From species to species there is variation in detail but it is easy to misinterpret developmental changes in the genital organs as specific differences.

Pedal gland: This enigmatic gland is found on many (maybe all) species of *Pleurobranchus, Berthella* and *Pleurobranchaea*. It was first described by Vayssière (1885), who attributed the first record of it to Delle-Chiaje (1828). Vayssière (1885: 111) was unable to suggest any function for the gland. Some steps have recently been made towards an understanding of the function of this gland. It is becoming clear that it has a sexual function. Thompson & Slinn (1959) showed that in *Pleurobranchus membranaceus* it is absent in juveniles but present in older specimens. Macnae (1962) noted the gland only in fully mature, sexually active specimens.

My observations on *Pleurobranchaea maculata* suggest that the appearance of this gland indicates the onset of sexual maturity. *P. maculata* reaches full size towards midwinter but there is no pedal gland. It appears when the water temperature rises in spring and remains until the animal dies the following summer. It thus seems that development of the gland takes place with attainment of sexual maturity. The shape of the gland varies considerably between species. I suggest that the gland produces a species-specific pheromone that diffuses through the water and draws members of the same species together to mate. Pleurobranchs tend to be solitary and widely scattered within one area, as dictated by their food; individuals are seldom found together except when copulating; it is possible, therefore, that there are chemical cues.

HIGHER CATEGORIES

Historical Overview

Pilsbry (1896) attempted the first synthesis of the Notaspidea. His system was based mostly on external characteristics. He recognized the Umbraculidae, with two genera and two subgenera, and the Pleurobranchidae, with six genera and two subgenera. Vayssière (1898,1901) published a large monograph dealing at length with the known pleurobranchs; his classification scheme had appeared earlier (Vayssière, 1896). The Pleurobranchidae were divided into four genera, one (*Pleurobranchus*) being further split into four subgenera. A second large monograph was written by Bergh (1897–1905), this work being produced quite independently from that of Vayssière. Bergh gave detailed anatomical accounts of specimens examined; however he did not treat all the known species as Vayssière had done. Bergh recognized six genera which he did not subdivide further. I have followed Winckworth (1946) in citing the dates of the five parts of Bergh's *Sempers*

Reisen, Malacologische Untersuchungen that deal with pleurobranchs.

Odhner (1926) presented another scheme, again based on external characteristics—pedal gland, gill rachis and mantle margin; within the Pleurobranchidae he recognized two subfamilies with a total of five genera and two subgenera. Marcus (1971) has translated Odhner's key into English. With some small alterations Odhner's classification has been kept by later reviewers (Thiele, 1931; Burn, 1962; Franc, 1968; Thompson, 1976); I use it here because it allows incorporation of the greatest number of features, including obvious external characteristics. Here I divide the Pleurobranchidae into two subfamilies with eight genera between them; recognition of subgenera is, in my opinion, premature at present. Mention must be made of Gardiner's (1936) brief paper, which because it reorganized generic nomenclature, was of great significance.

The order Notaspidea Fischer

All notaspideans have a single, elongate, plume-like gill on the right side of the body between the mantle and foot. All are carnivorous and have a broad radula with many teeth. All have rhinophores that are scroll-like and with a lateral, longitudinal slit. No species possesses parapodia. The order contains "tectibranchs" (with large, external, patelliform shells (Umbraculidae)), and "nudibranchs" (with shells that are internal or absent (Pleurobranchidae)). The Notaspidea form a transitional link between the lower (e.g. the shell-bearing Cephalaspidea) and higher (e.g. the shell-less Doridacea) opisthobranch groups. Evidence suggests that the Notaspidea represent an intermediate grade of organization. The shelled, umbraculiform members are so different anatomically from the pleurobranchs that both may well have reached the notaspidean state by independent paths from tectibranch ancestors.

Recently Thompson (1970, 1976) and Edmunds & Thompson (1972) have called this order Pleurobranchomorpha (originally a tribe of the order Opisthobranchia of Pelseneer (1906). I prefer to keep the name Notaspidea because it is brief and has been used consistently throughout the literature. Also, the other tribes which Pelseneer placed within the Opisthobranchia have been largely dismantled since. For example, Pelseneer included the Lophocercidae (now in Oxynoeidae, Sacoglossa) and Limacinidae, Cymbuliidae, Cavoliniidae (now in Thecosomata) in the tribe Bullomorpha. He included the Pneumodermatidae, Clionopsidae, Notobranchaeidae, Thiptodontidae, Clionidae, Halopsychidae (now in Gymnosomata) in the tribe Aplysiomorpha. It would appear more appropriate to use something other than Pleurobranchomorpha if a new name should be needed.

Thiele (1931) combined the Notaspidea with the Nudibranchia *sensu lato* in a new order, Acoela, to reflect their close relationships, the nudibranchs having risen from the pleurobranchs (Odhner 1939). But since the Notaspidea are so different from other groups of opisthobranchs they should be given equal ranking with the Nudibranchia. Furthermore, the homogeneity of the order Nudibranchia itself is in doubt (Minichev, 1970; Minichev & Starobogataov, 1978). Hyman (1967) noted that Thiele's taxonomy had failed to gain general acceptance, and most authors who have recently considered the arrangement of higher groups within the Opisthobranchia have given the Notaspidea ordinal status equal to that of the Nudibranchia *sensu lato* (Hyman, 1967; Minichev, 1970; Morton, 1958, 1972; Nordsieck, 1972; Taylor & Sohl, 1962; Thompson, 1976). Franc (1968), however, lists the order separately as Pleurobranchacea Deshayes, 1830.

Members of the order have long been recognized as falling into two distinct series, and I agree with Thompson (1976) that each warrants no less than subordinal ranking. The first (Umbraculacea) includes the family Umbraculidae Gray (= Umbrellidae auct.) with three genera: *Umbraculum* Schumacher, 1817; *Tylodina* Rafinesque, 1819; *Tylodinella* Mazzarelli, 1897.

The Umbraculidae is here diagnosed as follows:

Shell external, limpet-like, with protoconch minute and hyperstrophic, apex near centre, interior with a closed or incomplete muscle scar, periostracum often dense; body smaller or much larger than shell, mantle thin, margin serrated or tentaculate; foot with large flat sole, upper surface smooth or tuberculate; head with a pair of enrolled rhinophoral tentacles with sessile eyes at their bases, mouth with two pairs of small oral tentacles. Gill a long plume lying between mantle and foot on anterior and right side, adnate and bearing numerous bipinnate branches for greater part of its length, posterior end free and bipinnate;

anal papilla projecting behind attached portion of gill, penis anterior—external, lying in anterior sinus of foot, in median line in front of and below head (*Umbraculum*) or retractile, on right side in front of gill (*Tylodina, Tylodinella*). Radula very broad, bearing a great number of similar, crowded, needle-like teeth, with recurved simple cusps which lack subdenticles; buccal armature consisting of lightly cornified polygonal plates which lack subdenticles.

Some authors (Pruvot-Fol, 1954; Burn, 1959) divide the Umbraculidae into two families—Umbraculidae Dall and Tylodinidae Gray, on the basis of the proportions of the shell and body, and the position of the head (either projecting or included in an anterior sinus of the foot), and differences in external genitalia, shell periostracum and muscle scars, radula and buccal armature.

The Pleurobranchidae Menke

The second series (suborder Pleurobranchacea) constitutes the family Pleurobranchidae with seven recognized genera:

Pleurobranchus Cuvier, 1805 (*sensu* Thompson, 1970 and Baba & Hamatani, 1971)
Berthella Blainville, 1825
Pleurehdera Marcus & Marcus, 1970
Berthellina Gardiner, 1936
Pleurobranchaea Meckel in Leue, 1813
Euselenops Pilsbry, 1896
Pleurobranchella Thiele, 1925

The present work adds one more—*Bathyberthella* n. gen.

The Pleurobranchidae are defined as follows:

Gill on right side of body, extending backwards in groove between mantle and foot, rachis smooth or tuberculate, side pinnae subdivided into pinnules, anterior part attached to body by a basement membrane, posterior end free; prebranchial aperture in front of gill. Shell internal beneath mantle, either small or absent; when present the shell is haliotiform or spatulate. Mantle smooth or tuberculate, either large and separated from foot (*Pleurobranchus, Berthella, Pleurehdera, Berthellina, Bathyberthella*) or smaller than foot and merging with it anteriorly and/or posteriorly (*Pleurobranchaea, Euselenops, Pleurobranchella*). Head with trapezoidal oral veil projecting above mouth, its lateral edges longitudinally grooved; a pair of rolled rhinophores above oral veil, also laterally grooved, rhinophores arising either together mid-anteriorly or separately where mantle merges anteriorly into oral veil. Gut with extensible oral tube and muscular pharyngeal bulb inside which lie jaws and radula; two jaws placed laterally, composed of numerous similar imbricated mandibular elements; radula broad, with or without rachidian; gut with unpaired, dorsal oral gland opening anteriorly into pharyngeal bulb and a pair of salivary glands; tubules from oral gland ramify to a greater or lesser extent throughout the body; stomach a large, unthickened, mid-ventral sac; anus opening on right side. Animals hermaphroditic with diaulic or triaulic reproductive systems; penis retractile, stout or long and slender, sometimes with enclosing flaps externally and with or without penial gland internally, smooth or papillose; vas deferens with or without prostatic portion; eggs laid in loosely arranged, or coiled, spawn bands; larva hatching as a pelagic veliger. Distributed worldwide in tropical and temperate (rarely in cold) areas. The Pleurobranchidae have two subfamilies.

Pleurobranchinae Menke, 1828

Shell internal; distinct mantle with free edges all round; rhinophores arising together mid-anteriorly; radula without a rachidian; mandibular elements cruciform with simple or denticulate blades; generally low activity (but a few species are able to swim).

Pleurobranchaeinae Pilsbry, 1896

No shell; mantle reduced and continuous anteriorly with the oral veil, rhinophores separate, dorso-lateral; radula with or without rachidian; mandibular elements polygonal or scale-like and denticulate; generally high activity.

Burn (1962) elevated both to familial rank essentially on the presence or absence of a shell; but as Edmunds & Thompson (1972) have shown for *Berthellina citrina*, this character is not constant even within a species. My opinion is that these groups show such a unity of body design and gill plan and structure of the gut and reproductive system as to warrant placing them within a single family. These similarities suggest their derivation from a common ancestor.

Burn also split the Pleurobranchidae (*sensu* Burn, 1962) into two subfamilies—the Pleurobranchinae (large and with tuberculate gill rachis and pedal gland), and the Berthellinae (smaller with a non-tuberculate rachis

and no pedal gland). These divisions are unjustified on the characters chosen since small species of Pleurobrachinae do exist, e.g. *Pleurobranchus ovalis* Pease (Thompson, 1970); some species of *Berthella* (subfamily Berthellinae) do have weak tubercles on the gill rachis, e.g. *B. ornata* (Cheeseman) (present observations); and several (if not all) species of *Berthella* have a pedal gland when they are sexually mature. Even though these groups do not warrant separation at the subfamilial level as advocated by Burn I agree with his distinctions and consider the "pleurobranchine" and "berthelline" groups represent natural lineages. The two monotypic genera *Pleurehdera* and *Bathyberthella* (the new genus described herein) together display all the characters required to span the gap between *Pleurobranchus* and *Berthellina*. I do not wish to imply, however, that either *Pleurehdera* or *Bathyberthella* is ancestral to either the "pleurobranchine" or the "berthelline" groups.

Relationships of genera within the Pleurobranchinae remain confused. I recognize only four (*Pleurobranchus, Berthella, Berthellina, Pleurehdera*) and add a fifth (*Bathyberthella*). I support Thompson (1970) and Baba & Hamatani (1971) in considering *Pleurobranchus* to encompass *Oscanius* Leach and *Susania* Gray. Two conflicting classifications for this subfamily have emerged from past studies; one based on radular characteristics unites *Berthella* and *Pleurobranchus* (e.g. Vayssière, 1896, 1898; Odhner, 1926), the other relying on the nature of the mantle, gill and shell unites *Berthella* and *Berthellina* and excludes *Pleurobranchus* (Burn, 1962). The two systems are incompatible. As stated above, my studies so far have led me to support the latter classification. Even though this stance is adopted, I acknowledge that *Pleurobranchus* and *Berthella* are close to each other. At present the only satisfactory character differentiating them is presence or absence, respectively, of tubercles on the mantle and gill rachis. When this character is set aside it seems impossible to draw a hard and fast line between them; indeed their radular and jaw structures seem identical. According to this criterion some species remain poised between *Pleurobranchus* and *Berthella* (the New Zealand *Berthella ornata* (Cheeseman) and North American *Berthella americana* (Dall) are examples). I suggest that difficulty of separating them has arisen more through lack of critical appraisal of species than lack of distinguishing characters. Species belonging to both genera require further examination (particularly for characters related to mantle, radula, jaws, reproductive and alimentary systems) that will enable the boundary to be cut more sharply if these genera are to remain separate.

Pleurobranchus and *Berthella* have long been upheld as separate. Both are enormous genera, easily the largest in the order, each has more than 50 named species although no one is sure how many biological species exist.

Pleurehdera (with its type species, *P. haraldi,* still known from only a single specimen from the Tuamotu Archipelago) and *Bathyberthella* are particularly important because they possess characters linking them with both pleurobranchine and pleurobranchaeine genera. One or both could be a key between the major genera or subfamilies. An appraisal of *Bathyberthella* with regard to other genera appears later in this work in connection with the description of the new species from New Zealand.

Despite the natural affinity of *Berthellina* and *Berthella* there are sufficient characters presently recognized to adequately diagnose them as separate. All species of *Berthellina* have immediately recognizable radulae, their shells too are distinctive; they have a prostate gland and jaws with (almost always) smooth mandibular elements. Were it not for its mandibular elements and pedal gland, *Pleurehdera haraldi* would be classified as a *Berthellina.* In all these characters *Berthellina* species differ from the *Pleurobranchus-Berthella* group, which warrants their separation, but I would not remove *Berthellina* any further than recognizing it as a separate genus. There are probably not more than four valid species of *Berthellina.*

The three genera in the Pleurobranchaeinae (*Pleurobranchaea, Euselenops, Pleurobranchella*) are well separated from each other by several characters. *Pleurobranchaea* has numerous species, many poorly defined (Marcus & Marcus, 1966). *Euselenops* is monotypic—*E. luniceps* (Cuvier, 1817) being widespread. The deepwater *Pleurobranchella* has possibly four species (Willan, 1977).

SYSTEMATICS

Berthellina Gardiner, 1936

Berthella Vayssière, 1896: 115 (*non Berthella* Blainville, 1825).

Berthellina Gardiner, 1936: 198. Type-species by original designation: *Berthellina engeli* Gardiner, 1936.

Definition

Relatively small pleurobranchs, body elliptical and convex; mantle large, smooth, simple and free all round, without an anterior crenulation; pedal gland never present; gill rachis smooth; anus at posterior end of gill membrane; shell beneath mantle, small (1/4–1/5 length of body), triangular or ovate, carried anteriorly, occasionally absent; teeth or radula elongate, lamelliform, serrated on distal section of posterior edge; mandibular elements cruciform on inner surface of jaw, smooth or indistinctly denticulate; vas deferens dilated into prostate gland; penis without accessory leaves.

Remarks

Species with these characters were attributed to *Berthella* Blainville, 1825 by Pilsbry (1896), Vayssière (1896, 1989) and Odhner (1926). Later authors used *Berthellina* for this genus (Gardiner, 1936; Odhner, 1939). The reasons for the change are given in the following summary.

Blainville (1825) created *Berthella* to include notaspidean opisthobranchs with lamellate teeth and smooth jaw elements; he designated *Berthella porosa* Blainville, 1825 as type-species. Vayssière (1898: 271) pointed out that *B. porosa* Blainville was a junior objective synonym of *Berthella plumula* (Montagu, 1803) and therefore Montagu's *Bulla plumula* took precedence as type-species. But a re-examination of the characters of *Berthella plumula* by Gardiner (1936) showed that this species has smooth, hook-like radular teeth and denticulate jaw elements. These characters had been applied to *Bouvieria* Vayssière, 1896, so *Bouvieria* became a synonym of *Berthella*. Gardiner (1936) created *Berthellina* for those species with lamellate radular teeth.

It is probable that some of the six species of *Berthellina* recognized by Burn (1962) are not valid. Indeed Edmunds & Thompson (1972) and Thompson (1976) amalgamated *B. engeli* with *B. citrina* (Rüppell & Leuckart), thus giving that species' distribution as Indo-Pacific, Mediterranean and marginally North Atlantic (e.g., Britain). Marcus & Marcus (1967a) named a new subspecies (*Berthellina engeli ilisima*), found from San Diego, California, to Guaymas, Mexico, but it was rejected soon afterwards (Bertsch, 1970). If *B. engeli* does exist in North America and is synonymous with *B. citrina*, then the one species would have an almost world-wide distribution. Care is needed here, and I concur with Thompson (1977) that hasty lumping of *Berthellina* species at this stage would be unwise. I think some reinstatements may be necessary in future (e.g. *Berthellina engeli* Gardiner). Marcus & Marcus (1957) transferred *Pleurobranchus (Oscanius) amarillius* Mattox to *Berthellina* where it is now considered a synonym of *Berthellina quadridens* (Mörch) (Burn, 1962; Marcus & Hughes, 1974; Thompson, 1977). One species overlooked in Burn's (1962) list is *Berthellina africana* Pruvot-Fol (1953) from the Atlantic coast of Morocco.

Abbott (1974) disrupted accepted nomenclature by replacing *Berthellina* with *Gymnotoplax* Pilsbry. But the type-species of *Gymnotoplax* (*Pleurobranchus americanus* Verrill) is undoubtedly a species of *Berthella.* Abbott's alteration is therefore unacceptable (Willan, 1978).

Berthellina citrina (Rüppell & Leuckart, 1828) (Figs. 1–6, 9, 10, 13–19)

There are at least eight synonyms for this species. I do not give a synonymy here for three reasons. Burn (1962) has already presented an adequate synonymy and discussion; also the species is so widespread that a full synonymy would be very lengthy. Finally, in New Zealand this species has been confused with another pleurobranch (*Berthella mediatas* Burn) and references do not discriminate between the two.

Recent investigations have uncovered three more names to add to the synonymy of *Berthellina citrina*:

Pleurobranchus cuvieri Bergh, 1898: 129–131, pl. 11, figs. 19–27.

Berthella borneensis Bergh, 1905b: 69–70, pl. 5, fig. 3; pl. 11, figs. 45–47;

Berthella minor Bergh, 1905b: 70–71; pl. 13, figs. 1–3.

Marcus & Marcus (1970) redescribed *Berthellina cuvieri* (Bergh) from Madagascar, but their material easily falls within the range of variation of Indo-Pacific specimens of *B. citrina.* Narayanan (1970) studied material from the Gulf of Kutch, India, that he had pre-

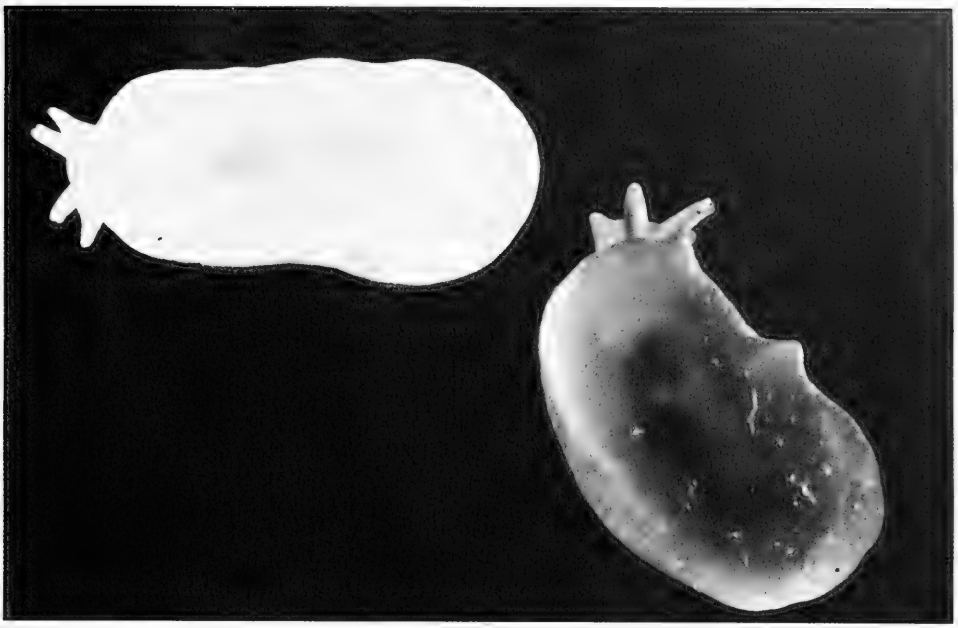


FIG. 5. *Berthellina citrina.* Lengths of both specimens approx. 40 mm. From 3–4 m, Leigh Harbour, North Auckland, 22 Nov. 1973. Photograph: G. W. Batt.

viously assigned to *Berthellina minor* (Narayanan, 1969) and placed that name also in the synonymy of *B. citrina.*

Live animal (Fig. 5)

During this study over 80 living specimens of *Berthellina citrina* have been examined; this is an adequate number to permit a description encompassing the natural variability of the species over its geographic range in New Zealand.

Shape elliptical, mantle wrapping entire upper part of body except rhinophores and oral veil which project anteriorly; rhinophores exposed from just behind their point of fusion; foot generally does not appear behind posterior edge of mantle when slug is crawling. Colour varies from deep apricot-orange to pale lemon; white spots almost always on surface, generally more numerous on sides and back, less apparent in largest individuals (approx. 50 mm long). Rhinophores and oral veil same colour as mantle surface. Mantle very delicate, smooth, soft, and translucent; anterior reddish-brown shell and posterior digestive gland, which appears as a black smudge, can be seen easily from dorsal aspect. When mantle margins are removed (e.g. through predation by *Pleurobranchaea maculata*), white spots become larger and more numerous after 5–6 days.

Rhinophores elongate, diverging by 50–60°, raised above oral veil, consisting of a spirally-wound sheet of tissue of 1½ whorls, open at last turn to leave a narrow lateral slit. Distal margin of rhinophoral sheet can be moved to control size of slit. At posterior-lateral corner, slit expanded to form a pyriform aperture, water enters along slit and is expelled through distal aperture. Eyes lie near, and just behind, basal aperture, covered by anterior edge of mantle.

Oral veil extending forward from head as a trapezoidal sail, anterior margin broad, wavy—but not nearly as sinuous as in *Berthella mediatas*; thickened lateral borders of oral veil have a deep furrow that expands into a cavity internally at base of veil.

Gill rachis smooth and cylindrical, basement membrane attaching gill to body for two-thirds of the gill's length; 18–28 pinnae on upper side of gill, first pinna always on upper side; mean number of pinnae for 15 specimens 23.7. Anus on dorsal side of gill, at hind end of basement membrane, opening on a

slightly raised papilla; longitudinal folds in interior of rectum visible within anus. Prebranchial aperture opens just in front of, and slightly above, front of rachis; a much smaller aperture, the renal pore, opens below and behind base of rachis.

In specimens preserved in alcohol, mantle is creamish in colour. The delicate, translucent tissue becomes opaque, apparently thicker, and velvet-like, frequently obscuring shell.

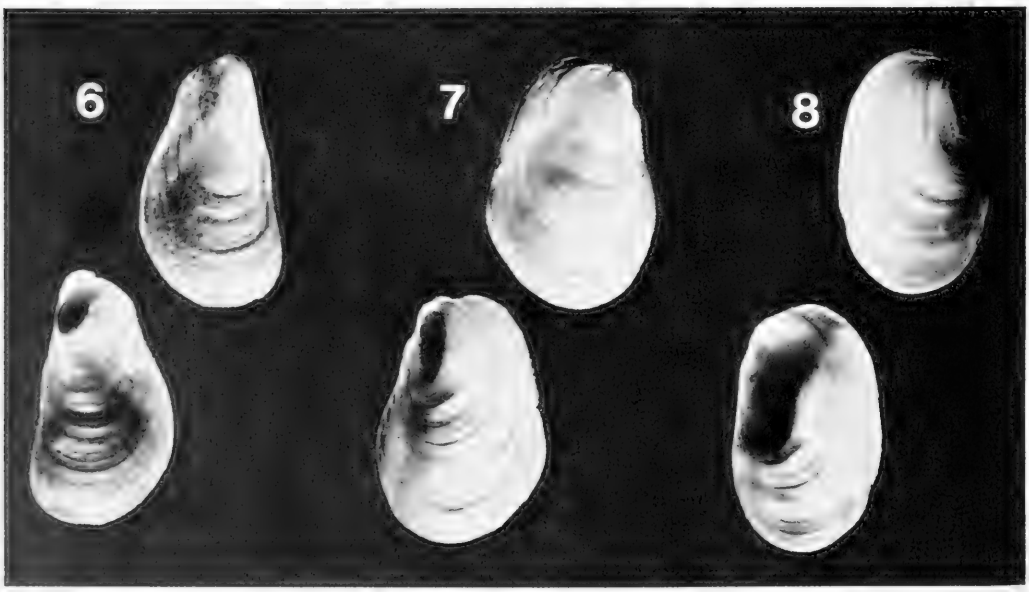
Shell (Figs. 6, 9, 10)

Shell small (1/4–1/5 length of extended body), thin, flattened, triangular; lying above pericardium in front of digestive gland; visible through mantle in living specimens. Consisting of two distinct, and clearly demarcated regions—protoconch of 1¼ to 1½ whorls and teleoconch; protoconch translucent, lacking regular sculpture but possessing some irregular calcification externally (Fig. 9); teleoconch spatulate, golden-brown or rich reddish-brown, older shells thicker and darker, clear apical region always present on teleoconch; first-formed teleoconch weakly convex and quadrangular, later growth unequal, producing eventually a triangular, spatulate form. Shell entirely covered by glistening, transparent periostracum that flakes off when shell dries; periostracum does not extend beyond shell margin.

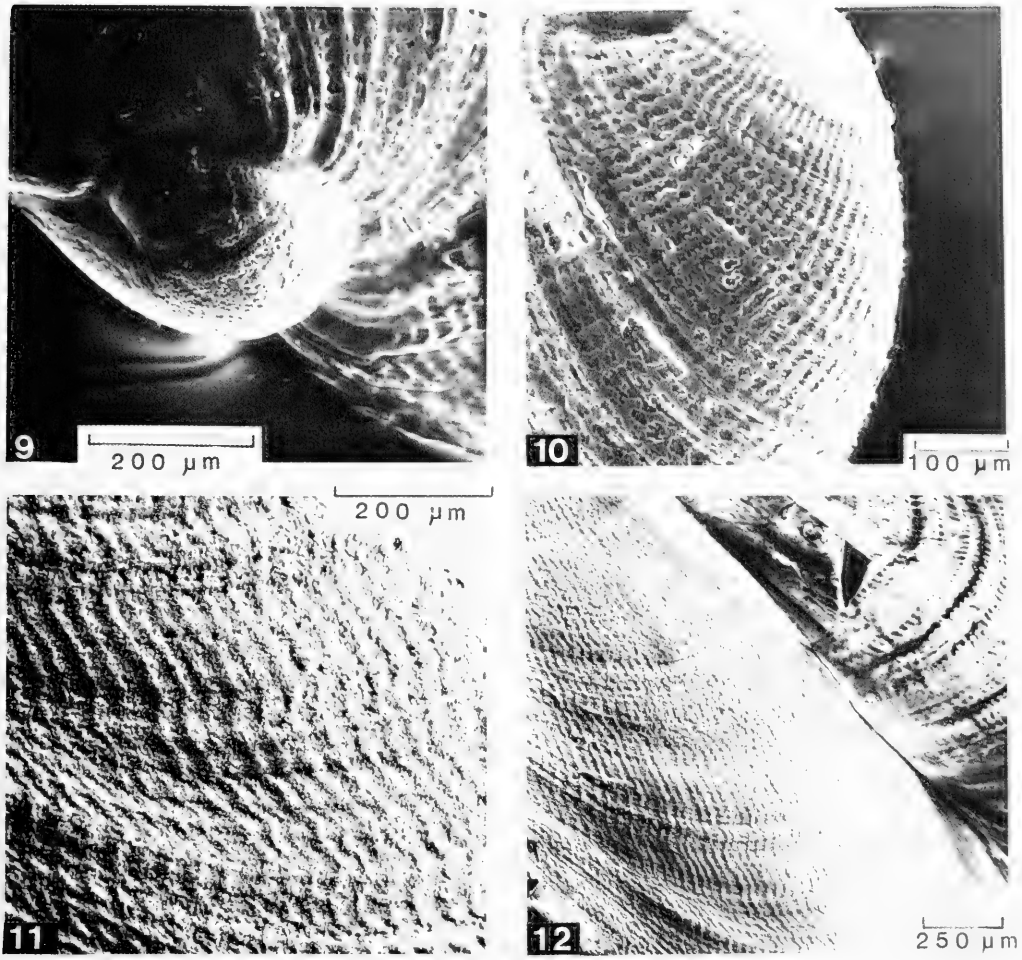
To the naked eye the surface sculpture is of irregular, concentric growth rings coalescing towards the margin in the adult shell to produce broad, flat ridges. Fine surface sculpture is most obvious near teleoconch apex (Fig. 10) and appears as a series of radiating, punctate depressions separated by flattened areas. Punctae occur in radial files, with some concentric organization; occasionally three to five merge within the radial rows. However, concentric fusion (which corresponds with growth checks) can also occur, becoming more obvious in older portion of shell, obliterating much of the regular radial organization.

All specimens of *Berthellina citrina* that I have examined (up to 5 cm) have had shells; this finding is contrary to that of Thompson (1970). He claimed that larger specimens (2 cm and over in extended body length) lacked shells. In New Zealand, *B. citrina* does not show the linear relationship between shell length and body length that has been shown for this species in the Red Sea (Gohar & Abul-Ela, 1957).

Five shells from specimens of *Berthellina citrina* taken at Leigh Harbour have been de-



FIGS. 6–8. Shells of New Zealand pleurobranchs. 6. *Berthellina citrina*. Length 6.1 mm × width 3.3 mm. Specimen from Pananehe Is., Spirits Bay, 14 Jan. 1972; 7. *Berthellina ornata*. 13.8 × 9.5 mm. Specimen from Army Bay, Whangaparoa Pen., 10 Jan. 1974; 8. *Berthella mediatas*. 10.3 × 6.4 mm. Specimen from Army Bay, Whangaparoa Pen., 10 Jan. 1974.



FIGS. 9–12. Scanning electron micrographs of shells. 9. *Berthellina citrina*, detail of protoconch. 10. *Berthellina citrina*, sculpture on apical region of teleoconch. 11. *Berthella ornata*, sculpture on exterior of teleoconch. 12. Same, periostracum has peeled off and appears at top right.

posited in the mollusc collection, Auckland Institute and Museum.

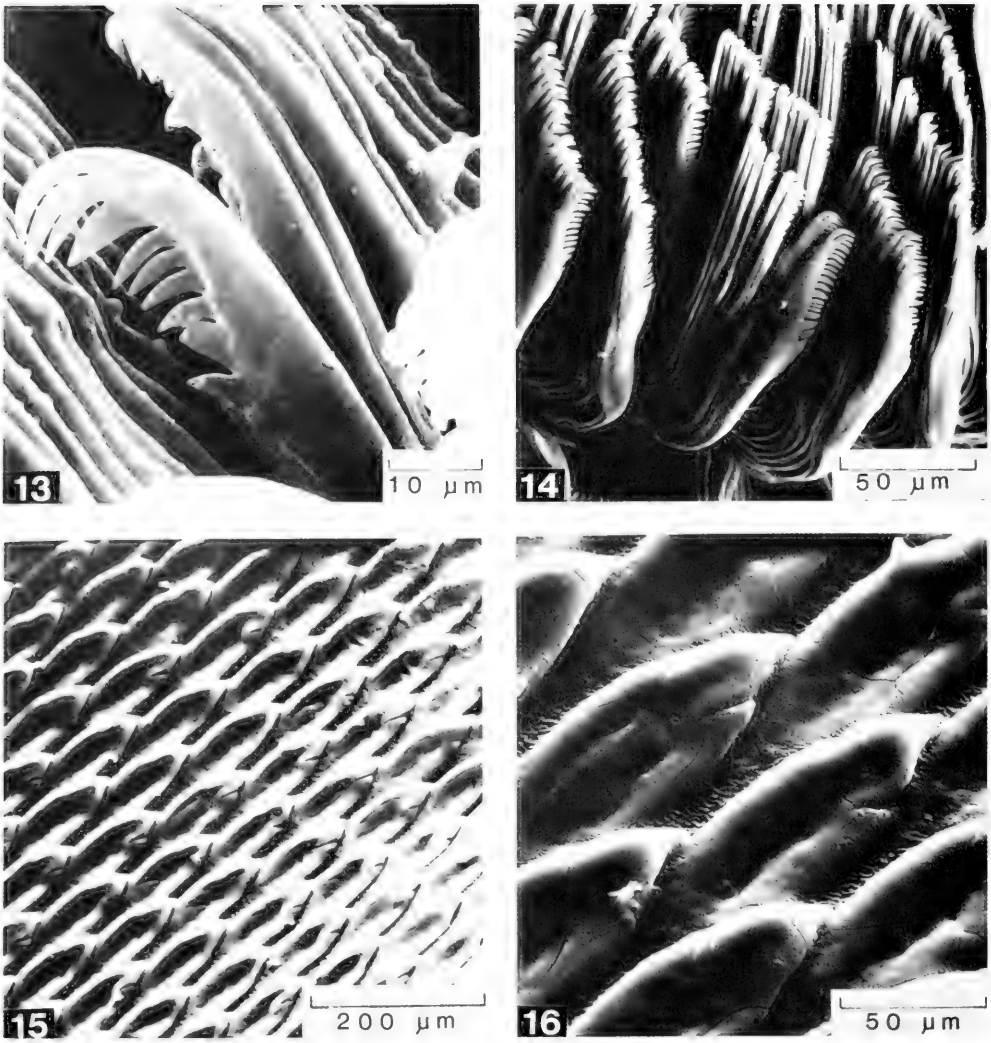
Radula (Figs. 13, 14)

Radula broad with two symmetrical halves, each forms one side of a V. The following radular description is applicable to an adult animal.

Central tooth lacking. Lateral teeth all similar, but showing gradual changes in length and denticulation across rows. Lateral teeth near midline 90 µm high, elongate, weakly denticulate on upper third of posterior face, terminal cusp differentiated from rest of tooth by a relatively deep groove. Base of each tooth curving sharply backwards and slightly thickened at its attachment to radular membrane.

Middle lateral teeth (Fig. 14) very large—140 µm high, comb-like, 15–20 strong denticles present on upper half of posterior face of blade; again terminal cusp longer than remaining denticles, all denticles project in same plane. There is a group of one to three broader denticles immediately below cusp; remaining denticles narrow, either needle-like or rounded, and frequently bifid at tips.

Outermost lateral teeth (Fig. 13) shorter (approx. 100 µm) than middle ones, and recurved towards posterior face; 15–20 denticles on upper half of posterior face, smaller



FIGS. 13–16. Radula and jaws of *Berthellina citrina*. 13. Detail of cusp and denticles on posterior face of a single tooth from middle lateral region of radula. 14. Outer lateral teeth and basal supporting structure within each row. 15. Mandibular elements on inner face of jaw. 16. Detail of same.

than those on middle teeth, with weaker grooves separating them, seldom bifid; terminal cusp projects in plane of tooth, tip slightly flexed upwards, and separated by a relatively deeper groove from rest of denticles, which project obliquely from posterior face, although still in same plane.

Examination of teeth with the SEM shows that the surfaces of the blade are smooth and there are no secondary structures on the denticles. There is a compression furrow on the shaft parallel to the anterior face. The furrow and the broad dorsal ridge beside it lock the blade in position laterally alongside adjacent teeth; there is thus a system for lateral support along the rows during the feeding movements.

Each tooth has an enlarged basal portion—a flange forming an oblique angle with the blade. On this flange, towards the base of the anterior face of the blade, is a raised ridge with a shallow depression at its base. A corresponding ridge on the next tooth in the same row fits into this depression, and into the depression on the flange of this tooth locks a ridge on the next tooth and so on. SEMs show

the overlap of successive ridges down the row (Fig. 13).

Any single formula for *Berthellina citrina* would be inadequate since the number of rows and the number of teeth per row increase as the animal grows. Specimens examined had radular formulae in the range of 40 × 150.0.150 to 90 × 180.0.180. This is approximately the same as Burn's (1962) range (60 × 120.0.120 to 95 × 200.0.200).

Thompson (1970) published the first SEM photos of radular teeth of *Berthellina citrina*.

Jaws (Figs. 15, 16)

Two jaws, one on each side of the radula, are joined anteriorly at top and bottom by labial cuticle; anterior edges of cuticle recurved. Jaws rectangular, narrowing posteriorly to a point.

Jaws composed of columns of interlocked small elements; at jaw surface elements appear cruciform, with broad bases and elongate blades. Midway between base and blade are two rounded, lateral processes, one on either side; each process abuts against a similar process in the same row. "These processes are not exactly opposite, those on one side being slightly in advance of those on the opposite side, thus determining the slight obliquity of the rows. Toward the dorsal and ventral margins of the mandible, the elements become somewhat more irregular in form and depart from the typical shape found in the central areas." (MacFarland, 1966; part of description of *Berthellina engeli* Gardiner, but applicable to all species of *Berthellina*.)

A mid-ventral spike on flattened base of each element locks into place behind lateral processes of two adjacent elements so supporting them from behind; overlapping tip of blade of element in the next row posteriorly acts to enforce connection from above. Blade of one element fits between base and lateral processes of two adjacent elements; raised and with dorsal flange so that dorsal surface presents only a series of similar flattened blades. SEM photos (Figs. 15, 16) give a view of this top surface.

Mandibular elements are approx. 80 μm long and up to 30 μm wide at centre of jaws, base slightly narrower; element widens in a smooth curve to blunt tips of lateral processes, element constricts just above lateral processes and broadens anteriorly to form blade. Blade elongate, straight-sided, narrowing gradually to a pointed tip.

All jaws but one had no denticulation on the blade. The specimen in Figs. 15 and 16 had one or two definite denticles developed near the apex of many of the mandibular elements. Most had a single cusp, but a few showed a weaker cusp in a similar position on the other side of the blade. These weaker cusps lay in the plane of the mandibular elements and did not break the smooth outlines of the blade. They were not separated at their bases from the rest of the dorsal surface of the element and narrowed rapidly. Baba (1937) and Burn (1962) have also noticed these structures on the mandibular elements of *Berthellina citrina*.

The presence of these rudimentary and irregular cusps on the mandibular elements of *Berthellina citrina* invalidates, in a sense, the diagnostic character of smooth-sided jaw elements for the genus; but they are so different from those of *Berthella* and *Pleurobranchus* that the mandibular elements are still an important taxonomic character.

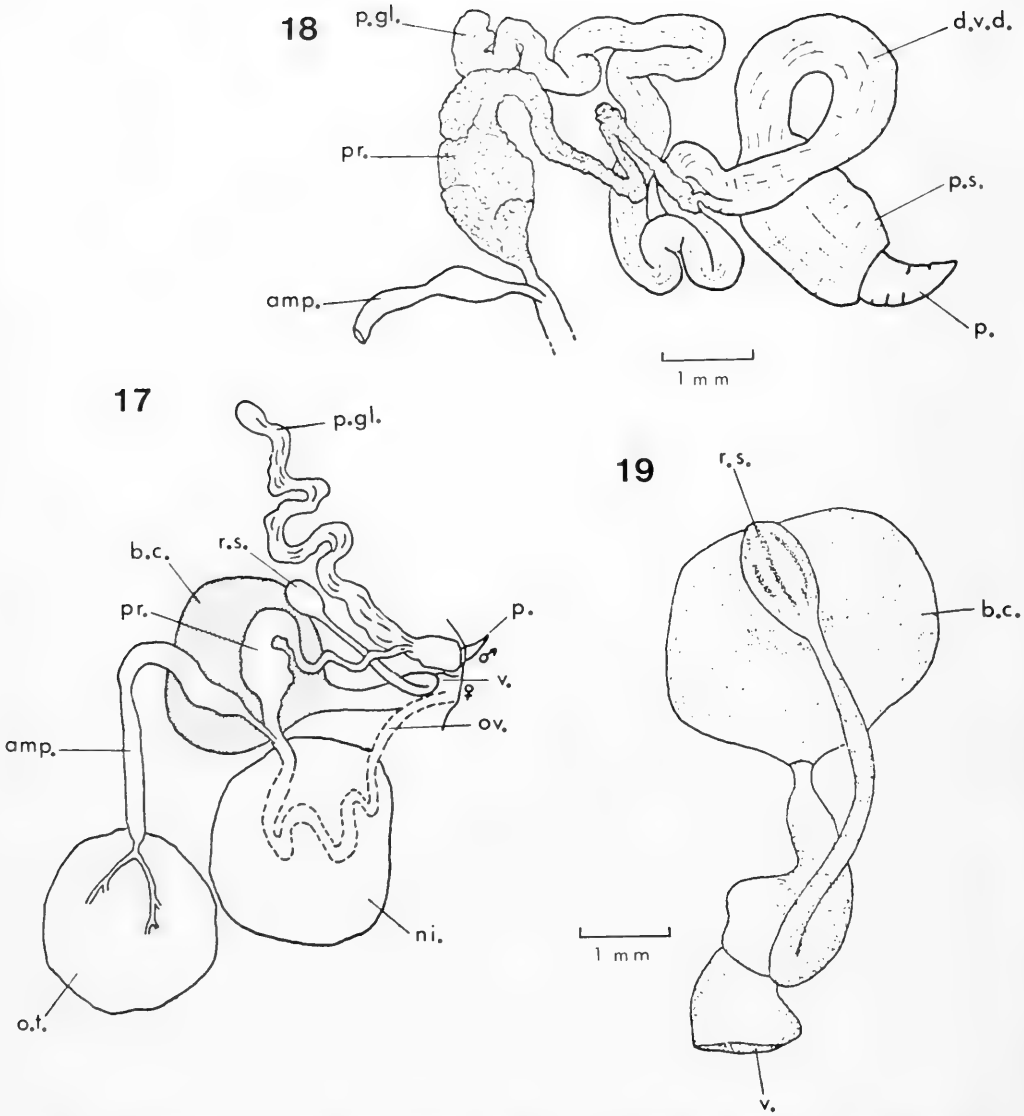
Reproductive system (Figs. 17–19)

Reproductive apertures located on right side just in front of anterior end of gill, surrounded by a low, collar-like ridge. Most preserved specimens examined had terminal portions of the reproductive ducts everted to some degree. Penial opening foremost, middle opening marks vagina, and behind that is large aperture of oviduct. When genital papilla is everted, oviduct and vagina are separated by a broad flap of tissue projecting upwards from surrounding collar, but in retracted state they share a common atrium.

Ovotestis yellowish, large, lying against right side of digestive gland, appearing glandular because of layers of immature eggs. Hermaphrodite duct divides into two as it enters ovotestis; each branch fine, white; after a short distance each divides repeatedly.

Upon leaving ovotestis, hermaphrodite duct enlarges into an ampullar region which crosses beneath looped intestine and rises to dorsal surface of visceral mass where it appears as a prominent tube, passing anteriorly across fluffy, yellow mucous gland. At anterior end of mucous gland, hermaphrodite duct constricts and forms a T-junction with a larger duct, anterior limb of the latter being proximal vas deferens; posterior limb enters nidamental gland complex after a very short distance.

Proximal vas deferens short, rapidly enlarging to yellow prostate gland; distal vas



FIGS. 17–19. Reproductive system of *Berthellina citrina*. 17. Composite view of structure of the reproductive organs. 18. Detail of structures associated with distal section of vas deferens and penis. 19. Detail of seminal receptacles in a mature animal. Abbreviations: amp. = ampulla; b.c. = bursa copulatraix; d.v.d. = distal section of vas deferens; ni. = nidamental glands; o.t. = ovotestis; ov. = oviduct; p. = penis; p.gl. = penial gland; pr. = prostate gland; p.s. = penial sheath; r.s. = receptaculum seminis; v. = vagina.

deferens much narrower, undergoing several loops and enters base of penis; a larger, much-coiled penial gland located there too (penial gland has also been called accessory prostate gland—Vayssière, 1898, MacFarland, 1966); penial gland elongate, thick-walled with longitudinal ridges, coiled about distal vas deferens, blind-ending. Beyond entrance of penial gland vas deferens is tightly looped several times at base of penis. Penis short and curved, unarmed, projecting forward when everted, surrounded by stout muscular sheath. When everted, penis has weak concentric ridges on its surface and ends in a sharp tip. Fig. 18 gives a diagram of the anterior organs of the male section of the pallial gonoduct.

Two prominent sacs open into vagina—

bursa copulatrix which is spherical and thin-walled, and receptaculum seminis, which is smaller and thick-walled. Receptaculum seminis has a terminal, club-shaped dilation and a curved canal looping to enter vagina near its opening, where another swelling is present (see Fig. 19). No connection of vagina with oviduct exists, save through the external vaginal opening into its common exit close to the nidamental gland complex.

Distribution

The geographic range of *Berthellina citrina* in New Zealand is entirely northern (Aupourian). It extends down the east coast of the North Island, from Northland to the Bay of Plenty. There are no records so far of its occurrence on the west coast (see Appendix).

Elsewhere *Berthellina citrina* has been recorded under various names from Australia (Quoy & Gaimard, 1832; O'Donoghue, 1924; Dakin, 1952; Burn, 1962; Thompson, 1970), New Caledonia (Risbec, 1928), Hawaii (Kay, 1979), Palau Islands (Marcus, 1965), Japan (Baba, 1937, 1949, 1969; Hirase, 1937; Usuki, 1969), Sri Lanka (Kelaart, 1883), South Africa (Macnae, 1962), Indonesia and Mauritius (Macnae, 1962; Marcus & Marcus, 1967a), Gulf of Kutch, India (Narayanan, 1970), Gulf of Elat (Marbach & Tsurnamal, 1973), and Red Sea (Gohar & Abul-Ela, 1957). Apart from *B. oblongata* (Audouin) and *B. saidensis* (O'Donoghue) from the Red Sea, *B. citrina* is the only species in this genus known in the Indo-Pacific (Burn, 1962).

I can confirm Burn's (1962) and Thompson's (1970) records of *Berthellina citrina* from New South Wales because I have collected specimens there myself. I have also collected *B. citrina* in southern Queensland and Vanuatu (New Hebrides). Dr. M. C. Miler found this same species in the Solomon Islands during the Royal Society B.S.I.P. Expedition of 1965.

Discussion

The identity of *Berthellina citrina* in New Zealand cannot be doubted. In *B. citrina* the combination of a small, spatulate shell, elongate radular teeth with denticulate posterior edges, mandibular elements with smooth (or weakly denticulate) blades, and prostatic dilation of the vas deferens are thoroughly distinctive features allowing easy identification and separation from all other New Zealand pleurobranchs.

Biologists, following Bergh (1900), have applied the name "*Bouvieria aurantiaca* (Risso)" indiscriminately to any yellow or orange pleurobranch from New Zealand that lacked the dorsal, spotted pattern of *Berthella ornata,* so the two species answering this description (*Berthellina citrina* and *Berthella mediatas*) have not been hitherto distinguished. Table 2 lists the major characters which separate these two species. The only "*B. aurantiaca*" in the New Zealand literature that can be identified as *Berthellina citrina* is that by Morton & Miller (1968). This is because these authors give an excellent colored illustration of a living animal (pl. 11, fig. 6). The inclusion of *B. citrina* by Gordon & Ballantine (1977, in Appendix 3), was on my advice. "*Bouvieria aurantiaca* (Risso)" auct. is a valid species of *Berthella* from the Mediterranean Sea, and from the available literature it would appear close to *Berthella mediatas* Burn.

Several recent publications have treated the biology of *Berthellina citrina*. A summary of development was presented by Gohar & Abul-Ela (1957). Usuki (1969) studied reproduction, development and life history of Japanese specimens. Marbach & Tsurnamal (1973) made observations on feeding and acid secretion of specimens from the Gulf of Elat (Red Sea).

I have not included any distribution records for *Berthellina engeli* Gardiner in the geographic range list given above because I feel that it is premature to synonymize that species with *B. citrina*. In specimens of *B. engeli* that I have examined, the shells have been consistently more oval in shape, proportionately larger and positioned more posteriorly on the visceral mass.

Berthella Blainville, 1825

Berthella Blainville, 1825: 370. Type-species by original designation: *Berthella porosa* Blainville, 1825 (= *Bulla plumula* Montagu, 1803).

Cleanthus "Leach MS., 1819" Gray, 1847: 163. Published in the synonymy of *Berthella* Blainville, 1825.

Bouvieria Vayssière, 1896: 66. Type-species by subsequent designation (Odhner, 1926: 22): *Pleurobranchus aurantiacus* Risso, 1818.

Gymnotoplax Pilsbry, 1896: 20. Type-species by subsequent designation (Willan, 1978:

339): *Pleurobranchus americanus* Verrill, 1885.

Berthellinops Burn, 1962: 135. Type-species by original designation: *Berthellinops serenitas* Burn, 1962.

Abbott (1974) placed *Cleanthus* Gray as a synonym of *Pleurobranchus* Cuvier, 1804. Elsewhere (Willlan, 1978) I have already shown that the holotype of *Pleurobranchus americanus* Verrill, upon which *Gymnotoplax* Pilsbry is based, is a typical *Berthella*. Apart from a supposedly opposite (instead of alternate) arrangement of pinnae on the gill, *Berthellinops* Burn is a *Berthella*. Burn himself later examined additional material and found this arrangement not to persist, and accordingly would now treat *Berthellinops* as a synonym of *Berthella* (R. Burn, personal communication, July 1977).

Definition

Shelled pleurobranchs; body elliptical and convex; mantle large, simple and free, without an anterior indentation; pedal gland present; shell ovate, large (at least half length of body); gill rachis smooth or weakly tuberculate; radula with simple, curved or erect teeth; mandibular elements with smooth or denticulate blades; reproductive system lacking prostate gland.

Remarks

Members of the genus approach *Berthellina* in their relatively small size and (generally) smooth gill rachis; but in characters of the radula, jaws and reproductive system they appear to show close affinities with *Pleurobranchus*.

Two *Berthella* species are found in New Zealand; both range throughout the country. There is the endemic *Berthella ornata* (Cheeseman), and secondly *Berthella mediatas* Burn, a species shared with temperate southern Australia.

Berthella ornata (Cheeseman, 1878)
(Figs. 7, 11, 12, 20–31)

1879. *Pleurobranchus ornatus* Cheeseman: 175, pl. 15, figs. 1, 2.—1879, Cheeseman: 378, pl. 16, figs. 1, 2.—1880, Hutton: 124.—1896, Pilsbry: 206, pl. 47, figs. 22, 23.—1898, Vayssière: 337, pl. 14, figs. 18, 19.—1913, Suter: 550 (in subgenus *Berthella* Blainville).—1915, Suter: pl. 77, fig. 6.

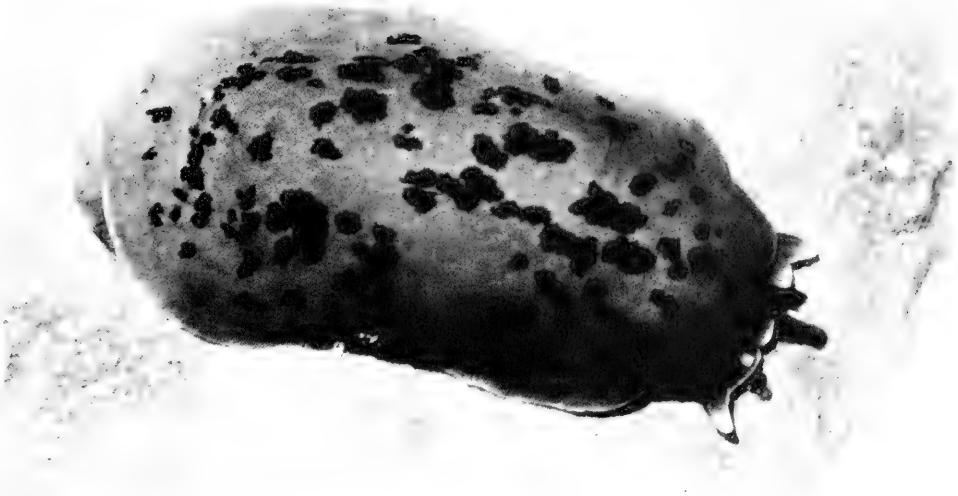
1924. *Bouvieria ornata* Cheeseman; Odhner: 51, 86.—1926. Odhner: 22.—1937. Powell: 89, No. 1404.—1953, Milligan: 134, 1139.—1957, Powell: 114.—1961, Powell: 107.—1964, Williams: 19, illust.—1968, Morton & Miller: 167, 576, pl. 11, fig. 7.—1976, Powell: 112.—1977, Gordon & Ballantine: 112.—1979, Powell: 282, pl. 51, fig. 3.

The description of *Pleurobranchus ornatus* Cheeseman (and *Pleurobranchaea novaezealandiae* Cheeseman) first appeared in *Proceedings of the Zoological Society of London* for the year 1878. An identical description was printed in the Transactions and Proceedings of the New Zealand Institute for the same year (but not published until 1879) (Pilsbry, 1896). Cheeseman's descriptions were each accompanied by a figure, meticulously drawn by his sister Evelyn (A. W. B. Powell, personal communication, 1977). Powell (1939b) designated these drawings as iconotypes and he has recently republished them in colour (Powell, 1979). These iconotypes are held in the Malacology Department, Auckland Institute and Museum.

Live animal (Figs. 20, 21)

Body oval to elongate; mantle smooth and soft, broad and circular. In actively crawling specimens (Fig. 22a) rhinophores and oral veil project beyond front edge of mantle, mantle margin just covers point of fusion of rhinophores; sole of foot often visible posteriorly. Mantle covers body as a broad cloak, lateral areas extend beyond foot to sweep the ground. In resting animals, tail and oral veil tuck beneath mantle, and rhinophores barely show (Fig. 22b).

Anterior edge of mantle truncate, raised in midline to allow outward extension of rhinophores, but not indented mid-anteriorly; posterior edge of mantle broadly rounded. Mantle thick in central area, thinner towards margins, not delicately translucent as in *Berthellina citrina*; margin entire, but often sinuous—particularly on right side where upthrown posterior-lateral edge forms a temporary exit for exhalant water current. Most animals have an apparently smooth mantle surface, but in some, large pores can be detected by eye on the surface. When an animal is removed from its substrate, the broad lateral areas of the mantle bend round the foot and hug the sides



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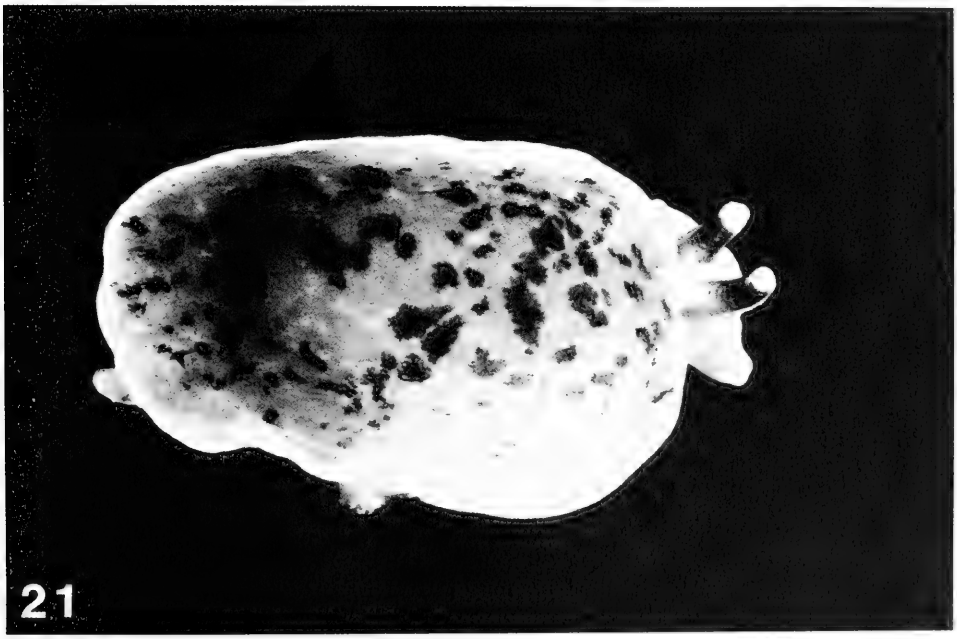

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FIGS. 20, 21. *Berthella ornata*. 20. Length = 40 mm. From intertidal reef platform to left of Matheson Bay, near Leigh, North Auckland, 5 Oct. 1975. Photograph: R. C. Willan. 21. Length = 38 mm. From Pacific Bay, Tutukata, Northland, 8 March 1974. Photograph: G. W. Batt.

of the body to give a cylindrical and protective form.

Colour characteristic, consisting of an even ground shading from pure white to pale (or rich) reddish-brown to orange-brown. Overlying this are irregular blotches and spots, all a rich, dark, red-brown and very variable in shape and size. Some individuals show few (approx. 30) markings over mantle, more often entire mantle is covered. Markings tend to be largest and more concentrated on mid-dorsal region of mantle (area over shell and digestive gland), they decrease in size and number outward from the centre, and are small and scattered on anterior and lateral marginal areas. In some animals, particularly small ones (up to 10 mm in extended length), these spots extend out to mantle margins; adults have a more or less broad zone next to mantle margin that possesses nothing but background coloration; margin itself is opaque white. Digestive gland appears as a dark central smudge; shell not visible through mantle.

When the animal is handled roughly and repeatedly, or when the mantle is prodded, the mantle produces a thick, milky-white repugnatory fluid; discharge can be localized or occur over the entire surface; secretion becomes incorporated with clear body mucus and is left behind as slug crawls away.

Foot large, oval, broad-soled, truncated in front and rounded behind; border minutely crenulate all round but crenulations are weakest at anterior margin; sole sticky. The posterior quarter of sole in fully grown specimens has a large, but indistinct, circular pedal gland. All specimens examined had a creamy-white foot, with a thin, opaque white line at margin. No markings present on the foot sole, but lightly-pigmented blotches sometimes present on dorsal surface near tail.

Oral veil broad, with thickened, moderately pronounced edges that are grooved laterally; anterior border sinuous, but not nearly as deeply embayed as in *Berthella mediatas*; very weakly crenulate along anterior margin. Oral veil creamy-white, always without markings, though an indistinct white line along anterior border is present. Undersurface (in mid-anterior region) and lateral edges periodically touch the substratum in an exploratory manner as animal crawls.

Rhinophores Y-shaped, joined at bases and diverging by 30–45°; raised upwards at 90° from horizontal in an actively crawling animal, and at 45–60° when animal quiescent; each limb can be moved independently. Tips of rhinophores always white; in live specimens tips are active and mobile; very flexible as they move to test oncoming water. There is some degree of variability in coloration of lower regions of rhinophores; these regions are often the same colour as mantle. Specimens with darkly-pigmented mantles usually have dark, reddish-brown or chocolate pigment in this region; extreme tips and fused basal areas are unpigmented. Small specimens (and adults with pale dorsal coloration) have rhinophores lightly pigmented with buff-brown or cream. Coloration of rhinophores changes to some extent with size of the individual. Most small specimens (less than 20 mm extended length) had cream rhinophores (e.g. nine from Goat Island Bay, Leigh; May 1974). Larger specimens (greater than 30 mm extended length frequently had rhinophores bearing darker pigmentation (e.g. 10 from Waiwera; January, July, September 1974). In living specimens rhinophores appear smooth externally, but in the preserved state strong contraction of these organs produces deep transverse grooves.

When magnified, both oral veil and rhinophores are seen to be covered with white, conical papillae. These papillae are evenly distributed over oral veil and are very dense towards tips of rhinophores. Sparse, white papillae are also present on mantle and tail.

Berthella ornata has an exceptionally long gill, reaching backwards almost to level of tip of tail; gill attached to body wall for over half its length by basement membrane. Number of pinnae ranges from 18–25, first pinna always arises on upper side of rachis; mean number of pinnae counted on 12 adults—22; approximately half the pinnae arise from posterior free end of gill. Pinnules are particularly large and give the gill a feather-like appearance. All specimens examined had creamy-white gills, devoid of markings.

In living, and some well-fixed specimens, it is possible to see that the rachis is not smooth, but regularly covered with weak ridges or knobs where the pinnae join. These ridges continue on to the surface of the pinnae which appear smooth. It would seem that these weak knobs are homologous with the better-developed tubercles of *Pleurobranchus* Cuvier. Most preserved *B. ornata* appear to have smooth rachises to their gills.

Anus opens at side of body, on dorsal side of gill at hind end of basement membrane (i.e.

about half-way along gill's total length); anus opens on a low, conical, backward-projecting papilla.

Numerous glassy spicules (Fig. 23) are embedded in mantle, particularly at base of rhinophores where limbs diverge; spicules are 50–75 μm in greatest total length; spicules consist of 2, 3, or 5 separate rays, each is straight or slightly curved, and terminates in a blunt tip, nearly all rays are equal in length. Although rays of spicules project in all directions they do not diverge at right angles. Spicules very brittle, contraction of animal on death is enough to shatter most of then. Spicules effervesce in dilute acid suggesting they are, like the shell, calcareous.

Shell (Fig. 7)

Shell always present, generally rectangular and flattened, sides constricted towards apical (posterior) part; slightly convex with anterior and posterior margins elevated from horizontal. Protoconch of 1½ whorls, on left posterior-lateral corner; posterior flange of shell higher than level of protoconch. Lateral margins of shell parallel, or showing a slight divergence anteriorly; anterior margin broadly rounded. Macroscopic sculpture is of irregular concentric folds, flattened, narrow and compressed towards margins. Microsculpture (Figs. 10, 11) consists of numerous, radiating ridges interspersed with broad depressions; ridges and depressions are parallel, directed in wavy lines that cross concentric folds; surface granular in texture. This sculpture is present only towards shell apex and becomes progressively weaker until, over the anterior third of teleoconch, it is obsolete.

Outer surface of shell covered with a thin, iridescent periostracum (Fig. 12) which readily peels off; it is dull, whitish and chalky on shells of preserved specimens; periostracum does not exactly duplicate underlying wavy sculpture of shell, but appears punctate towards apex and smooth anteriorly.

Shell thin, white towards apex, pale buff over remainder; interior brownish-orange towards anterior edge, with a faint, red-brown streak towards left corner. Interior sculptured with raised, concentric ridges that narrow towards anterior margin.

Four shells from specimens of *B. ornata* taken at Mahurangi Island, off Waiwera Beach, have been deposited in the mollusc collection, Auckland Institute and Museum.

Radula (Figs. 24, 28, 29)

Pale yellow-brown, broad and rectangular; maximum length 7.0 mm, width 4.0 mm when flattened. No rachidian in any row. Lateral teeth small and simple; inner and middle laterals (Fig. 28) 40–60 μm high, hook-like, curved, with sharp-pointed apices; all have a relatively broad blade, both edges of which are smooth. Outer lateral teeth erect, straight-sided, with relatively narrow blades and rounded apices; outermost lateral teeth decrease regularly from 45 μm to 30 μm (for extreme outer lateral tooth). There are many rows of teeth in the radula, giving the following range of radular formulae for adult specimens: 90 × 65.0.65 to 140 × 140.0.140. These formulae are high compared to those of other *Berthella* species.

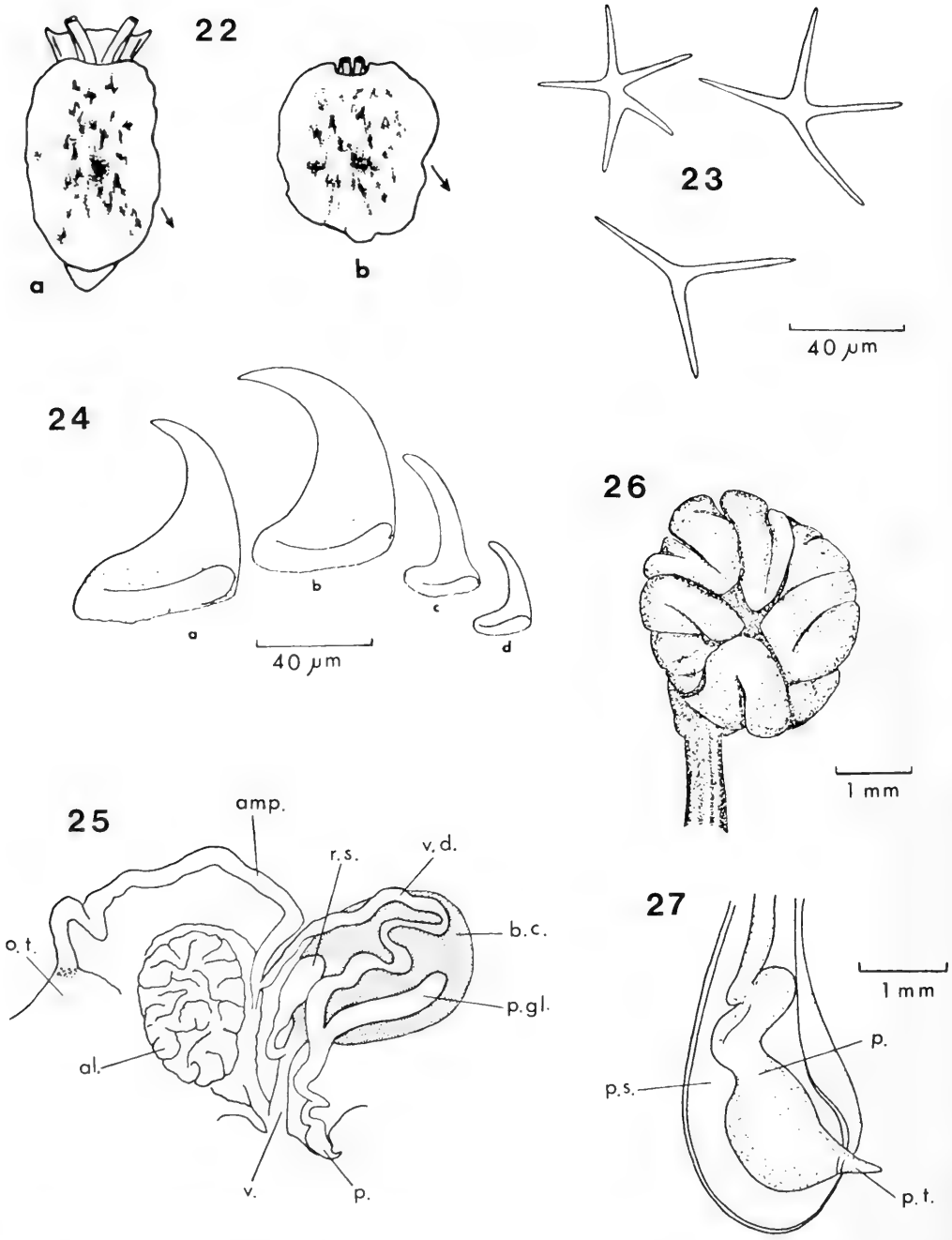
Jaws (Figs. 30, 31)

Relatively small (approx. 2.0 mm long in adults), oval, with both ends rounded; jaws composed of numerous, cruciform, and relatively squat elements, each being 60–65 μm long. Jaw elements broad-based, bases concave, widening to lateral processes; sides of blade smooth; each element terminates at a blunt, pointed apex (Figs. 30, 31); very rarely some elements have a weak denticle on side of blade about half way between a lateral process and apex. Apical thickenings on all mandibular elements are very apparent in both light microscopical views and SEM photos; thickenings rise from plane of top surface of element (i.e. that on inner surface of the jaw) and terminate in a sharp cusp; edges of blade near apex can also be raised. Such terminal thickenings have not been previously recorded for pleurobranch mandibular elements. The jaw elements of *Berthella ornata* are broader and squatter than those of *Berthellina citrina.*

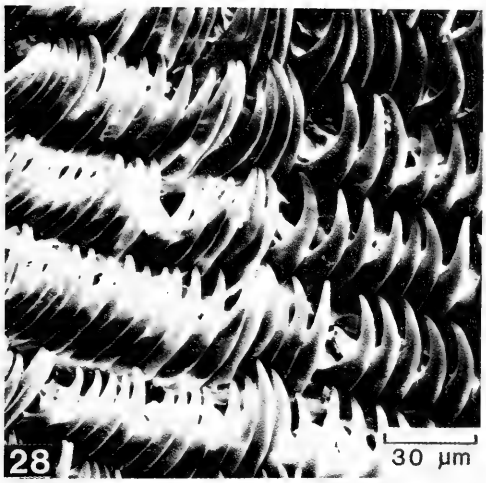
Reproductive system (Figs. 25–27)

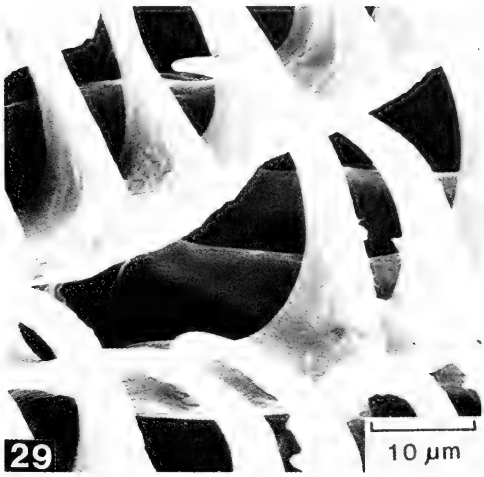
Similar to that of *Berthellina citrina*, but with no prostatic enlargement of the vas deferens. The reproductive system of *B. ornata* differs in several other points from that of *B. citrina,* and these details are reported because of their specific importance.

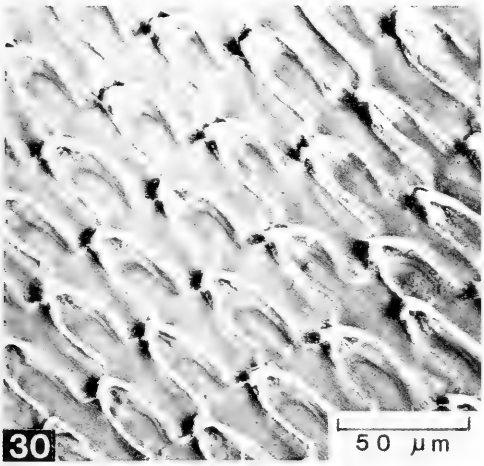
Although size of nidamental glands varies with age and maturity, two regions are discernible. A dorsal, white and compact albu-

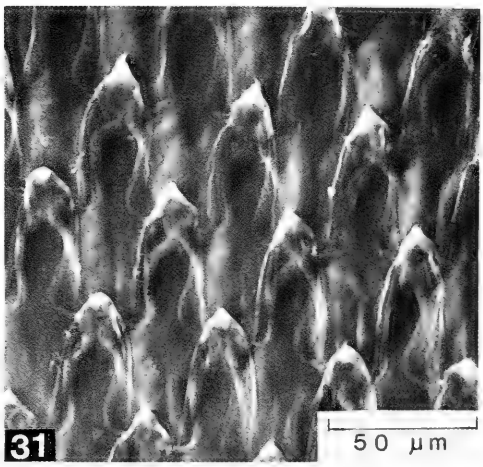


FIGS. 22–27. *Berthella ornata*. 22. Whole animal from above, a = crawling, b = resting. Arrow indicates direction of exhalant current; 23. Spicules from mantle; 24. Radular teeth, a = inner lateral, b = middle lateral, c = outer lateral, d = outermost lateral; 25. Composite view of structure of reproductive organs; 26. Detail of albumen gland from ventral surface; 27. Detail of penis and penial sheath, the latter cut open. Abbreviations: al. = albumen gland; amp. = ampulla; b.c. = bursa copulatrix; o.t. = ovotestis; p. = penis; p.gl. = penial gland; p.s. = penial sheath; p.t. = apex of penis; r.s. = receptaculum seminis; v. = vagina; v.d. = vas deferens.









FIGS. 28–31. Radula and jaws of *Berthella ornata*. 28. Inner and middle lateral teeth showing change in structure across a row. 29. Detail of a single middle lateral tooth. 30. Mandibular elements on inner face of jaw. 31. Detail of the same.

men gland (Fig. 26), consisting of a tightly-coiled tubular mass that is convoluted in external apperance, and the mucous gland. This latter gland lies below the albumen gland; it is flattened, solid and cream in color.

The vagina is grooved internally and passes as a long, straight canal to two seminal receptacles; vagina opens directly into a large sac-like bursa copulatrix whose dark-colored contents can be seen through the thin wall. A side branch arises from vaginal duct just before base of bursa copulatrix and loops to smaller receptaculum seminis. This concentration is well back from vaginal aperture. There is a dilation in front of receptaculum seminis; receptaculum solid, roughly conical in shape, pressed against bursa copulatrix when in its natural position.

Vas deferens much-convoluted, thin-walled, of a slightly larger diameter than hermaphrodite duct, pressed against membrane surrounding bursa; vas deferens lacks a prostatic glandular region. A penial gland arises from vas deferens at level of bursa; it is finger-like, flattened, thin-walled, not extensively coiled. Penial gland pressed against

membrane of bursa copulatrix, although not entwined with vas deferens. Beyond junction with penial gland, vas deferens runs beneath vagina, both lie within a common membranous sheath. Penis unarmed, of a smaller diameter than hermaphrodite duct; when retracted, penis remains coiled inside its sheath.

Distribution

Berthella ornata is found in clean, rocky situations on open or partially sheltered coasts throughout New Zealand. It ranges from the intertidal to shallow subtidal depths (approx. 15 m).

Discussion

A distinctive species of pleurobranch, *Berthella ornata* is the only endemic shallow-water New Zealand member of the order Notaspidea. It can be distinguished from its New Zealand congener, *B. mediatas* Burn, by colour, texture of mantle surface, gill length, and position of anus. *B. ornata* has smooth-bladed mandibular elements and relatively large, curved, middle lateral radular teeth; *B. mediatas* has strongly denticulate blades to the mandibular elements and relatively small, less curved, middle lateral teeth.

Berthella ornata has several other characteristics important from a taxonomic view and which may prove significant in future studies of phylogeny within the genus: mantle coloration; gill; mandibular elements; radula; reproductive system.

Other than *Berthella ornata,* few members of the genus have distinctive colour patterns on the mantle. Most species are uniformly dull yellow-orange or pale fawn, the mantle being more or less translucent with the dark digestive gland and light ovotestis showing through. A literature search revealed five other *Berthella* species with conspicuous darker markings on the mantle surface:

Berthella ocellata (Delle-Chiaje, 1828) from the Mediterranean Sea. This species has a yellow-ochre to reddish-brown ground colour overlain with large, white or yellowish blotches; it has a much narrower shell than *B. ornata,* with a tall, projecting spire.

Berthella stellata (Risso, 1826) from the Mediterranean Sea. It has numerous, small spots on a bright yellow background; there is an unpigmented central area in the form of a cross. *B. stellata* has denticles on the blades of the mandibular elements.

Berthella scutata (Martens, 1880) from Mauritius. This species is yellowish with large spots of dark purple-brown on the mantle and a few smaller spots on the oral veil, rhinophores and sides of the foot; the mantle is evenly and finely granulose all over; like the previous species, *B. scutata* has denticulate mandibular elements. *B. scutata* may be a species of *Pleurobranchus*; Marcus (1977) considers it a *nomen dubium.*

Berthella kaniae Sphon, 1972 from Panama and Mexico. Ground colour is deep yellow (Thompson, 1970). It has a whitish or pale fawn mantle, patterned over the entire surface with minute, pale brown, polygonal markings; this species has two short denticles on either side of the blade of each mandibular element.

Berthella kaniae Sphon, 1972 from Panama and Mexico. Ground colour is deep yellow to almost white; the mantle, gill, and veil, as well as the tips of the rhinophores and the area around the genital apertures are spotted reddish-brown. Like *B. ornata*, it has smooth blades to the mandibular elements, but in *B. kaniae* the teeth are of similar size and shape across each row of the radula, and the living animal has the capacity to autotomize parts of the mantle edge (Sphon, 1972)—a behaviour never observed in *B. ornata.*

Two of the smaller *Pleurobranchus* species also have darker markings on the mantle—*Pleurobranchus tessellatus* Pease, 1868 from Polynesia, and *P. ovalis* Pease, 1868 from Tahiti and Australia.

In *Berthella ornata* the gill plume is particularly long, extending for more than half the body length, the rachis is not smooth but weakly knobbed where a pinna branches off. These knobs appear homologous with the rachal tubercles of *Pleurobranchus s.s.* Further detailed studies must be made of the gills of *Berthella* and *Pleurobranchus* to see if a separation based on this single characteristic is as clear as has previously been assumed.

Both Burn (1962) and Thompson (1970) diagnose *Berthella* as having denticles on the blades of the mandibular elements. Yet there are none in *B. ornata.* A small group of other *Berthella* species has also been reported to have smooth-edged elements; these are: *B. ocellata* (Delle-Chiaje) (Vayssière, 1898: 288); *B. kaniae* Sphon; *B. tupala* Marcus (Bertsch, 1975).

Berthella mediatas Burn, 1962
(Figs. 8, 32–44)

1900. *Pleurobranchus aurantiacus* Risso; Bergh: 210, pl. 20, figs. 34–38 (*non Pleurobranchus aurantiacus* Risso, 1818).
1924. *Bouvieria (Pleurobranchus) aurantiaca* (Risso); Odhner: 51.—1939a, Powell: 217, No. 1232 (*non Pleurobranchus aurantiacus* Risso, 1818).
1955. *Bouvieria aurantiaca* (Risso); Powell: 118 (*non Pleurobranchus aurantiacus* Risso, 1818).
1957. *Pleurobranchus punctatus* Quoy & Gaimard; Burn: 15 (*non Pleurobranchus punctatus* Quoy & Gaimard, 1832).
1962. *Berthella mediatas* Burn: 142.—1966b, Burn: 271, No. 26.—1969, Burn: 80, No. 15 (listed as "*Berthella mediata* Burn (1962)").

Live animal (Fig. 32)

Extended body length to 30 mm. Mantle broad, with lateral margins extending beyond sides of foot; when animal is crawling, foot exceeds mantle in length; rhinophores and oral veil project well in advance of raised anterior margin. Mantle relatively thick, dull cream or pale brownish-orange, with a slight central darkening due to the underlying digestive gland; occasionally a few small, diffuse, white spots are present as well; shell not usually visible. Mantle highly porous, pores large and very numerous over entire surface, visible in strong light without magnification. Glassy spicules similar to those of *B. ornata* are present amongst pores on mantle but require magnification to be seen. Foot hidden all round by mantle except posteriorly; dirty yellow-orange in color and unspotted; anterior mucous groove makes front border darker; pedal gland present on sole, positioned towards one side, appears as a darker, thickened, triangular region, with base at back of foot.

Oral veil large, narrow at insertion and broadening towards anterior border; tips of grooved lateral areas noticeably forward-projecting. Anterior margin deeply sinuous, with a mid-anterior embayment. Oral veil pale, dirty yellow, lateral areas darker.

Rhinophores long, projecting well beyond mantle's anterior border; pale yellow or orange.

Gill relatively small, never extending behind tail when the animal is crawling, always covered by mantle, attached for half its length to body wall, rachis narrow, smooth; 18–23 pinnae arise from upper surface and alternate

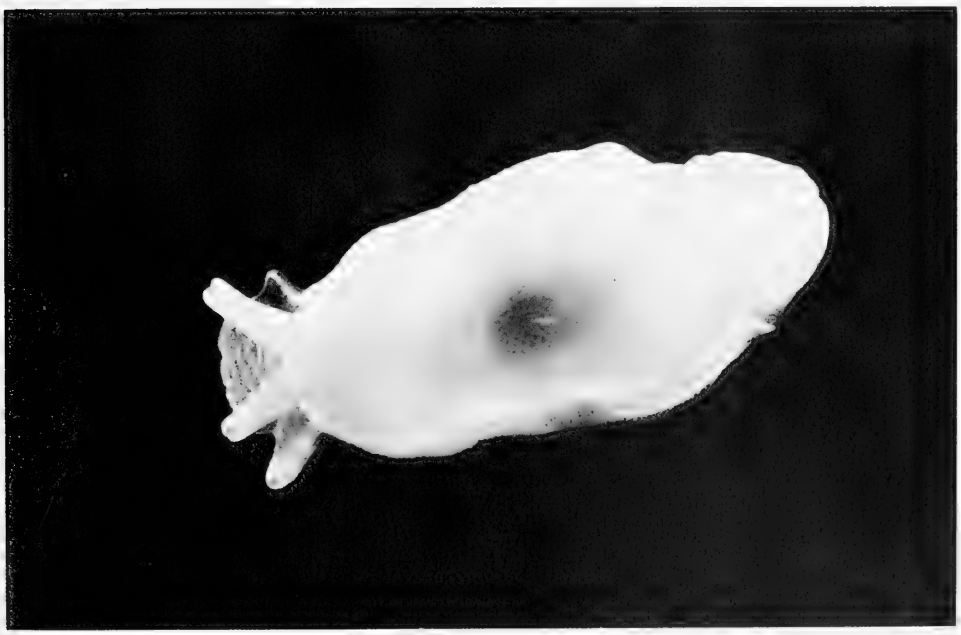


FIG. 32. *Berthella mediatas*. Length = 17 mm. From Cape Egmont, Taranaki, 16 July 1974. Photograph: G. W. Batt.

with a similar number below; pinnae bear smaller pinnules (in a specimen from Portobello I counted 12 pinnules on anterior edge of the fourth pinna). Anus opens forward of middle of gill membrane on upper side of gill, approximately between the second and third pinnae; anus not raised on a papilla.

Shell (Fig. 8)

Shell large, about half total body length; moderately convex, rectangular and not constricted towards apical (posterior) end; lateral margins nearly parallel; anterior and posterior margins rounded and a little produced beyond level of protoconch (Fig. 8). Protoconch small, white, just over one whorl, not projecting posteriorly but confluent with dorsal surface.

One important distinguishing feature of shell is a whitish flange that projects beyond margin on columellar side (i.e. on left back corner when shell is seen dorsally). This expansion is produced below the spire; it is only weakly developed in juvenile shells but very prominent in large shells.

Sculpture on outside of shell is of numerous, concentric ridges; they often form broad, raised areas with flattened tops. Concentric sculpture is most strongly developed on early and middle regions of teleoconch. Ridges frequently intersect each other; each ridge therefore is of a changing width; ridges run parallel with anterior and right margins, but arch strongly towards posterior margin and left hand edge. Sculpture most clearly visible on smooth area near apex, very similar to that of *B. ornata*; consisting of numerous, fine, wavy ridges and hollows that become obsolete towards the middle part of the shell. I have not yet examined shells with SEM. Shell light golden-brown, with faint, broad, radial streaks of orange-brown; covered externally with a glistening, iridescent periostracum. Shell concave internally, inner surface raised into numerous, irregular, rounded concentric folds separated by flattened areas.

Three shells from specimens taken at Army Bay, Whangaparoa Peninsula, have been deposited in the mollusc collection, Auckland Institute and Museum.

Radula (Fig. 42)

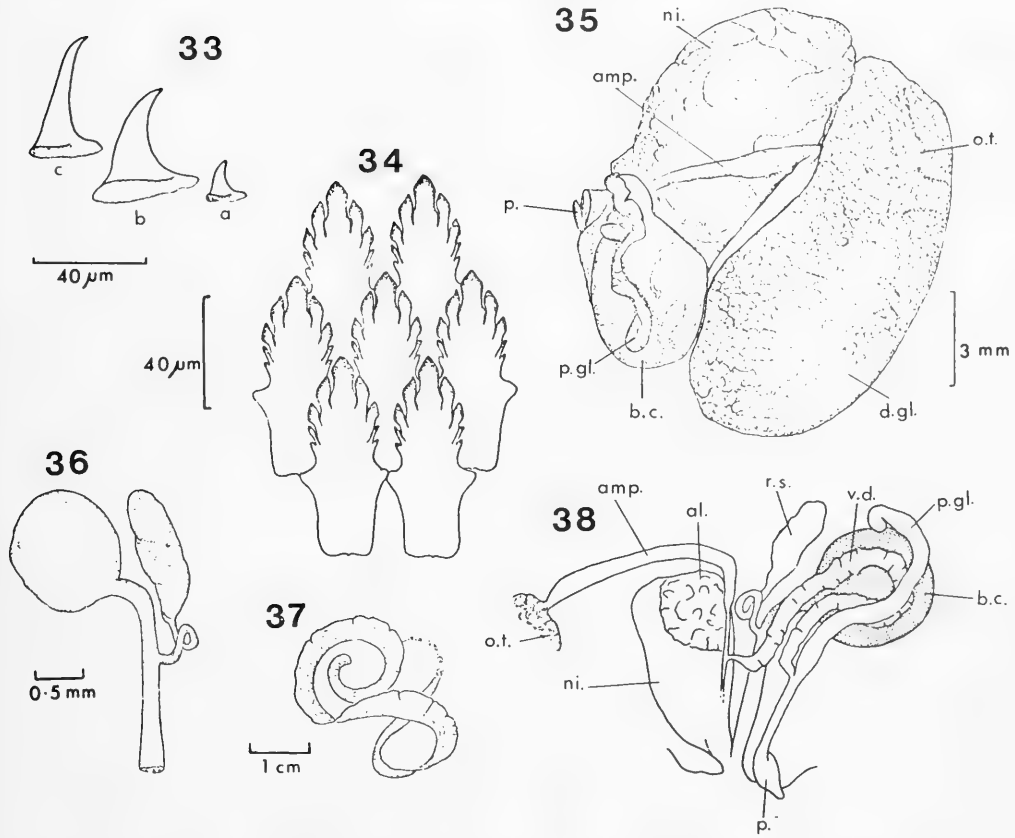
Radula broad, rectangular, expanded towards youngest end; rather small in comparison with radula of similar-sized *Berthella ornata* (largest radula measures—3.0 × 1.8 mm). Rachidian lacking. Lateral teeth numerous, small and without denticulations; inner and middle laterals (Figs. 39–41) similar, broad-based, erect, and ending in a smooth, hooked apex. Lateral teeth increase progressively from the innermost (8 μm high) towards middle (20–30 μm), then outer laterals increase to approx. 35 μm high (Fig. 42). Outer laterals more erect than inner or middle laterals, blades long, straight, apices sharp, only slightly recurved. Extreme outer lateral teeth needle-like, getting progressively smaller.

Numbers of teeth have been remarkably consistent in all radulae examined, with a range of 61 × 65.0.65 to 98 × 76.0.76 (10 radulae). The formula of 56 × 52.0.52 given by Burn (1962) would not appear significantly outside this range.

Jaws

Jaws approximately rectangular, anterior edges rounded. Mandibular elements (Figs. 43, 44) cruciform, sides of blades strongly denticulate. Base of each element narrow (approx. ¼ of total length of 65–70 μm), distance between the lateral processes is 30–35 μm. Strong denticles, which completely occupy sides of blade above lateral processes are most characteristic feature of jaws; denticles coarse, numbers equal or unequal on either side (varying from 3 to 5); denticles point forward, separated by deep grooves; largest denticles immediately adjacent to sharp-pointed apex. Tips of lateral denticles and apices of elements themselves thickened.

Specimens from the North Island and most South Island localities I have examined show little variation in the denticulation of the mandibular elements, and Bergh (1900) illustrated identical elements in a specimen taken near the Chatham Islands. However, two of the jaws from South Island specimens (from Akaroa Harbour and Portobello) have mandibular elements without the characteristic denticulation of the blades and these elements are relatively broader (50–60 μm long, 30–35 μm wide). Sides of blades are irregular but not denticulate, apices are blunt and unthickened; sides taper towards tips. Lateral processes have a relatively larger area of contact with each other than in denticulate elements. Many more jaws require study before it is possible to say whether these variations in



FIGS. 33–38. *Berthella mediatas.* 33. Radular teeth, a = outer lateral, b = middle lateral, c = inner lateral. 34. Group of mandibular elements on inner face of jaw. 35. Reproductive organs *in situ* from the undersurface. 36. Detail of seminal receptacles in a mature animal. 37. Spawn coil. 38. Composite view of structure of reproductive organs. Abbreviations: al. = albumen gland; amp. = ampulla; b.c. = bursa copulatrix; d.gl. = digestive gland; ni. = nidamental glands; o.t. = ovotestis; p. = penis; p.gl. = penial gland; r.s. = receptaculum seminis; v.d. = vas deferens.

mandibular elements are discontinuous as it would appear, or continuous.

Reproductive system (Figs. 35, 36, 38)

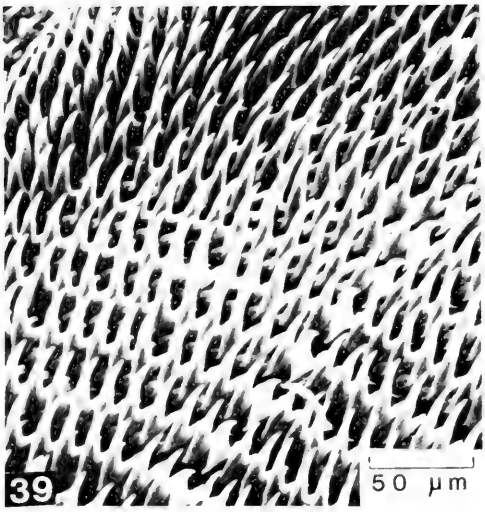
General arrangement similar to that of *Berthella ornata*. Receptaculum seminis of *B. mediatas* an irregular, club-shaped organ arising relatively farther back up vagina, its duct is longer and the receptaculum itself relatively larger. Penial gland elongate, thin-walled, with a recurved tip, relatively longer than that of *B. ornata.*

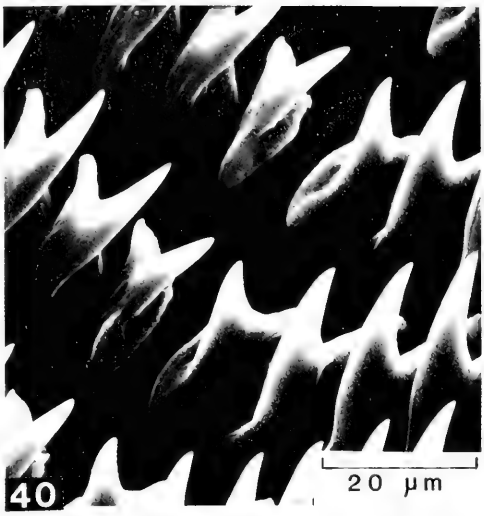
Egg coil (Fig. 37) loosely spiralled, of about two whorls, flanged, white despite the yellow or brown coloration of *B. mediatas* animals.

Distribution

Berthella mediatas has a continuous distribution throughout New Zealand. Most specimens have been collected from the undersides of intertidal stones and so far there are very few sublittoral records. I suspect the species does not extend deeper than 20 m.

There are several literature records of "*Bouvieria aurantiaca* (Risso)" from southern New Zealand localities: Port Pegasus, Stewart Island (Odhner, 1924); near the Chatham Islands (Bergh, 1900); Masked Island, Auckland Islands (Odhner, 1924); Auckland Islands (Powell, 1955). I have not been able to examine these specimens and


39
50 µm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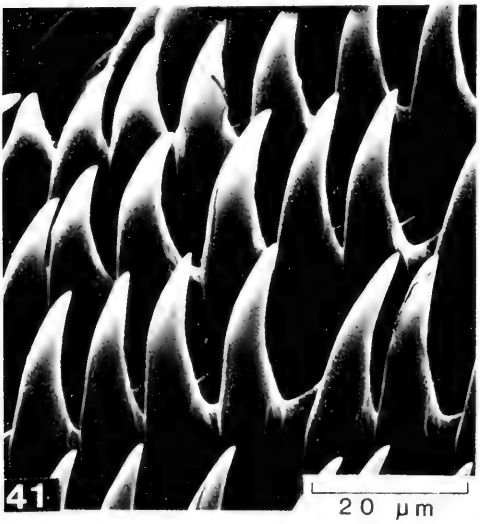
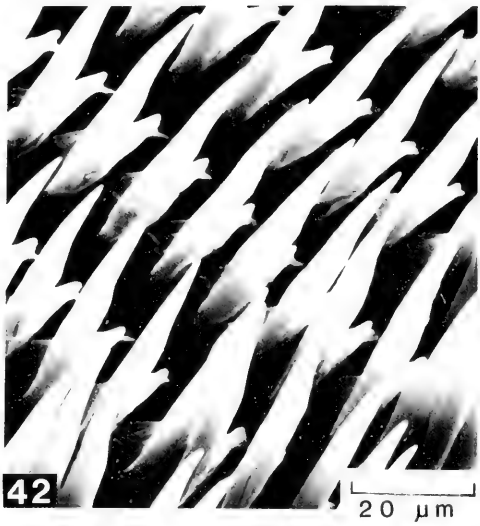
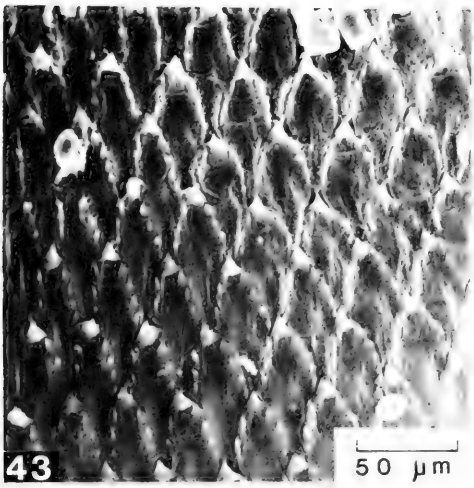
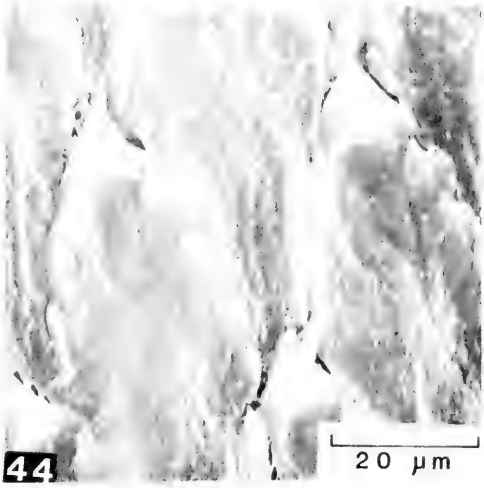
20 µm


41
20 µm


42
20 µm


43
50 µm


44
20 µm

the only description is Bergh's. However, these are probably all *Berthella mediatas* because the only other yellow or orange pleurobranch from shallow water, *Berthellina citrina,* does not extend farther south than East Cape (North Island). Bergh's (1900) description and figure of his specimen (preserved in sublimated picric acid) are quite sufficient to identify it as *B. mediatas*, although his description is incorrect regarding the site of the anus. Bergh's Chatham Islands locality was confirmed by the specimen taken on Rangitira Island in 1977.

Berthella mediatas also occurs in Australia: Tasmania; South Australia; Victoria; south Western Australia (Burn, 1962, 1966b, 1969, and personal communication). It is the commonest pleurobranch along the Victorian coastline. Thompson (1970) did not record it from eastern Australia, and I found none there during visits in 1975, 1979 and 1980. The New Zealand specimens represent a new locality record. Since *B. mediatas* is already known to have such a wide distribution around the temperate Australian coastline, I would still expect it to be found in southern New South Wales.

Discussion

Confirmation of the presence of *Berthella mediatas* in New Zealand has been achieved by examination of three Australian specimens kindly sent by Mr. R. Burn. Data are as follows: Lorne, Victoria—1 under a stone in a channel on rock platform, R. Burn, 24 Nov. 1974; Warneet, Westernport Bay, Victoria—2 on undersides of stones in sandy area, R. C. Robertson, 15 Sept. 1968. The preserved specimens measured 10.2, 17 and 17 mm long respectively. Not only do they agree with the New Zealand material, but also they have enabled clarification of several points raised by the original description (Burn, 1962). Pedal glands were present on both the larger specimens and the mandibular elements of all specimens had three to five strong denticles on either side of the blade. Both these observations contradict the original description and remove objections regarding the similarity of Australian and New Zealand material. In the Australian specimens the anus opens within the anterior third of the gill basement membrane, generally close to the insertion of the third pinna. This is even further forward than originally described by Burn, he mentioned the anus as being "at the mid-length of the gill membrane."

The name "*Bouvieria aurantiaca* (Risso, 1818)" which has previously been applied indiscriminately in New Zealand to both *Berthellina citrina* (Rüppell & Leuckart) and *Berthella mediatas* Burn should be reserved for a species of *Berthella* from the Mediterranean Sea. The association of the name *Berthella aurantiaca* (Risso) with New Zealand stems from Bergh's (1900) usage. Since then the name has become entrenched and appears in works by Suter (1913), Odhner (1924), Powell (1937, 1939a, 1946, 1955, 1957, 1961, 1976, 1979) and Morton & Miller (1968). Bergh (1900) held the view that the occurrence of *B. aurantiaca* in New Zealand was indicative of one of the more widely-spread forms of opisthobranchs. Probably because of Bergh's reputation and lack of further detailed studies by others, this identification was never challenged. Bergh missed the subtle but significant characters that separate *B. aurantiaca* from *B. mediatas*, and then Suter (1913) compounded the errors by confusing *B. mediatas* and *Berthellina citrina*. Holding no doubt to the same view expressed above, Bergh (1898) reported *Berthella aurantiaca* from Norway, but this record was also subsequently rejected (Odhner 1939).

The two orange pleurobranchs that have been confused in New Zealand can easily be separated since they differ in numerous characters, the most significant of which are summarized in Table 2.

Berthella mediatas shows considerable similarity to some of its congeners in other parts of the world. Fortunately, in New Zealand its separation from *B. ornata* is straightforward. *B. mediatas* appears to have its closest relations in Australia and Europe; separation from these species can be made by reference to Odhner's (1939) and Burn's (1962) thorough studies. The only species that I will distinguish it from is *B. aurantiaca* (Risso). According to descriptions given by Vayssière (1898) and Odhner (1939), *B. aurantiaca* is apricot-orange when alive, al-

FIGS. 39–44. Radula and jaws of *Berthella mediatas*. 39. Curved inner lateral teeth near midline of radula (midline at top left); note single row of malformed teeth. 40. View of rows of innermost lateral teeth on either side of central row viewed from above; note absence of central row. 41. Detail of curved inner lateral teeth; midline is at upper left; weak serrations on teeth in foreground are artifacts caused by scanning beam. 42. Detail of erect outer lateral teeth. 43. Low-power view of mandibular elements on inner surface of jaw. 44. Detail of same.

TABLE 2. Distinguishing features between *Berthellina citrina* (Rüppell & Leuckart) and *Berthella mediatas* Burn.

Character	*Berthellina citrina*	*Berthella mediatas*
Mantle texture	Smooth, small pores	Many large pores
Shell	Small, triangular, approx. 1/5 body length	Larger, auriculate, approx. 1/2 body length
Anal opening	Hind end of gill membrane	Anterior third of gill membrane
Anterior margin oral veil	Almost straight	Sinuous, deeply cleft mid-anteriorly
Pedal gland	None	Present in adults
Radula	Teeth erect, elongate, denticulate on upper third	Teeth shorter, curved, smooth
Mandibular elements	Blades generally smooth, occasionally with weak denticles	Both sides of blade usually strongly denticulate
Prostate gland	Present	None
Gill	Brownish-cream	Golden-yellow
Spawn coil	White	Golden

most reddish on top. The mantle is translucent and reveals the very large shell beneath; the shell may be up to one-half of the length of the living animal. The anus is at the hind end of the gill membrane. *Berthella aurantiaca* appears to be restricted to the Mediterranean Sea (Bergh, 1892; Vayssière, 1898; Schmeckel, 1968; Barash & Danin, 1971).

Bathyberthella Willan, n. gen.

Definition

Small pleurobranchs; abyssal; body elliptical; mantle smaller than foot, free all round, without an anterior crenulation, smooth; pedal gland present; rhinophores arising together mid-anteriorly; anus at posterior end of gill membrane; shell internal, very large—covering entire viscera, flexible, cuticular, without any calcification; radular teeth very numerous, narrow, erect, smooth, similar across each row, rachidian lacking; mandibular elements oval or elliptical at jaw surface, anterior margin irregularly denticulate; vas deferens dilated into a prostate gland; penis smooth, without accessory structures, penial gland present.

Type-species: *Bathyberthella zelandiae* Willan, n. sp.

The description of this new pleurobranch is included in this present paper so that it is complete for all the known New Zealand Notaspidea. *Bathyberthella zelandiae* is not likely to be taken often because of the depth at which it lives and its fragility.

All known specimens were collected by the New Zealand Oceanographic Institute's research vessel "Tangaroa" whilst it was sampling the benthos of the Bounty Trough on the "Canyon Coral '79" cruise. All the specimens came from two stations close to each other in abyssal depths (>1600 m); they were taken with an epibenthic sled sampling device. A total of 43 specimens was sorted from the stations.

I was fortunate to be present when these pleurobranchs reached the surface in the trawl and were washed from the substratum of light grey ooze. All the animals were moribund when they were sorted from the sample. Those that appeared least damaged were placed in fresh sea water but they did not recover. Despite their moribund condition (all had partially everted buccal masses), the specimens were not dead and it was possible to give an account of the live animal from those that were recovered intact.

Bathyberthella zelandiae Willan, gen. & sp. nov. (Figs. 45–56)

Live animal

Extended body length up to 40 mm. Specimens of all sizes from 5 to 40 mm were repre-

sented in this collection. Body oval, globose, very flaccid. Mantle rather delicate, with a definite free border all round, edges thin; surface smooth, no pores apparent; spicules absent; mantle shorter than rhinophores in front, or tail behind. Foot spongy, pedal gland present on sole; pedal gland a large thickened area occupying posterior section of sole but not extending to foot margin, oval, with posterior end wider, oriented at right angles to longitudinal axis of body. Eyes conspicuous, at base of rhinophores, usually large for an abyssal mollusc.

Rhinophores rather short, covered with minute papillae. Oral veil short, anterior margin smooth (i.e. lacking digitations); weakly embayed mid-anteriorly; upper surface papillate (papillae smaller and fewer than on rhinophores).

Gill relatively small in proportion to body length, never extending to level of hind end of mantle; free for about half its length; rachis narrow, smooth; 19–23 pinnae on (upper side of) rachis (mean for 8 specimens—21 pinnae). Anus opens on upper side of gill at hind end of basement membrane; interior longitudinally ridged.

Body creamish, salmon anteriorly; mantle translucent, cream, marked with small, vague, white flecks and speckles, yellow spots occasionally present; digestive gland appearing as a black smudge posteriorly; gill brownish; proboscis salmon.

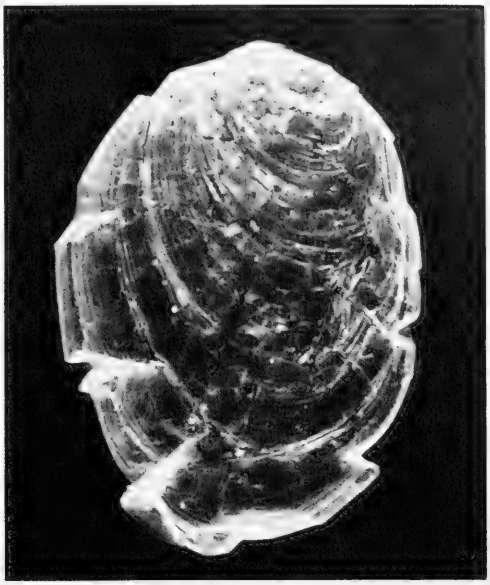


FIG. 45. Shell of *Bathyberthella zelandiae*. Length 23 mm × width 18 mm. Specimen from 1676 m, northern side of Bounty Trough, 26 Oct. 1979.

Shell (Fig. 45)

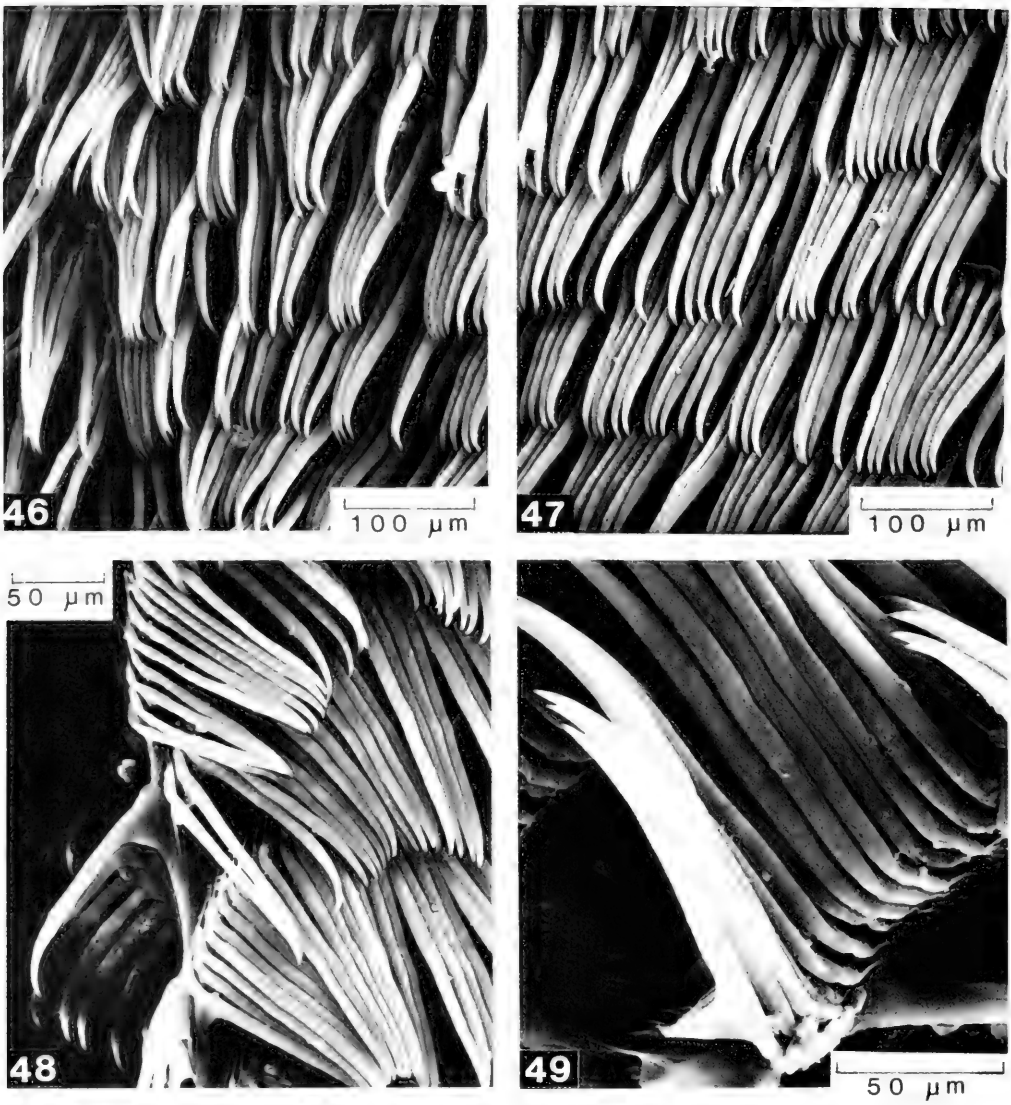
Shell present beneath mantle in all specimens examined; very large (e.g. 25 × 16 mm in a specimen of 30 mm preserved length); covering entire visceral mass (i.e. it reaches level of front of rhinophores anteriorly). Shell entirely cuticular (without any calcification), very thin and fragile, easily deformed in any direction, disturbance of liquid surrounding shell causes crumpling. Shell concave, broadly oval, a little narrower posteriorly; protoconch not produced beyond posterior margin; shell lacks a posterior flange. Surface flat, concentric growth lines are the only sculpture present, chitin shows localized crumpling caused by compression of overlying mantle during fixation, radial folds present towards margin (particularly anteriorly); a series of regular, undulating ridges present over a small area near apex but ridges are absent over rest of teleoconch. Shell not connected to body muscles or underlying integument. Shell shining, transparent or faintly yellow.

Two shells from paratypes (NZOI Stn. S152) have been deposited in the wet section of the mollusc collection, National Museum of New Zealand.

Radula (Figs. 46–49)

Buccal mass extremely long, able to be protruded up to half body length. Radula square in appearance, rather short (up to 5 mm in length) and extremely broad through having a very great number of teeth per row. Innermost 40 rows slope very acutely towards midline, remaining rows nearly perpendicular to midline. Central tooth absent. Lateral teeth very numerous, fine, similar in structure across rows, tall and elongate, tapering gradually to a slightly recurved apex; no denticles on blade whatsoever; base triangular, with a thickened posterior area and thin, forward-produced extension, base about five times as broad as middle section of blade.

Inner lateral teeth average 120 μm high (Figs. 46, 47). Middle laterals proportionately higher (155 μm) having base not significantly enlarged. Outermost laterals shorter than middle laterals (95–115 μm); teeth in outer-



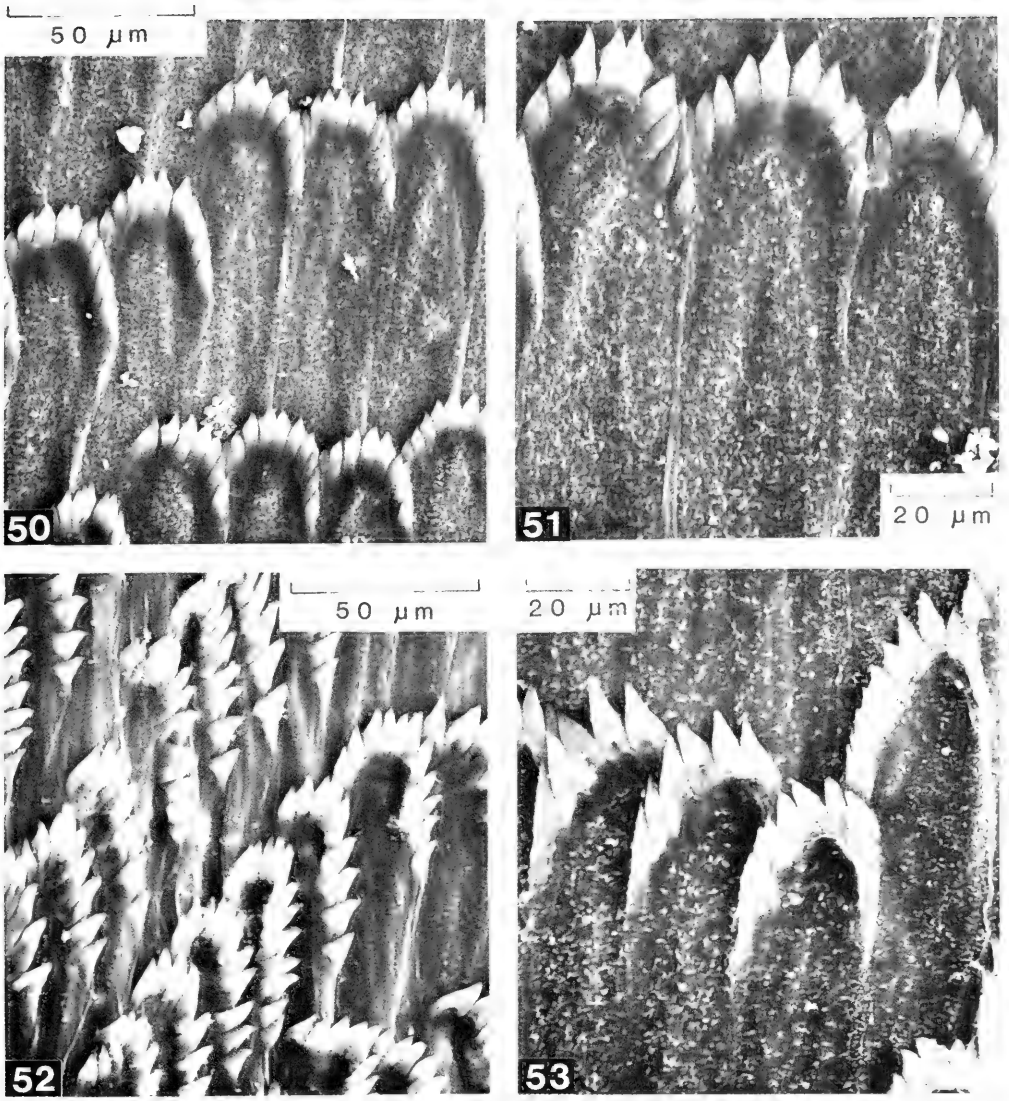
FIGS. 46–49. Radula of *Bathyberthella zelandiae*. 46. Lateral teeth near midline of radula (midline is just to left of centre). 47. Lateral teeth contiguous to those in Fig. 46. 48. Outermost lateral teeth from extreme edge of radula. 49. Detail of same.

most 20 rows become very narrow (only 6 µm wide) and acicular (Figs. 48, 49).

Mean number of rows is 62.1 (standard error = 1.4, radulae from six adults examined). The enormous number of closely-packed laterals prevents an accurate count being made of numbers of teeth per row with a light microscope. Counts of two radulae using a SEM have shown there to be between 210 and 240 lateral teeth. Therefore *Bathyberthella zelandiae* exhibits the following range of radular formulae for adults: 58 × 210.0.210 to 67 × 240.0.240.

Jaws (Figs. 50–53)

Pair of jaws lines buccal mass, about 5 mm in length. Jaws lightly chitinized, blunt anteriorly, tapering to a rounded point posteriorly. Jaws composed of numerous rows of mandibular elements; rows very irregular at surface of jaws, some elements run parallel or slightly



FIGS. 50–53. Jaws of *Bathyberthella zelandiae*. 50. Group of mandibular elements of jaw from a region near centre. 51. Detail of same. 52. Group of mandibular elements towards edge of jaw; note asymmetry of denticles. 53. Detail of same.

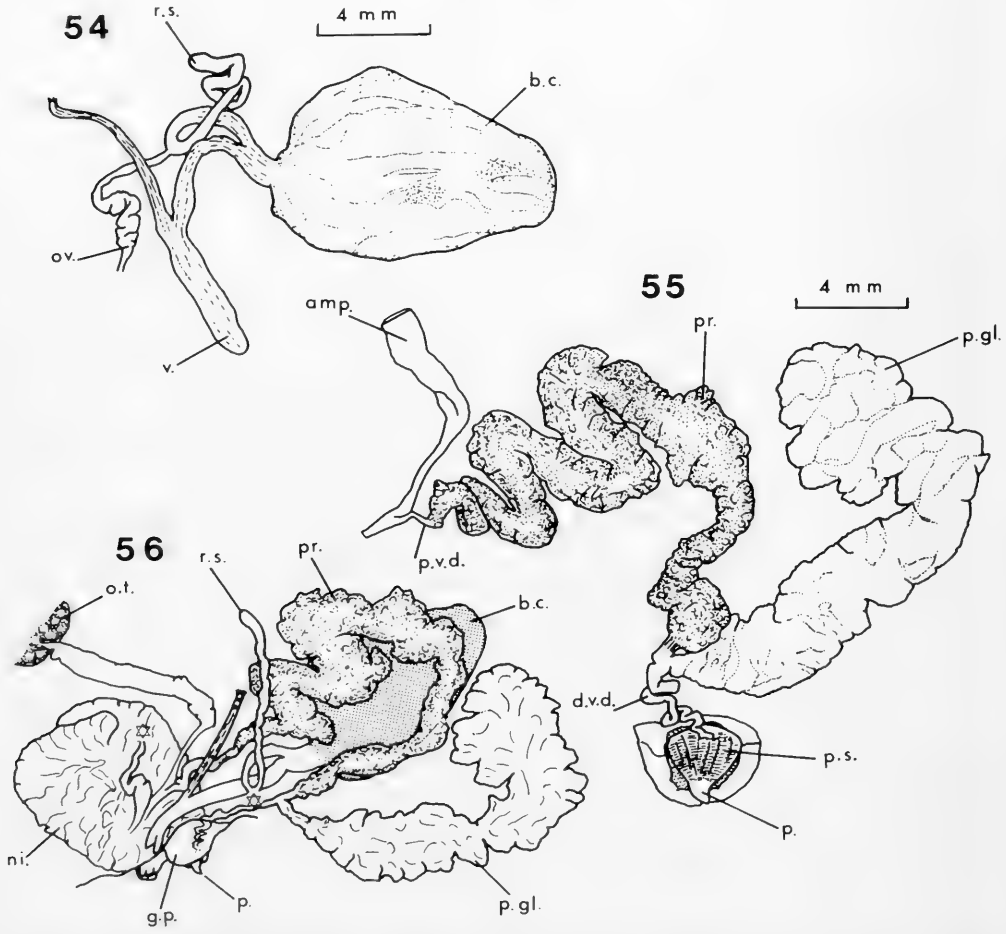
obliquely in groups of 10–12, other elements are considerably displaced from their neighbors to produce highly disorganized patterns; impression of inner surface of jaws is of rows of erratic elements (shagreened appearance). Elements themselves closely-packed (110 µm apart at centre of jaw), elongate or polygonal, bearing a series of thickened, conical denticles along curved, anterior border, the median ones being strongest; elements at centre of jaw possess 4–10 strong denticles arranged symmetrically (Figs. 50, 51); elements towards edges of jaw possess 7–14 weaker denticles that are disposed asymmetrically on leading edge (Figs. 52, 53); denticles towards edges are taller and narrower than those near centre, those towards edges curve away from surface in a claw-like manner (Fig. 53). Denticle numbers are very variable, contiguous elements often have differing numbers; all denticles lack smaller subsidiary denticles.

Reproductive system (Figs. 54–56)

All specimens died with tip of penis protruded from summit of genital papilla; vaginal and oviduct apertures separate. Ampullar region of hermaphrodite duct rather long, smooth-walled, white, flattened, pressed against nidamental glands; it constricts abruptly as it penetrates genital mass between nidamental glands and bursa copulatrix; hermaphrodite duct gives rise to proximal vas deferens before terminating within nidamental complex.

Proximal vas deferens enlarges after a short distance to large prostate gland; prostate gland entwined with penial gland and both are compressed onto bursa copulatrix which they ensheath; prostate moderately thick and spongy; penial gland elongate, tubular, exceedingly thin-walled, fragile, translucent, much-convoluted, dilated distally, wall puckered into pockets; penial gland entirely different in structure to prostate gland. Distal vas deferens long, narrow, thick-walled, it passes into large, conical penial sheath; sheath covered with papillae on outside.

Vagina moderately long, walls ridged internally, possessing a long, narrow muscle slip medially; passing to both bursa copulatrix and receptaculum seminis. Bursa copulatrix dis-



FIGS. 54–56. Reproductive system of *Bathyberthella zelandiae*. 54. Detail of vagina and seminal receptacles. 55. Detail of male section of pallial gonoduct and associated organs. 56. Composite view of structure of reproductive organs, two points marked with asterisks are connected to each other. Abbreviations: amp. = ampulla; b.c. = bursa copulatrix; d.v.d. = distal section of vas deferens; g.p. genital papilla; ni. = nidamental glands; o.t. = ovotestis; o.v. = oviduct; p. = penis; p.gl. = penial gland; pr. = prostate gland; p.s. = penial sheath; p.v.d. = proximal section of vas deferens; r.s. = receptaculum seminis; v. = vagina.

coidal or club-shaped (Fig. 54), walls exceedingly thin, sometimes with weak longitudinal folds. Receptaculum seminis narrow, tubular, much-coiled, approximately equal in diameter to vas deferens; a narrow twisted oviduct originates at base of receptaculum seminis and travels behind retractor muscle to lie at centre of nidamental glands.

Distribution

Bathyberthella zelandiae is only known from the northern slope of the Bounty Trough, southwest of New Zealand. Its bathymetric range is from 1640 to 1676 m.

Type Data

HOLOTYPE: 1676 m, 45°52.3′S; 174° 04.9′E, northern side of Bounty Trough, S.W. of New Zealand (Stn. S152), R.C.W. on G.R.V. "Tangaroa," 26 Oct. 1979 (NZOI, Reg. no. H-342).

PARATYPES: seven specimens (all juveniles), 1640 m, 45°46.0′S, 174°24.5′E, northern side of Bounty Trough, S.W. of New Zealand (Stn. S150) R.C.W. on G.R.V. "Tangaroa," 26 Oct. 1979 (NZOI, Reg. no. P-571); 10 specimens collected with holotype at Stn. S152 (NZOI, Reg. no. P-572); 25 specimens collected with holotype at Stn. S152 (NM).

Discussion

Bathyberthella is a most significant genus within the Pleurobranchidae. This is because *B. zelandiae* possesses an unexpected amalgam of characters some of which are peculiar to it alone and others which relate it to genera of both the accepted pleurobranch subfamilies. The enormous, uncalcified internal shell separates *Bathyberthella* at once from all other genera, but the morphology of the shell is probably related to the abyssal existence of the species. *B. zelandiae* has the external appearance of a member of the subfamily Pleurobranchinae, particularly a species of *Berthella* or *Berthellina*. The reproductive system recalls *Berthellina,* the teeth are smooth as in *Berthella* yet elongate and numerous as in *Berthellina.* These pleurobranchine features strengthen Burn's (1962) contention that the small pleurobranchs (with smooth, non-emarginate mantles and smooth gill rachises) are closer to each other than either is to *Pleurobranchus*. The mandibular elements of *B. zelandiae* show a great likeness to those of *Pleurobranchaea* (subfamily Pleurobranchaeinae).

The bulk of characters favour placing *Bathyberthella* in the Pleurobranchinae. Within that subfamily is another equally anomalous, yet important, monotypic genus—*Pleurehdera. Pleurehdera haraldi* is small; it has a smooth mantle and gill rachis; it possesses a pedal gland, prostate gland and penial gland; its teeth are elongate, most possess a single denticle near the apex but the outermost laterals are smooth for about one quarter of the row; the mandibular elements are variable (Marcus & Marcus, 1970). Some of the variation shown by the mandibular elements of *Pleurehdera haraldi* resemble those of *Bathyberthella zelandiae.* Even allowing for the abyssal habitat of *B. zelandiae,* the differences between shells, radulae and reproductive systems of *Pleurehdera* and *Bathyberthella* indicate they are not congeneric.

I interpret *Pleurehdera* and *Bathyberthella* as terminations of narrow lines produced during the radiation that followed the acquisition of the pleurobranch grade of organization by opisthobranchs. Both these genera stem from near the *Berthella/Berthellina* dichotomy. The genera *Euselenops* and *Pleurobranchella* illustrate analogous cases; they probably represent terminations of narrow side lines that originated on the pleurobranchaeine side of this radiation. I cannot interpret any of these monotypic (or very small) genera as ancestral to any large present day genus. This is because all the small genera possess a mosaic of characters many of which are quite unlike those of their presumed derivatives. One point that emerges is that the oval type of mandibular elements with denticulate anterior borders (as in the Pleurobranchaeinae) preceded the cruciform type of elements (as in the Pleurobranchinae).

It is unlikely that *Bathyberthella zelandiae* could be confused with either of the two small, yellow or orange pleurobranchs from New Zealand that look superficially similar (*Berthella citrina, Berthella mediatas*) since both the latter occur in shallow water on the continental shelf. Listing the many points of difference between these three species would involve repetition, in a comparative context, of diagnostic characters for each genus and reconstruction of a table similar to Table 2 (p. 248) to incorporate *B. zelandiae.* Comparisons of the significant characters between these three species will be presented in the key and repe-

tition of the more subtle points of distinction is unnecessary in view of the abyssal habitat of *B. zelandiae* and the scarcity with which it is likely to be encountered.

Pleurobranchaea Meckel in Leue, 1813

Pleurobranchaea Meckel in Leue, 1813: 11. Type-species by subsequent monotypy (Blainville, 1825: 376): *Pleurobranchidium meckelii* Blainville, 1825.
Pleurobranchidium Blainville, 1825: 372, 376. Type-species by monotypy: *Pleurobranchidium meckelii* Blainville, 1825.
Koonsia Verrill, 1882: 545. Type-species by monotypy: *Koonsia obesa* Verrill, 1882.
Pleurobranchillus Bergh, 1892: 27. Type-species by subsequent designation (Willan, 1977: 153): *Pleurobranchillus morosus* Bergh, 1892.

Meckel (in Leue, 1813) established *Pleurobranchaea* without including nominal species. Blainville (1825) was the first author to describe the species *meckelii* in the genus *Pleurobranchaea,* and it is this species *ipso facto* which becomes the type of *Pleurobranchaea* by subsequent monotypy. According to the International Code of Zoological Nomenclature, the spelling must revert to *meckelii* (from *meckeli*) since this is the correct original spelling (Art. 32(1), I.C.Z.N. 1961). I have considered the synonymy of *Koonsia* and *Pleurobranchillus* elsewhere (Willan, 1977). Bergh (1897: 3, note 2; 1898: 64) himself subsequently recognized *Pleurobranchillus* as a synonym of *Pleurobranchaea* (see Vayssière, 1901: 74; and Marcus & Marcus, 1957: 25).

Definition

Moderate-sized to large pleurobranchs with oval or oblong bodies that are blunt anteriorly; mantle reduced, smaller than foot, merging with oral veil anteriorly and tail posteriorly, covered with low tubercles; rhinophores far apart, inserted on either side of head at base of oral veil; oral veil with digitate processes along anterior margin; pedal gland present on posterior part of foot sole in sexually mature specimens; some species possess a caudal spur on dorsal side of tail; anus towards rear of gill basement membrane; shell absent; buccal mass relatively large; radula with central row, laterals curved, smooth-sided, most with an accessory denticle—either strongly or weakly developed; mandibular elements polygonal or rounded at jaw surface, denticulate along anterior edge; penis without accessory leaves; prostate gland present.

Remarks

As with all the Notaspidea, species of *Pleurobranchaea* often show considerable intraspecific variability and this has frequently resulted in the creation of spurious species. This cause for uncertainty has been compounded by inadequate description of new species based on poorly fixed material. Nevertheless there are subtle and consistent differences separating the valid species.

Marcus & Marcus (1957) and Marcus (1957) listed the 18 described species of *Pleurobranchaea*; they later claimed that *P. algoensis* Thiele and *P. japonica* Thiele were unrecognizable (Marcus & Marcus, 1966). Four species have been added since the Marcus' first lists: *P. hamva* Marcus (1957); *P. gemini* Macnae (1962), *P. californica* MacFarland (1966); *P. gela* Marcus & Marcus (1966). More material, however, has forced *P. hamva* to be incorporated into the synonymy of *P. hedgpethi* Abbott (Marcus & Marcus, 1967b).

Much more work needs to be done on this genus; for example, to determine the status of such species as *Pleurobranchaea capensis* Vayssière, 1898, *P. gemini* Macnae, 1962, *P. brocki* Bergh, 1897 and *P. agassizi* Bergh, 1897. Some new species are known and await description.

Because of their large size, high level of activity and variety of relatively complex behaviours, species of *Pleurobranchaea* are now frequently used in physiological research, e.g. Davis and Mpitsos (1971), Davis *et al.* (1977), and this is further reason to re-examine the taxonomic status of the various entities.

Pleurobranchaea maculata (Quoy & Gaimard, 1832) (Figs. 57–70)

1832. *Pleurobranchidium maculatum* Quoy & Gaimard: 301, pl. 22, figs. 11–14.
1878. *Pleurobranchaea novaezealandiae* Cheeseman: 276, pl. 15, fig. 3.—1879, Cheeseman: 378, pl. 16, fig. 3—1880, Hutton: 124.—1896, Pilsbry: 227, pl. 53, fig. 87.—1897, Bergh: 150–152, No. 31 and 154–155 No. 33.—1913, Suter: 553.—1915, Suter: pl. 36, fig. 2.—1933, Allan: 446.—1949, Baba: 133, pl. 10, figs. 31, 32,

34.—1957, Burn: 12, 15.—1957, Marcus & Marcus: 26.—1965, Guang-Yu & Si: 266, 275; 1966, Marcus & Marcus: 177.—1969, Baba: 191.—1976, Powell: 112.—1979, Powell: 282, pl. 51, fig. 2.

1896. *Pleurobranchaea maculata* (Quoy & Gaimard); Pilsbry: 227, pl. 53, figs. 88, 89.—1897, Bergh: 153–154, No. 32.—1901, Vayssière: 49–56, pl. 5, figs. 238–247.—1913, Suter: 552.— 1915, Suter: pl. 23, fig. 17.—1924, Odhner: 52.—1937, Powell: 85, No. 1236.—1954, Pruvot-Fol: 33.—1957, Marcus & Marcus: 26.—1958, Burn: 6.—1966a, Burn: 271.—1966b, Burn: 106.—1969, Burn: 80.—1970, Thompson: 192–195, fig. 10.—1976, Powell: 112.—1977, Gordon & Ballantine: 40, 112.—1979, Powell: 283.

1898. *Pleurobranchaea novaezelandiae* [sic] Vayssière: pl. 15, fig. 28.—1901, Vayssière: 69–72.—1900, Bergh: 208, pl. 20, figs. 56, 57, pl. 21, fig. 69.—1920, Mestayer: 170–171.—1924, Odhner: 52.—1937, Powell: 85, No. 1234.—1961, Powell: 107.—1964, Williams: 20.—1968, Morton & Miller: 167, 576, pl. 11, fig. 8.—1969, Batham: 78.—1970, Thompson 196.—1972, Morton: 346.—1973, Miller & Batt: 19, fig. 78 (image reversed during printing).—1977, Willan: 154.—1977a, Ottaway: 217–218.—1977b, Ottaway: 125–130, fig. 1 (error pro *P. novaezealandiae* Cheeseman, 1878).

1900. *Pleurobranchaea novaezelandiae* var. *granulosa* Bergh: 209.—1913, Suter: 554 (as ssp. *granulosa*).—1937b, Powell: 85, No. 1235 (as ssp. *granulosa*).—1961, Powell: 107 (as ssp. *granulosa*).

1933. *Pleurobranchaea dorsalis* Allan: 445, pl. 56, figs. 4, 5.—1957, Marcus & Marcus: 26.

1950. *Pleurobranchaea maculata dorsalis* Allan: 208, fig. 1.

1976. *Pleurobranchaea granulosa* Bergh; Powell: 112.—1979, Powell: 282.

The original description of *Pleurobranchidium maculatum* by Quoy & Gaimard (1832) was brief and dealt only with external features. The description was greatly expanded by Vayssière (1901) after a thorough re-examination of the type material. Vayssière (1901) detected a mistake made by Quoy & Gaimard (1832) with respect to *Pleurobranchaea maculata.* Vayssière had examined the (five) specimens collected by "l'Astrolabe" and he expressed astonishment that "Nouvelle-Zélande" was given as the locality on the labels on three of the five original specimens. Quoy & Gaimard (1832) stated that all had originated from Port Western to Jarvis Bay, Australia. Vayssière suspected that Quoy & Gaimard had erroneously labelled those three specimens; he did note, however, that New Zealand was included as part of the original locality citation for *Pleurobranchaea maculata.*

My investigations have shown that there is only a single species of *Pleurobranchaea* in New Zealand, for which the name *Pleurobranchaea maculata* (Quoy & Gaimard) has priority. No consistent differences can be found to warrant the continued separation of New Zealand specimens under the names of *P. novaezealandiae* Cheeseman or *P. novaezelandiae granulosa* Bergh (see below). Several other authors have already anticipated the synonymy of *P. novaezealandiae* with *P. maculata.* In her discussion, Pruvot-Fol (1954: 33) concluded by reiterating Vayssière's remark that the two might well be variants of a single species. Burn (1958) synonymized *P. novaezealandiae* and the Australian *P. dorsalis* Allan with *P. maculata.* Thompson (1970) suggested that all the Australian records of *Pleurobranchaea* are based on *P. maculata* (Quoy & Gaimard).

Live animal (Fig. 57)

Body large, elongate, oval, covered dorsally by a relatively small mantle beneath which foot projects all round. Mantle confluent anteriorly with oral veil, posteriorly it is continuous, over a relatively narrow area, with tail; sides of mantle free, left border held close to body, right slightly larger, held away from body to accommodate gill underneath. Mantle smooth, soft, upper surface (and that of oral veil) entirely covered with minute puckers and folds. Ground colour varies from pale grey to almost ash-black, broken by irregular, pale, grey-white areas to give a dappled-reticulate appearance. Raised areas sprinkled with numerous, minute, and almost microscopic, white dots; hollows between these raised areas darker. Some individuals uniformly dark, others have paler areas and a patchy appearance. Auckland east coast populations appear to show approximately equal proportions of dark and dappled morphs. Undersurface of free mantle borders smooth, grey,

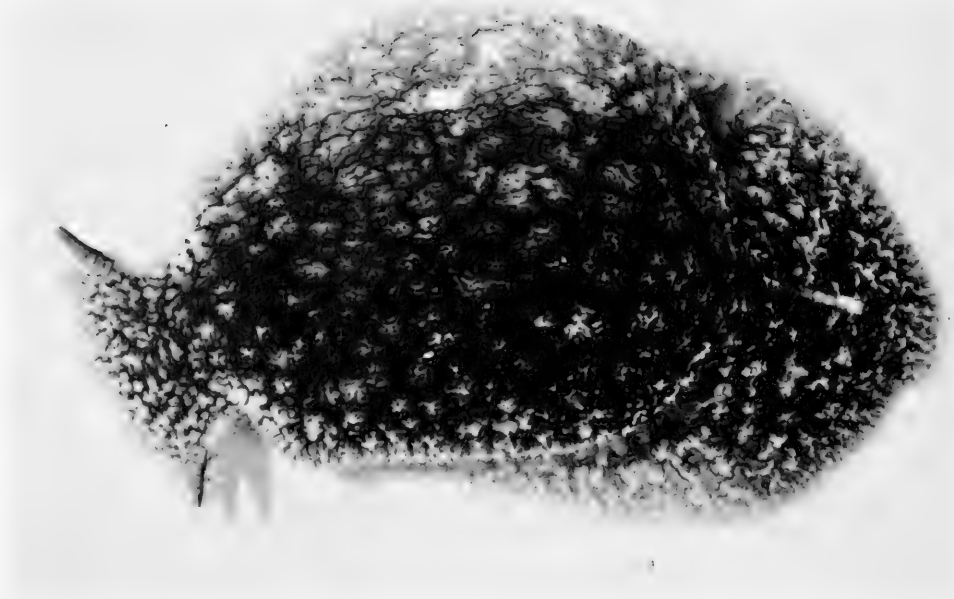


FIG. 57. *Pleurobranchaea maculata* (Quoy & Gaimard). Length = 132 mm. Specimen from 7–11 m, off middle head of German Bay Hill, Akaroa Hbr., Banks Pen., May 1962. Photograph: M. C. Miller.

regularly speckled with white dots. Mantle discharges a clear fluid of pH = 1.

Oral veil trapezoidal, lower surface smoother than upper surface; it bears some raised pustulose areas forming weak reticulations; anterior border of veil broad, straight, wider than widest part of body when animal is crawling. Anterior corners project laterally beyond front edge. Lateral areas marked dorsally by a white line. On undersurface, a groove extends length of lateral areas; here each area appears as a brownish streak. All along anterior edge of oral veil are many, small, branched processes; therefore oral veil of *Pleurobranchaea* species is more elaborate than that of *Berthellina* or *Berthella* species. The increased structural complexity is accompanied by increased activity; when animal is crawling actively, anterior border constantly ripples as it explores substratum in different areas.

Rhinophores arise laterally where the mantle passes into oral veil; mottled grey and white, tipped with white.

Gill large, conspicuous, generally its hind end extends beyond overlying right mantle flap; basement membrane attached to gill for approximately half its length.

Gill rachis bears 20–28 alternating pinnae (mean number for 9 specimens 23.0); pinnae relatively longer than in species of *Berthellina* or *Berthella*; pinnae bear large numbers of closely-packed pinnules (25–30 on anterior pinnae); rachis itself broad, flattened, with small, irregular knobs and lumps; however, rachises of pinnae round, smooth. In living specimens, gill is light ash-grey, with rachis and rachises of pinnae overlain with tiny black specks. This superficial speckled layer rubs off with ease in newly dead specimens, revealing a subepidermal layer of larger, opaque, white spots which does not rub off. Anus opens a short distance in front of hind end of the gill basement membrane. Prebranchial aperture conspicuous, on a papilla before front of gill rachis, interior ridged.

Foot broadly rounded anteriorly, more pointed behind; upper surface similar in colour and pattern to mantle; foot borders thin and with white puckers that are more regular than on mantle. Anterior dorsal surface with a clearly demarcated, broad, greyish mucous groove; this area being wider than area of the foot overhung by oral veil. Mucous groove has a deep, mid-anterior cleft perpendicular to its axis. Sole of foot very large, smooth, pale ash-grey, lightly speckled with white, margin

white. Pedal gland present posteriorly in sexually mature individuals, lying beneath foot epithelium, length about 1/6 total length of the foot; pedal gland somewhat asymmetric and triangular with base (closest to tail) shorter than sides; base does not reach extremity of tail. The white buccal mass and dark digestive gland are visible through transparent tissue of foot sole. Caudal spur never present on dorsal surface of tail.

Pleurobranchaea maculata is able to float upside-down on the meniscus in a still container of water, then lateral and posterior foot margins show slight indentations.

Animals are normally encountered at rest on undersides of stones, foot and body contracted and humped up; mantle rounded, gill extending beyond right border, pinnae twitching occasionally quite independently of each other; oral veil contracted against anterior mantle edge; rhinophores held laterally in groove formed by oral veil and mantle.

Radula (Figs. 58–63)

Radula large (8.4 mm long for a specimen 37 mm in extended length), relatively broad, deeply grooved in midline, with many rows of

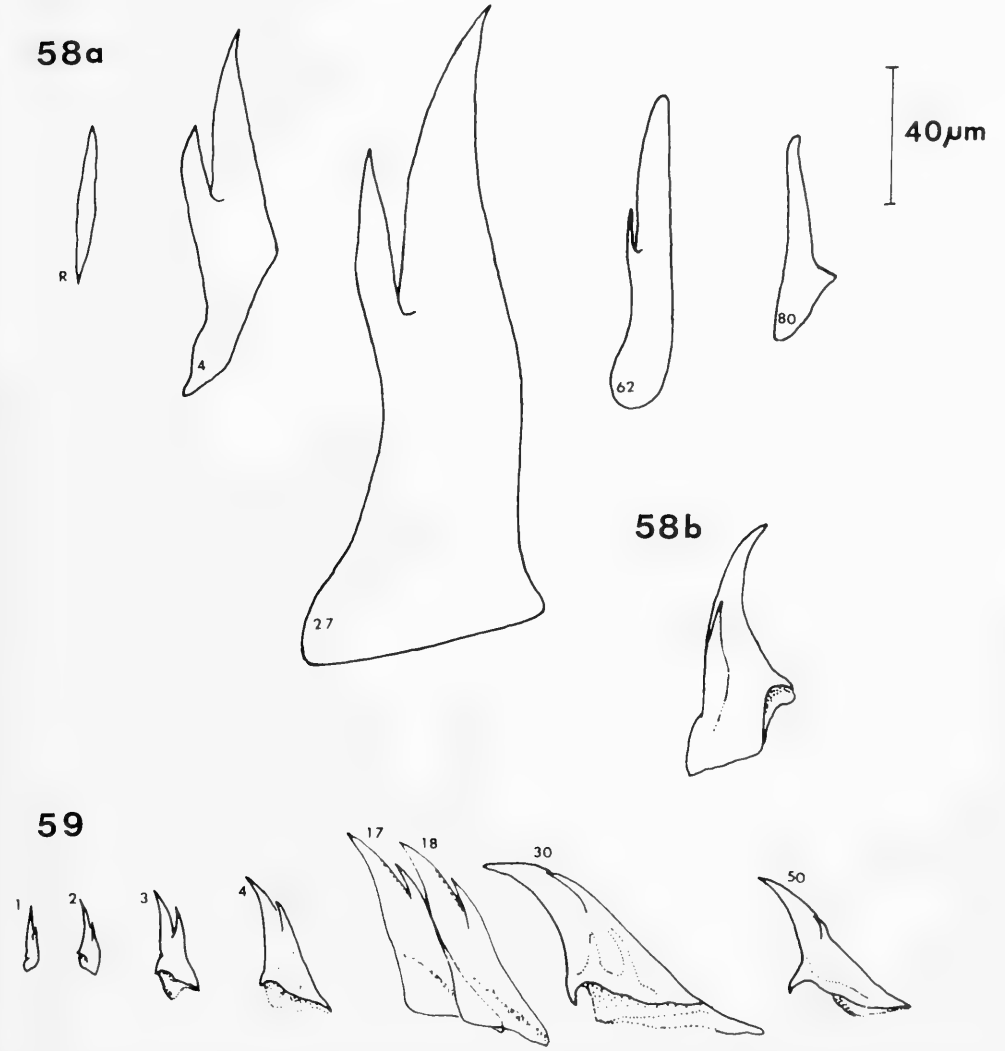


FIG. 58, 59. Radula of *Pleurobranchaea maculata.* 58a. Representative teeth from a half row; 58b. Middle lateral tooth showing detail of basal attachment region; 59. Teeth from a half row (after Vayssière, 1901, pl. 5, figs. 238–240, 242).

lateral teeth symmetrically on either side of central row. Radula of adult *P. maculata* has 40–50 rows. Rachidian inconspicuous, short (approx. 30 μm high), narrow, with a rounded base and pointed apex; apparently constant in length in all rows. Careful preparation is necessary to keep rachidian on radula; despite its presence in all rows, it fell away in most cases during preparation of radula.

There are 70–80 lateral teeth within each half-row; laterals undergo characteristic changes in size and structure across rows. Outermost lateral teeth (Fig. 63) small, peg-like, narrow, rounded apically with apices slightly recurved; extreme outermost teeth (number 80 counting from midline) smallest, approx. 48 μm high. By 65th lateral, a small spike is present on inner side near base of tooth, cusp of tooth itself sharper; at this point teeth are up to 60 μm high. Teeth continue to get larger, and lateral spike enlarges to a strong accessory denticle increasing in size, so that by 41st tooth (approx. mid-way across row) denticle is 64 μm high, the tooth itself being 298 μm high. In this middle region teeth have large, broad bases, sides parallel to point where denticle arises, beyond there sides curve inwards to sharp apex; lateral denticle straight-sided and erect (Figs. 61, 62). To the inside of this region, teeth again slowly decrease progressively although lateral denticle remains nearly same height. Close to rachidian there is a rapid decrease, teeth and denticles decreasing proportionately, second lateral approx. 60 μm high and still bears a small accessory denticle.

A radular formula typical of an adult is 43 × 74.1.74. The range of radular formulae for adult New Zealand specimens examined is 40–50 × 70–80.1.70–80. Radular formula increases as *P. maculata* grows; a juvenile (crawling length 5 mm) from Cape Three Points, Akaroa Harbour, had a formula of 25 × 39.0.39; tooth height also increases proportionately with growth.

System for attachment of teeth to basement membrane like that of *Pleurobranchaea californica* (MacFarland, 1966: 97), although the basal facets are knobbed rather than hook-like in *P. maculata* (Figs. 61, 63). System for support of teeth along rows also present; outer laterals (Fig. 63) have a socket towards base where right-angled elbow of base of tooth in front fits, same interlocking arrangement present between middle lateral teeth (Fig. 61).

Tooth structure is a very constant and characteristic feature of *Pleurobranchaea maculata,* particularly the strong, accessory lateral denticle on middle lateral teeth. *P. californica* has very small, weak denticles (MacFarland, 1966). I have redrawn Vayssière's (1901, pl. 5) illustration of isolated radular teeth from specimens of the type lot of *P. maculata* (Fig. 59); they show a strong resemblance to New Zealand material.

Jaws (Figs. 64, 65)

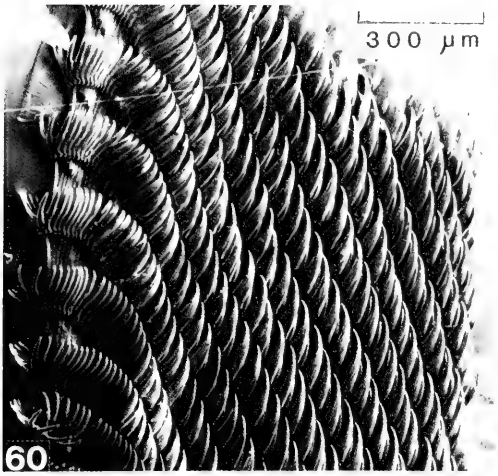
A pair of elongate, chitinous jaws lines inside of buccal bulb; rectangular; hind portion expanded and rounded, anterior margin deeply sinuate, with a forward-projecting spike towards centre. Many tightly-fitting, polygonal elements, in the form of closely-packed columns make up jaws; surfaces of elements flattened, resembling interlocking paving stones; elements arranged in alternating rows (about 60 per row), mostly hexagonal though some are pentagonal or round. From the surface, base and sides of elements appear smooth, sides are parallel, slightly longer than the base; there are no lateral projections. On broadly convex anterior face are 5–12 small, pointed denticles; denticles not positioned symmetrically about anterior edge. Central area on inner face is irregularly pustulose (Fig. 65). Average dimensions of elements are: length 40 μm; width 60 μm.

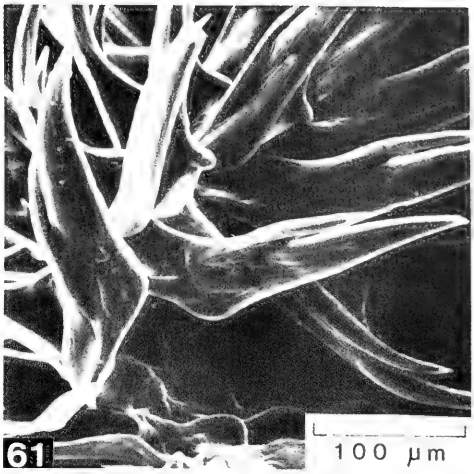
MacFarland (1966) studied the development of mandibular elements in *Pleurobranchaea californica.*

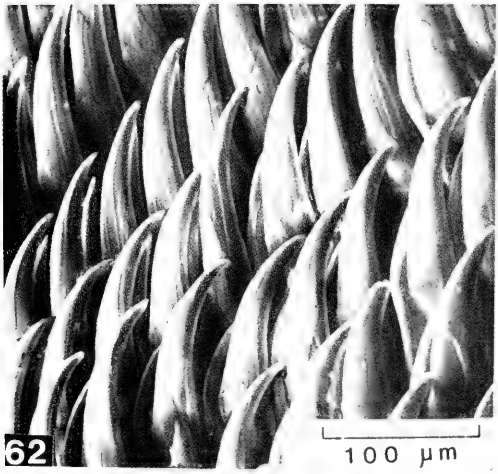
Reproductive system (Figs. 66–69)

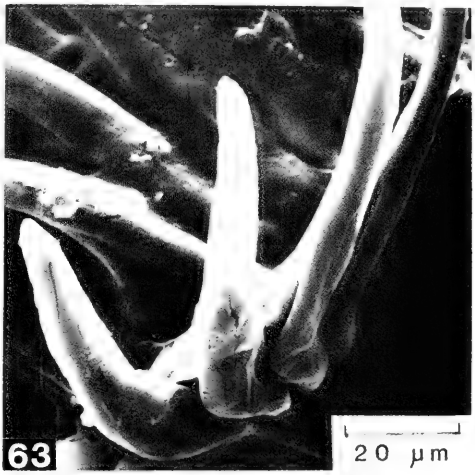
To expose the reproductive system in dissection, the network of anastomosing tubules of dorsal accessory gland must first be removed; these fine tubules surround all organs of reproductive system and gut. Because the

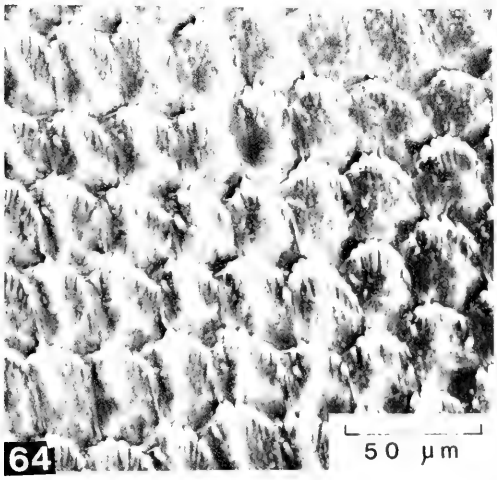
FIGS. 60–65. Radula and jaws of *Pleurobranchaea maculata.* 60. Teeth on one half of the radula; outermost laterals are on the extreme left. 61. Detail of large, middle lateral tooth; note accessory denticle on each tooth. 62. Detail of same; longitudinal striations are probably dried mucus. 63. Detail of small, peg-like outermost lateral teeth. 64. Mandibular elements on inner face of jaw. 65. Detail of the same.

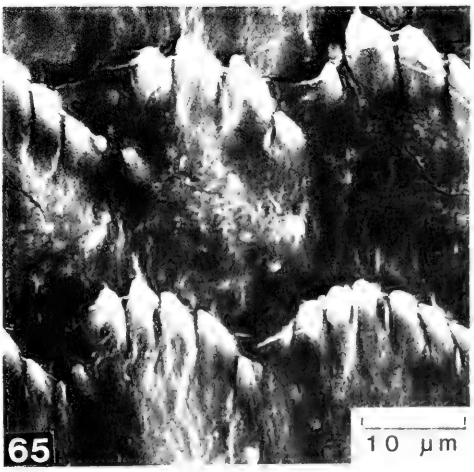

300 µm
60

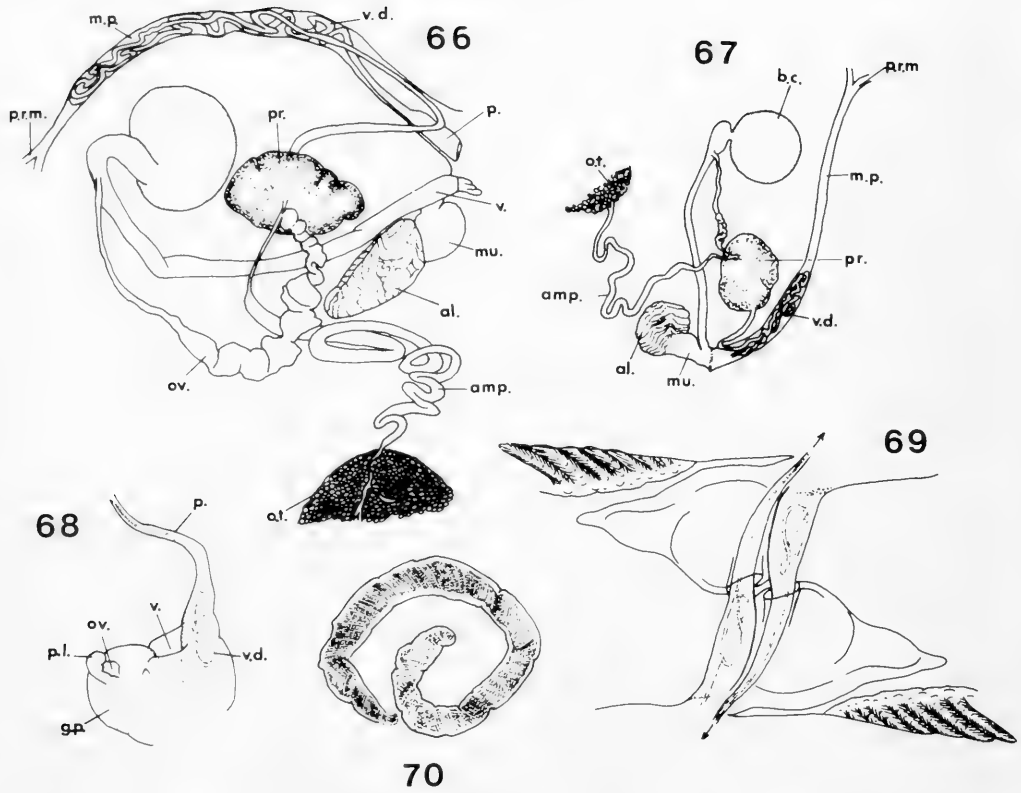

61
100 µm


62
100 µm


63
20 µm


64
50 µm


65
10 µm



FIGS. 66–70. Reproductive system of *Pleurobranchaea maculata*. 66. Composite view of structure of reproductive organs. 67. Reproductive system of a paratype (after Vayssière, 1901, pl. 5, fig. 247). 68. Detail of fully everted genital organs. 69. Orientation of genital organs during reciprocal copulation, arrows indicate direction of sperm injection into bursa copulatrix of partner. 70. Spawn mass. Abbreviations: al. = albumen gland; amp. = ampulla; b.c. = bursa copulatrix; g.p. = genital papilla; m.p. = muscular pocket for vas deferens; mu. = mucous gland; o.t. = ovotestis; ov. = oviduct; p. = penis; p.l. = posterior lobe of everted genital papilla; pr. = prostate gland; p.r.m. = penial retractor muscle; v. = vagina; v.d. = vas deferens.

structure of the reproductive organs differs in many ways from that of species of the Pleurobranchinae, it is described here in full.

Ovotestis dorsal, applied to, but not interlobed with, digestive gland; creamish-white, compact, appearing granular because of elongate acini stacked side by side. Inside ovotestis, fine white collecting ducts can be traced amongst acini; these smaller ducts join larger and larger ducts, finally to a still slender, sinuous, white hermaphrodite duct with ovotestis on its anterior ventral surface. Immediately upon leaving ovotestis, hermaphrodite duct greatly increases in diameter and wall thickness to form ampulla. Ampulla lies next to foot musculature, much coiled and convoluted; it gradually enlarges as it passes towards ventral midline. Near midline, ampulla turns forward (below nidamental complex), and abruptly narrows to about ¼ of its previous diameter, now becoming quite straight; it branches into two, and, after becoming a very short, but slightly wider proximal vas deferens, enters prostate gland.

Oviduct is the other of these branches; at its point of origin from hermaphrodite duct (beside the prostate gland), oviduct is large, its walls deeply constricted, of a greater diameter than hermaphrodite duct, thickest approx. ⅓ of the distance along its length, after that diameter is halved. Oviduct next travels as a straight, whitish tube beneath bursa copulatrix to join much larger vagina before the latter passes into bursa copulatrix.

Bursa copulatrix varies in position and shape; it lies, *in situ*, in midline, either dorsally, above the buccal mass and visceral ganglia, or ventrally, below and next to them.

Bursa generally discoidal or club-like, flat beneath and convex above. Vagina completes a half loop on dorsal side of bursa before passing into it; end of the vagina connected to bursa is neither swollen nor constricted. Recepaculum seminis absent.

Vagina slowly increases in diameter as it passes, in a semicircle, beneath all the reproductive organs, to exterior. Vagina is tube of greatest width in reproductive system; it is whitish, its walls are tough and unconstricted. Nidamental gland complex situated besides vagina—mucous gland orange, relatively thin-walled; albumen gland larger, more solid, cone-shaped, walls much-convoluted.

Vas deferens is a continuation of hermaphrodite duct from the point at which oviduct departs; proximal vas deferens is visible for only a very short distance before entering prostate gland. Prostate gland irregular in outline and size, spongy, consisting of many tightly-packed vesicles. Distal vas deferens leaves prostate gland, its walls thickened and shining. Just beside the penis, distal vas deferens curves backwards into an elongate pocket of clear and tough tissue; pocket passes backwards above prostate gland, female glands and digestive gland, then travels to foot where retractor muscles attach it to floor of body cavity; this pocket is the most conspicuous part of entire genital system. Distal vas deferens lies within pocket, it often loops over itself, it retains its same diameter throughout entire course after leaving prostate gland. Towards the end of its course back inside pocket vas deferens expands gradually into penis. Penis is long, narrow, smooth-walled, quite smooth, capable of eversion to a great distance.

The organization of the reproductive system in New Zealand *Pleurobranchaea* material presents one of the strongest reasons for believing it to be conspecific with *P. maculata* (Quoy & Gaimard). The system detailed above (Fig. 66) is almost identical with that described by Vayssière (1901) for specimens from the type lot (Fig. 67).

The reproductive system in this genus is highly diagnostic for each species. In *Pleurobranchaea meckelii* the pocket for the vas deferens is shaped like a large, inverted cone and the retractor muscle is inserted on the body wall; in this species too, the oviduct has a short proximal tube followed by two ovoid swellings; the shape of the bursa copulatrix is also different. The enigmatic caudal spur is present on the post-dorsal surface of the foot.

Behaviour at copulation and oviposition

Not only is the organization of genital organs important in species recognition, but also reproductive behaviour is highly species-specific. *Pleurobranchaea* species tend to be solitary, so mating encounters occur seldom and have been reported very infrequently in the literature. Davis & Mpitsos (1971) give details of copulatory behaviour in *P. californica*. The following account describes the mating behaviour of *P. maculata* in detail.

When two mature specimens of *Pleurobranchaea maculata* encounter each other, one of two behaviour patterns results. Upon contact one animal rapidly everts its oral tube and makes feeding thrusts towards the other; often removing a piece of flesh. Most attacks of this kind appear to be directed at the tail region. This type of behaviour most often results from initial contact of the oral veil of one specimen, with some other part of the other specimen. The attacked animal may let go of the substratum and swim with head-to-tail flexions to avoid further attacks.

The second response often occurs when two specimens meet head first. After the oral veils contact each other, forward motion slows, both partners raise their right mantle edges, and partially evert their genital organs. This is a very characteristic sexual posture. It is often followed by mutual circling during which the genital organs are fully everted, and the long, whip-like penis is rapidly everted and thrust in the general region of the reproductive apertures and gill.

In the laboratory, following an encounter of this type, one partner frequently fails to reciprocate and crawls away. But if both animals are receptive at that time, circling and penial thrusting continue. The penis is retracted and fully everted repeatedly but the posterior swelling that exposes the vagina remains everted and distended. Behind the vagina this swelling becomes lobed and narrows to a tail-like extremity (Fig. 68).

When the penis of one partner is correctly thrust towards the vaginal entrance, the penis enters and passes a considerable distance into the vagina (the tip probably reaches the base of the bursa copulatrix). Copulation is most often reciprocal by this stage, and Fig. 69 shows the position of everted genitalia and penial insertion in such a reciprocal copulation. Copulation lasts less than two minutes.

Oviposition lasts one or two hours. The egg

mass (Fig. 70) is usually in the form of a loose and irregular coil of approximately 1½ whorls, but this pattern is not circumscribed as in the Pleurobranchinae; some egg masses are irregulary looped, others open. Larger individuals lay larger coils. The egg mass is circular in cross section and is composed of a clear, gelatinous outer area inside which the long string of white eggs is tightly coiled; coiling is generally oblique to the outer wall. The egg mass is attached to the substratum by a narrow, opaque-white strip which frequently persists after the mass has disintegrated. Mestayer (1920) and Graham (1941) have recorded observations and given photographs of aquarium spawning in *Pleurobranchaea maculata*.

Larvae hatch after approximately 10 days; they are planktonic veligers with a small cup-like shell and operculum. Veliger shells are 180–200 μm long and approx. 120 μm wide. In aquaria, settlement was observed after six days but could be delayed for at least one week. The larvae can feed in the plankton; larvae, experimentally fed with a suspension of *Dunaliella primolecta*, ingested these cells. Further aspects of the planktonic life and settlement of veligers were not studied.

Distribution

Pleurobranchaea maculata occurs throughout New Zealand. It lives both intertidally and subtidally (to at least 300 m) and appears to be equally abundant through that entire bathymetric range. Specimens are most frequently encountered nesting in depressions on the undersurfaces of stones; when uncovered they immediately start crawling to safety.

Pleurobranchaea maculata is more tolerant than other pleurobranchs of waters carrying suspended silt, and thus appears in harbours and estuaries where the others are absent. This is probably also correlated with food; *P. maculata* is an opportunistic carnivore and can take advantage of a wide range of prey species, whereas other pleurobranchs feed on species of sponge or ascidians which are themselves confined to clear water situations.

Pleurobranchaea maculata also occurs in southern Queensland (Burn, 1966a), New South Wales (Allan, 1950; Thompson, 1970), Victoria, Australia (Burn, 1957, 1966, 1969), China (Tchang-Si, 1934; Guang-Yu & Si, 1965), Sri Lanka (White, 1948) and Japan (Baba, 1937, 1949, 1969; Baba & Hamatani, 1952).

Discussion

Some discrepancies exist in the description of specimens of *Pleurobranchaea* from New Zealand. For instance, in the diagnosis of *P. novaezealandiae*, Cheeseman (1878) stated: "branchial plume often over an inch in length, and free for half that distance; pectinations about 17, finely ciliated." This number of pinnae is well below the average (23) for the species based on material I have examined, but the distal pinnae are so small Cheeseman probably missed them in his count.

Quoy & Gaimard's (1832) original figure of *Pleurobranchaea maculata,* later reproduced by Pilsbry (1896), Vayssière (1901) and Suter (1915), was inexact. It was drawn from a moribund, or dead, specimen as evidenced by the everted penis. Vayssière (1901) gave a detailed account of the reproductive system, jaws, and radula of the type material, all of which correspond well with the same organs for New Zealand specimens. Cheeseman's (1878, 1879) original illustration of *P. novaezealandiae,* reproduced subsequently by Pilsbry (1896), Vayssière (1898) and Powell (1979), is slightly inaccurate too; the mantle appears not to be confluent with the oral veil, but this is merely due to the angle from which the animal has been drawn.

Bergh (1900) originally differentiated *Pleurobranchaea novaezelandiae* var. *granulosa* on the basis that the back and tail were covered with small, round and oval granulations (0.5–1.0 mm diameter). This difference in surface texture only reflects the degree of variation of this character in *P. maculata* and it is accentuated by the fixation procedure adopted. Baba (1937) realized this and included *P. novaezelandiae* var. *granulosa* in *P. novaezealandiae*. Other differences do exist between var. *granulosa* and *novaezealandiae* according to Bergh (1900) in the shape of the jaws and ampullae of the salivary glands; but these differences do not merit taxonomic separation. They can be explained by intraspecific variation and by fixation techniques respectively. Bergh gave no illustration for his single specimen from French Pass. I have examined specimens in the Suter Collection (National Museum of New Zealand) from Te Onepoto, labelled as *P. novaezealandiae granulosa,* and can find no consistent differences from typical *P. maculata.*

Pleurobranchaea maculata is similar to the Mediterranean species *P. meckelii*, but there are several points of difference: sexually mature *P. meckelii* has a caudal spur, there is

none in *P. maculata*; in *P. maculata* the retractor muscle is attached posteriorly to the floor of the body cavity whereas in *P. meckelii* it is attached to the body wall dorso-laterally; there is a significantly greater number of denticles (11–15) along the anterior edge of the mandibular elements in *P. meckelii*; the shape of the rachidian tooth is also different in the two species. *Pleurobranchaea maculata* differs from *P. californica* in details of the radula and reproductive system.

An Ectoparasitic Nematode from *Pleurobranchaea maculata*

Whilst examining the external features of specimens of *Pleurobranchaea maculata* collected from beneath subtidal stones in Leigh Harbour (23 Nov. 1973), I discovered that the slugs each carried several ectoparasitic nematodes. One had 18 nematodes, most were on the undersurface of the foot with a few on the mantle, none was on the rhinophores, oral veil or gill. Later I found more than 30 embedded in the foot sole of a *P. maculata* from Goat Island Bay.

The nematode has a long and relatively broad body which is bent to form a loop. There are pronounced adhesive organs on the tail. Specimens had their tails buried a few millimeters in the *P. maculata* tissue, and when removed most carried away some mollusc flesh.

Dr. W. C. Clark examined the nematodes and kindly informed me they probably belonged to the order Monhysterida but this could not be confirmed because all were immature. The presence of these nematodes on *Pleurobranchaea maculata* raises questions regarding their life history patterns and possible specificity.

KEY TO NEW ZEALAND NOTASPIDEA

1. Shell external, circular and flattened, limpet-like; body large and warty ..... *Umbraculum sinicum* (Gmelin, 1791)

   Shell internal or absent; body elongate, slug-like 2

2. Shell present beneath mantle; rhinophores arise together; mantle large, smooth; simple border to oral veil 3

   Shell absent; rhinophores widely separated, at sides of head; mantle small, greyish, its surface puckered and wrinkled; anterior border of oral veil with numerous branched papillae ..... *Pleurobranchaea maculata* (Quoy & Gaimard, 1832) Fig. 57

3. Shell relatively large (up to ½ body length), auriculate; radular teeth simple, without denticulations 4

   Shell relatively small (1/5 to 1/4 body length), triangular; radular teeth with a series of denticulations on posterior margin; mandibular elements (generally) smooth; prostate gland present; mantle lemon-yellow to apricot with scattered white specks; no pedal gland on sole of foot ..... *Berthellina citrina* (Rüppell & Leuckart, 1828) Figs. 1, 5

4. Mantle uniform in colour, dull orange or creamish-yellow with a few white specks; mandibular elements denticulate 5

   Mantle spotted with large, chocolate-brown blotches; anus at rear of gill membrane; mandibular elements smooth ..... *Berthella ornata* (Cheeseman, 1878) Figs. 20–22

5. Mantle with numerous, large pores; shell calcareous; anus near front end of gill membrane; mandibular elements cruciform with denticulate blades; prostate gland absent, found intertidally and shallowly subtidally (to ca. 20 m) .. *Berthella mediatas* (Burn, 1962)

   Mantle smooth, not conspicuously porous; shell very large, cuticular and without calcification; anus at rear of gill membrane; mandibular elements oval with denticles on anterior border; prostate gland present; occurs only in very deep water (>1000 m) ..... *Bathyberthella zelandiae* Willan, gen. et sp. nov.

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APPENDIX

This Appendix lists the New Zealand pleurobranch material that I have examined during this study. Habitat data are included where possible. Molluscan collections housed in the National Museum of New Zealand, Wellington (NM), New Zealand Oceanographic Institute, Wellington (NZOI), Auckland Institute and Museum (AIM), and Geology Department, University of Auckland (AUG), have been inspected. Dr. M. C. Miller of the Zoology Department, University of Auckland, has generously given me access to his opisthobranch collections. Comparative Australian material was kindly provided by Mr. R. Burn of Victoria. I collected most of the live specimens on which the morphological descriptions here are based, both intertidally, and subtidally by scuba diving, and these are signified by the letters "R.C.W." in this Appendix.

Berthellina citrina

NORTH ISLAND: Spirits Bay, Northland—1 washed live on to beach near Pananehe Island, R.C.W., 14 Jan. 1972; 18 m, Black Point, N.E. side of Karikari Pen.—2 copulating, and a freshly-laid egg coil, R.C.W., 11 Feb. 1978; 10 m, off N. side of Jolliffe Point, Matai Bay, N. end of Doubtless Bay—1 beneath a stone, sand substrate, R.C.W., 30 March 1978; 11 m, off S.W. side of Motutapere Is., Cavalli Is.—1 beneath a stone, R.C.W., 29 Dec. 1978; 8.5 m, large bay at N. side of Hamaruru Is., Cavalli Is.—1 beneath a stone, R.C.W., 31 Dec. 1978; 15 m, Nursery Cove, Aorangi Is., Poor Knights Is.,—abundant on undersides of stones and also several copulating pairs, beneath kelp forest; 20 m, entrance to Riko Riko Cave, Aorangi Is., Poor Knights Is.—4 on undersides of stones, fine bryozoan substrate, R.C.W., 10 Dec. 1973; 15 m rock slope to left of Riko Riko Cave, Aorangi Is., Poor Knights Is.—abundant on undersides of stones, R.C.W., 31 Dec. 1978; 60 m, off Deep Water Cove, Bay of Islands—1 shell, dredged in grey, muddy ooze, R.C.W., 7 April 1973; 6–8 m, off Castle Rock, Taurikura, Whangarei Hbr.—1 dredged amongst deposit of *Glycymeris, Pecten, Oxyperas, Tawera,* some fine broken shell and small stones, also ascidians, bryozoans and hydroids, M. C. Miller, 18 May 1961; McGregor's Bay, Whangarei Heads—2 on 14 May 1961 and 3 on 5 May 1961, both M. C. Miller; High Is., Whangarei Hbr.—on undersides of rocks at low water level, 2 on 14 Jan. 1959, 3 on 15 May 1961 and 3 on 15 Dec. 1961; Ocean Beach, Whangarei Heads—4 (including one copulating pair), beneath intertidal stones, M. C. Miller, 14 Dec. 1961; 15–16 m, Northern Bay, Little Barrier Is.—2 on undersides of stones, R.C.W., 25 Jan. 1977; 15–18 m

"Sponge Garden" and "North Reef," off N.W. tip of Goat Is., Leigh—2 on 7 Aug. 1973, 22 on 11 May 1974, 5 on 14 May 1974, 6 on 21 Sept. 1975, 1 on 27 May 1976, several on 28 Sept. 1976 and several on 29 Sept. 1976, all R.C.W., 20 m, "The Canyon," at S.E. corner of Goat Is., Leigh—2 and several spawn coils, R.C.W., 24 Dec. 1975; "Echinoderm Reef Flat," Goat Island Bay, Leigh—5 on 19 Dec. 1960, 3 on 28 Aug. 1961, 2 on 26 Oct. 1961, and several on 21 May 1967, M.C. Miller; "Echinoderm Reef Flat," Goat Island Bay, Leigh—1 on 14 May 1974, 9 on 24 May 1974, all R.C.W., all specimens found on undersides of stones and rocks in shallow pools and channels near low water mark; 12–15 m, near "Knot Rock," c. 100 m offshore from W. end of Goat Island Bay, Leigh—2 on 25 June 1977, 1 on 5 July 1977, 3 on 29 Aug. 1977, and 1 on 20 Sept. 1977, all R.C.W.; 3–4 m, Leigh Hbr.—9 on 22 Nov. 1973 and 4 on 14 March 1974, all found on the undersurfaces of stones on substrate of gravel and coarse sand, R.C.W.; 4.5 m, Matheson Bay, Leigh—1 and spawn coil, R.C.W., 19 Jan. 1976; Ti Point Channel, Leigh—4 on undersides of stones, on coarse sand-gravel substrate, R.C.W., 16 May 1974; 43 m, Aldermen Is.—1, 21 Nov. 1971 (NM); 59–74 m, off E. side of Mayor Is.—1 shell, amongst pebbles, shell grit and algae, 22 Jan. 1979 (NM); Omaio Bay, E. Bay of Plenty—1, R. K. Dell, 12 March 1962 (NM); little bay before Cape Runaway, East Cape—1, on underside of an intertidal stone, K. R. Grange, 29 March 1975.

Berthella ornata

NORTH ISLAND: Henderson Pt., S. end of Rarawa Beach, Northland—1 on underside of a low-tidal, weed-covered rock, R.C.W., 18 Jan. 1970; Mahinepua Peninsula, near Whangaroa—1, R.C.W., 7 Jan. 1966; "The Gap," Mahinepua Peninsula, near Whangaroa—2 on undersides of stones in the sublittoral fringe, R.C.W., 7 Jan. 1970; Church Bay, Tutukaka—4 on undersides of low-tidal stones, J. D. Willan, 17 Oct. 1970; Pacific Bay, Tutukaka—1 on underside of a stone, E.L.W.S. level, C. Grange, 8 March 1974; High Is., Taurikura, Whangarei Hbr.—3 on undersurfaces of rocks at low-water mark, 7 Jan. 1958, 1 on 14 Jan. 1959, 2 on 16 Jan. 1959, several pairs copulating on 13 Dec. 1961 and 2 on 15 May 1961, all M. C. Miller; 6 m, E. side of High Is., Taurikura, Whangarei Hbr.—1 on undersurface of a stone, R.C.W., 21 June 1975; McGregor's Bay, Whangarei Heads—1 beneath an intertidal stone, M. C. Miller, 14 May 1961; "Echinoderm Reef Flat" Goat Island Bay, Leigh—3 on 28 Aug. 1961 and 1 on 26 Oct. 1961, both M. C. Miller, and 1 on underside of a stone on substrate of clean, coarse sand, middle of low-tidal platform in a rock pool, 22 May 1974 and 9 on 24 May 1974, both R.C.W.; 6 m off Goat Is., Leigh—1 on underside of a stone on substrate of coarse gravel and sand, D. Rowe, 30 June 1974; 12–15 m near "Knot Rock," c. 100 m offshore from W. end of Goat Island Bay, Leigh—1 on underside of a stone, R.C.W., 29 Aug. 1977; Omaha, Leigh—1 on underside of a low-tidal stone, E. N. Gardner, 16 Sept. 1974; Kawau Is., Hauraki Gulf—1, C. Wormald, 13 Aug. 1967; Beehive Is., off Kawau Is., Hauraki Gulf—1, R.C.W., 26 Sept. 1965; Mahurangi Is., off Waiwera Beach—1 on 30 Aug. 1969, 8 on 12 Jan. 1974, 1 on 22 July 1974 and 1 on 15 Sept. 1974, all on undersides of stones in pools, at low-water level, R.C.W.; 11 m, eastern side of Tiritiri Matangi Is., Hauraki Gulf, 1 on underside of a stone, R.C.W., 29 Feb. 1976; Army Bay, Whangaparoa Pen.—2 under a rock ledge. E.L.W.S. level, in a rock pool, under *Cystophora retroflexa,* R.C.W., 10 Jan. 1974; Takapuna, Auckland—1, H. Suter (Suter Colln., NM M17843); Beacon Rocks, Mount Maunganui, Bay of Plenty—2 shells, E. N. Milligan (AGU): Breaker Bay, Wellington—1 under a stone in brown algal association, W. R. B. Oliver, 15 Sept. 1923 (W. R. B. Oliver Colln., NM).

SOUTH ISLAND: Headland Pt. Portobello Pen.—1 on 21 Aug. 1962, crawling across muddy floor of a cave, 0.3–0.5 m below low-water level, and 1 on 22 Aug. 1962, on undersurface of a rock at low-water level, both M. C. Miller.

Berthella mediatas

NORTH ISLAND: Ocean Beach, Whangarei Heads—1, M. C. Miller, 14 Dec. 1961; High Is., Taurikura, Whangarei Hbr.—2 seen but only 1 collected, on undersurface of a rock, low tide level, M. C. Miller, 16 Jan. 1969; "Echinoderm Reef Flat," Goat Island Bay, Leigh—1 on the underside of a stone, E.L.W.S. level, R.C.W., 1 June 1977, and 1 in holdfast of *Laurencia* sp. in a rock pool on inner part of reef, R.C.W., 23 June 1979; Army Bay, N. side of Whangaparoa Pen.—4 on undersides of low tidal stones in rock

pools, R.C.W. and J. D. Willan, 10 Jan. 1974; Takapuna, Auckland—1, H. Suter (Suter Colln., NM M17843; specimen in lot with 2 *Berthella ornata*); Narrow Neck Reef, Auckland—1 spawning, H. Suter, 24 July 1906 (NM); Cape Egmont, Taranaki—1 on underside of low-tidal stone, K. R. Grange, 16 July 1974; Pencarrow Head, Wellington—2, W. F. Ponder, 20 Oct. 1956 (NM); Point Howard, Wellington—1, W. F. Ponder, 11 Nov. 1958 (NM); Lyall Bay, Wellington—4, R. K. Dell, 8 Jan. 1950 (NM).

SOUTH ISLAND: Cape Three Points, Akaroa—4 on 17 May 1962, 1 on 18 May 1962 and 1 on 22 May 1962, all on undersurfaces of rocks at low tide level, M. C. Miller; Aquarium Pt., Portobello, Otago Hrb.—4 on 17 Jan. 1961, 1 on 18 Jan. 1961 and 1 on 17 Aug. 1962, all on undersurfaces of rock at low water level, M. C. Miller; Quarantine Is., Portobello, Otago Hbr.—2 amongst moveable stones in a sheltered inlet, M. C. Miller, 20 Aug. 1962.

CHATHAM IS: East Bay, South East (Rangatira) Is.—1 under a stone, in low tidal rock pool, E. C. Young, 2 Jan. 1975.

Pleurobranchaea maculata

NORTH ISLAND: Russell, Bay of Islands—1, L. J. Mather, May 1965 (NM); 6–8 m, S. of Castle Rock, Tutukaka—1 on substratum of coarse broken shell and sand, M. C. Miller,19 Jan. 1959; N. end of Oakura Beach—1 on underside of a stone at low tide, M. C. Miller, 17 Jan. 1960; beach at end of reclamation, near channel, N. side of Tutukaka Hbr.—1 crawling on mud amongst rocks; low water level, K. R. Grange, 14 May 1972; 8–10 m, "Waterfall Reef," coast near Goat Is., Leigh—several small juveniles on undersides of stones on 26 March 1977 and many on 22 Dec. 1978, both R.C.W.; 18–20 m, "Deep Point" and "The Canyon" at southeastern tip of Goat Is., Leigh—2 and several egg masses on 21 Sept. 1975, 1 juv. on 24 Dec. 1975, many and fresh spawn on 4 June 1976 and many on 12 Dec. 1978, all R.C.W.; 9–17 m, "North Reef," off N.W. tip of Goat Is., Leigh—1 on underside of a stone on 11 May 1974 and 1 juv. on underside of a stone on 7 May 1977, both R.C.W.; 18 m, "Sponge Garden," off N.W. tip of Goat Is., Leigh—2, R.C.W., 28 Sept. 1976; 6–7 m, off S.W. tip of Goat Is., Leigh—1, D. K. Rowe, 30 June 1974; "Echinoderm Reef Flat," Goat Island Bay, Leigh—2 on undersurfaces of stones at low-tide level, 28 Aug. 1961 and several seen on undersurfaces of rocks, in pools just above low-tide level, 26 Oct. 1961, both M. C. Miller; 3 m, Leigh Hbr.—5, R.C.W., 22 Nov. 1973; 9 m, Matheson Bay, Leigh—many, R.C.W. and M.S. Leighton, 21 Dec. 1978; 2–3 m, Ti Point Channel, entrance to Whangateau Hbr., Leigh—1 on 14 May 1974 and 1 on 22 May 1978, both R.C.W.; Mahurangi Is., Waiwera—many on undersurfaces of low-tidal stones in pools, 30 Nov. 1973 and many in similar habitats on 12 Jan. 1974, both R.C.W.; Army Bay, N. side of Whangaparoa Pen.—2 on undersurfaces of stones in low-tidal pools on 27 Dec. 1973 and many in similar habitats on 10 Jan. 1974, both R.C.W.; Matakatia Bay, S. side of Whangaparoa Pen.—1 on undersurface of a stone in a low-tidal pool, R.C.W., 30 Nov. 1973; Surfdale, Waiheke Is.—8, R.C.W., 25 Nov. 1973; 5–6 m, between Waiheke Is. and Pakatoa Is.—1, P. R. Bergquist, 14 March 1974; 8–9 m, Rakino Channel—1 on substratum of mixed shell and sand, M. C. Miller, 16 Nov. 1960; Rangitoto Channel, off North Head—1 and several spawn masses, M. C. Miller, 16 Sept. 1967; Takapuna Beach—1, H. Suter (Suter Colln., NM); West Tamaki Reef, Waitemata Hbr.—1 on underside of a stone at low-tide level, M. C. Miller, 8 Dec. 1962; Tamaki Str.—1 in bottom fish trawl, W. Tong, Sept. 1974; bay E. of Little Blowhole, South Head, Manukau Hbr.—1 found at E.L.W.S. level, D. Brambley, 2 Sept. 1966; 4.5 m, Bowentown entrance, Waihi—W. Salmons, Oct. 1977; 14 m, approx. 1.2 km offshore from Tataraimaka Historic Site Headland, S. of New Plymouth, Taranaki—1 juv. on undersurface of a stone, J. Nicholson, 15 Feb. 1978 and 1 juv., amongst fouling hydroids and algae on buoy rope, close to surface, R.C.W., 5 May 1978 and 6 juvs., 10.5–12 m on undersurfaces of stones, R.C.W., 4 July 1979; Island Bay, Wellington—1, N. M. Adams, 5 Oct. 1970 (NM); Lyall Bay, Wellington—1, C. Hale, Nov. 1952 (NM); 120 m, Cook Str. trawling grounds, 1 taken by M. V. "Thomas Currell," 27 Feb. 1964 (NM).

SOUTH ISLAND: Tasman Bay, Nelson—1, M. Young, Nov. 1934 (NM); 12 m, Golden Bay, Nelson, N. Z. Marine Dept. (NM); Port Underwood, Marlborough Sounds—3, R. Ponder, May 1962 (NM); Port Levy, Banks Pen.—1, W. R. B. Oliver, 1 April 1907 (Oliver Colln., NM); Te Onepoto, Lyttelton—1 (Suter Colln., NM); 8–12 m, off Middle Headland of German Bay Hill, Akaroa Hbr.—2 on sub-

stratum of mud and coarse, broken shell, M. C. Miller, 11 May 1962; Cape Three Points, Akaroa Hbr.—several under stones at low water, and crawling amongst algae below low water, M. C. Miller, 22 May 1962; Oputereinga Pt., Akaroa Hbr.—1 in a large pool amongst mussels, M. C. Miller, 20 May 1962; Yacht Club shore, Akaroa Hbr.—several on undersides of stones, M. C. Miller, 21 May 1962; 280 m off Cape Campbell—1, F. Abernethy, 14 Nov. 1962 (NM).

STEWART ISLAND: Golden Bay, Paterson Inlet—1, R. K. Dell, 29 Oct. 1948 (NM); 7 m, Big Glory Cove, Paterson Inlet, M. V. "Munida" Stn. 67–37. E. J. Batham, 15 Feb. 1967 (NM); 20 m off southern corner of Native Is., Paterson Inlet—1 and several spawn masses, R.C.W., 8 Feb. 1977.

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SEXUALITY WITH RESPECT TO SHELL LENGTH AND GROUP SIZE IN THE JAPANESE OYSTER *CRASSOSTREA GIGAS*

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ABSTRACT

The sex ratios for two populations (Big Beef Creek and Dabob Bay, Hood Canal, Washington) of the Japanese oyster, *Crassostrea gigas,* were determined with regard to shell length and group size (i.e., the number of oysters attached together as a group). In this study the proportion of males was found to decrease with increasing shell length as is the case for protandrous hermaphrodites and was also found to increase with increasing group size.

Also, an investigation was made of individual sexuality with respect to group size. In addition to singleton oysters (i.e., one oyster with no shell attachment to conspecifics), a wide variety of group sizes was found in both populations ranging from two to sixteen oysters per cluster. This study revealed that for doubleton oysters (i.e., two oysters per group) there was a highly significant incidence of male-female individuals as opposed to male-male and/or female-female individuals. This social relationship found among doubleton oysters is not apparent for oysters contained in group sizes greater than two individuals per cluster. The male-female relationship in doubletons is discussed with respect to its evolutionary significance.

Key words: female-male doubleton; group size; oyster; sex ratio; social behavior.

INTRODUCTION

The determination of sex in oysters has been and still is a controversial area of research (Coe, 1932a, 1932b, 1934, 1940; Haley, 1977). However, recent advances have been made for oysters (Haley, 1977) as well as for other marine invertebrates (Hoagland, 1978). In sex-labile marine invertebrates such as the oyster, it is thought that the duration of a sex phase (male or female) results from simple Mendelian segregation of multiple sex genes, whose action is additive (Bacci, 1965). Haley (1977) has provided evidence that the American oyster, *Crassostrea virginica* (Gmelin, 1791), displays at least three polymorphic sex loci. Each locus segregates two (male and female) alleles which have an additive effect in the individual. This type of model predicts that when male and female alleles of an individual are in equal number, the individual has the ability of being either sex and perhaps readily prone to sex reversal. It follows that when there are incidences of an unbalanced number of male or female alleles, the sexual phase of an individual would be dictated by the sex allele in greatest number (Bacci, 1965). However, there exists a large volume of literature which indicates that sex determination in sex-labile marine invertebrates is also influenced by the environment (Bacci, 1965; Hoagland, 1978; Reinboth, 1975) and the social behavior of individuals (Hoagland, 1978).

Sex determination in *Crassostrea* species has been influenced by such environmental conditions as the amputation of gill lamellae (Amemiya, 1936), crowding (Burkenroad, 1931, 1937); Coe, 1932a; Needler, 1932), nutrition (Coe, 1932a, 1934; Amemiya, 1925), geographical location (Katkansky & Sparks, 1966), and steroids (Mori et al., 1969). Indications of social interactions in sex determination of oysters can only be inferred from the available data (Burkenroad, 1931); however, studies involving social behavior between individuals of oyster populations have not been conducted. I have in this paper presented evidence of social influence on the sex determination of the Japanese oyster, *Crassostrea gigas* (Thunberg, 1795), as well as examined individual sexuality with respect to shell length and location to conspecifics.

In the Pacific Northwest of the United States of America, *C. gigas* spends the nonplanktonic period of its life as a sessile intertidal adult. Sexual reproduction occurs when gametes of the two sexes are broadcast into the surrounding water where fertilization occurs upon contact. Consequently, for suc-

cessful fertilization to occur it is necessary for these sedentary adults to be in close proximity as well as exhibit sexual heterogeneity between contiguous individuals. Planktonic larval development lasts from two to three weeks which provides ample opportunity for dispersion (Quayle, 1969). When the time of settlement approaches, the gregarious (Cole & Knight-Jones, 1949; Knight-Jones, 1952; Hidu, 1969) oyster larvae select suitable attachment sites (usually near conspecifics) where they will settle, metamorphose, and grow into adults.

Due to the gregarious nature of the planktonic larvae during settlement, this species consists of dense populations. The physical structure of these populations can be partitioned into different group sizes. The group size will be defined as the number of oysters which are attached together by shell fusion and/or attached to the same substratum. A particular group or cluster of oysters may develop by: (1) new recruits settling on an adult; and/or, (2) two or more recruits settling in juxtaposition and fusing their shells together as they grow older. An oyster that is not physically attached to another individual can be denoted a singleton. Two oysters whose shells are fused together can be considered a doubleton (or given a group size of two); three oysters can be considered a tripleton, and so on.

MATERIALS AND METHODS

Oyster samples were collected during the reproductive season (July through August) of 1975 and 1976 from Big Beef Creek and Dabob Bay, Hood Canal, Washington. The sex and length of each animal within the various groups were determined. The shell length of an individual was measured from the umbo to its ventral valve edge. The sex of an individual was determined by microscopic examination to be either male (m), female (f), or undetermined (ud), depending on whether sperm, eggs or no gametes, respectively, were found in the gonad. The population sex ratio (i.e., males divided by the total number of males and females) has been recorded for various shell lengths. Also, the observed and expected number of groups with particular sex ratios were determined. The expected number of groups was computed from the expected group frequency, which is obtained from the expression $[p + (1 - p)]^n$, where p is the frequency of males in the sample, $(1 - p)$ is the frequency of females, and n is the group size.

RESULTS

Sex Ratio, Shell Length and Group Size

The data from this study have been tabulated with regard to group size and the corresponding shell length of the oysters within the populations (Tables 1 and 2). The proportion of males in the two populations (for consecutive years) declined with increasing shell length class. The data support the known pattern of sexuality in *Crassostrea* species: protandry (Amemiya, 1925, 1929; Burkenroad, 1931; Coe, 1934; Needler, 1932).

In addition to providing support for protandry in *Crassostrea gigas,* the data in Tables 1 and 2 record the proportion of males per group size. As an example, for group

TABLE 1. The relationship between oyster sexuality, shell length, and group size for *Crassostrea gigas* from the Big Beef Creek population, Hood Canal, Washington. The sex ratio (s/r) is recorded with respect to shell length and group size. The number of males (m), females (f), and undetermined sex types (ud) are recorded for each shell length and group size. n is the total number of individuals.

Shell length (cm)	Sex	1975 Group size							1976 Group size						
		1	2	3	4	5–9	n	s/r	1	2	3	4	5–16	n	s/r
1.60–3.99	m	0	0	0	1	2	3	1.000	3	6	1	6	20	36	1.000
	f	0	0	0	0	0	0		0	0	0	0	0	0	
	ud	0	0	1	0	9	10		12	7	14	22	46	101	
4.00–4.99	m	0	0	0	0	1	1	0.500	10	4	3	8	7	32	0.970
	f	0	0	0	0	1	1		0	0	1	0	0	1	
	ud	0	0	0	1	2	3		0	2	1	2	7	12	
5.00–5.99	m	1	0	3	1	3	8	0.800	4	7	4	4	4	23	0.920
	f	0	1	1	0	0	2		0	1	0	0	1	2	
	ud	0	0	0	0	0	0		0	0	1	1	0	2	

TABLE 1 (Continued).

Shell length (cm)	Sex	1975 Group size							1976 Group size						
		1	2	3	4	5–9	n	s/r	1	2	3	4	5–16	n	s/r
6.00–6.99	m	0	2	2	0	3	7	0.636	9	11	5	5	4	34	0.850
	f	0	1	2	1	0	4		3	2	0	1	0	6	
	ud	0	0	0	0	0	0		1	1	0	1	0	3	
7.00–7.99	m	0	6	2	0	0	8	0.727	14	11	6	2	0	33	0.750
	f	0	1	0	0	2	3		2	4	1	1	3	11	
	ud	0	0	0	0	0	0		1	1	0	0	0	2	
8.00–8.99	m	0	3	1	0	1	5	0.357	17	15	6	3	4	45	0.703
	f	0	4	2	1	2	9		7	8	0	2	2	19	
	ud	0	0	0	0	0	0		1	0	1	0	0	2	
9.00–9.99	m	0	8	1	0	1	10	0.417	11	17	4	8	4	44	0.657
	f	2	9	2	0	1	14		9	7	2	3	2	23	
	ud	0	0	0	0	0	0		2	1	0	0	0	3	
10.00–10.99	m	0	4	2	1	2	9	0.375	15	7	5	5	2	34	0.420
	f	2	9	1	1	2	15		21	14	4	3	5	47	
	ud	0	0	0	0	0	0		1	0	0	0	0	1	
11.00–11.99	m	0	5	1	0	1	7	0.304	10	7	0	5	1	23	0.411
	f	0	11	1	2	2	16		17	9	3	0	4	33	
	ud	0	0	0	0	0	0		2	0	1	0	0	3	
12.00–12.99	m	1	0	1	0	2	4	0.308	9	6	1	1	2	19	0.388
	f	1	4	3	1	0	9		19	6	0	2	3	30	
	ud	0	0	0	0	0	0		0	0	0	0	0	0	
13.00–13.99	m	0	0	0	0	0	0	0.000	10	7	0	0	2	19	0.475
	f	1	2	2	0	0	5		11	6	2	2	0	21	
	ud	0	0	0	0	0	0		1	0	0	1	0	2	
14.00–14.99	m	0	0	0	1	0	1	0.200	3	1	1	0	0	5	0.185
	f	1	1	0	1	1	4		13	3	1	3	2	22	
	uf	0	0	0	0	0	0		1	0	0	0	0	1	
15.00–15.99	m	0	0	0	0	0	0	0.000	3	3	1	2	1	10	0.400
	f	0	0	0	0	0	0		5	2	1	2	5	15	
	ud	0	0	0	0	0	0		0	1	0	1	0	2	
16.00–16.99	m	0	0	0	0	0	0	0.000	4	0	0	0	0	4	0.308
	f	0	0	0	0	0	0		6	2	0	0	1	9	
	ud	0	0	0	0	0	0		1	0	0	0	0	1	
17.00–17.99	m	0	0	0	0	0	0	0.000	1	0	1	0	1	3	0.300
	f	0	0	0	0	0	0		4	1	1	0	1	7	
	ud	0	0	0	0	0	0		0	0	0	0	0	0	
18.00–18.99	m	0	0	0	0	0	0	0.000	0	0	0	0	1	1	0.200
	f	0	0	0	0	0	0		4	0	0	0	0	4	
	ud	0	0	0	0	0	0		0	0	0	0	0	0	
19.00–19.99	m	0	0	0	0	0	0	0.000	1	0	0	0	0	1	0.500
	f	0	0	0	0	0	0		0	0	1	0	0	1	
	ud	0	0	0	0	0	0		0	0	0	0	0	0	
20.00----	m	0	0	0	0	0	0	0.000	1	0	0	0	0	1	0.071
	f	0	0	0	0	0	0		6	4	2	0	1	13	
	ud	0	0	0	0	0	0		1	0	0	0	0	1	
TOTALS:															
Males		2	28	13	4	16	63		125	102	38	49	53	367	
Females		7	43	14	7	11	82		127	69	19	19	30	264	
Undet.		0	1	0	1	11	13		24	13	18	28	53	136	
Total		9	72	27	12	38	158		276	184	75	96	136	767	
[m/(m+f)]		.22	.39	.48	.36	.59	.43		.49	.59	.67	.72	.64	.58	

TABLE 2. The relationship between oyster sexuality, shell length, and group size for *Crassostrea gigas* from the Dabob Bay population, Hood Canal, Washington. The sex ratio (s/r) is recorded with respect to shell length and group size. The number of males (m), females (f), and undetermined sex types (ud) are recorded for each shell length and group size.

Shell length (cm)	Sex	1975 Group size							1976 Group size						
		1	2	3	4	5–10	n	s/r	1	2	3	4	5–16	n	s/r
1.40–3.99	m	0	0	0	2	0	2	1.000	0	0	1	0	0	1	1.000
	f	0	0	0	0	0	0		0	0	0	0	0	0	
	ud	2	1	4	2	8	17		1	6	6	5	44	62	
4.00–4.99	m	2	6	1	0	1	10	0.526	1	2	1	1	1	6	0.857
	f	6	2	0	0	1	9		0	0	0	1	0	1	
	ud	3	1	3	2	4	13		0	1	0	1	12	14	
5.00–5.99	m	3	4	1	1	7	16	0.667	1	1	1	1	5	9	0.643
	f	4	2	1	0	1	8		1	0	2	0	2	5	
	ud	6	2	3	3	6	20		0	0	1	1	4	6	
6.00–6.99	m	2	7	7	0	2	18	0.563	5	1	3	3	6	18	0.667
	f	7	2	3	0	2	14		4	3	0	0	2	9	
	ud	1	0	3	0	2	6		0	1	2	0	5	8	
7.00–7.99	m	1	6	4	4	0	15	0.484	3	16	9	1	7	36	0.571
	f	6	7	2	0	1	16		13	7	2	0	5	27	
	ud	1	1	0	0	0	2		0	2	0	0	4	6	
8.00–8.99	m	1	7	3	2	0	13	0.520	10	23	20	1	17	71	0.664
	f	7	3	2	0	0	12		10	15	5	0	6	36	
	ud	0	2	1	0	0	3		1	4	1	0	1	7	
9.00–9.99	m	3	3	5	2	1	14	0.400	10	28	10	5	15	58	0.576
	f	10	4	3	2	2	21		17	14	13	1	5	50	
	ud	0	1	0	0	1	2		0	2	4	0	0	6	
10.00–10.99	m	1	5	7	0	1	14	0.438	9	19	7	3	10	48	0.527
	f	8	6	3	1	0	18		8	18	8	4	5	43	
	ud	1	0	0	0	0	1		1	0	0	1	2	4	
11.00–11.99	m	9	4	2	2	0	17	0.386	2	12	8	4	12	38	0.447
	f	15	6	4	1	1	27		6	21	7	3	10	47	
	ud	2	1	0	0	0	3		1	1	1	1	2	6	
12.00–12.99	m	3	3	6	0	0	12	0.375	0	7	4	4	7	22	0.647
	f	9	5	3	3	0	20		0	8	0	0	4	12	
	ud	3	0	1	0	0	4		1	0	1	1	0	3	
13.00–13.99	m	4	2	0	0	2	8	0.276	3	1	4	0	8	16	0.500
	f	3	8	7	0	3	21		0	7	2	2	5	16	
	ud	1	0	0	0	0	1		2	1	0	1	2	4	
14.00–14.99	m	1	2	2	0	0	5	0.417	1	2	2	0	6	11	0.579
	f	1	6	0	0	0	7		2	2	2	1	1	8	
	ud	1	0	0	0	0	1		0	0	1	0	0	1	
15.00–15.99	m	2	0	0	0	0	2	0.400	3	5	0	0	3	11	0.688
	f	0	2	0	1	0	3		0	1	1	0	3	5	
	ud	1	0	0	0	0	1		0	0	0	0	1	1	
16.00–16.99	m	1	0	0	0	0	1	0.500	0	0	0	0	0	0	0.000
	f	0	1	0	0	0	1		1	1	1	1	2	6	
	ud	0	0	0	0	0	0		0	0	0	0	0	0	
17.00–17.99	m	0	0	0	0	0	0	0.000	0	1	1	0	2	4	0.571
	f	0	0	0	0	1	1		1	1	0	0	1	3	
	ud	1	0	0	0	0	1		0	1	0	0	0	1	
18.00–18.99	m	0	0	0	0	0	0	0.000	0	0	0	0	1	1	0.333
	f	0	0	0	0	0	0		2	0	0	0	0	2	
	ud	0	0	0	0	0	0		0	0	0	0	0	0	

TABLE 2 (Continued).

Shell length (cm)	Sex	1975 Group size 1	2	3	4	5–10	n	s/r	1976 Group size 1	2	3	4	5–16	n	s/r
19.00–19.99	m	0	0	0	0	0	0	0.000	0	1	0	0	1	2	0.400
	f	0	0	0	0	0	0		0	1	0	0	2	3	
	ud	0	0	0	0	0	0		0	0	0	0	0	0	
20.00----	m	0	0	0	0	0	0	0.000	2	0	0	0	2	4	0.250
	f	0	0	0	0	0	0		2	1	1	1	7	12	
	ud	0	0	0	0	0	0		0	0	0	0	0	0	
TOTALS:															
Males		33	49	38	13	14	147		50	119	71	23	103	366	
Females		76	54	28	8	12	178		67	100	44	14	60	285	
Undet.		23	9	15	7	21	75		5	19	17	11	77	129	
Total		132	112	81	28	37	400		122	238	132	48	240	780	
[m/(m+f)]		.30	.47	.57	.62	.54	.45		.43	.54	.52	.62	.63	.56	

sizes of one individual the population sex ratio is below 0.50, indicating a greater incidence of female oysters. But, with increasing group size, the population sex ratio rises above 0.50, indicating an increase in male individuals. Keeping this in mind as well as the decrease in the proportion of males with increasing shell length, it appears that large singleton oysters are more often female than male and that clusters of oysters usually consist of large females with many small male individuals.

Tables 1 and 2 also give sex ratios in each of the two populations for consecutive years. The proportion of males was less in 1975 for both populations than it was in 1976 (for the same populations). Three possibilities can account for these annual differences in sex ratio. They are: (1) new recruitment into the population in 1975, which would reflect an incease in the number of males for 1976 according to the protandry theory; or, (2) delayed sex change from male to female in 1976 perhaps due to unfavorable environmental conditions (i.e., a depressed food resource); or, (3) early sex change from male to female in 1975, due to favorable environmental conditions. The data from Tables 1 and 2 favor the first alternative because of the large number of oysters from the smaller shell length found in 1976 (cf. Big Beef Creek) as compared to 1975.

Sexuality and Group Size

The sexuality of oysters within the various group sizes (i.e., singleton, doubleton, triplet and quadruplet groups) were studied (Table 3). Samples of these group sizes were again collected from the Big Beef Creek and Dabob Bay populations in 1975 and 1976. In this study the observed number of males and females per group was compared with the expected group frequency assuming a random distribution of both sexes. Of the four group sizes examined only the doubleton group size (i.e., two oysters per group) exhibited a significant deviation between observed data and expected values (Table 4). The doubleton group size consistently showed a greater abundance of the male-female type than expected for a random distribution.

Group Size Distribution

The distribution of the various group sizes was examined by random quadrat sampling of the big Beef Creek and Dabob Bay population in 1976 (Table 4). The number of oysters per group ranged from one to sixteen. The observed number of groups along with the expected number of groups (assuming a geometric distribution) has been tabulated as well as the observed and cumulative frequency of the groups for each population.

These data indicate that the observed group size frequencies between the two populations coincide well with each other. Also, the observed group size frequencies clearly indicate that the number of groups decreases with increasing group size. Singletons occurred in the greatest number. Assuming a geometric distribution for the number of various groups expected within a population, it

TABLE 3. Four different group sizes of the Japanese oyster *Crassostrea gigas* were studied with respect to the observed and expected (i.e., based on group frequency) number of males (m) and females (f) per group. The chi-squared values for goodness-of-fit of observed to expected values, degrees of freedom, and probability of occurrence are listed. Where p is the proportion [males/(males + females)] of males in the population and (1 − p) is the proportion of females. n is the number of groups sampled.

SINGLETON GROUP	m		f	
Group frequency	p		(1−p)	
Year sampled	1975		1976	
Population	BBC	DB	BBC	DB
Chi-squared	2.778	16.963	0.016	2.470
df	1	1	1	1
Probability	>.050	<.001	>.800	>.100
n	9	109	252	117

DOUBLETON GROUP	mm	mf	ff	
Group frequency	p^2	$2p(1-p)$	$(1-p)^2$	
Year sampled	1975		1976	
Population	BBC	DB	BBC	DB
Chi-squared	14.535	29.666	20.486	37.068
df	1	1	1	1
Probability	<.001	<.001	<.001	<.001
n	44	57	79	117

TRIPLET GROUP	mmm	mmf	mff	fff
Group frequency	p^3	$3p^2(1-p)$	$3p(1-p)^2$	$(1-p)^3$
Year sampled	1975		1976	
Population	BBC	DB	BBC	DB
Chi-squared	3.632	4.614	4.588	1.474
df	2	2	1	2
Probability	>.100	>.050	>.020	>.300
n	10	19	14	32

QUADRUPLET GROUP	mmmm	mmmf	mmff	mfff	ffff
Group frequency	p^4	$4p^3(1-p)$	$6p^2(1-p)^2$	$4p(1-p)^3$	$(1-p)^4$
Year sampled			1976		
Population		BBC		DB	
Chi-squared		1.020		0.113	
df		1		1	
Probability		>.300		>.700	
n		11		5	

would appear that a deficiency of doubletons exists for both populations. This deficiency appears to be offset by an excess in group sizes of greater than two oysters per cluster. The deviation of the observed data from the expected geometric distribution is statistically significant for both populations.

DISCUSSION

As in other sessile marine invertebrates which have a free-swimming larval stage in their life histories (Scheltema, 1971), *Crassostrea* oyster larvae terminate their planktonic period by testing and finally choosing an attachment site on which to metamorphose and commence a secondary life. The location or proximity of the attachment site to conspecifics would appear to be very important for the propagation of oyster species. Evidence for gregarious behavior in planktonic oyster larvae during settlement is documented for *Ostrea* species (Cole & Knight-Jones, 1949; Knight-Jones, 1952) as well as *Crassostrea* species (Hidu, 1969). This type of behavior between oyster larvae, and between larvae and adults would most certainly infer a communication system (e.g., via chemotactic secretion) (Galtsoff, 1930; Mackie & Grant, 1974; Crisp, 1974). The evolution of a communication system as exhibited

TABLE 4. The distribution of oysters per group size in 1976 is tabulated for two oyster populations in Hood Canal. The observed number of oyster/group was obtained by random sampling of each population: four one-quarter square meter quadrats. The expected numbers were obtained by assuming a geometric distribution with means of 0.588 and 0.561 for the Big Beef Creek and Dabob Bay population, respectively. The chi-square values for goodness-to-fit of observed to expected values, degrees of freedom, and probability of occurrence are given. Also, the cumulative frequencies of observed groups are recorded.

	Big Beef Creek				Dabob Bay			
	Number of groups		Frequency		Number of groups		Frequency	
Oyster/Group	Obs.	Exp.	Obs.	Cumul.	Obs.	Exp.	Obs.	Cumul.
1	114	114.0	0.588	0.588	88	88.0	0.561	0.561
2	30	47.0	0.155	0.742	25	38.7	0.159	0.720
3	18	19.4	0.093	0.835	16	17.0	0.102	0.822
4	17	8.0	0.088	0.923	6	7.4	0.038	0.860
5–16	15	5.6	0.077	1.000	22	5.9	0.140	1.000
TOTAL	194	194.0			157	157.0		
Chi-square	32.117				49.000			
df	4				4			
Probability	<.001				<.001			

by such gregarious behavior and the synchronized release of gametes by males and females (Galtsoff, 1938a, 1938b; 1940) facilitates successful reproduction in oyster populations. The study of group sizes with regard to the individual sexualiy of oysters within the groups provides additional evidence (i.e., the high incidence of heterosexual doubletons) in support of a communication system.

The sexuality of contiguous individuals prior to reproduction could be influenced if these individuals were of dissimilar size or age. For example, the male-female doubleton phenomenon (Table 3) described in this study could result from a sex or age dominance relationship as long as the appropriate sex alleles are present. If the opposite sex alleles are not present in the two individuals of the doubleton, then incidences should occur where the same sex is represented in these doubletons regardless of dissimilar shell size or age. Consequently, it is possible that females could sequester (via chemical secretions) the partner of the doubleton into becoming a male for a given reproductive cycle. Previous work with steroids on *C. gigas* indicate that estrogen greatly facilitates ovary and testes respiration while testosterone does not activate respiration in either gonad type (Mori, 1968). Therefore, estrogen (or some derivative), when secreted by the female, may be responsible for stimulating gonad development in other individuals of a group.

Other evidence for the existence of a communication system within doubletons surfaced when the observed group-size distribution was compared with that expected from a geometrical distribution (Table 4). A deficiency of doubletons and an excess of group sizes greater than two oysters per group in the group-size distribution suggests that the two-unit (doubleton) group-size is a short-lived phenomenon. That is, the male-female relationship established through communication may be responsible for attracting planktonic oyster larvae to settle on the doubleton and thereby generating larger group-sizes.

When populations of *C. gigas* are partitioned according to their permanent attachment to other conspecifics, an interesting similarity appears between this species and the marine mesogastropod *Crepidula fornicata*. That is, they both form multi-individual stacks; however, with respect to getting the sexes together there is an important difference between the two species. *C. fornicata* is mobile as an adult young male while *C. gigas* is sedentary. Other similarities are also evident. Both species are gregarious, display a planktonic larval stage of development, are sex-labile, are protandrous hermaphrodites, and have environmentally- and socially-influenced sex determination and hence sex ratios. These characteristics appear to be typical of mollusc species which disperse as larvae, are largely sedentary as adults and have patchy substrates over time and space (Hoagland, 1978).

When fertility increases more rapidly with age or size in one sex than in the other, it

would be to the individual's advantage (i.e., an increase in fitness) to assume that sex last (Ghiselin, 1969; Leigh et al., 1976). This may be the case for some oyster genera (e.g., *Crassostrea, Saccostrea*) where the evidence supports protandry, but, some other relationship between sex and age exists in *Ostrea* where species display rhythmic sexual cycles. Usually when age- (or size-) specific sex ratios are investigated in protandrous hermaphrodites, there is often a fairly sharp change in the sex ratio at a particular age (Warner, 1975). However, this sharp change does not appear in species where environmental and/or social influences in sex determination is evident (e.g., *Crepidula fornicata*; Hoagland, 1978; *Crassostrea gigas*, Tables 1 and 2). In fact evidence has been presented where sex reversal in *C. gigas* and *C. virginica* has occurred in the reverse direction from female to male (Amemiya, 1929; Burkenroad, 1937; Needler, 1942), while *Ostrea* species exhibit a rhythmic sex change cycle (Orton, 1927; Hopkins, 1937). However, it should be pointed out that these incidences occur after the initial sex reversal from male to female has already been completed. Evidently, after age-specific sex determination has been invoked, there is a time in the oyster's life when communicating individuals can influence sex determination (i.e., at least in doubletons).

In conclusion, it appears that the Japanese oyster is like other *Crassostrea* species with respect to individual sexuality. That is, *C. gigas* exhibits a protandrous hermaphroditic pattern of sexuality by change in sex types (i.e., from male to female) with increasing shell length (or age) and increasing group size. Finally, it would appear that sex determination in this oyster is influenced to some extent by a social relationship between some contiguous individuals.

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BIOLOGY OF THE NORTHEASTERN PACIFIC TURRIDAE. I. *OPHIODERMELLA*

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ABSTRACT

Behavior, environment, and reproduction of *Ophiodermella inermis* and *O. cancellata* were studied. *Ophiodermella inermis* was found to be a specialist vermivore upon the polychaete *Owenia fusiformis*. It lives in intertidal to shallow subtidal habitats characterized by the nearby presence of dense *O. fusiformis* beds. Predation by *O. inermis* may locally affect the *Owenia* populations, and the polychaete assemblage in general, as it can eat from 8–16% of the standing crop of this dominant polychaete. *Ophiodermella inermis* shows little capability for distance chemoreception, and finds prey by moving up-current until it encounters a worm. Predation on *O. inermis* by *Cancer gracilis* and *C. productus,* which may limit recruitment, is significant. The snail has a size refuge from *C. gracilis* but not *C. productus*. In the intertidal area where both crabs are common, recruitment is very rare and adult mortality is high, resulting in a declining population. Growth is rapid when the animals are small and effectively ceases when they reach 31 mm in length. Rapid juvenile growth may be an adaptation to heavy juvenile mortality. *Ophiodermella inermis* reproduces from October to July, with a peak of egg capsule deposition in February. Each female deposits from one to nine egg capsules per night and each capsule contains several hundred eggs. Encapsular development time is about 50 days. There are no nurse eggs, and all eggs develop to leave the capsule as planktotrophic veligers, which develop for at least five more weeks. Settlement was not observed.

Ophiodermella cancellata is only found subtidally in silty or shell fragment habitats. It is also a specialist predator upon an oweniid polychaete, *Myriochele oculata*. In the subtidal habitat examined, recruitment is temporally patchy. Adult mortality is continuous, relatively few snails exceeding 12.0 mm long. Alternative hypotheses explaining this mortality are proposed. This species appears to mate in summer and settle in winter, but capsule deposition was not observed.

Both species occur in habitats characterized by stable sediment particle distributions. Polychaete assemblages may change drastically and turrid predation may be a causative agent for these changes in the subtidal habitats.

Environmental stress effects are unimportant subtidally, but drastically affect the intertidal population of *O. inermis*, causing behavioral changes and some mortality.

Key words: *Ophiodermella*; Turridae; ecology; diet; habitat; subtidal; vermivores.

INTRODUCTION

Toxoglossan gastropods are often important components of tropical ecosystems, and the biology of *Conus*, in particular, is reasonably well known (Kohn, 1959, 1967, 1968; Kohn & Nybakken, 1975; Leviten, 1976, 1978; Nybakken, 1978). In many tropical ecosystems the most obvious toxoglossans are in the families Conidae and Terebridae, and members of a third family, the Turridae, are less evident. In colder water, boreal, and deep-sea ecosystems, however, the Turridae are the only toxoglossan family represented and many turrids are important components of the fauna (Hartman, 1955; Jones, 1950; Parker, 1964; Wade, 1972; Rex, 1976). While the morphology of a few turrids has been examined (Franc, 1952; Robinson, 1960; Smith, 1967a, 1967b; Sheridan et al., 1973; Shimek, 1975), the immense size of the group has precluded an adequate understanding of the relationships within it (Shimek & Kohn, 1981). Ecological information is lacking, with few published observations on feeding, habitats, or reproduction (Pearce, 1966).

In some habitats of the Puget Sound region of Wasington, turrids are relatively abundant. The most common turrids in these areas are generally members of the genus *Ophiodermella*. I examined turrid populations to determine habitat, dietary requirements, and aspects of predatory and reproductive behavior. Several major questions were addressed: 1) What is the relationship of diet to the potential dietary resources present? 2)

Are these animals dietary specialists or generalists? 3) What is the effect of these predators upon their prey populations? 4) Are there any peculiar traits that limit their choices of habitats or prey? 5) Is there any obvious relationship between reproductive biology and distribution? In addition to attempting to answer these specific questions, I made observations of natural history attributes to try to make some generalizations on turrid biology.

MATERIALS AND METHODS

Two species of *Ophiodermella* are found in the region: the smaller, *O. cancellata* (Carpenter, 1864) ranges from southern Alaska to California (Grant & Gale, 1931); the larger can be referred to as *O. inermis incisa* (Carpenter, 1864), type-locality Neah Bay, Washington, but for this study I will refer to it as *O. inermis*. There is some doubt whether *O. incisa* is a separate species (McLean, personal communication). *Ophiodermella inermis* (Hinds, 1843) ranges from southern California to the Pacific Northwest (Grant & Gale, 1931; Cernohorsky, 1975). (Fig. 1).

Study sites

Ophiodermella inermis was studied most intensively at an intertidal location on the east shore of Port Washington Narrows (47° 32′ 39″ N, 122° 39′ 15″ W) and in a shallow subtidal location near Windy Point, Dyes Inlet (47° 37′ 25″ N, 122° 40′ 30″ W). Additional *O. inermis* animals were collected at Alki Point in Seattle (47° 39′ 48″ N, 122° 26′ 06″ W) (Fig. 2).

The Port Washington Narrows (PWN) study site is oriented north-south, is about 180 m long and averages 20 m wide, although it is almost 40 m wide at the widest point. This site was sampled in six areas: high, +0.5 m; middle, 0.0 m; and low, −0.5 m to −0.8 m; in both sand and cobble areas. All tidal heights are in respect to datum, 0.0 m, defined as mean lowest low water. The sampling areas were chosen to cover all habitats where turrids were seen in preliminary observations. The beach was bounded at both ends by rocky, reef-like areas and subtidally by an area of high currents and moving "sand dunes" interspersed with areas of large shell fragments. Repeated preliminary observations in the rocky and subtidal areas indicated that no turrids are found outside the beach area, and therefore the turrid population was treated as a closed population. The beach itself could be divided into sandy or cobble areas. The cobble area, in the center of the beach and bounded on each side by sand, is 60–80 m long and consists of stones 0.5–2.0 m in diameter embedded in sand or gravel substrate.

The Windy Point (WP) area is completely subtidal, from −1.5 m to −9.0 m below MLLW and is bounded on the upper edge by a dense bed of sand dollars, *Dendraster excentricus* (Eschscholtz, 1831), and on the lower edge by a bed of sea pens, *Ptilosarcus gurneyi* (Gray, 1860). No turrids were found among the sand dollars and only an occasional individual was seen in the sea pen bed. The site was not bounded laterally, and appeared to extend over 2 km with no noticeable change. Four habitats were sampled: a gently sloping 20–30 m wide upper area (subarea upper bench) from −1.5 m to −3.0 m; a 10 m wide slope from −3.0 m to −6.0 m (subareas upper and lower slope) and a gently sloping 50 m wide lower area from −6.0 m to −9.0 m (subarea lower bench). The subareas or habitats were determined by depth and steepness of slope. The upper and lower benches are virtually level. The steeply sloping area was divided by depth into upper and lower halves based upon differences in algal cover. The upper bench and slope areas were covered in summer with a thick, 1.0 to 1.5 m deep at high water, mat of ulvoid algae, mostly *Enteromorpha*. The algal cover on the lower slope and bench consisted of scattered *Neoagardhiella baileyi* (Kütz.) Wynne & Taylor, 1973, *Anhfeltia concinna* J. Agardh, 1847, and *Desmarestia ligulata* (Lightfoot, 1777).

Ophiodermella cancellata was studied most intensively subtidally off the University of Washington Friday Harbor Laboratories dock, San Juan Island, Washington (48° 32′ 38″ N, 123° 00′ 50″ W) (Fig. 2). This study site (FHL) was the most topographically diverse of the major study sites containing at least five visually distinct habitats: areas of rock, wood chips, shell fragments, and upper and lower areas of silty mud.

This site was located in Friday Harbor Bay at depths of −10 m to −25 m below MLLW, and consisted of two permanent 100 m transect lines and the nearby substrate, up to 100 m from the lines. No discontinuities limited the site except at the upper edge where the boundary was the lower edge of an eelgrass, *Zostera marina* L., 1753, bed. Prelimi-

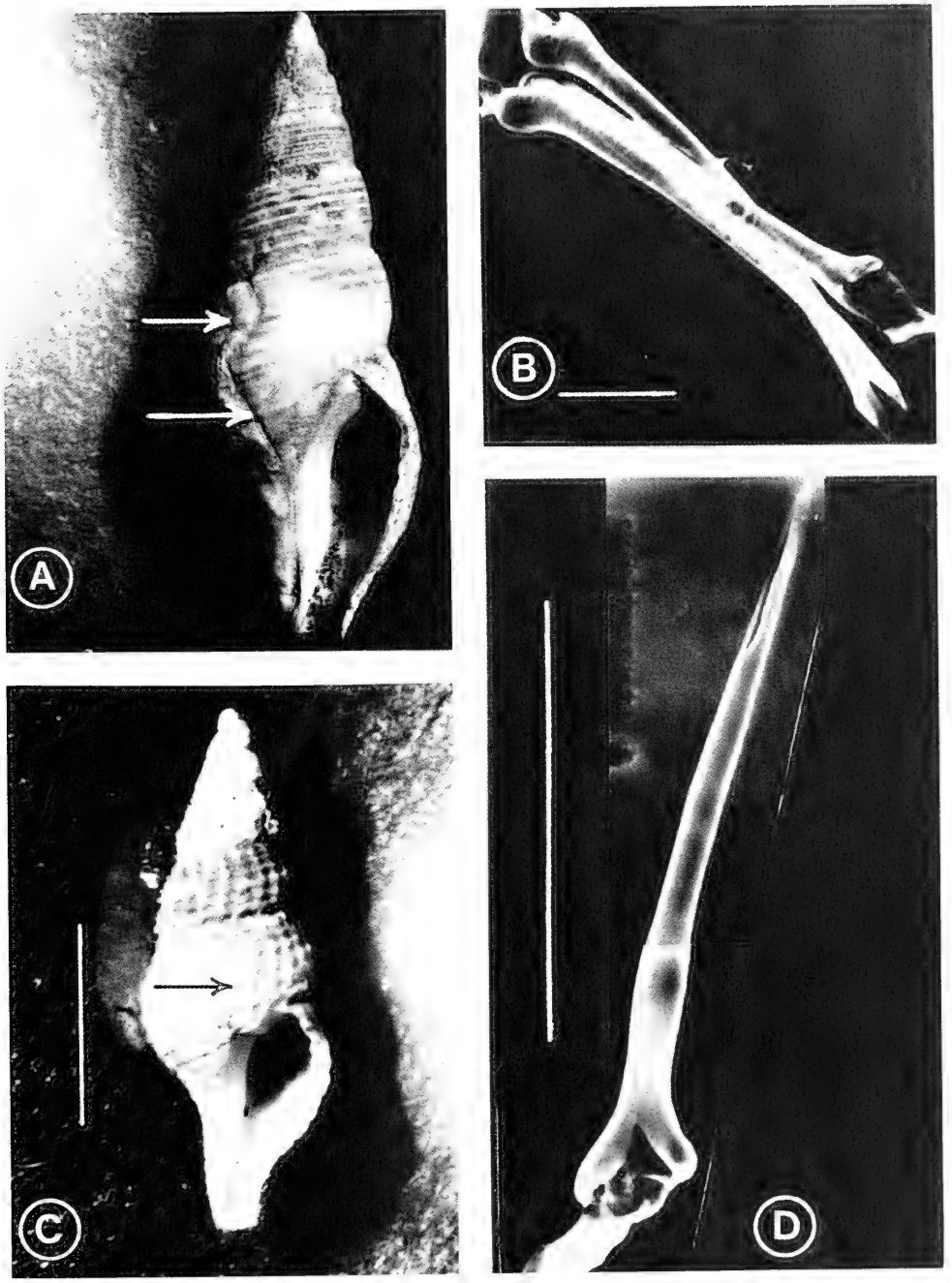

FIG. 1. A. *Ophiodermella inermis*. B. Radular teeth of *O. inermis*. C. *O. cancellata*. D. Radular tooth of *O. cancellata*. Scale bars in A and C are 5 mm; in B and D, 100 μm. Arrows indicate healed fractures.

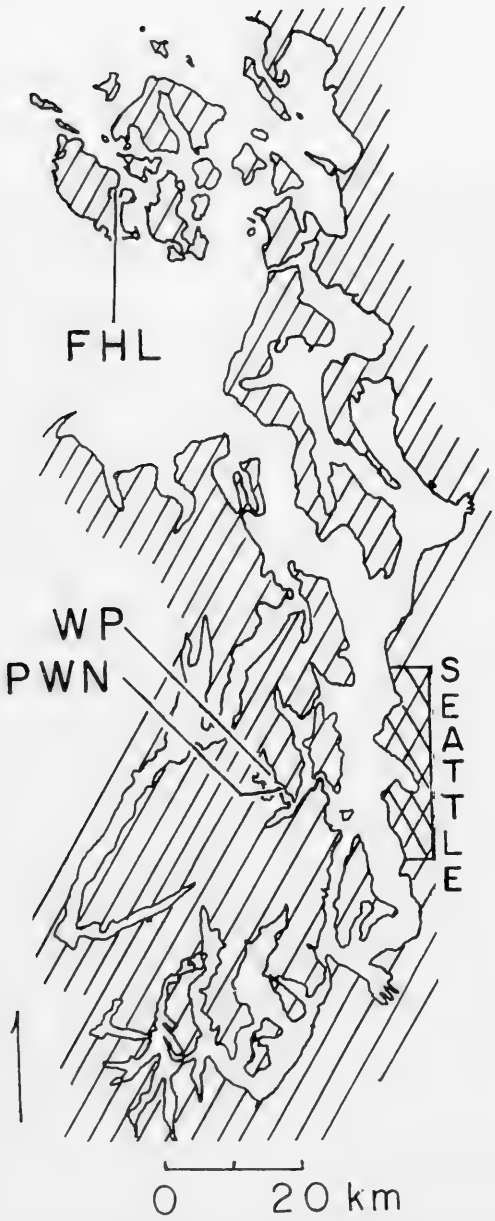

FIG. 2. Major study sites in Washington state. FHL = Friday Harbor Laboratories, San Juan Id.; PWN = Port Washington Narrows; WP = Windy Point.

nary observation established that no turrids were found in the eelgrass bed.

Preliminary observation also indicated no turrids, indeed virtually no macroscopic biota of any sort in the wood chip areas, consequently no sampling was done in those areas, otherwise the substrate appeared acceptable to turrids at least 100 m from the transects. No infaunal sampling was done in the rocky areas, characterized by large boulders and areas of bedrock, often several meters across, even though some turrids were seen in those areas. The tops of boulders and bedrock areas, the cracks in them, and the interstices between them were covered with silt, generally in very shallow layers. These pockets of sediment were too small to sample effectively.

The remaining habitats were all sampled quantitatively for infaunal polychaetes and turrid distribution. The upper (−10 m to −12 m) and the lower (−17 m to −20 m) silt areas, distinguished on the basis of depth related biotic factors such as diatom and algal cover, were otherwise similar topographically. These areas were not often confluent, being separated by shell fragment and rocky areas, but when they were they intergraded. The shell fragment area was similar to the silt areas, but with many more visible shell fragments. The shell fragment zones, usually near the rocky areas, could be found as far as 30 m from the rocks. No shell fragment areas were found above −13 m, and they seemed to be found only in areas of gentle slope.

Habitat analyses

At the three major study sites the physical and biological components of the major habitats were examined in detail. Two bimonthly replicate 0.018 m^2 cores were taken about 1 m apart to a depth of 10 cm in each habitat and fixed in 2–5% sea water formalin with Rose Bengal. Sediment analysis was performed by removing an aliquot of each sample, sieving it to remove all particles greater than 1.8 mm, which were then separated into size classes determined by standard Ø notation (Holme & McIntyre, 1971). The remainder of each aliquot was subsampled and two or three replicates were analyzed with a settling tube (Emery, 1938). A weighed subsample of the aliquot was sieved to remove the silt-clay fraction which was dried and weighed. Samples were analyzed graphically (Inman, 1952) and median particle size and sorting coefficient were computed.

The remainder of the sample was washed through a 0.5 mm sieve and the animals were collected and sorted by taxon. Gastropods and polychaetes were counted and identified to species when possible. Other taxa were

identified to class and counted but not detailed further. The two replicate samples were pooled for further biological analysis making the total area per sample 0.036 m^2.

Sediment parameters were tabulated and compared between the areas using the Wilcoxon Rank-Sum method (Hollander & Wolfe, 1973). Seasonal variability of the sediment parameters was insignificant, so no statistical comparison on the seasonal basis was done.

In situ measurements of PWN snail and habitat temperatures were taken with a YSI portable electric thermometer with a 3 mm diameter probe.

Polychaete assemblage abundances for each habitat were determined by quantitative infaunal sampling. The assemblages were compared between and within habitats on a seasonal basis using the "D" index (Whittaker, 1952; Whittaker & Fairbanks, 1958; Schoener, 1968; Pielou, 1974) to measure similarity and H″ (Kohn & Nybakken, 1975) to measure heterogeneity. The June and December polychaetes from each habitat were divided into prey and non-prey fractions based upon turrid fecal determination, dried, weighed, incinerated at 550°C, and reweighed.

Turrid distribution, collection and processing

At the three major sites periodic transect studies from November, 1973, until December, 1975, were used to determine turrid distributions, seasonal or other changes in those distributions, and to provide a reference for all quantitative infaunal samples. At the PWN sites, the transects were temporary because of human disturbance. Intersampling period deviation was minimized by placing the transects with reference to mapped landmarks. At the FHL site two 100 m transects were permanently emplaced. Habitat types and turrid positions relative to the transects were noted.

Turrids were collected, washed in sea water, placed individually into marked jars filled with filtered sea water, and maintained at 10°C for two to seven days. The animal was then removed from the jar, its length, width, and aperture length measured with calipers to the nearest 0.1 mm, and blotted dry. The shell was cleaned and an identifying code was applied with standard drawing ink which was covered with a layer of clear fingernail polish. After this layer had dried the animal was returned to fresh sea water at 10°C and observed to assure no noticeable effects of the measuring and marking procedure. An ink line was placed at the outer edge of the outer lip of the aperture to assess growth. This line was not covered with fingernail polish, as the solvent in the polish was lethal in some cases if applied near the aperture. The animal was then transferred to a "holding" aquarium where it was maintained in an artificial habitat similar to the normal one. All apparently healthy animals were returned to their habitat, although seldom to their point of capture, within two weeks. Measuring and marking mortality was less than one percent.

Recaptured marked snails allowed estimation of growth rates. Changes in total length were used to calculate growth rate. Shell shrinkage was caused by fracturing the outer lip and siphonal canal by crabs, and/or apical erosion. Body whorl height, as measured by aperture length, and total length were highly correlated; but as the aperture length is short, similar changes in length were relatively larger and more variable. Animals were assigned size classes on the basis of pregrowth size, and the mean growth rate in μm day^{-1} was calculated. For *O. inermis,* the WP population allowed a second determination of growth rate, as the size-frequency histogram of the population contained a number of distinct peaks, presumbly corresponding to yearly recruitment. This histogram was assumed to be the result of several overlapping normal distributions, and was separated into component distributions following the method given in Bliss (1967). A similar procedure was used to calculate growth rate for *O. cancellata* at FHL. The recapture rate of the WP population was so low that no independent verification of these assumptions could be made.

Mark-recapture data also allowed estimation of population size at the PWN area which was geographically bounded, and where the population was considered to be closed. Population size was estimated by Jolly's Stochastic Multiple-Recapture Method (Poole, 1974). These data were plotted and analyzed graphically. Similar analyses were not attempted at either the WP or FHL areas, as they were not bounded, and too few recaptures per unit time were made to make the population estimates meaningful.

At PWN seasonal snail distribution was determined by plotting snail position on maps of the areas. A 60 $\times$ 15 m scale rectangle was plotted on the same maps with the vertical midline being the boundary between the sand and cobble habitats, and the horizontal mid-

line being the −0.3 m contour line. These lines divided the center of the sampling area into four equal area rectangles, each 30 × 7.5 m, one each in the sand and cobble area from −0.8 m to −0.3 m, and −0.3 to +0.3 m. Seasons were defined as summer (May, June, July) and winter (November, December, January). The number of turrids plotted in each area was summed for each season, divided by the number of surveys per season, and tested using the log-likelihood ratio to determine any seasonal differences between substrates or heights.

Following measuring and marking, any particulate material remaining in the collecting jar was placed on a slide, dried, mounted in polyvinyl lactophenol (A. Kohn, personal communication), and examined. Identification of all fecal material was attempted. Feces consisted of mucus, radular teeth of the same animal, diatom frustules, and polychaete remains. Preliminary gut analysis by dissection

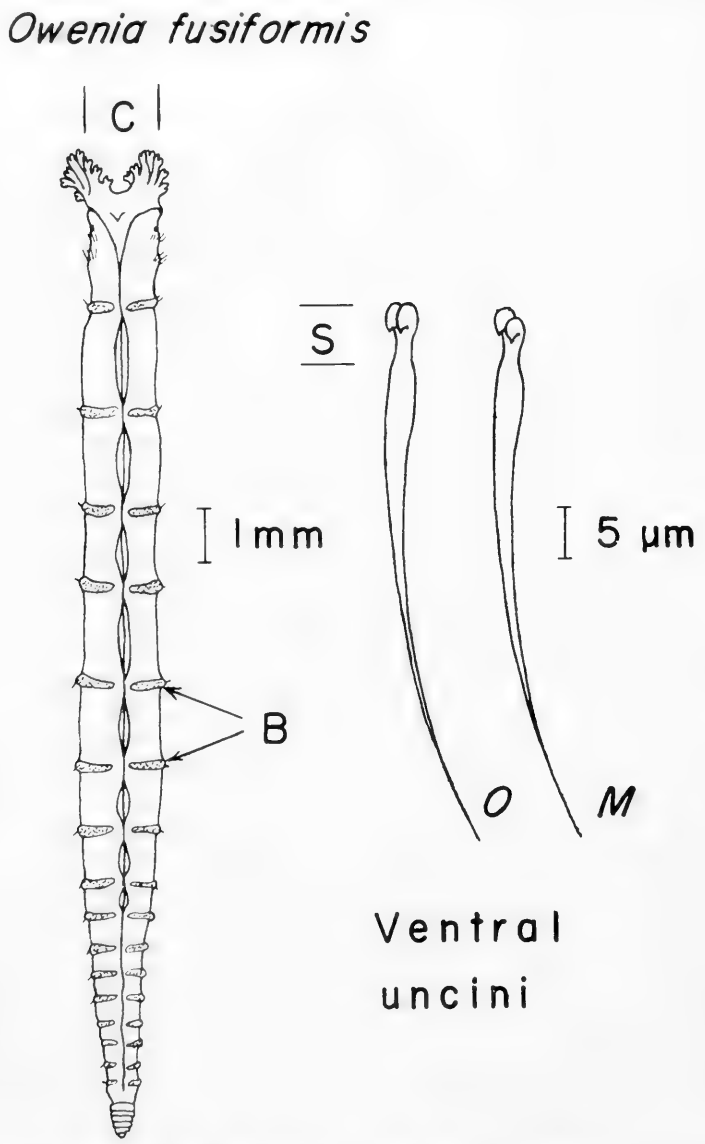

FIG. 3. *Owenia fusiformis,* ventral view, modified from Dales (1957); and ventral uncini from *Myriochele oculata, M*; and *O. fusiformis, O.* B = bands of uncini. C = collar width. S = shoulder length of an uncinus.

indicated that polychaetes swallowed whole were the only prey. Animals collected for these gut analyses were preserved immediately after capture in 70% ethanol and boiled for 10 minutes upon return to the laboratory. It is unlikely that any rapidly digested animals were undetected by these analyses. Thus, only polychaete remains consisting of setae, jaws, and occasional cuticular strips were accepted as indicators of feeding. These remains were identified by comparison with slides prepared of known polychaetes, and comparison with description and drawings of Berkeley & Berkeley (1952), Woodwick (1963), Blake (1966, 1971), Hartman (1969), Blake & Woodwick (1971), Foster (1971), Banse (1972), Banse & Hobson (1974), and Hobson & Banse (1981).

Some captured *O. inermis* were starved for one week, placed in a dish with their most common prey, *Owenia fusiformis* delle Chiaje, 1844, and the time from ingestion to defecation was noted.

Owenia fusiformis diameter at the top of the collar (Fig. 3) was measured for five large and five small worms. In addition the shoulder lengths of 10 uncini from each worm were measured. The uncini shoulder length correlated well with the collar diameter; $r^2 = 0.96$; (worm collar diameter (μm) = $-1963 + 396$ (uncinus shoulder length (μm))). To test whether different sized worms were being eaten by different *O. inermis* populations, fecal samples from each population were randomly chosen, examined, and uncini shoulder lengths were measured. Sample means were compared with a "t"-test.

Laboratory experiments

Ophiodermella inermis was tested in a "Y" choice chamber (Fig. 4) to determine its response to currents and ability to sense prey. In the response to prey experiments, 15–20 *Owenia fusiformis* were placed in a small cloth bag in a randomly chosen arm of the apparatus, with an empty bag in the other. Ten snails were placed at the base of the stem of the "Y," a current of 2.5 cm sec^{-1} was applied to both arms, and the experiment was run for at least eight hr. Similar experiments were run without bags or worms to test the response to current, and an additional set of unbaited experiments was run with a current of 5.0 cm sec^{-1} in one arm with no current in the other. The animals were scored as having made a choice when they were more than two centimeters into an arm at the completion of the run. The tube was cleaned between each set of snails by scrubbing to remove all traces of mucous trails. Results were compared with log-likelihood tests and cumulative binomial probabilities.

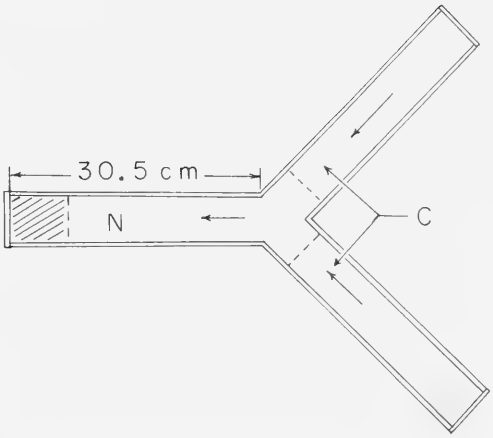

FIG. 4. "Y" choice chamber for chemo- and rheotaxis experiments. Arrows indicate the direction of water flow. N = No-Choice area. C = Choice areas. All arms were 30.5 cm long. Cross-hatched area is the starting position for the snails.

Animals from PWN were offered to two potential predators, *Cancer gracilis* Dana, 1852, and *C. productus* Randall, 1839, to determine if the crabs would eat the snails. Individual *O. inermis* were placed in an aquarium with an individual crab. After 24 hr the aquarium was examined to see if the crab had eaten the snail, or chipped the shell attempting to eat it. The crabs had been starved for one week prior to the experiments, and most of the crabs attempted to eat the snails, consequently no post-experimental verification of crab hunger was attempted.

Attempted unsuccessful crab predation can be assessed by counting the number of healed fractures (Vermeij et al., 1980). Randomly chosen *Ophiodermella inermis* from both PWN and WP areas were assessed for the number of healed fractures by the method of Vermeij et al. (1980). The total number of healed fractures (Fig. 1) was determined, and subdivided into those in the top 10 mm of the shell, measured from the apex to the suture line 10 mm from the apex, and those in the remainder of the shell. Only shells of the WP habitat size range, 15.0 mm to 30.6 mm, were

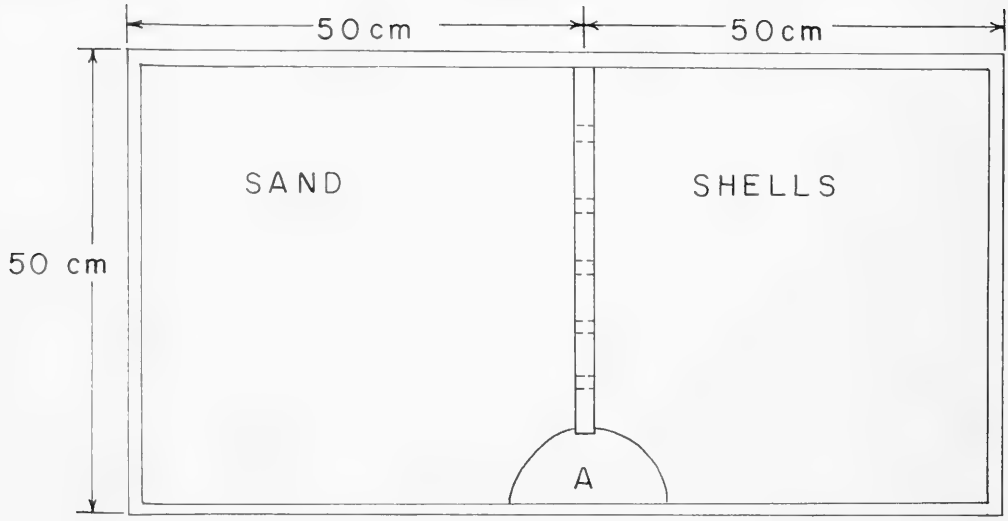

FIG. 5. Choice chamber for substrate choice experiments. A. Starting arena.

compared. The difference in the means of these samples were compared using a "t"-test.

A substrate choice chamber was constructed from an aquarium with a plexiglas partition extending from one side to 8 cm from the other side on the midline, thus dividing the aquarium into two equal area chambers. The partition had holes drilled in it to insure access from one chamber to the other. A starting arena cleared of sediment was maintained at the partition (Fig. 5). One chamber was filled to a depth of 2 cm with sand, sieved to insure particle size distribution from 0.250 mm to 0.500 mm. The other half had shell fragments sieved to insure particle size distribution in excess of 2.00 mm. Both sediment types had all noticeable biota removed. Turrids of one species were placed in the starting arena, and one week later they were collected and their positions noted. These data were analyzed using cumulative binomial probabilities, excluding all animals in the starting arena, on the walls, or partition.

Egg capsules were collected in the field or from containers that turrids were stored in. Capsule dimensions (Fig. 6), the egg number per capsule, and the egg diameter were measured. Developmental stages were determined for field collected capsules. The capsules were examined periodically and upon hatching, the veligers were maintained in filtered sea water containing either *Isochrysis* sp. or *Dunaliella* sp. Water in the cultures was changed periodically. The apparently mature veligers were given a variety of potential substrates to metamorphose upon.

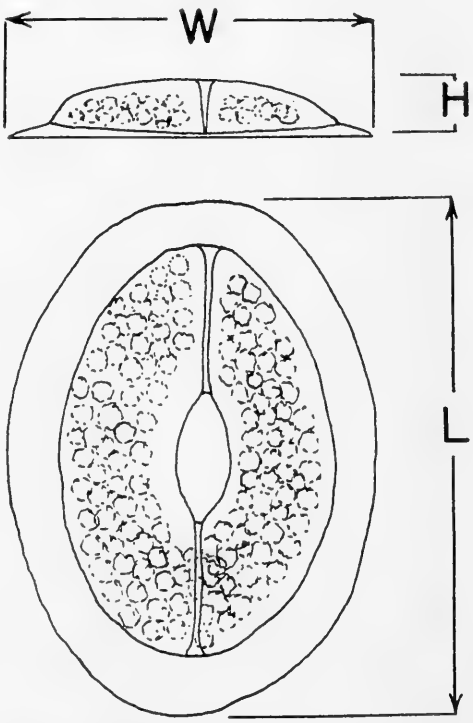

FIG. 6. Egg capsule of *Ophiodermella inermis* showing measurements taken. H = height. L = length. W = width. Dotted lines indicate embryos.

RESULTS

Habitat descriptions

Port Washington Narrows:

The PWN study area is on the east shore of Port Washington Narrows about 2 km N of Bremerton, Washington. Algal cover on the beach varied seasonally. In the summer, ulvoids in a single layer covered most of the beach up to +0.3 m. In the winter, algal cover was less than 5% in most areas. In the lowest intertidal areas, patches of *Gigartina papillata* (C. Agardh, 1821, and *Opuntiella californica* (Farlow, 1877) are found, and in the cobble areas are occasional patches of *Hedophyllum sessile* (C. Agardh, 1824). This study site is a narrow tidal channel characterized by periods of high currents, up to 7.5 km hr^{-1}, and thus the sediment is well aerated and no black, sulphide-smelling anoxic areas were encountered, except occasionally at a depth of nine to ten centimeters in the summer in the higher areas. Substrate temperatures are highest in summer (Fig. 7). During the winter, substrate temperatures approximated water temperatures and averaged about 9°C. No periods of freezing were encountered during the study.

Sediment physical parameters, median particle size and sorting coefficient were substantially different among the six PWN habitats. Compared with one another on a habitat by habitat basis (Tables 1, 2), the results indicate that the beach is patchy in both particle size distribution and sorting. The high sand areas contain some rocks, and all areas have holes dug by clammers, up to several hundred per tidal period, which are filled with unconsolidated sediments. Generally, the higher

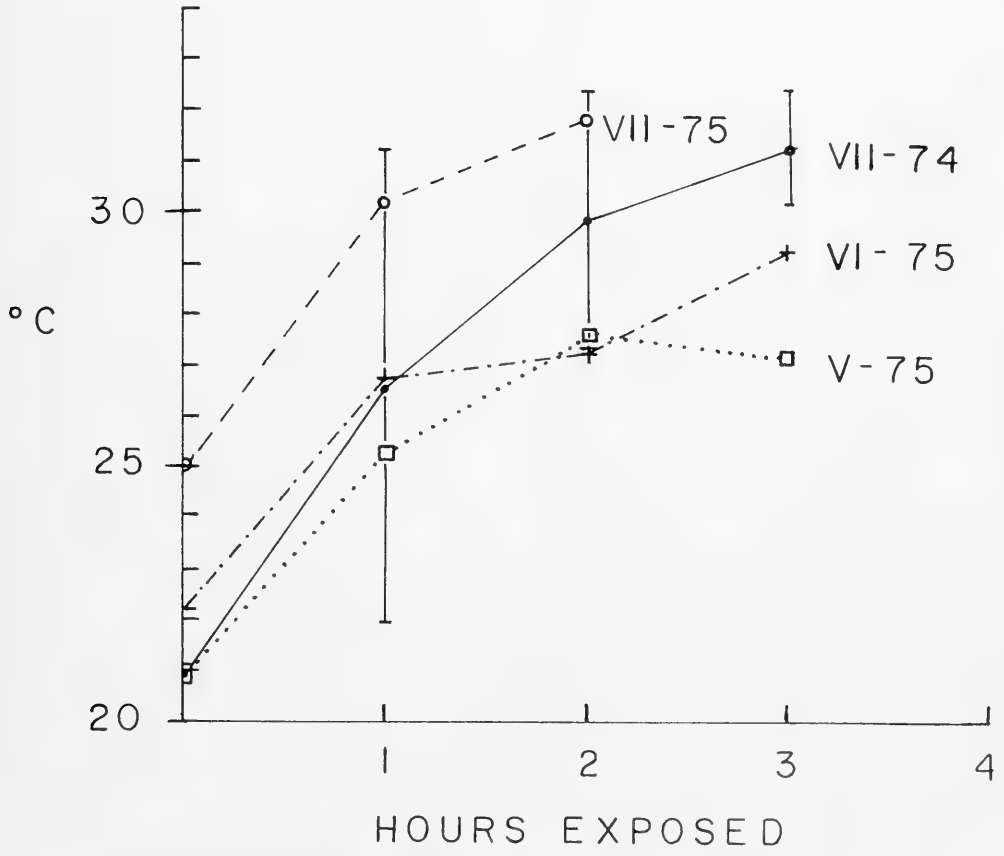

FIG. 7. Temperature of the exposed substrate at Port Washington Narrows. Values were taken on sunny days only in the months indicated and are the means of from two to five values. The range of values is shown for July, 1974 only. Similar ranges were seen for all months.

TABLE 1. Physical parameters—all habitats.

Habitat	Median particle size (mm)	Sorting coefficient	N
Port Washington Narrows (PWN)			
High cobble (HC)	2.11	3.79	10
Middle cobble (MC)	1.02	2.91	15
Low cobble (LC)	0.42	1.65	13
High sand (HS)	1.10	2.92	13
Middle sand (MS)	0.46	2.20	19
Low sand (LS)	0.43	2.49	15
Windy Point (WP)			
Upper bench (UB)	0.42	1.51	12
Upper slope (US)	0.29	1.54	11
Lower slope (LS)	0.36	1.45	11
Lower bench (LB)	0.40	1.31	12
Friday Harbor Laboratories (FHL)			
Upper mud (UM)	1.25	2.69	9
Shell fragments (SF)	1.10	2.75	12
Lower mud (LM)	0.71	2.79	10

TABLE 2. Comparison of differences in physical parameters. Tested with Wilcoxon Rank-Sum. O = not signifcant; + = significant at $\alpha = 0.05$; ++ = significant at $\alpha = 0.01$. Habitat abbreviations as in Table 1.

		Habitat													
		HC	MC	LC	HS	MS	LS	.	UB	US	LS	LB	.	UM	SF	LM	.
PWN	HC		+	++	++	++	++		++	++	++	++					
	MC	++		++	0	+	+		++	+	++	++					
	LC	++	0		++	+	+		0	0	0	0					
	HS	0	++			+	0		++	++	++	++					
	MS	++	0	++	++		0		++	++	+	++					
	LS	++	0	++	++	0			++	++	++	++					
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WP	UB	++	0	0	++	++	0			0	0	0		++	++	++	
	US	++	+	0	++	++	0		+		0	0		++	++	++	
	LS	++	0	0	++	++	+		0	0		0		++	++	++	
	LB	++	0	0	++	++	0		0	0	0			++	++	++	
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FHL	UM								++	++	++	++			0	0	
	SF								++	++	++	++		0		0	
	LM								++	++	++	++		0	+		
	.																

Sorting coefficient ($\sigma \phi$)

Median particle size (Md ϕ)

sediments are coarser and less sorted than the lower ones. Superimposed on this pattern is the tendency for the cobble area sediments to be coarser and less sorted than those at the corresponding tidal height in the sand areas, although these differences are not always significant.

The beach topography did not change seasonally. The area is well protected from wave action; most waves are caused by boat traffic in the adjacent channel. There is some fresh water runoff and erosion, primarily in the winter when channels up to five centimeters deep are dug several times each winter. Generally, these channels were filled in by tidal action within two days.

Seasonal polychaete trends are summarized on a habitat by habitat basis (Tables 3, 4). The sand and cobble areas have different polychaete assemblages, although overlap is high. In summer, the high cobble area has a very high density of *Pygospio elegans* Claparède, 1863 and a low diversity of organisms in general. The remainder of the summer PWN samples are all similar, $\bar{D} = 0.637$, reflecting the proportions of *Owenia fusiformis, Platynereis bicanaliculata* (Baird, 1863), *Malacoceros glutaeus* (Ehlers, 1897), *Axiothella rubrocincta* (Johnson, 1901), and capitellids. In winter, the assemblages become more distinct. The high cobble assemblage is still characterized by high densities (>25,000 m^{-2}) of *Pygospio elegans.* The high sand assemblage loses many of the rarer species and the *O. fusiformis* density drops from over 500 m^{-2} to less than 20 m^{-2}. The middle and lower areas remain similar, $\bar{D} = 0.620$, with *O. fusiformis, Axiothella rubrocincta,* and capitellids as the dominant organisms. The mean size of *O. fusiformis,* the principal turrid prey, peaks in late summer. A large component of small worms is added to the population in late summer-early autumn, coincident with the mortality of large individuals (Fig. 8), thus *O. fusiformis* appears to be an annual species. The summer prey biomass is concentrated in large individuals, while the winter populations contain many small worms.

Windy Point:

The Windy Point sediment physical parameters (Tables 1, 2) indicate that the WP sub-

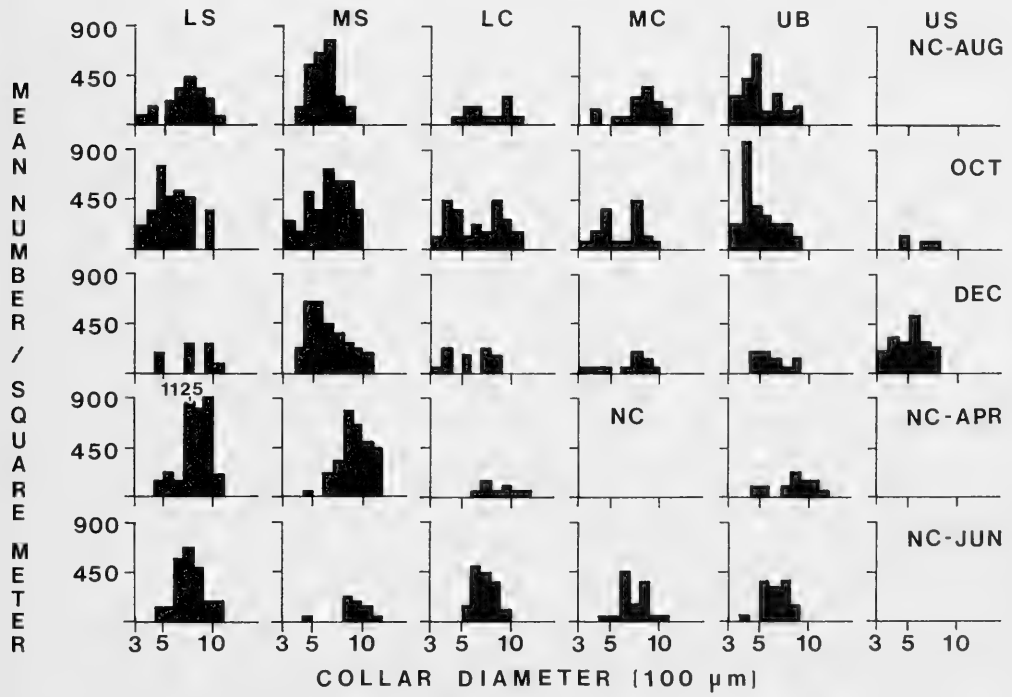

FIG. 8. Size frequency distributions of *Owenia fusiformis* in Puget Sound habitats. Port Washington Narrows habitats: LS = lower sand, MS = middle sand, LC = lower cobble, MC = middle cobble. Windy Point habitats: UB = upper bench, US = upper slope. NC = none collected.

TABLE 3. Habitat × habitat × season polychaete assemblage overlaps. Overlap index = "D" (Schoener, 1968). Habitat abbreviation as in Table 1.

		PWN						WP				FHL		
		HC	MC	LC	HS	MS	LS	UB	US	LS	LB	UM	SF	LM
PWN	HC	0.824	0.136	0.053	0.199	0.062	0.048	0.045	0.028	0.045	0.030			
	MC	0.156	0.718	0.553	0.551	0.645	0.523	0.456	0.319	0.297	0.283			
	LC	0.149	0.731	0.574	0.152	0.682	0.609	0.673	0.511	0.315	0.328			
	HS	0.194	0.686	0.589	0.361	0.283	0.145	0.164	0.122	0.084	0.107			
	MS	0.282	0.694	0.595	0.585	0.538	0.760	0.631	0.469	0.329	0.286			
	LS	0.114	0.668	0.585	0.648	0.586	0.754	0.673	0.511	0.315	0.328			
WP	UB	0.112	0.440	0.445	0.497	0.339	0.500	0.605	0.690	0.448	0.452	0.297	0.275	0.250
	US	0.043	0.155	0.164	0.267	0.183	0.185	0.512	0.507	0.518	0.522	0.274	0.258	0.288
	LS	0.087	0.245	0.204	0.290	0.294	0.271	0.584	0.673	0.483	0.655	0.281	0.241	0.332
	LB	0.059	0.300	0.207	0.288	0.284	0.304	0.571	0.557	0.711	0.487	0.341	0.299	0.383
FHL	UM							0.116	0.229	0.238	0.223	0.494	0.555	0.374
	SF							0.162	0.239	0.292	0.243	0.593	0.615	0.403
	LM							0.105	0.218	0.256	0.220	0.602	0.552	0.453

Summer

Winter

TABLE 4. PWN polychaete assemblage analysis; habitat abbreviations as in Table 1.

A. Numerical rank, top ten species. Habitat	HC		MC		LC		HS		MS		LS	
Season	S	W	S	W	S	W	S	W	S	W	S	W
Species												
Capitella capitata (Fabricius, 1780)	8	7	–	–	–	10	–	8	–	–	–	–
Mediomastus ambiseta (Hartman, 1947)	6	3	2	1	7	3	2	3	1	4	2	4
Notomastus tenuis (Moore, 1909)	3	2	6	9	3	4	9	–	2	10	–	6
Hemipodus borealis Johnson, 1901	2	5	–	–	–	–	10	5	5	–	–	–
Glycinde picta Berkeley, 1927	–	–	8	10	9	–	8	6	7	8	5	9
Axiothella rubrocincta (Johnson, 1901)	9	4	4	2	5	9	7	–	–	7	7	5
Nephthys caecoides Hartman, 1938	–	–	9	8	8	8	–	–	–	6	8	7
Platynereis bicanaliculata (Baird, 1863)	–	–	3	5	1	1	1	–	9	5	3	2
Armandia brevis (Moore, 1906)	–	–	–	7	–	6	–	–	10	2	–	3
Scoloplos pugettensis (Pettibone, 1957)	–	–	–	–	10	–	–	–	–	–	6	–
Owenia fusiformis delle Chiaje, 1844	4	10	1	6	2	2	4	7	3	1	1	1
Eteone longa (Fabricius, 1780)	5	8	10	–	6	–	3	4	6	9	–	–
Malacoceros glutaeus (Ehlers, 1897)	7	9	5	3	–	5	5	1	8	4	4	8
Pygospio elegans Claparède, 1863	1	1	7	4	–	–	4	2	4	–	9	–
Others	10	6	–	–	4	7	–	–	–	–	10	10
B. Assemblage data—all polychaetes.												
Mean number m^{-2}	13,667	28,612	2,472	3,269	2,806	2,833	1,222	872	2,668	8,397	2,500	2,437
H″	1.00	0.43	2.46	2.58	2.40	2.83	2.45	1.77	2.55	2.53	2.18	2.30
Mean ash free Dry weight (gm^{-2})												
Turrid prey spp.	0.7	<0.1	1.4	0.3	1.7	0.4	0.2	0.2	1.8	1.5	3.9	1.0
Non-prey spp.	18.7	3.6	7.2	5.5	9.6	10.4	7.4	0.6	2.3	6.9	3.9	19.4

TABLE 5. WP polychaete assemblage analysis; habitat abbreviations as in Table 1.

A. Numerical rank, top species. Habitat	UB		US		LS		LB	
Season	S	W	S	W	S	W	S	W
Species								
Capitella capitata (Fabricius, 1780)	–	10	3	–	6	–	–	–
Mediomastus ambiseta (Hartman, 1947)	3	4	8	7	2	7	2	5
Notomastus tenuis (Moore, 1909)	8	–	–	–	10	–	6	–
Tharyx multifilis Moore, 1909	–	–	5	4	7	1	8	1
Glycinde picta Berkeley, 1927	–	6	–	–	9	6	9	8
Lumbrineris spp. Blainville, 1828	–	–	6	3	–	3	3	2
Axiothella rubrocincta (Johnson, 1901)	7	3	9	–	–	8	–	10
Nephthys caecoides Hartman, 1938	–	5	–	6	–	–	–	–
N. ferruginea Hartman, 1940	9	–	4	9	4	–	5	9
Platynereis bicanaliculata (Baird, 1863)	4	2	7	5	5	4	4	3
Scoloplos pugettensis (Pettibone, 1957)	6	7	2	2	3	5	7	4
Owenia fusiformis delle Chiaje, 1844	2	1	–	1	–	–	–	–
Eteone longa (Fabricius, 1780)	10	8	–	–	–	10	–	–
Malacoceros glutaeus (Ehlers, 1897)	5	–	–	–	–	–	–	–
Polydora socialis (Schmarda, 1861)	1	9	1	8	1	2	1	7
Others	–	–	10	10	8	9	10	6
B. Assemblage data—all polychaetes.								
Mean number m^{-2}	3,611	1,722	2,750	3,519	2,472	2,630	2,792	1,750
H″	2.22	2.52	2.39	2.84	2.83	2.59	2.58	3.02
Mean ash free Dry weight (g m^{-2})								
Turrid prey spp.*	4.0	0.8	3.0	0.9	3.3	5.4	1.0	0.3
Non-prey spp.	3.6	16.8	1.5	7.5	0.6	3.8	3.4	6.7

*Including *Polydora socialis*.

areas are similar to one another and to the PWN lower and middle cobble, and lower sand subareas.

The polychaete fauna of the upper slope and bench areas is similar to the PWN lower habitats, particularly in winter (Tables 3, 5). There is little overlap between the lower WP habitats and any of the PWN habitats particularly as regards the important prey species, *Owenia fusiformis.* Only in the upper bench area was *O. fusiformis* in any appreciable densities. The secondary prey species, *Polydora socialis* (Schmarda, 1861), was abundant in all WP habitats especially in the summer, although it was less common in the winter when *O. fusiformis,* if present, was more abundant. Particularly in the summer, *P. socialis* contributes substantially to the prey biomass, which can be somewhat misleading, as it is a distinctly minor food component. All of the prey biomass in the lower areas was due to *Polydora socialis.* As at PWN, the polychaete assemblages of the upper and lower areas became more distinct in winter.

Friday Harbor Laboratories:

The sediment analyses of the FHL habitats indicate that the sampled areas are quite homogeneous (Tables 1, 2). The lower mud area is the only one of the unconsolidated sediment areas to be somewhat different, with a distinctly smaller median particle size. However, the difference is not statistically significant. Even with many more visible shell fragments, the median particle size of the shell fragment area is similar to that of the upper mud area. The sorting coefficients of all the areas are similar.

The other physical factors affecting the FHL areas do not vary between the habitats. Temperature varied yearly from 6° to 11°C. Salinity was not measured *in situ,* but measurements at the nearby laboratories indicated that the normal salinity of 27–29‰ did not vary widely. Some seasonal variation in salinity was present due to flooding of some large local rivers. However, such events were not of long duration, and were generally surface phenomena not reaching the −10 m depths necessary to impact these areas. Current velocity 1 m above the bottom seldom exceeds 20 cm sec^{-1}, peaking at about 70 cm sec^{-1} only during periods of maximum tidal exchange.

The FHL polychaete fauna is diverse, and

TABLE 6. FLH polychaete assemblage analysis; habitat abbreviations as in Table 1.

A. Numerical rank, top ten species.

Habitat	UM		SF		LM	
Season	S	W	S	W	S	W
Drilonereis falcata Moore, 1911	–	–	–	–	6	4
Capitella capitata (Fabricius, 1780)	–	9	–	–	–	–
Mediomastus ambiseta (Hartman, 1947)	–	6	5	–	–	8
Chaetozone setosa Malmgren, 1867	5	–	2	4	4	6
Cirratus cirratus Müller, 1776	3	–	10	9	–	5
Tharyx multifilis Moore, 1909	6	3	4	3	3	1
Axiothella rubrocincta (Johnson, 1901)	1	2	1	1	1	–
Nephthys ferruginea Hartman, 1940	4	–	–	7	–	2
Pholoe minuta (Fabricius, 1780)	7	–	–	–	–	3
Laonice cirrata (Sars, 1861)	8	4	8	6	5	7
Prionospio cirrifera Wirén, 1883	–	–	7	10	–	–
P. steenstrupi Malmgren, 1867	2	1	3	2	7	9
Polycirrus caliendrum Claparède, 1868	9	5	6	8	2	–
Terebellides stroemi Sars, 1863	–	10	–	5	8	–
Others	10	7,8	9	–	9,10	10
B. Assemblage data.						
All polychaetes.						
Mean number m^{-2}	778	1,056	1,264	723	1,556	1,204
H″	2.95	2.56	2.66	2.79	3.11	3.22
Mean ash free Dry weight (g m^{-2})						
Turrid prey spp.	All less than 0.1					
Non-prey spp.	3.1	8.8	3.1	2.5	0.8	46.0

relatively sparse (Tables 3, 6). The most abundant species, such as *Axiothella rubrocincta* and *Tharyx multifilis* (Moore, 1909), are present in many of the samples. Less common but often very important species such as *Myriochele oculata* Zachs, 1922, the major turrid prey, are often poorly represented. The similarity between the infaunal samples from all of the habitats is high in summer, but except for the shell fragment habitat, there are many changes by winter. Coupled with this discrimination some species are associated with only one habitat, such as *Drilonereis falcata* Moore, 1911 in the lower mud, and *Prionospio cirrifera* Wirén, 1883, in the shell fragments.

Biology of *Ophiodermella*

Ophiodermella inermis is the only turrid found at PWN, although it was associated with two other turrids, *Oenopota levidensis* (Carpenter, 1864) and *Kurtziella plumbea* (Hinds, 1843) at WP. An estimate of *O. inermis* population size is available only from PWN, where the population was effectively contained by physical factors. The population apparently varied annually from a low in midwinter to a high in late spring or early summer (Fig. 9). Few juveniles were collected during the sample period, only 5 of the 1125 animals collected for fecal sample examination were less than 15 mm long. Juveniles were probably not missed during the sampling; turrids as small as 3.0 mm long were regularly collected in other sampling areas. At WP where smaller *O. inermis* animals were found, they looked and appeared to behave much like the adults, supporting the contention that few juveniles were present at PWN. The PWN population consists of a narrow shell length distribution with few individuals differing from the mean length by more than 5.0 mm (Fig. 10). Mean shell length did not vary significantly from season to season, and remained the same for at least 15 months. Recaptured *O. inermis* at PWN allowed estimation of growth rate. Animals were assigned size classes on the basis of pregrowth sizes, and the mean growth rate, in μm day^{-1}, was calculated. Two growth trends are evident; smaller animals grow much faster, and individuals reach a determinate adult size, approximately 31 mm in length, which few individuals exceed. The decrease in growth rate with increasing length is described by a power curve: growth rate (μm day^{-1}) = $5.14 \times 10^{11} \times$ (pregrowth length (mm))$^{-7.751}$; $r^2 = 0.79$. Exclusion of values of shrinkage and zero growth changes the curve only slightly: growth rate (μm day^{-1}) = 6.33

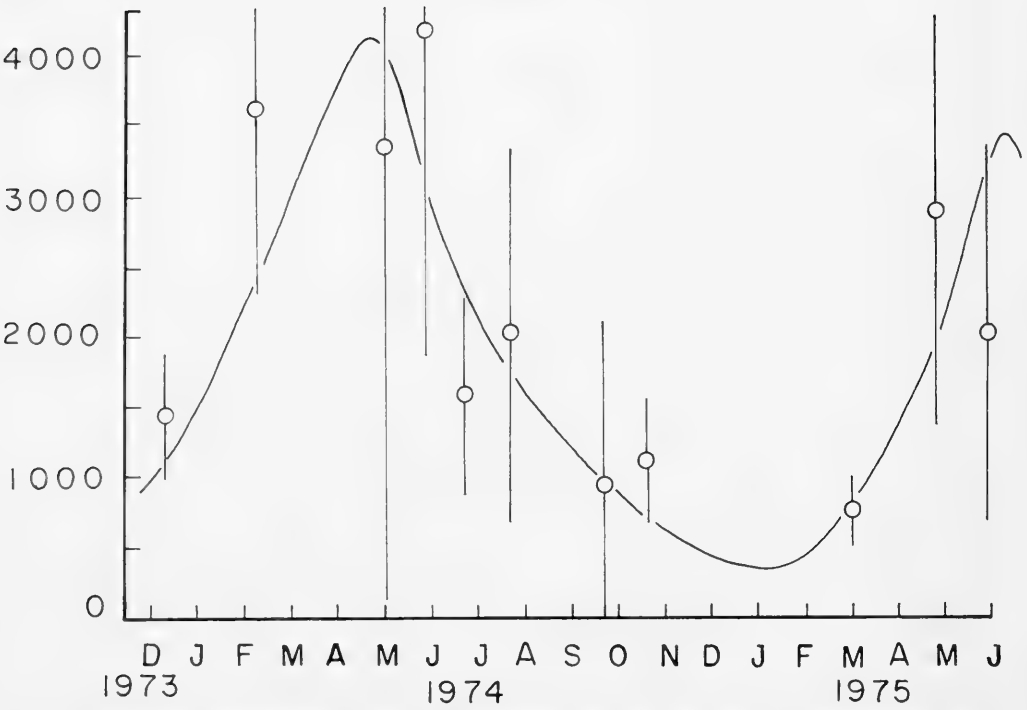

FIG. 9. Population size estimates of the Port Washington Narrows population of *Ophiodermella inermis*. Bars are one standard error on either side of the estimated population size. The curve was fitted by a series of power curve equations.

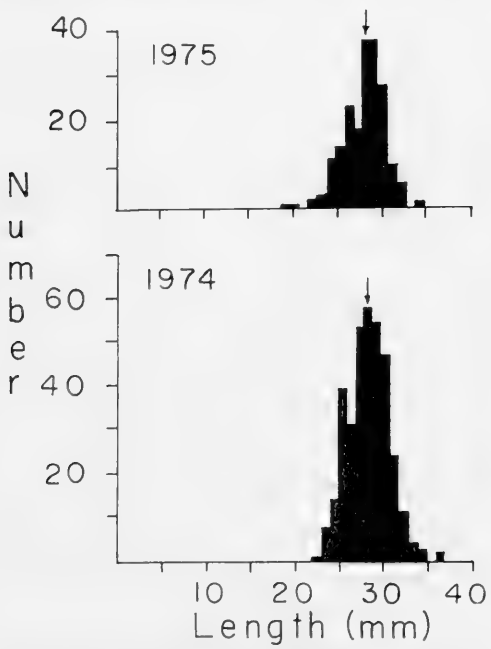

FIG. 10. Size frequency distribution of *Ophiodermella inermis* collected at PWN. Data are for the three month period ending 31 January, of the indicated year. Lower N = 360, Upper N = 195. Arrows indicate the mean size.

$\times 10^9 \times (\text{pregrowth length (mm)})^{-6.260}$; $r^2 = 0.86$. These curves are based upon a relatively narrow size range, the smallest recaptured animals at PWN had a pregrowth length of about 23 mm, and are probably not valid for smaller sizes. Nonetheless, they indicate the growth rate of small animals may be rapid.

Examination of the WP population size frequency distribution shows several peaks (Fig. 11), and the histogram can be separated into a series of normal distributions with distinctly different means. If the PWN population is assumed to be a mature population with little recruitment, and the characteristics of that size frequency distribution are used as the basis for separating the larger "mature" component of the WP population, a series of arbitrary distributions result (Fig. 11). If the peaks of these distributions can be assumed to represent successive yearly recruitments, and if the animals at WP can be assumed to grow at the same rates as the nearby PWN population, both sets of data can be pooled to compute growth rates for a larger suite of animals. A logarithmic equation describing the change in growth rate related to size was calculated by fitting the above data to a formula of the general form: $y = a + b \ln x$, where y = growth rate (μm day^{-1}), x = pregrowth rate

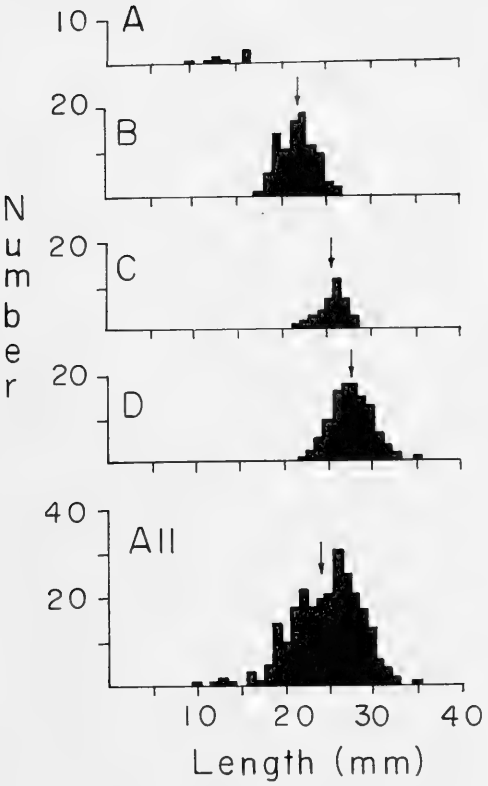

FIG. 11. Size frequency distribution of *Ophiodermella inermis* at Windy Point. All = Total number collected, N = 254, 1975. A–D Hypothesized recruitment cohorts from previous years. A = 1974, N = 7; B = 1973, N = 95; C = 1972, N = 38; D = 1971 and earlier, N = 114. Arrows indicate means.

(mm), and a and b are constants. The resulting equation: $y = 80.463 - 22.371 (\ln x)$, describes the change in growth rate well, $r^2 = 0.964$, over the larger range of size classes, from 21 to 30 mm.

If this curve is an accurate representation of the change in growth rate with increasing size, the time necessary to grow from 21.5 mm to 25.4 mm is 1.1 years, and from 25.5 mm to 27.7 mm is 0.9 years. These sizes are the mean sizes of cohorts in Fig. 11. More importantly, this more general equation can provide a better estimation of growth rates for smaller sizes than could be done with data from the PWN population alone. If this relationship is valid down to the smallest size, and if a shell length of 1.5 mm is assumed as the size of the settling veliger, it will take 0.56 years to reach 10.0 mm in length, and 2.3 years to reach 21.5 mm. Consequently, growth is probably very rapid in young *O. inermis,* and slows as they mature. No definitive measure of size at sexual maturity is available, but the smallest female *O. inermis* to spawn was 22.5 mm in total length.

Many of the "mature" snails collected at PWN 500–700 days after marking showed essentially no change in length, growing less than 1.0 mm, and they were only slightly more likely to increase than to decrease in length. Decreases were caused by apical erosion, probably from mechanical abrasion, and aperture destruction by unsuccessful predatory attempts by crabs. Mechanical erosion of the shell was also caused by the use of the shell as burrow substrate by spionid polychaetes of the genus *Polydora*. In some cases, the shell was substantially eroded by the worms. In two cases, I collected snails whose shells were perforated by the worm burrows exposing the digestive gland. Although these snails survived the measuring and marking procedure apparently without harm, they were not recovered subsequently.

The growth pattern exhibited by *O. cancellata* is not similar to that in *O. inermis*. Mark-recapture data were too sparse to indicate confidently growth rates or changes in growth rates. Many of the data indicated shrinkage, caused primarily by apical erosion. If apical erosion was occurring at a similar rate in the two species, the effect of increasing error would be greater on the smaller *O. cancellata*. There is a trend for growth rate in length to slow as the animals get larger, but it is not significant because of the large variances involved. Measurements of lip growth were unsuccessful with *O. inermis,* presumably as mechanical abrasion of the beach sand removed the marks. Lip growth measurements were obtained for a few *O. cancellata,* however, and they show no correlation with the growth rate in shell length, and a negative correlation with increasing length (Fig. 12).

Examination of the size frequency data from the FHL *O. cancellata* population indicates that the population size frequency distribution changed dramatically over the course of the study (Fig. 13). The mean size of the population in summer, 1974, is significantly larger than the mean size in the summer of 1975. The intervening winter population is distinctly bimodal, indicating both mortality of the larger individuals, and the recruitment into the population of the smaller ones. The rate of change in the mean size of the smaller size cohort over the year is about half as much as is necessary to grow from the mean of the small sized cohort to that of the

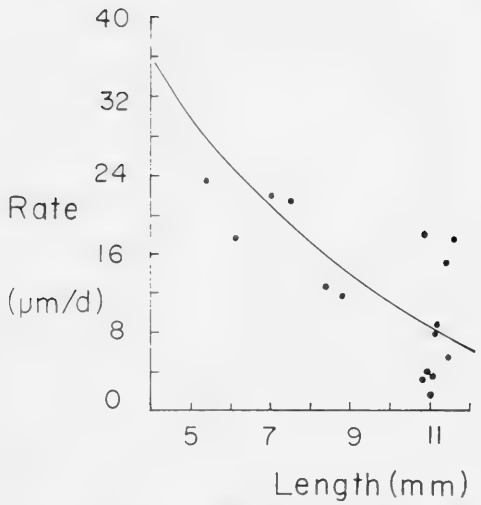

FIG. 12. Outer lip growth rate versus pregrowth length for field recaptured *Ophiodermella cancellata*. Calculated curve: $y = 60.92 - 21.46 \ln x$, $r^2 = 0.77$.

larger size cohort in only one year. If the two cohorts of winter 1974–1975 are taken to be successive recruitments they must be at least two years apart. If the larger sized cohort is assumed to be mature animals, and the small sized cohort is assumed to be recruited at least two years later, the summer, 1975, distribution can be divided into its components (Fig. 13). This assumes a relatively constant adult mortality which seems to be borne out by the gradual decrease in the size of the modal group of larger individuals. My collection of *O. cancellata* was size dependent; I seldom collected any animals smaller than 3.0 mm, as they were difficult to distinguish from the surrounding sediment particles. It is evident by the large group of smaller animals crossing that collection threshold in the summer of 1975 that recruitment was good for that cohort, which probably represents individuals that settled the previous winter.

Predation

Large crabs, *Cancer productus* and *C. gracilis* are present at the PWN and WP study sites, and both will attempt to eat *O. inermis* (Table 7). Successful predation by *C. gracilis* upon adult *O. inermis* is unlikely, but *C. productus* can eat them. Both crabs are abundant at PWN and although no quantitative estimates of abundance could be obtained as they tended to remain below the water line, *C. gracilis* appeared more common than *C. productus*. Ranging over the en-

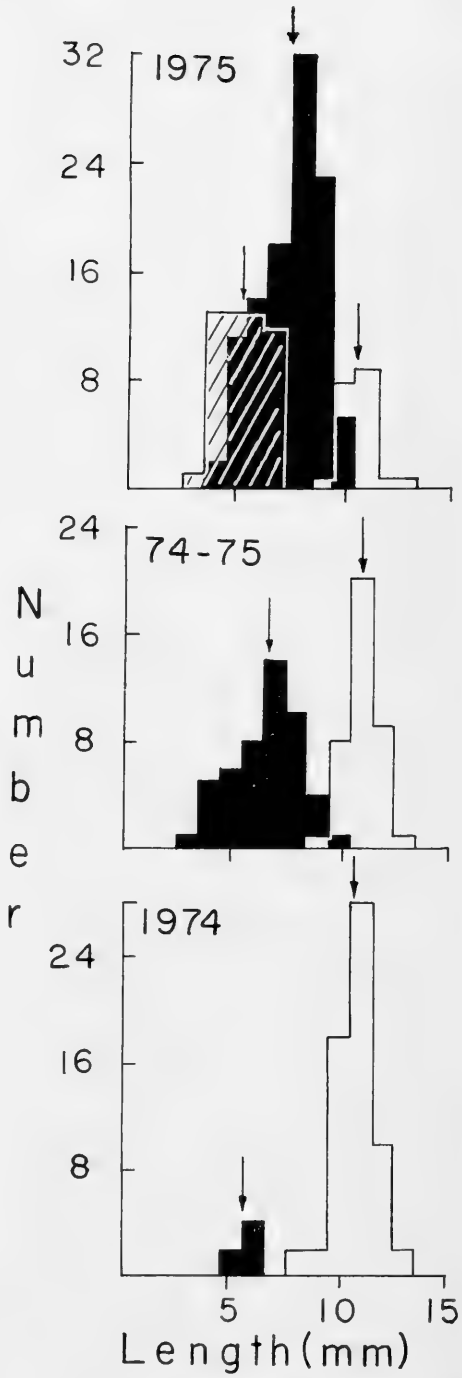

FIG. 13. Size frequency distributions of *Ophiodermella cancellata* at FHL from animals collected in summer, 1974, N = 68; winter, 1974–75, N = 88; and summer, 1975, N = 177. Hypothesized recruitment cohorts are indicated; the 1970 and earlier cohorts are white, 1972 is black, and the 1973 cohort is cross-hatched. Means are indicated by arrows.

TABLE 7. Results of crab predation experiments.

Ophiodermella inermis	Crab: *Cancer gracilis*	Crab: *Cancer productus*
Eaten	0	5
Not eaten		
Attacked	1	2
Not attacked	7	5

G = 9.51, $P < 0.005$. Thus, *C. productus* is significantly more successful at attacking and eating *O. inermis* than is *C. gracilis.*

tire beach at high tide, they have access to any *O. inermis* present, perhaps excluding buried individuals. There is no evidence that *O. inermis* is any more attractive to either crab than other gastropods present at PWN. Nonetheless, substantial predation pressure could be inferred due to several factors. First, *O. inermis* is relatively large, only *Nucella lamellosa* (Gmelin, 1791) and *Polinices lewisii* (Gould, 1847) are larger gastropods on this beach. Second, it is abundant, having densities up to 0.9 animals m^{-2}. Third, the frequency of repaired shell fractures indicates that attempted predation is common. Fourth, these frequencies of shell repair are amongst the highest recorded (Vermeij et al., 1980).

Attempted crab predation as measured by healed shell fractures (Fig. 1) is significantly more common at PWN than at WP (Table 8). *Cancer productus* is less abundant at WP, restricted to areas where debris in the habitat creates suitable refuges. Near these areas crab predation may be important. *Cancer gracilis* is seasonally abundant (Table 9) but is probably not a predator on adult *O. inermis,* although it may be an important mortality factor for juveniles.

An *O. inermis* shell that has a total length of 15 mm or a calculated age of about one year, has an apical length of 10 mm if the apical length is measured from the apex of the shell to the shoulder suture at the top of the body whorl. The number of healed fractures in the top 10 mm of apical length therefore corresponds to the number of healed fractures in animals up to 15 mm total length. The number of healed fractures is not significantly different for the two areas and is relatively low (Table 8), indicating few unsuccessful attacks on juveniles. The number of healed fractures on the remainder of the shell is significantly greater at PWN. It is likely that this rate of increased attempted predation on PWN adults is reflected in increased successful predation on small PWN individuals, which probably accounts for their rarity in the area.

Predatory pressure on the *O. cancellata* population was difficult to assess. Large crabs are rare in the lower mud habitat where *O. cancellata* is most common, only two adult *C.*

TABLE 8. Repaired crab-fracture frequency.

Area	Mean number of repaired fractures: Top 10 mm of spire	Mean number of repaired fractures: Remaining spire	Number examined	Shell length range (mm)
PWN	1.58 ± 1.12	6.97 ± 2.54	31	15.0–30.6
WP	1.09 ± 1.33	4.24 ± 2.46	34	15.2–30.6
t-test on difference of means:	t = 1.642; n.s.	t = 4.364; $P < 0.001$.		

TABLE 9. Distribution of *Cancer gracilis* at Windy Point, 1975.

Month	Mean number of *C. gracilis* observed per 25 m^2: J	F	M	A	M	J	J	A	S	O	N	D
UB	0	1	–	1	2	1	5	3	0	0	0	0
US	2	1	–	0.5	14	0.5	4.5	1.5	0	2	0	1
LS	1	1	–	1	3	1	5.5	0	3	1	0	0
LB	0	0	–	0.5	2	1	2.5	0	1	0	0	0
Number of surveys	2	2	0	2	3	2	3	2	1	1	1	1

magister Dana, 1852 were seen in over 150 hr of subtidal observation, and no adult *C. productus* were ever seen in the habitat, although both were relatively common less than 200 m away. Juvenile *C. productus* are seen in the area occasionally, and presumably they could be attacking the snails. Six of the 60 *O. cancellata* recaptured after marking showed evidence of recent shell fracturing, and older fractures were not uncommon (Fig. 1). Large hermit crabs, *Pagurus ochotensis* Brandt, 1851, and *P. armatus* (Dana, 1851), are common in the habitat, and might attack the snails. Thirteen of the 48 *O. cancellata* shells collected with hermit crabs in them had been drilled by naticids, and both *Natica clausa* Broderip & Sowerby, 1829, and *Polinices pallidus* Broderip & Sowerby, 1829, are found in the area, but most shells showed no evidence of the cause of death. Fish predation may be a factor; the ratfish, *Hydrolagus colliei* (Lay & Bennett, 1839) is sometimes found in the area and is known to prey on gastropods (Miller et al., 1978), but none could be collected for gut analysis. A large asteroid, *Luidia foliolata* Grube, 1866, occurs sporadically in the lower mud and ingests small gastropods and infauna. Five were collected and their gut contents examined: no turrid remains could be identified, although other small gastropods characteristic of the habitat were represented. It is also possible that my manipulation was resulting in mortality of *O. cancellata.* Of the 48 *O. cancellata* shells that were collected with hermit crabs in them, only two were previously marked, however, indicating that this factor is probably not major.

Environmental stress effects have little influence at either of the subtidal sites; however, some physical factors can have a pronounced effect at PWN. Temperature effects are especially important. During the first summer low tide with uninterrupted sunlight in 1974 (20-VI-74), 42 of 111 snails collected were dead or moribund. Following this initial sunny low tide, few animals were collected exposed on the beach during the remaining summer low tides. Subsequent observations determined the temperatures on the beach (Figs. 7, 14). Shell temperatures closely approximated substrate temperatures, and although tests were not done to determine temperature tolerances, animals collected with shell temperatures in excess of 30°C or from substrates of similar temperatures were generally dead or dying. No periods of freezing were observed during the three winters (1973–74, 1974–75, 1975–76) that PWN was visited. I was present on the beach during all winter tides when the low tide exposed snails, consequently, cold stress is probably less important than heat stress.

Many *O. inermis* at PWN were observed and subsequently collected in areas of fresh water runoff. These animals appeared to suffer no ill effects upon their return to a normal saline environment. Due to beach topography, the maximum time for fresh water immersion was about three hr. No apparent effects were observed upon snails left in fresh water in the laboratory at ambient temperatures (5°C, winter; 20°C, summer) for up to three hr.

Habitats

Ophiodermella inermis ranges completely over all habitats at PWN, and although there are some significant seasonal differences in distribution (Table 10), it is unlikely that these changes are due to tracking of the prey population. Although there is a slight positive correlation between prey and turrid distribution in the winter, there is none in the summer. The causative agent for the shift in distribution remains unclear, but it may be an artifact of the summer behavioral change.

Ophiodermella inermis was the most abundant turrid at WP, although *Oenopota levidensis* and *Kurtziella plumbea* are also present. Of the 254 *O. inermis* whose positions were determined, 71 (28%) were seen on the upper bench, 65 (26%) on the upper slope, 57 (22%) on the lower slope, and 61 (24%) on the lower bench. Each habitat accounted for 25% of the surveyed area. Thus, there is no defined use of one habitat.

The turrid assemblage at FHL is large and diverse. In addition to *O. cancellata, Kurtziella plumbea, Oenopota elegans* (Möller, 1842), *O. excurvata* (Carpenter, 1864), *O. fidicula* (Gould, 1849), *O. levidensis, O. turricula* (Montagu, 1803), *O. pyramidalis* (Strom, 1788), and *Clathromangelia interfossa* (Carpenter, 1864) were also found. Transect studies indicate that *O. cancellata* does not use all of the habitats equally. Within 1 m of the FHL transects are 144 m^2 of the lower mud or silt habitat, 138 m^2 of the shell fragment habitat, 20 m^2 of the rock habitat, and 48 m^2 of the upper mud habitat. During the period of the study, these habitats represented 36%, 34%, 5%, and 25%, respectively, of the area surveyed. Of the *O. cancellata* found

within 1 m of the transects: 277 (68%) were found in the lower mud, 112 (28%) were found in the shell fragments, 11 (3%) were found in the rocks, and 7 (2%) were found in the upper mud. The distribution of *O. cancellata* is significantly different from the distribution of the habitats ($G = 253.6$; $P < 0.005$), and shows a distinct bias for the lower mud habitat. A similar result is apparent in the minor sites where *O. cancellata* is found (Table 11), all of which are similar to the lower mud.

During the substrate preference tests *O. inermis* showed a slight, but insignificant, tendency to avoid areas of shell. The results of

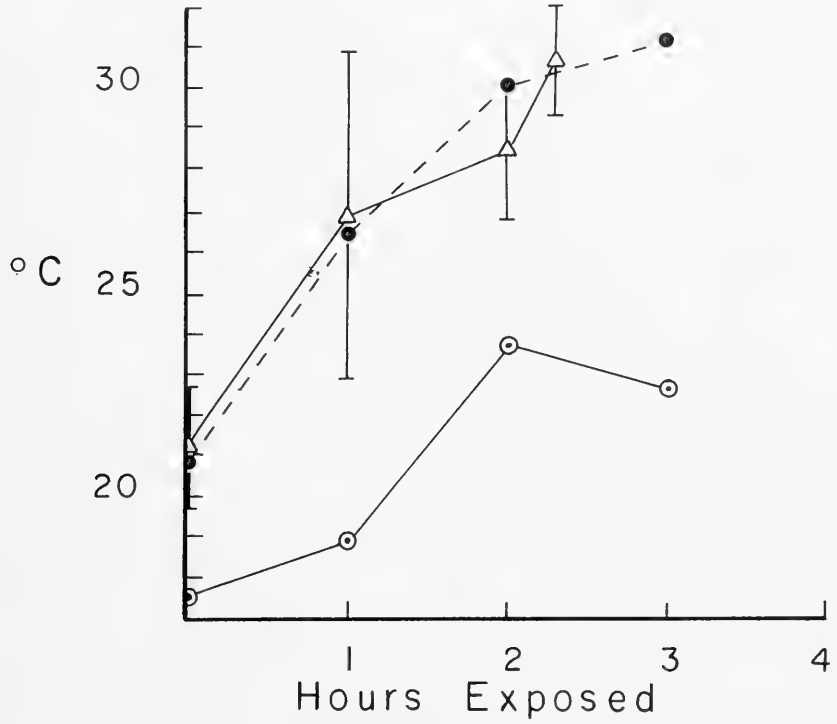

FIG. 14. Exposed shell, substrate, and air temperatures, of *Ophiodermella inermis* at PWN. Full mid-day sun, no wind, 18 July, 1974. Standard deviations shown for shell only, similar variations were seen for all values. All points are means of five values. • = substrate. Δ = shell. ⊙ = air.

TABLE 10. *Ophiodermella inermis* distribution differences at PWN. Three level nested analysis of variance comparing habitats (sand, cobble); heights within habitats (middle, low); and seasons within heights (summer, winter).

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F	
Among habitats	1	23.040	23.040	1.426	n.s.
Among heights within habitat	2	32.320	16.160	0.168*	n.s.
Among seasons within heights	4	218.740	54.685	4.976	$P < 0.001$**
Within seasons,					
Error	92	1012.860	11.009		
Total	99	1286.960			

*Calculated with a recomputed MS (MS′ = 96.026) due to mixed model ANOVA (Sokal & Rohlf, 1969).
**$F_{0.001(4,120)} = 4.95$.

TABLE 11. Minor study sites with *Ophiodermella cancellata*.

Area	Position	Depth (m)	Bottom type	Abundance
Iceberg Point, Lopez Id.	48° 24′ 54″ N, 122° 53′ 24″ W	73– 91	Silt, shell fragments	+
Lopez Sound	48° 28′ 48″ N, 122° 50′ 06″ W	49	Silt	+
Smallpox Bay, San Juan Id.	48° 32′ 26″ N, 123° 09′ 47″ W	18	Silt	+
Parks Bay, Shaw Id.	48° 33′ 45″ N, 122° 59′ 20″ W	9	Silt	+++
Upright Channel	48° 34′ 18″ N, 122° 52′ 30″ W	36– 55	Silt, shell fragments	+
Potato Patch	48° 35′ 00″ N, 122° 50′ 42″ W	42– 55	Silt, shell fragments	+
North Shore, Shaw Id.	48° 35′ 29″ N, 122° 57′ 28″ W	18– 40	Silt	++
McConnell and Reef Is.	48° 36′ 00″ N, 123° 01′ 04″ W	15– 22	Silt, shell fragments	+
Jones and Yellow Is.	48° 36′ 00″ N, 122° 02′ 00″ W	22–115	Silt, shell fragments	+
North Pass	48° 36′ 36″ N, 123° 00′ 42″ W	18– 29	Silt	+
Deer Harbor, Orcas Id.	48° 36′ 54″ N, 123° 00′ 09″ W	15– 22	Silt	+
Cowlitz Bay, Waldron Id.	48° 41′ 30″ N, 123° 03′ 18″ W	9– 55	Silt	+

+ = present; ++ = common; +++ = abundant

TABLE 12. Results of substrate choice experiments.

Turrid	Substrate		p*
	Sand	Shells	
Ophiodermella inermis	13	9	0.523
O. cancellata	8	11	0.648

*Two tailed cumulative binomial probability of a deviation as large or larger if P(sand) = P(shells).

the tests with *O. cancellata* show a slight preference for shell fragments over the sand (Table 12). These results are probably more useful for *O. inermis* than *O. cancellata,* as the sand versus shell choice is more characteristic of the substrate choices at the PWN and WP sites, than at FHL. However the particle size distribution in the upper mud approximates the particle distribution in the sand area of the choice chamber, and the shell portion of the choice chamber is representative of some portions of the lower bench habitat at WP and the shell fragment habitat at FHL.

Diet

During the study, 1125 *O. inermis* were collected from PWN and their feces, if any, were microscopically examined (Table 13). Of these, 369 had feces containing polychaete remains; 363 of these were identifiable, and 353 of these were from *Owenia fusiformis.* This polychaete has only two types of setae, long capillary notosetae, and minute bidentate hooked uncini (Fig. 3). There may be several hundred thousand of these characteristic uncini per worm (MacIntosh, 1915). As the notosetae of *O. fusiformis* are not absolutely distinctive, a fecal sample was recorded as identified only if both uncini and notosetae were present. *Owenia* was fed to eight *Ophiodermella inermis* and the mean time from ingestion to defecation at 8–10°C was determined as 1.3 ± 0.5 days. Using this information, the fecal sample data, and the estimate of population size, an estimate of the mean yearly predation upon *O. fusiformis* by *Ophiodermella inermis* can be calculated (Table 14).

As at PWN, *O. inermis* at WP is primarily eating *Owenia fusiformis* (Table 13). The overlap between the diets from the two sites is 0.994. Examination of worm uncini from the feces of snails collected at the two sites, and calculation of worm collar diameter indicates that the snails from both sites are eating worms in the same size range (Table 13). Because of the small sample size for certain months at WP, assessment of the impact of the snails on the polychaete population is not possible. Nonetheless, *O. inermis* appears to be feeding at a relatively constant rate similar to the rate at PWN.

Twelve *O. inermis* animals were collected from a sandy beach on the south side of Alki Point, Seattle, Washington, on 10 July, 1975. Two defecated and had eaten *O. fusiformis.* Two individual *O. inermis* were collected in the San Juan Islands, both subtidally from sandy substrates superficially similar to the WP area. Neither produced any identifiable fecal remains.

Ophiodermella cancellata is also a vermivore, but it feeds less often than *O. inermis* (Table 15). Although the diversity of diet is

TABLE 13. Dietary information—*Ophiodermella inermis*.

A. Identified fecal samples per month.

	J	F	M	A	M	J	J	A	S	O	N	D	Total	H″*
PWN														
Number of turrids examined	112	164	0	68	88	98	76	9	4	49	337	120	1125	
Prey species														0.15
Owenia fusiformis delle Chiaje, 1844	55	47		21	50	26	18	0	1	21	67	47	353	
Polydora limicola Annenkova, 1934							1						1	
P. quadralobata Jakobi, 1883				1									1	
P. socialis (Schmarda, 1861)					1	2	2	1		2			8	
Unidentified	2	1			1		1			1			6	
% eating	50.9	29.3	–	32.4	59.1	28.6	28.9	11.1	25.0	49.0	19.9	39.2	33.1	
WP														
Number of turrids examined	6	69	0	35	53	35	20	0	7	0	17	2	244	
Prey species														0.11
Owenia fusiformis delle Chiaje, 1844	1	27		15	14	9	5		2		10		83	
Polydora socialis (Schmarda, 1861)					1	1							2	
Unidentified				1	1	1	1						4	
% eating	16.7	39.1	–	45.7	30.2	31.4	30.0		28.6		58.8	0.0	36.5	

Habitat dietary overlap from fecal samples: D = 0.994

B. Calculated mean collar diameter of *Owenia fusiformis* eaten.

Area	Number of fecal samples examined	Calculated mean worm collar size (μm)
PWN	7	943.95 ± 408.87
WP	7	853.57 ± 234.46

No significant difference in worm sizes.

*Calculated from identified prey items only.

TABLE 14. Estimate of the effect of *Ophiodermella inermis* predation.

A. *Owenia fusiformis* population

	Beach area (m^2)	Mean number *Owenia* (m^{-2})	Total estimated mean population of *Owenia*, 1974–1975
Sand	2.3×10^3	8.1×10^2	1.9×10^6
Cobble	3.3×10^3	3.4×10^2	0.4×10^6
Total	3.6×10^3		2.3×10^6

B. *Ophiodermella inermis* population

Mean yearly fraction eating *Owenia*: 0.314
Mean digestion time per *Owenia*: 1.3 ± 0.5 days, at 8–10°C.

Date	Estimated *Ophiodermella* population size
May, 1974	4193
May, 1975	2023

C. Predation effects

	Estimated number of *Owenia* eaten if digeston time is:	
	1 day	2 days
Per day, May, 1974:	1318	659
Per day, May, 1975	635	318
From May, 1974 to May, 1975:	3.6×10^5	1.8×10^5
Fraction of total *Owenia* population:	0.16	0.08

somewhat greater than in *O. inermis, O. cancellata* is also a dietary specialist, and also primarily eats an oweniid polychaete, *Myriochele oculata*. The uncini of *M. oculata* are also quite distinctive which facilitated fecal analysis (Fig. 3). Far fewer *O. cancellata* are feeding per unit time compared to *O. inermis*, and relatively more prey items are unidentifiable, perhaps reflecting the increased dietary diversity.

Chemo- and rheoreception

Ophiodermella inermis from PWN were tested in a choice chamber to determine the extent of distance chemoreception and/or current effects (Table 16). The results are unambiguous; current is needed for any response; in the absence of current the animals do not move. The response is greater, however, if both bait and current are present. They move up current, do not make any choices that lead them to the baited arm, and they respond randomly in the choice chamber.

Ophiodermella inermis reproduction

Collection and confinement for fecal sample examination act as stimuli to egg capsule deposition. The peak of capsule deposition was during February, although deposition occurred from October through July (Table 17). After fecal sample examination and marking, the animals were released into an aquarium for up to two weeks prior to their return to their own areas, and many deposited egg capsules. The capsules were identical to those collected in the field. Since the behavior in the holding tank corresponded to the observed natural behavior, the deposition of egg capsules probably corresponded with natural deposition.

Encapsular development averaged about seven weeks. Trochophore and early veliger stages are passed in the capsule, growing and becoming very mobile within the capsule. There are no nurse eggs, and the number of veligers hatched appeared to be the same as the original number of eggs in the capsule.

After hatching, the veligers are very mobile

TABLE 15. Dietary information—*Ophiodermella cancellata.* Identified fecal samples per month.

	J	F	M	A	M	J	J	A	S	O	N	D	Total	H″*
Number of turrids examined	50	57	64	95	132	50	74	43	43	34	14	26	682	
Prey species														0.31
Myriochele oculata Zachs, 1922	3	2	6	5	5	3	1		6	2	2	2	37	
Polydora socialis (Schmarda, 1861)										1			1	
Syllid, unidentified			1		1								2	
Unidentified polychaete	1	1		2			1	1	1	2			9	
% eating	8.0	5.3	10.9	7.4	4.5	6.0	2.7	2.3	16.3	14.7	14.3	7.7	7.2	

*Calculated from identified prey items only.

TABLE 16. Choice experiments.

A. Chemotaxis.

Conditions	No choice	Chosen arm baited	Unbaited	Total run
i. No bait—equal water flow in both arms*	78	10	12	100
ii. Bait—equal water flow in both arms	30	38	32	100
iii. Bait—no water flow in either arm	79	12	9	100

Test	Choice	No choice	p**
Ai.	22	77	7.95×10^{-9}
Aii.	70	30	3.93×10^{-5}
Aiii.	21	79	2.62×10^{-9}

B. Rheotaxis.

Conditions	No choice	Chosen arm flow	No flow	Total run
i. No bait—water flow in one arm	75	22	3	100
ii. No bait—no water flow in either arm*	95	4	1	100

Test	Choice	No choice	p**
Bi.	25	72	2.82×10^{-7}
Bii.	5	95	6.26×10^{-23}

C. Probabilities of choices made.

Test	Potential choices A	Potential choices B	2-tailed cumulative binomial probability If $P_{(A)} = P_{(B)} = 0.5$	
Ai. Control	10	12	0.832	Non-significant
Aii.	38	32	0.664	Non-significant
Aiii.	12	9	0.550	Non-significant
Bi.	22	3	1.57×10^{-5}	Highly significant
Bii.	1	4	0.776	Non-significant

* = Choices are: right vs. left arms.
** = 1-tailed cumulative binomial probability if $P_{(choice)} = P_{(no\ choice)}$

and feed actively for up to five weeks. They live for another two weeks in a relatively inactive condition (Table 18). This inactive period probably corresponds to the time when the animals would normally settle. I was not able to induce metamorphosis. A variety of settlement substrates were offered: sand, silt, empty *O. fusiformis* tubes, *Phyllochaetopterus* tubes, and bare glass, but no settlement occurred.

Spawning and development of *O. cancellata* were not observed although copulation was seen several times in the field from July through September. The smallest *O. cancellata* collected was 1.8 mm long and was collected on 20-II-75. Most (53 of 85, 64.4%) small (3.0 to 5.5 mm) *O. cancellata* were collected from April through June, suggesting a settlement time of late autumn to mid-winter.

DISCUSSION

Both species of *Ophiodermella* are predatory specialists upon oweniid polychaetes. The degree of specialization in these species is uncommon, yet there does not appear to be a close correspondence between turrid distribution with any polychaete species on the fine scale in any of the habitats examined. For example, at WP *O. inermis* was common in all habitats, while *Owenia* was concentrated in

TABLE 17. *Ophiodermella inermis* reproductive information.

A. Field observations

	Area:	Date:	Number seen:
I. Copulation	PWN	II-XI-73	"several"
	PWN	25-XI-73	3
	PWN	9-XII-74	1
	PWN	19-XI-74	1
	WP	24-V-73	1
II. Capsule deposition			
	PWN	26-V-74	3
	PWN	13-V-76	1

B. Laboratory observations

I. Capsule deposition

Month	Number deposited	Number of *O. inermis* examined
October	2	49
November	0	354
December	0	122
January	1	118
February	20	193
March	4	40
April	4	103
May	6	141
June	1	133
July	1	96
TOTAL	39	1253

TABLE 18. *Ophiodermella inermis* egg capsule characteristics.

	Number of capsules examined
Mean dimensions (mm)	
length 4.68 ± 0.99	25
width 3.95 ± 0.77	25
height 1.44 ± 0.29	25
Mean egg	
number 208 ± 61	14
diameter (μm) 222 ± 15	90
Mean number of days encapsular period: 50.6 ± 13.05	18
Maximum number of days of post-hatching survival: 50	

the upper bench area, and was only sporadically found elsewhere. *Ophiodermella inermis* is very mobile; during one 30 min period, I observed one marked individual to move 4 m along a transect. Consequently, the snails could easily vary their habitat and forage in the upper areas from the lower ones. Although the chemo- and rheotaxis experiments indicate scant powers of distance chemoreception (Table 16), the tendency of the animals to move up current if prey are present presumably aids them in locating prey.

Ophiodermella inermis clearly does not select habitats due to sediment particle size except in a gross manner; it is seldom found on large (diameter $>$ 50 cm) boulders, and was never seen in bedrock areas. It does respond to degree of exposure: after encountering the high temperatures typical of the first summer low tides, the behavior patterns of the animals change drastically. Prior to this

exposure, most of the population is uncovered at low tide. After the first few exposed tidal periods, the upper limit of the population apparently drops from +0.5 m to +0.2 m, and the majority of the population remain buried through the low tide period, emerging shortly before the incoming tide passes over them. During the late winter and early spring, the animals do not bury and are left stranded around the beach for the duration of the low tide. Collection of the animals is facilitated in late winter and spring when the animals are exposed and visible. In many cases, they appeared to be actively foraging while exposed. This seasonality of behavior results in the apparent cycling on the population (Fig. 9). This behavior pattern is triggered by exposure on sunny warm days, and presumably is maintained by repeated periods of high insolation. It is obviously a protective behavior pattern, and although some animals perish initially, relatively few are exposed to the worst of the summer heat.

If habitat and prey utilization patterns can be attributed to active preferences, the relevant cues can be hypothesized on the basis of comparing resources available and utilized. If this is so, *O. cancellata,* by virtue of its almost absolute specialization on *Myriochele oculata*, one of the rarest polychaetes sampled (2 out of 11,667 worms sampled), must be responding primarily to the distribution of its principal prey, and secondarily to any substrate parameters. This hypothesis is supported by the lack of any substrate preference in experimental conditions. Unfortunately the distribution of *M. oculata* is unknown here, hence no correlation with its predator's distributions can be made. While it cannot be conclusively shown that prey with no identifiable remains were not being eaten by *O. cancellata*, the extreme dietary specialization otherwise seen in both *O. inermis* and *O. cancellata* argues against this. On the basis of the *O. cancellata* distribution being strongly skewed to the lower mud, it can be assumed that *M. oculata* is most common there. Although distance chemoreception was not tested in *O. cancellata,* the rarity of the prey suggests that this species may have better capabilities in this regard than *O. inermis.*

These two turrids may react differently to prey population densities. The extreme specialization on abundant prey such as seen at PWN and WP is consistent with optimal foraging theory (Emlen, 1966, 1968; MacArthur & Pianka, 1966; Schoener, 1971; Hairston, 1973) which predicts that where food is not limiting, specialization should occur. The PWN site contains several years' supply of food, and presumably no food limitation exists at WP either, because of the abundance of prey in the upper bench and slope areas. Predatory effects are difficult to substantiate without experimental manipulation. Nonetheless, some of the annual drop in the WP upper slope *O. fusiformis* populations is likely the result of the turrid.

This is certainly unlike the situation at FHL, where *O. cancellata* preys upon the rare *Myriochele oculata.* The turrid is quite mobile, and I have starved some for up to two months without any apparent ill effects. Both of these properties would be expected in predators whose prey is rare, although many carnivorous gastropods survive prolonged starvation regardless of prey density. Furthermore, the low fraction of turrids feeding could be indicative of the low prey density and long search time. That the prey density is low there is no doubt; the cause of this low density is the pertinent question.

Is *O. cancellata* such an efficient predator that it can effectively keep its prey at a very low density? If so, is the turrid food limited? Or is *O. cancellata* simply adept at finding low density prey? The turrid population recruits well, but apparently not every year (Fig. 13). Since *Owenia fusiformis* is an annual (Fig. 8), it is reasonable to suggest that *M. oculata* is as well. If this is the case, perhaps the relationship between *O. cancellata* and its prey is a cyclic one where the predator regularly exterminates the prey and then itself goes extinct locally. This might result in the pattern seen in the *O. cancellata* size frequency distribution, and account for the missing year class. Perhaps veligers respond to the presence of the prey as a settlement cue, and during a low period in the population of *M. oculata* they do not settle. If the predators can starve for long periods, especially as prereproductive juveniles, when the energy requirements for gamete production would be non-existent, they might be able to survive from annual pulse to annual pulse of their relatively transitory prey. As the prey population would respond to predator population cycles, but out of phase, this cyclic interaction would be quite stable, and could result in periods of very low population of both the predator and the prey. The smaller individuals could devote more time and energy to seeking prey and might be substantially more suc-

cessful than the reproductive individuals at finding sufficient food to maintain growth. If the preproductive effort involved substantial energy utilization for mate seeking, gamete production, and egg capsule production, the reproductive success would be expected to decline, perhaps as precipitously as we see here, especially in a food limited situation.

The infaunal polychaete diversity is relatively high at all three major sites, and the mean polychaete size is small. The structure of the soft-sediment community may be largely dependent upon the activities of these organisms (Sanders, 1958, 1960; Woodin, 1974, 1976; Gray, 1974; Rhoads & Young, 1970). Turrid predation may substantially affect this assemblage of worms, especially if the turrids can render their prey rare, or if they can drastically affect the population of a dominant organism. *Owenia fusiformis*, a dominant component of the infauna at PWN and the upper WP areas, can certainly be affected by *Ophiodermella inermis,* at least on the small scale. These hitherto ignored predators of an essentially unknown trophic level (Trevallion et al.,1970) may be having substantial effects on the community as a whole.

The effects of predation upon both of these turrids is important and difficult to assess. Brachyuran predation is significant at PWN and at least on small individuals at WP. Mature *O. inermis,* greater than 27 mm long, probably have escaped predation by *Cancer gracilis*, although *C. productus* can certainly prey upon them. The relative rarity of *C. productus* at WP probably indicates that the large *O. inermis* there have a size refuge from predation that is lacking at PWN. Indeed, the relative abundance of small *O. inermis* at WP may be due largely to the lack of efficient predation from the larger crab. The highly compressed unimodal size frequency distribution of *O. inermis* at PWN appears largely due to predation by crabs upon new recruits and other small individuals. The population structure reflects a large successful settlement and recruitment swamping the predators, coupled with a rapid early growth rate. Indeed, the apparently rapid early growth rate of *O. inermis* would be strongly selected for by predation on smaller animals. That the larger animals have at least a partial refuge from predation is evident from the healed and repaired fractures of the kind caused by attempted crab predation. This model of crab predator assumes the smaller *C. gracilis* to be the most abundant predator, but still leaves the population at the mercy of *C. productus.* The burying behavior of intertidal *O. inermis* in the summer, and after prey ingestion, presumably also aids in escaping predation. Because of the ability of *C. productus* to eat turrids of any size, as well as the periodic early summer exposure mortality, the population at PWN depends upon massive periodic turrid recruitment or massive crab mortality to maintain itself. If no recruitment occurs for 5 or more years, the population is likely to go extinct. Subsequent to the majority of this study, the PWN was surveyed twice in the autumn of 1980, and a total of two *Ophiodermella inermis* were found for ten man-hours of search. In a comparable period of 1973, over 400 animals were collected, suggesting that the population is now effectively extinct.

The WP population, on the other hand, should be able to survive, due to the largely size selective nature of the predation, the lack of environmental stress effects, and the rarity of the larger predator. Surveys of this area in December, 1980, and January, 1981, showed an apparently normal population.

Predation effects upon *O. cancellata* at FHL are more difficult to determine. The snail population is obviously subject to some adult mortality factor; very few animals are 12.0 mm long or longer, and the larger animals consistently disappear from the population. Naticid predation is certainly a major factor. Because of the small size of the species, and the evidence of shell breakage in about 10% of the recaptured animals, crab predation is also a factor, although its magnitude is unclear. The fact that most of the empty shells recovered had no evidence of the cause of death made it impossible to unambiguously determine whether malnutrition induced by reproductive stress, asteroid predation, disease, or a determinate life span produced the observed distribution of dead snails.

The long larval stage of *O. inermis,* up to four months, half spent in an egg capsule, and half as a planktonic veliger, may be related to the extreme dietary specialization. The long larval stage may allow sufficient development of the specialized toxoglossan radula to permit the capture of *Owenia fusiformis* by the recently metamorphosed juveniles. If this is the case, settlement of the juveniles should coincide closely with the settlement of juvenile prey polychaetes, presuming that small snails must eat small worms. For *O. inermis* this appers to be the case, most of the turrids appearing to be ready to settle no earlier than

late spring and no later than mid-summer. This is the period when *Owenia fusiformis* shows a substantial increase in density, presumably from settling juveniles. Recruitment of *O. inermis* may be very patchy. The unimodality of the PWN site frequency data (Fig. 10) suggests one, or at most two closely spaced recruitments several years prior to 1973, and none since.

These turrids provide examples of dietary specialization unparalleled in temperate intertidal gastropods. Furthermore, the specialization upon tubicolous polychaetes of only one family, the Oweniidae, and the basic similarities of the radular teeth of these two turrids (Fig. 1), and other members of the subfamily Borsoniinae, invite investigation of the dietary requirements of other members of the subfamily, and functional analysis of this toxoglossan radular tooth. Because of the specialization of the turrids upon only one prey species per predator, very fine partitioning of the dietary resource base is possible, and coupled with the diversity of potential prey polychaetes in temperate and boreal unconsolidated sediment ecosystems, this may be responsible for the phenomenal adaptive radiation seen in many genera of boreal turrids.

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BURROWING AND THE FUNCTIONAL SIGNIFICANCE OF RATCHET SCULPTURE IN TURRITELLIFORM GASTROPODS

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ABSTRACT

Burrowing by some Indo-Pacific, high-spired gastropods is aided by low amplitude asymmetrical shell sculpture. This sculpture functions as a ratchet, resisting back-slippage when the snail is inserting its foot into the sediment while allowing free forward movement of the shell. In experiments, when ratchet sculpture is removed or covered the snails take longer and more burrowing cycles to burrow into the sediment, compared to unmodified animals or experimental controls. Species that have evolved ratchet sculpture are found in the Cerithiidae (the genus *Rhinoclavis* Swainson, 1840), Mitridae and Terebridae.

As in burrowing bivalves with sculpture serving a similar function, ratchet sculpture remains approximately constant in size during the ontogeny of the snail. Ratchet sculpture is highly distinctive because it combines negative allometry and strong asymmetry, suggesting that ratchet sculpture may also be used in inferring the life habits of fossil gastropods.

Key words: Gastropoda; functional morphology; sculpture; burrowing; *Terebra*; *Mitra*; *Rhinoclavis*.

INTRODUCTION

Infaunal bivalves have evolved a variety of shell sculptures that aid the bivalves in burrowing. These sculptures include growth-conformable concentric ridges (e.g. *Anomalocardia brasiliana* (Gmelin, 1791); Stanley, 1981), radial ribs (e.g. *Codakia* Scopoli, 1777; Seilacher, 1973), combinations of concentric and radial ribs (e.g. *Donax dentifer* Hanley, 1843; Seilacher, 1973) as well as discordant (neither spiral nor axial) asymmetrical ridges (e.g. *Divaricella* von Martens, 1880; *Strigilla* Turton, 1882; Stanley, 1969, 1970; Seilacher, 1972, 1973). Also, at least one genus of bivalves has evolved asymmetrical modifications of the periostracum which may aid in burrowing (*Sinonovacula* Prashad, 1924; Seilacher, 1972). Thus far similar sculptures have not been described in the Gastropoda, nor has the function of the sculpture actually been tested in the majority of cases.

Many turritelliform gastropods are active burrowers. Like most burrowing bivalves and gastropods (Trueman & Ansell, 1969, and references therein), turritelliform species burrow with a series of discontinuous movements. The shell and part of the animal act as a penetration anchor as the snail's foot probes the sediment. The foot then forms a terminal anchor and the shell and remainder of the animal is drawn forward. This burrowing cycle is repeated as the snail continues to burrow. A spike-like shell is not an optimal anchor, but the anchoring effect of the shell can be improved with sculpture. This sculpture would serve a ratchet-like function, increasing the effectiveness of the penetration anchor by reducing back-slippage while minimizing resistance to forward movement through the sediment. Several species of Indo-Pacific gastropods have evolved asymmetrical sculpture that appears to serve this ratchet function. The objective of this project was to test the hypothesis that shell sculpture aids in burrowing by turritelliform gastropods by increasing the anchoring effect of the shell.

PROCEDURES

I selected two relatively common species of infaunal Indo-Pacific gastropods for the experimental portion of this study. These species, *Terebra dimidiata* (Linnaeus, 1758) (Neogastropoda: Conacea) and *Rhinoclavis aspera* (Linnaeus, 1758) (Mesogastropoda: Cerithiacea), occur sympatrically in the Indo-Pacific region but subsist on different foods, burrow in different ways, and have different sculptures.

Terebra dimidiata is an infaunal predator

which feeds on enteropneusts (Miller, 1966, 1975). This species burrows solely with the foot, in a manner similar to that described for *Terebra gouldi* Deshayes, 1859 (Miller, 1975). The shell of this species bears a single cuesta or asymmetrical ridge on each whorl; a second cuesta is formed at the sutural ramp (Fig. 1A, B). The cuestas on each whorl are of approximately equal size and remain about the same size on successive whorls.

Eight specimens of *T. dimidiata* from Piti Bay, Guam, were used in the first experiment. The snails were maintained in running seawater aquaria with coarse carbonate sand substrates. Seawater temperature was fairly constant throughout the experiments (27–29°C). Each snail was timed for three trials burrowing into the substrate; the number of burrowing cycles required for the animals to bury themselves was also recorded. (For the purposes of this experiment, burrowing time was defined as the length of time from initial penetration of the sediment until the apex of the shell was drawn into the substrate.) This procedure was repeated for the subsequent trials. The sculpture of each snail was then

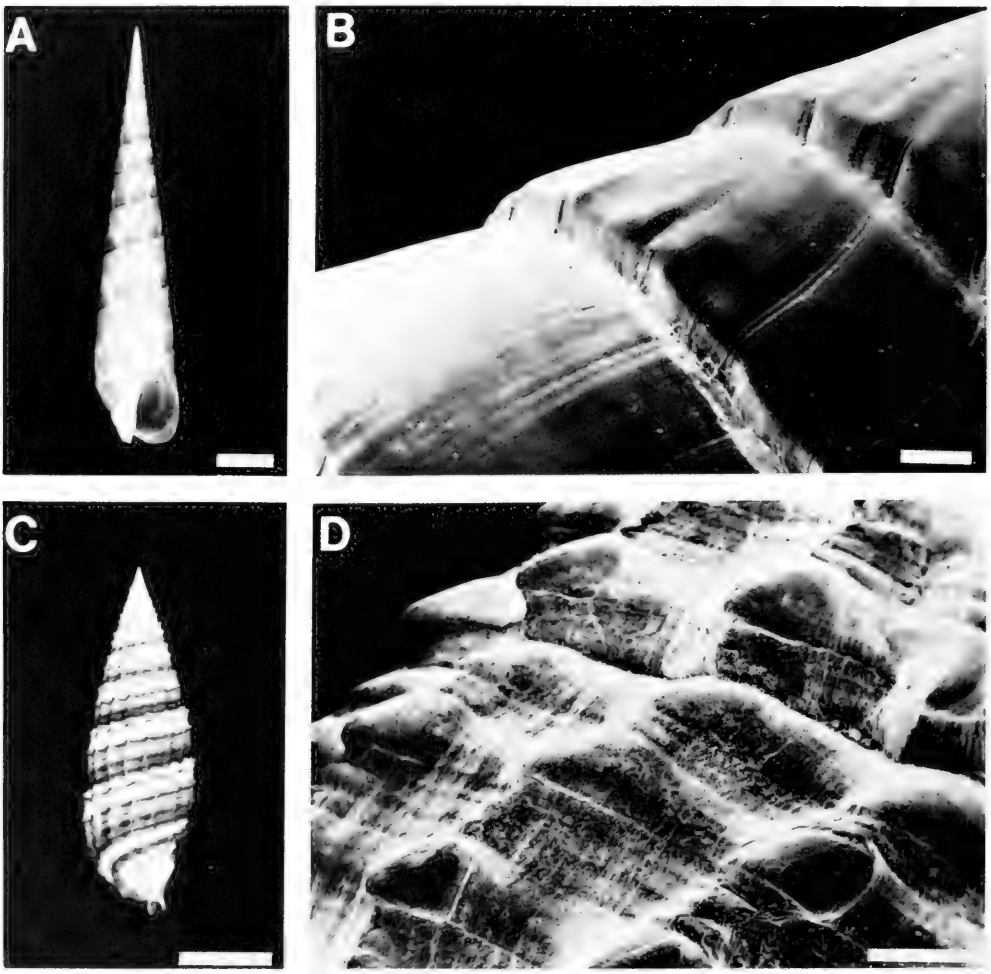

FIG. 1. A. *Terebra dimidiata* from Piti Bay, Guam. Scale bar is 1 cm. B. Scanning electron micrograph (SEM) of sculpture on *T. dimidiata*. Cuesta in center is the sutural ramp. Note cuestas are approximately equal in height. Cuestas are shingled toward the apex (to lower left), shallow slope is toward aperture. Scale bar is 1 mm. C. *Rhinoclavis aspera* from Pago Bay, Guam. Scale bar is 1 cm. D. SEM of *R. aspera* sculpture. Tubercles are inclined toward apex. Scale bar is 1 mm.

filled with a quick-drying paste and allowed to dry. Excess paste was removed and the paste was covered with a lacquer and allowed to dry. The animals were wetted periodically during this process, which lasted up to seven minutes, and were returned to the aquaria for one day prior to the burrowing trials. For a control on the experimental treatment, the lacquer and paste were removed, the animals rested one day, and then timed burrowing.

The experimental treatment added only a few percent to the weight of the animal. Any effect from this increase in weight was offset by an amount equal to the weight of the water displaced by the paste and lacquer. A second control, on lacquer alone, was not possible because the lacquer tended to collect in the cuestas and round out the sculpture. This bead of lacquer dried only very slowly and required keeping the snail out of the water for an extended period. The one control tests for possible long term effects of the total treatment on the snails. The behavior of the animals showed no obvious changes when the snails were coated with paste and lacquer.

Rhinoclavis aspera is an infaunal cerithiid (Fig. 1C) (Houbrick, 1978). The diet of this species is unknown but Houbrick (1978) has reported algae and sand grains in the stomachs of some specimens. This species' sculpture consists of asymmetrical tubercles mounted on transverse (collabral) ridges (Fig. 1D). *R. aspera* uses both its large spatulate head and foot to penetrate the sediment. In burrowing, the head is placed upon the anterior dorsal surface of the foot and both are inserted into the sediment. The head is lifted up, the foot thrusts down, and the animal pulls the shell and body forward. This cycle is repeated as burrowing continues.

Rhinoclavis aspera could not be treated with the same experimental technique used for *T. dimidiata* because of the high relief of the sculpture. To cover the tubercles would require large amounts of paste which would concomitantly increase the area of the anterior cross-section of the shell. This would increase the burrowing time regardless of the effect of covering the sculpture.

Fifty-one specimens of *R. aspera* were collected from Pago Bay, Guam, and divided into three groups of 17 specimens each. The snails were placed in running seawater aquaria with coarse carbonate sand substrate. The initial group was not modified. The tubercles were carefully filed from the second (experimental) group. About five minutes were required to gently file off the tubercles without removing the transverse ridges. In the third group of snails, the control group, the snails were filed for five minutes but only the sculpture on the dorsal side of the apex was removed. As the apex is the last part of the shell drawn into the substrate, scupture in this area should be the least important in burrowing. Each snail was rested for one day before burrowing trials commenced. The snails were timed for three trials each burrowing into the sand; the number of burrowing cycles was also recorded for each burrowing. Animals were rested briefly between burrowings to prevent tiring.

RESULTS

Analysis of the data from the experiment with *Terebra dimidiata* is complicated by a correlation between burrowing times and shell length ($r = .68$, $N = 19$, $P < .001$) (Fig. 2). The number of burrowing cycles is also correlated with shell length ($r = .62$, $N = 19$, $P < .002$). Therefore, the data were analyzed using ANOVA techniques treating shell length as a covariate of burrowing time.

Results show that *Terebra dimidiata* required more time and burrowing cycles to burrow when the sculpture was covered, compared to the initial or control treatments (Fig. 3). The F-values obtained in the pair-wise comparisons of burrowing times of the unmodified and control groups with the experimental group were 26.74*** and 29.19***, respectively ($N = 8$). There were no significant differences between the times and number of burrowing cycles taken to burrow by the unmodified and control groups (the F-value for the comparison of burrowing times between the unmodified and control groups was .817). Similar results were obtained in the analysis of the number of burrowing cycles taken to burrow by the different groups.

Rhinoclavis aspera also takes significantly more time and burrowing cycles to burrow when the sculpture is removed, compared to either the control or unmodified groups (Fig. 4). As with the previous experiment, the analysis treats burrowing time as a covariate of shell length. The F-values obtained in the pair-wise comparison of the unmodified and control groups with the experimental group were 9.20** and 5.92*, respectively ($N = 17$). Comparison of the unmodified and control group yielded an F-value of .008. Similar re-

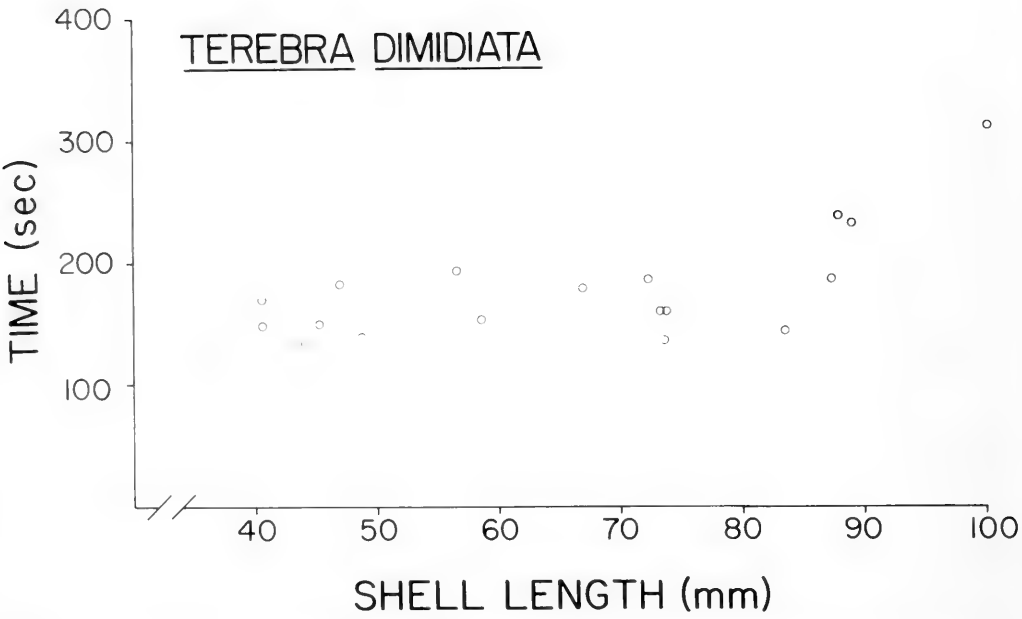

FIG. 2. Burrowing times for *Terebra dimidiata*. Shell length is highly correlated with burrowing time. Each point is the average of three burrowing trials for a single animal.

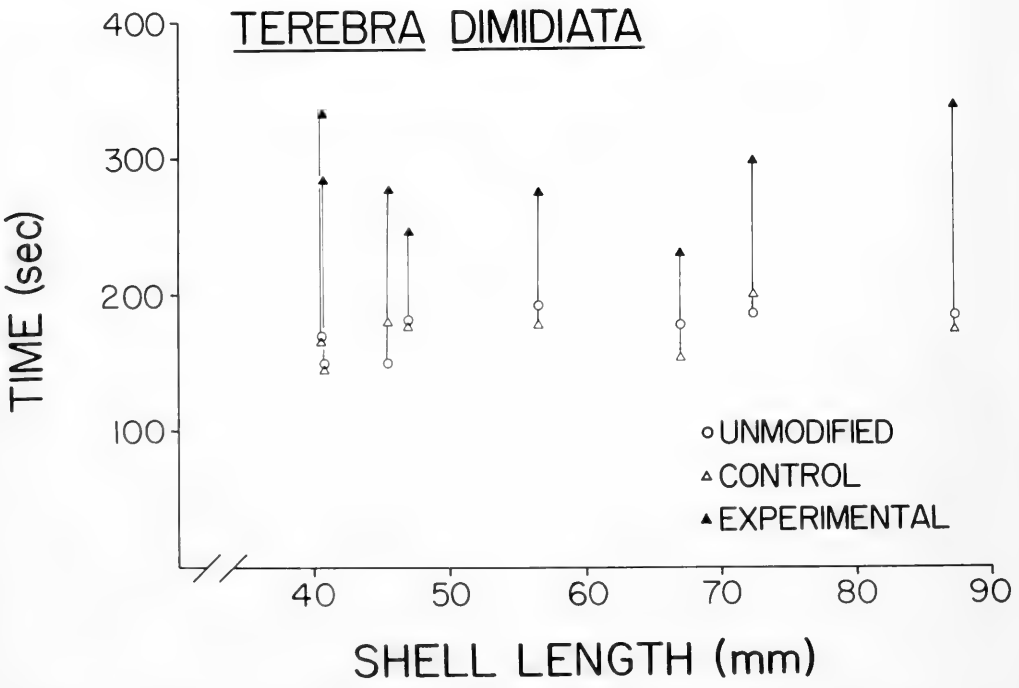

FIG. 3. Results of burrowing trials with artificially smoothened *Terebra dimidiata*. Note the uniform increase in burrowing times when the ratchet sculpture is covered (experimental group). Each point is the average of three burrowings by one individual. Vertical lines connect data from three treatments of one specimen.

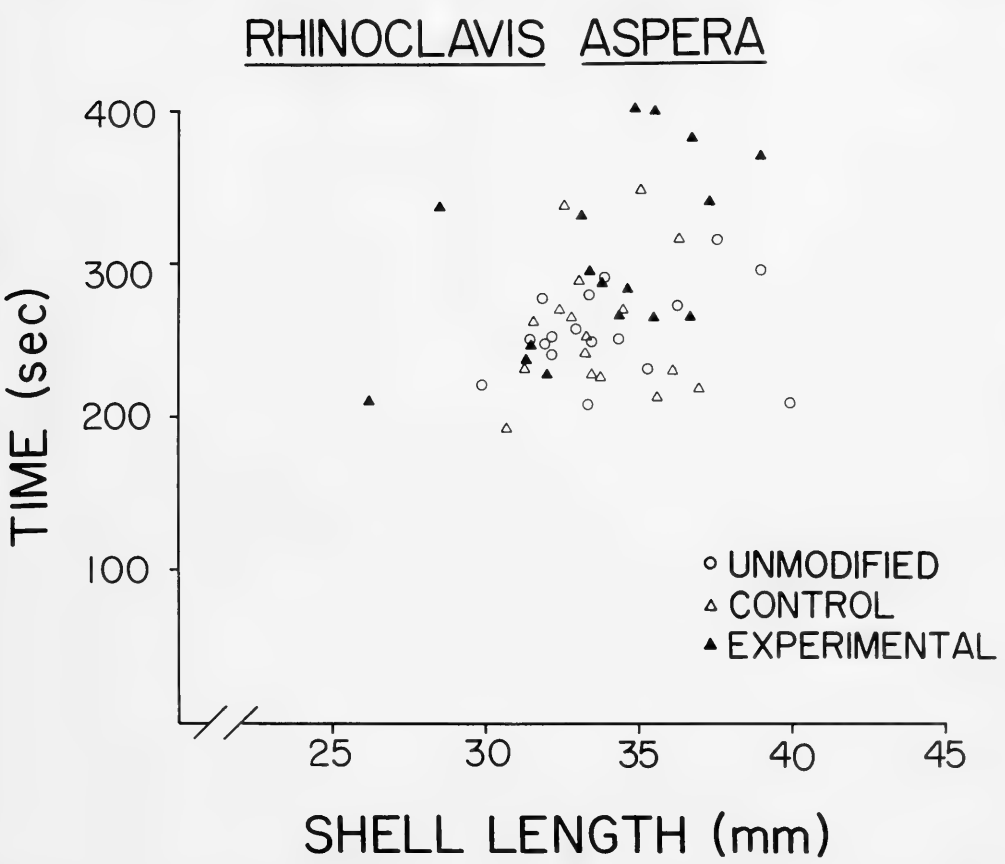

FIG. 4. Results of burrowing trials with artificially smoothened *Rhinoclavis aspera*. Each point is an average of three burrowings by one individual.

sults were obtained in the analysis of the number of burrowing cycles the snails required to burrow when the sculpture was removed.

DISCUSSION

The consistent results obtained with the two different experimental methodologies strongly supports the hypothesis that ratchet sculpture aids burrowing by increasing the effectiveness of the snail's penetration anchor. During the course of the experiments, I frequently observed the penetration anchor of smoothened snails to fail, causing the shell to slip backwards. Back-slippage was immediately followed by a brief cessation of burrowing, which partially accounts for the greatly increased burrowing times of artificially smoothened animals.

An alternative to the methodologies employed here would be to compare the length of single burrowing cycles between the unmodified, experimental and control groups. However, I did not utilize this methodology because the lengths of the burrowing cycles increase as the snails penetrate deeper into the sediment. Any analysis using length of burrowing cycles would have required an additional correction for the depth to which the snail had penetrated the sediment. This seemed an unnecessary complication to the experimental design.

Several other factors potentially could bias the results reported here. These factors are handling of the animals (Brown, 1971), time-specific activity patterns (Webb et al., 1959; Miller, 1966) and the use of artificial or reduced light in the experiments (Bell & Frey, 1969). In order to avoid these sources of bias, care was taken to ensure that the possible

effects of each of these factors were spread over all treatment groups.

Linsley (1978) noted that size does not seem to be a major factor determining locomotory rates in most gastropods. Apparently, this generalization does not extend to burrowing, at least by shell-draggers. Large *Terebra dimidiata* penetrate the sediment faster, in absolute terms, than small individulas (Fig. 2). This pattern in high-spired gastropods is similar to that in bivalves, where there is a strong correlation between shell length and burrowing time (Stanley, 1970).

The sculpture present on the species studied here is negatively allometric during the ontogeny of the organisms. The height of the sculpture remains approximately constant in size during ontogeny while the distance between tubercles or cuestas increases in constant proportion to other features of the shell. In *T. dimidiata,* the sculpture increases in height at a rate of about 1.1, while the whorl expansion rate of the species is about 1.2 (see Raup, 1966, for a discussion of whorl expansion rate). In *R. aspera* from Guam, the height of the tubercles increases until they reach about .5 mm and remain approximately constant in height thereafter. This pattern of constant sculpture size during ontogeny matches the pattern observed among many bivalves and crustaceans which have ratchet sculpture (see Seilacher, 1972, 1973).

One remaining puzzle is why so many species of burrowing snails (and bivalves!) lack ratchet sculpture. I have found ratchet sculpture on fewer than ten percent of the more than two hundred infaunal marine turritelliform species I have examined. The majority of the burrowing bivalves examined by Stanley (1970) lack any ratchet sculpture. Are most species failing to optimize or is ratchet sculpture advantageous only under limited circumstances? Stanley (1969) suggested that ratchet sculpture should not be expected in bivalves dwelling in muddy sediment because the mud would be too fluid to allow the sculpture to engage the substrate. Further research is necessary to resolve this problem.

Several burrowing gastropods have asymmetrical shell sculpture that does not remain constant in size during ontogeny (e.g. *Bullia* sp., *Terebra crenulata* (Linnaeus, 1758); Fig. 5). This sculpture may also aid in burrowing, but I have not been able to test this hypothesis. Savazzi (1981) has shown experimentally that the efficiency of ratchet sculpture is decreased when the sculpture becomes large relative to the sediment grain size. Savazzi's results suggest that asymmetrical, isometric, sculpture may serve some function besides assisting in burrowing.

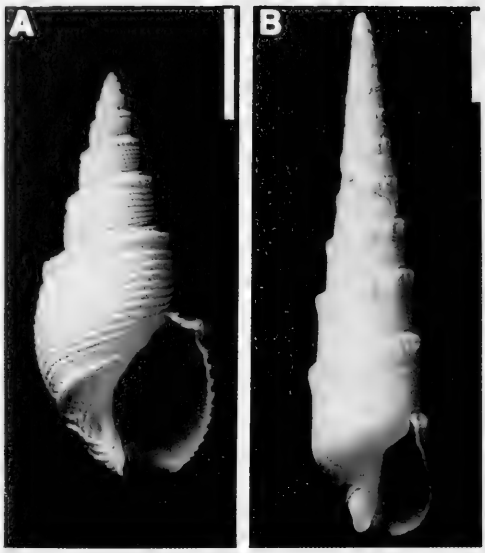

FIG. 5. Species with sculpture resembling ratchet sculpture. A. *Bullia* sp. B. *Terebra crenulata.* Scale bars are 1 cm.

Most species of burrowing turritelliform gastropods lack pronounced shell sculpture, presumably because the increased resistance to penetration of the sediment resulting from sculpture would inhibit burrowing. This problem is avoided by species with ratchet sculpture because ratchet sculpture is asymmetrical (smoothed in the direction of burrowing), in contrast to the symmetrical sculpture usually found on epifaunal species (see Savazzi, 1981, for a discussion of the relative merits of symmetrical and asymmetrical sculpture). I would expect epifaunal species which occasionally burrow (e.g. *Cerithium nodulosum* Bruguière, 1789) would burrow faster when artificially smoothened because of the reduction in friction with the sediment. This is exactly the opposite of the experimental results presented here, as burrowing times increase when ratchet sculpture is removed. Experimental modification of species with other types of sculpture remains to be attempted but it does not seem likely that symmetrical sculpture will aid in burrowing.

The combination of strong asymmetry and negative allometry during ontogeny make ratchet sculpture a highly distinctive morphol-

ogy. Thus far, I have observed ratchet sculpture only in actively burrowing species among the Mitridae, Terebridae and Cerithiidae (the genus *Rhinoclavis*). This suggests that ratchet sculpture will be useful in the interpretation of the life habits of fossil gastropods. Unfortunately, the use of ratchet sculpture in paleoecology will be severely limited because ratchet sculpture is comparatively uncommon. Some non-burrowing crabs (e.g. *Grapsus grapsus* (Linnaeus, 1758); Schmalfuss, 1978a) and bivalves (*Bankia setacea* (Tryon, 1863); Röder, 1977) have evolved sculpture similar to ratchet sculpture, which the organisms use to wedge themselves against hard substrates. However, this sculpture is not negatively allometric during ontogeny which, along with other adaptations to hard substrates, permits these organisms to be distinguished from burrowers.

As mentioned in the introduction, ratchet sculpture has also evolved in the Bivalvia. Stanley (1969, 1970) and Seilacher (1972, 1973) have documented occurrences of ratchet sculpture in a variety of modern and extinct infaunal species. Ratchet sculpture has also evolved in the Brachiopoda (Seilacher, 1972), Crustacea (Seilacher, 1972, 1973; Schmalfuss, 1978a; Savazzi, 1981) and Trilobita (Stitt, 1976; Schmalfuss, 1978b), and has, in most cases, been inferred to aid in anchoring the organism in an unconsolidated substrate. A similar structure has also been noted in the carpoid echinoderms (or calcichordates) (Jeffries, 1975; Derstler, 1975; Jefferies & Lewis, 1978). Particularly interesting is the observation that *Calappa* (Weber, 1795), a burrowing crab that preys on both *Rhinoclavis aspera* and *Terebra dimidiata* (Vermeij, 1978; Vermeij et al., 1980) has also evolved ratcheted sculpture on its carapace (Schmalfuss, 1978a). The evolution of ratchet sculpture among these different taxa is one of the unusual examples of convergent evolution among marine invertebrates.

CONCLUSIONS

Artificially smoothened specimens of *Terebra dimidiata* and *Rhinoclavis aspera* take more time and burrowing cycles to burrow compared to controls or to unmodified animals. The sculpture on these species appears to perform a ratchet function, preventing back-slippage by locking the shell to the substrate while the snail is inserting its foot into the sediment but allowing free forward movement of the shell through the sediment. Ratchet sculpture is distinct from other sculptures because it combines strong asymmetry with constant size during the ontogeny of the animal. Ratchet sculpture may be useful for interpreting life habits of fossil gastropods, because it is morphologically distinct and limited to actively burrowing species.

Other authors have documented convergent features which evolved in the Brachiopoda, Crustacea, Trilobita and possibly the Echinodermata. Thus, ratchet sculpture is a common solution to the functional needs of a diverse range of burrowing organisms.

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EFFETS DES CONDITIONS D'ÉCLAIREMENT SUR LE POTENTIEL REPRODUCTEUR DE *LYMNAEA STAGNALIS* (GASTÉROPODE PULMONÉ)

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RÉSUMÉ

La fécondité de *Lymnaea stagnalis* a été étudiée sous courte et longue photophase, sous différentes intensités lumineuses, sous cinq lumières colorées et à l'obscurité.

La fécondité cumulée des limnées élevées sous courte photophase (LD 7:17) est toujours plus élevée (9.6% à 89.5%) que celle des animaux élevés sous longue photophase (LD 16:8). Cette différence peut être expliquée par un accroissement de la longévité qui conduit à un plus grand nombre de pontes par animal.

La fécondité n'est proportionnelle à l'intensité lumineuse que sous longue photophase.

Par rapport à leur témoin respectif, la fécondité cumulée n'est réduite que sous la lumière jaune (−13.4%); elle est augmentée dans les 4 autres cas: +35.7% (bleu), +47.4% (vert), +61.8% (U.V.) et +77% (rouge). A l'exception du cas de la lumière jaune, la durée de la période de ponte et le nombre de pontes par animal sont augmentés. Nos résultats montrent que les cinq lumières colorées sont perçues par les limnées.

Une réduction de la fécondité, due essentiellement à un effet sur le nombre de pontes pondues par animal, est observée lors de l'élevage à l'obscurité.

Le "Potentiel Limnée" (P.L. = volume de la coquille × fécondité cumulée) est plus élevé quand les animaux sont élevés sous jours courts. Le P.L. n'est proportionnel à l'intensité lumineuse que chez les limnées élevées sous jours longs et il atteint une valeur paradoxale chez celles qui sont élevées à l'obscurité. Le P.L. est réduit chez les animaux élevés sous lumière jaune; il est au contraire augmenté dans les quatre autres cas de lumière colorée. Ceci met en évidence un effet spécifique de ces lumières.

Mots-clés: *Lymnaea stagnalis*; fécondité; photophases; intensités lumineuses; lumières colorées; obscurité.

INTRODUCTION

Dans des travaux antérieurs, nous avons montré, chez *Lymnaea stagnalis* (Linn.) l'existence d'importants troubles de la croissance et de la fécondité d'une part lors d'intoxications à long terme par divers toxiques (Bluzat et al., 1979; Bluzat & Seugé, 1981; Seugé & Bluzat, 1979 et sous presse), d'autre part lors de la variation d'un facteur du milieu tel que la minéralisation de l'eau (Seugé, 1980; Seugé & Bluzat, 1982a). Dans une étude précédente (Seugé & Bluzat, 1982b), nous avons examiné l'influence de diverses conditions d'éclairement sur la croissance de ce Mollusque; nous nous proposons ici d'exposer les effets sur sa fécondité de la photophase, de l'intensité lumineuse en lumière blanche, de l'obscurité et de cinq lumières colorées.

A notre connaissance, les données bibliographiques relatives à ces sujets sont peu nombreuses chez les Gastéropodes: Deschiens & Byan (1956), Deschiens (1957), Gaud (1958), de Witt & Sloan (1960), Yung (1911; Franc, 1968: 498), Graber (1884; Franc, 1968: 498, 499), Liche (1934a, 1934b) et Carmichael (1933; Franc, 1968: 499), analysées par Franc (1968), Van der Steen (1967), Bohlken et al. (1978) et Dogterom (1980): les informations qui peuvent en être recueillies sont souvent contradictoires. Les différences de méthodologie, la durée d'observation et l'espèce étudiée en particulier, peuvent expliquer, en partie, les divergences constatées mais les auteurs modernes (Van der Steen, 1967; Bohlken et al., 1978; Dogterom, 1980 & Seugé, 1980) s'accordent pour souligner, chez *Lymnaea stagnalis,* l'importance des conditions d'éclairement sur la reproduction.

MATÉRIEL ET MÉTHODE

L'animal utilisé est le Mollusque Gastéropode Pulmoné *Lymnaea stagnalis* élevé

depuis plusieurs générations au laboratoire dans l'eau de ville (pH: voisin de 7,5; dureté totale: 230 mg $CaCO_3$/litre; 20° ± 0,5 C; aération permanente); l'alimentation est assurée par des feuilles de laitue fournies en quantité suffisante et l'eau est renouvelée chaque semaine. Toutes les expériences exposées ci-dessous sont réalisées simultanément avec des animaux groupés dans un bac de verre contenant 2 litres d'eau. Trois cents jeunes limnées sont mises en expérience le jour de leur éclosion; à cinq semaines elles sont mesurées pour la première fois et seuls les 40 individus de plus grande taille sont conservés. Une deuxième sélection est opérée de la même façon un mois plus tard: chaque lot est alors constitué de 12 limnées qui sont maintenues en expérience jusqu'à ce que 50% d'entre elles soient mortes; l'étude de chaque lot est donc arrêtée quand le nombre de limnées vivantes est <5. Dans tous les cas les animaux sont exposés dès l'éclosion aux conditions lumineuses étudiées.

Des élevages sont réalisés sous lumière blanche (tubes Mazda "blanc industrie" TFR/40/BBL) soit en longue photophase LD 16:8 séries 16, soit en courte photophase LD 7:17 séries 7 et dans les deux cas sous différentes intensités lumineuses:

—1200 lux: séries 16-1 et 7-1,
— 180 lux: séries 16-2 et 7-2,
— 10 lux: séries 16-3 et 7-3,
— 50 lux: série 16-4.

En plus un lot a été élevé à l'obscurité quasi-permanente (série 0).

Les élevages sous lumières colorées ont tous été réalisés sous une photophase LD 16:8: les gammes de radiations sont obtenues par des tubes lumineux spéciaux entourés d'un filtre de rhodoïd "R.P." de 0,25 mm d'épaisseur. Les tubes et les filtres utilisés sont les suivants:

—lumière ultraviolette (U.V.): tubes TFA/4L/5 Mazda; pas de filtre; λ = 380–440 nm; 360 lux (témoin: 16-2).
—lumière bleue (B): tubes TF/40 Bleu Mazda; rhodoïd n° 2005; λ = 430–480 nm; 230 lux (témoin: 16-2).
—lumière verte (V): tubes TF/40 Vert Mazda; rhodoïd n° 2003; λ = 500–550 nm; 360 lux (témoin: 16-2).
—lumière jaune (J): tubes TF/40 Jaune Mazda; rhodoïd n° 222; λ = 550–630 nm; 1300 lux (témoin: 16-1).
—lumière rouge (R): tubes TL/40/15 Philips; rhodoïd n° 227; λ =630–670 nm; 50 lux (témoin: 16-4).

Dans tous les cas les intensités lumineuses ont été mesurées au niveau de la surface de l'eau; il est impossible d'apprécier avec exactitude la quantité de lumière reçue par chaque animal en fonction de sa position dans l'aquarium (dissimulation sous la salade, niveau de l'enfoncement entre autres).

Les pontes sont prélevées et dénombrées chaque semaine; le nombre des oeufs dans les pontes est compté sous stéréomicroscope. Les résultats sont donc enregistrés chaque semaine, à partir des premières pontes et jusqu'à la mort de 50% des animaux dans chacun des lots; les moyennes hebdomadaires (nombre d'oeufs/nombre d'animaux; nombre de pontes/nombre d'animaux; nombre d'oeufs/nombre de pontes) permettent de calculer pour chaque lot en effectuant la somme de 4 moyennes hebdomadaires la valeur mensuelle du:

—nombre moyen d'oeufs par animal (f),
—nombre moyen de pontes par animal.

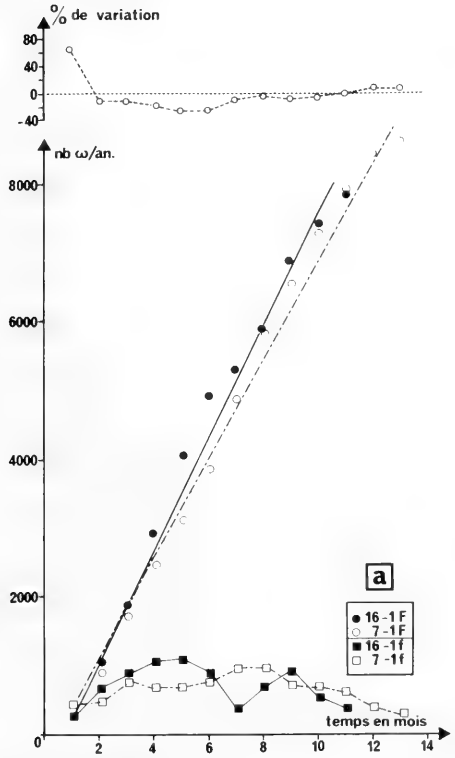
L'évolution de la fécondité cumulée (F) et du nombre cumulé de pontes (P) est ensuite étudiée en fonction de l'âge puis en fonction de la taille; par ailleurs, le nombre moyen d'oeufs par ponte (Nm) est calculé pour toute la période de ponte.

Le rendement biologique de l'espèce est enfin apprécié par le calcul du produit: volume de la coquille (V en mm^3) × fécondité cumulée (F), auquel nous donnons le nom de "Potentiel Limnée" (P.L.) (Seugé, 1980).

RÉSULTATS

La Fig. 1 montre l'influence de la durée de la photophase, de l'intensité lumineuse et de

FIG. 1. Analyse de la fécondité en fonction du temps (nb ω/an: nombre d'oeufs par animal): influence de la longueur de la photophase sous trois intensités lumineuses. 1a = intensité lumineuse 1200 lux; 1b = intensité lumineuse 180 lux; 1c = intensité lumineuse 10 lux et obscurité; Partie inférieure des graphiques; fécondité mensuelle (f); Partie médiane des graphiques: fécondité cumulée (F); Partie supérieure des graphiques: pourcentage de variation de F (LD 7:17 = séries 7)—par rapport à F (LD 16:8 = séries 16): 1a et 1b.—par rapport à F (0): 1c. 1d, e et f: variations mensuelles du nombre moyen de pontes émises par limnée sous longues (séries 16) et courtes (séries 7) photophases et sous différentes intensités lumineuses: 1200 lux (1d), 180 lux (1e), 10 lux et obscurité (1f).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a
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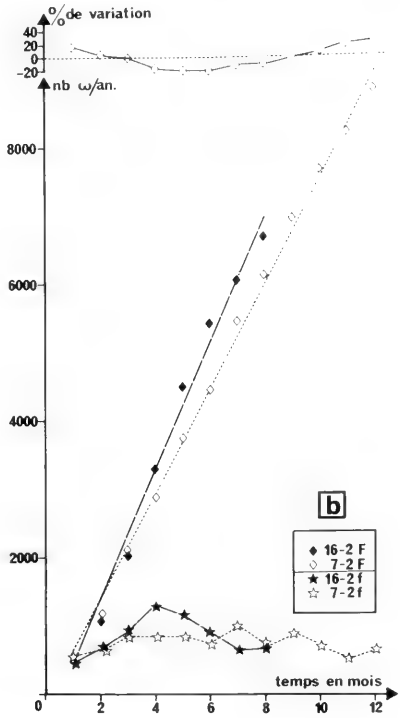
temps en mois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b
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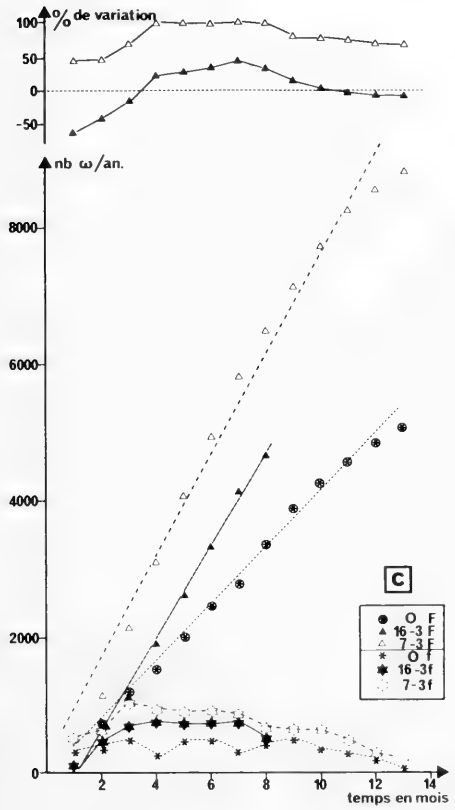
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7-3 F
O f
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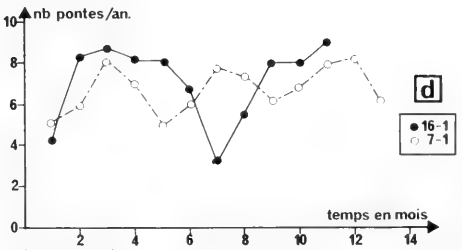
7-3 f
temps en mois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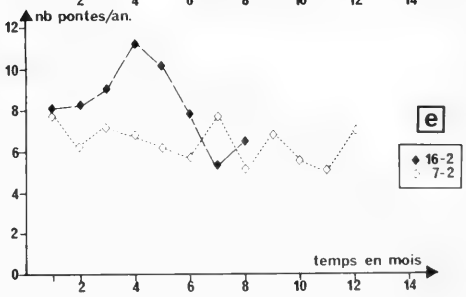
d
16-1
7-1
temps en mois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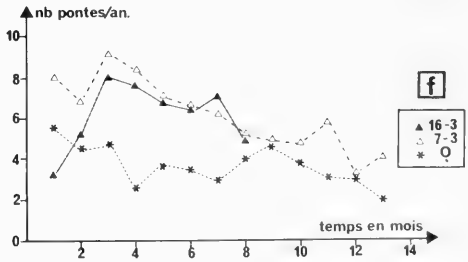
e
16-2
7-2
temps en mois


nb pontes/an.
f
16-3
7-3
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temps en mois

l'obscurité sur la fécondité: 1a partie inférieure du graphique 1a (1200 lux) indique que la fécondité mensuelle fluctue en fonction du temps sous les deux photophases; la partie médiane, fécondité cumulée en fonction de l'âge, souligne que les animaux 7-1 pondent au total plus que les autres.

De même les Fig. 1b (180 lux) et 1c (10 lux) amènent à des conclusions identiques dans le cas des faibles intensités; le graphique 1c présente en plus l'évolution de la fécondité des animaux élevés à l'obscurité et démontre qu'ils pondent au total un peu plus que les animaux 16-3 mais beaucoup moins que les animaux 7-3.

Une seule des deux composantes de la fécondité, le nombre moyen de pontes émises par animal, est réellement modifiée:

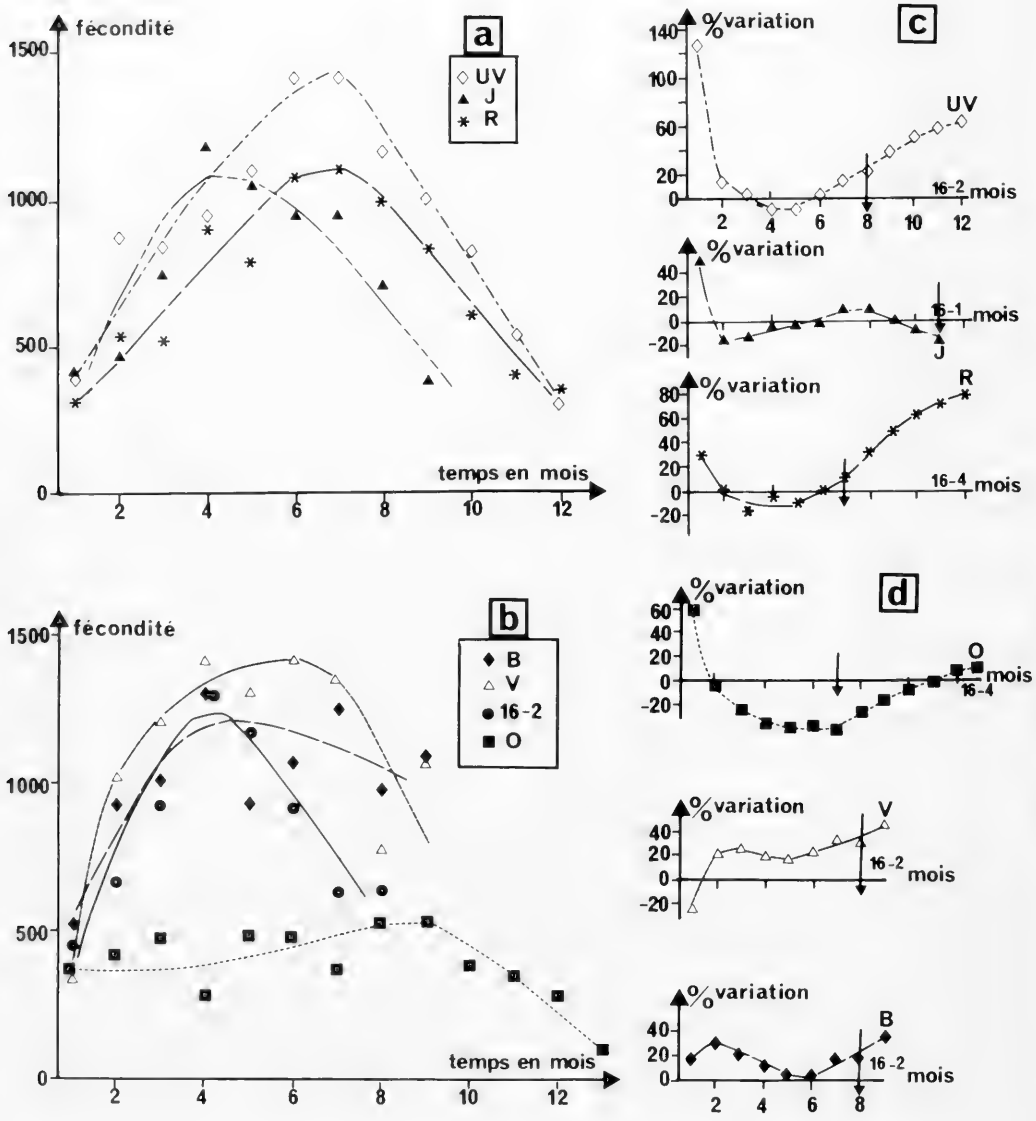

FIG. 2. Analyse de la fécondité en fonction du temps: influence de la couleur de la lumière. 2a et 2b: évolution de la fécondité mensuelle (f); 2c et 2d: pourcentages de variation de la fécondité cumulée en fonction du témoin respectif (le moment de la mort de celui-ci est précisé par une flèche).

les courbes 1d, e et f en présentent les variations mensuelles dans chaque cas.

La Fig. 2 montre l'influence des diverses gammes de longueurs d'onde sur la fécondité. Les graphiques 2a et 2b précisent l'évolution de la fécondité mensuelle dans les différents groupes. Dans trois cas (U.V., Jaune et Rouge, Fig. 2a) cette évolution se traduit, comme pour la lumière blanche, par la succession d'une phase ascendante, d'un maximum de fécondité et d'une phase descendante; ces trois phases n'existent pas distinctement quand les limnées sont élevées sous des lumières bleue et verte ainsi qu'à l'obscurité (Fig. 2b).

Les graphiques 2c et 2d indiquent, en pourcentages, les fluctuations de la fécondité cumulée en fonction du temps par rapport à celle du témoin respectif; à l'exception du cas de la lumière jaune ces variations sont importantes, le plus souvent dans le sens positif.

Les données de la fécondité cumulée en fonction de l'âge permettent, pour simplifier, de tracer des droites qui sont rassemblées dans la Fig. 3a: la fécondité des animaux élevés à l'obscurité se distingue nettement de celle des autres groupes.

Les graphiques 3b, c et d montrent l'évolution de la fécondité cumulée en fonction de la taille après transformation log-log: cette représentation souligne que pour une taille donnée la fécondité varie avec les conditions d'éclairement.

Le Tableau 1 permet de confronter le bilan de la fécondité des limnées élevées sous les différentes conditions d'éclairement; il rappelle en outre la taille moyenne maximale observée des coquilles (Seugé & Bluzat, 1982b) et indique enfin la valeur du "Potentiel Limnée" dans chaque cas.

Le Tableau 2 rassemble les résultats relatifs à la fertilité des oeufs, à la durée moyenne

TABLEAU 1. Fécondité des limnées élevées dans différentes conditions d'éclairement (12 limnées par groupe).

Groupes expérimentaux et témoins	Age début ponte (mois)	Durée ponte (mois)	F	z	P	Nm	T_{mx} ob (mm)	P.L. $\times 10^7$
16-1	2,25	11	7882	9,2	78,5	100,4	38,9	4,3
16-2	2	8	6696	8,8	67,4	99,3	36,5	3,4
16-3	2,5	8	4655	7,9	49	95	36,3	2,5
16-4	2,25	7	4723	7,7	54,5	86,8	36,9	2,4
7-1	3	13	8637	12,9	87,6	98,6	42	6,6
7-2	2,75	12	8915	9,5	78,2	114	41,7	7,2
7-3	2,5	13	8821	8,3	81,9	107,7	42,7	7,5
0	2,5	13	5107	5,7	49,2	103,8	45,9	5,3
U.V. (T 16-2)	2	12	10832	10,7	108,6	99,7	39,8	7,2
Bleu (T 16-2)	2	9	9089	14,1	94,6	96,1	35,5	3,8
Vert (T 16-2)	2	9	9871	14,1	85,6	115,3*	38,1	4,8
Jaune (T 16-1)	2	9	6827	10	81,2	84,1*	40,3	3,7
Rouge (T 16-4)	2	12	8362	10,2	92,9	90	42	6,3

Groupes 16-1, 16-2, 16-3 et 16-4: longue photophase (LD 16:8) pour des intensites 1200, 180, 10 et 50 lux. Groupes 7-1, 7-2 et 7-3: courte photophase (LD 7:17) pour des intensités de 1200, 180 et 10 lux. Group 0: élevage à l'obscurite. Groupes U.V., Bleu, Vert, Jaune et Rouge: lumières colorées (LD 16:8); pour chaque cas le témoin lumière-blanche est indiqué entre parenthèses. Valeurs moyennes par limnée de:

—F: fécondité cumulée;
—z: pente de la droite log (F) = z log (T) + log (a);
—P: nombre cumulé de pontes émises par animal;
—Nm: nombre moyen d'oeufs par ponte;
P.L.: "Potentiel Limnée."
—*: la comparaison des moyennes mensuelles montre que les séries J et 16-1 d'un côté, V et 16-2 d'un autre diffèrent significativement (test t avec $P < 0.01$).

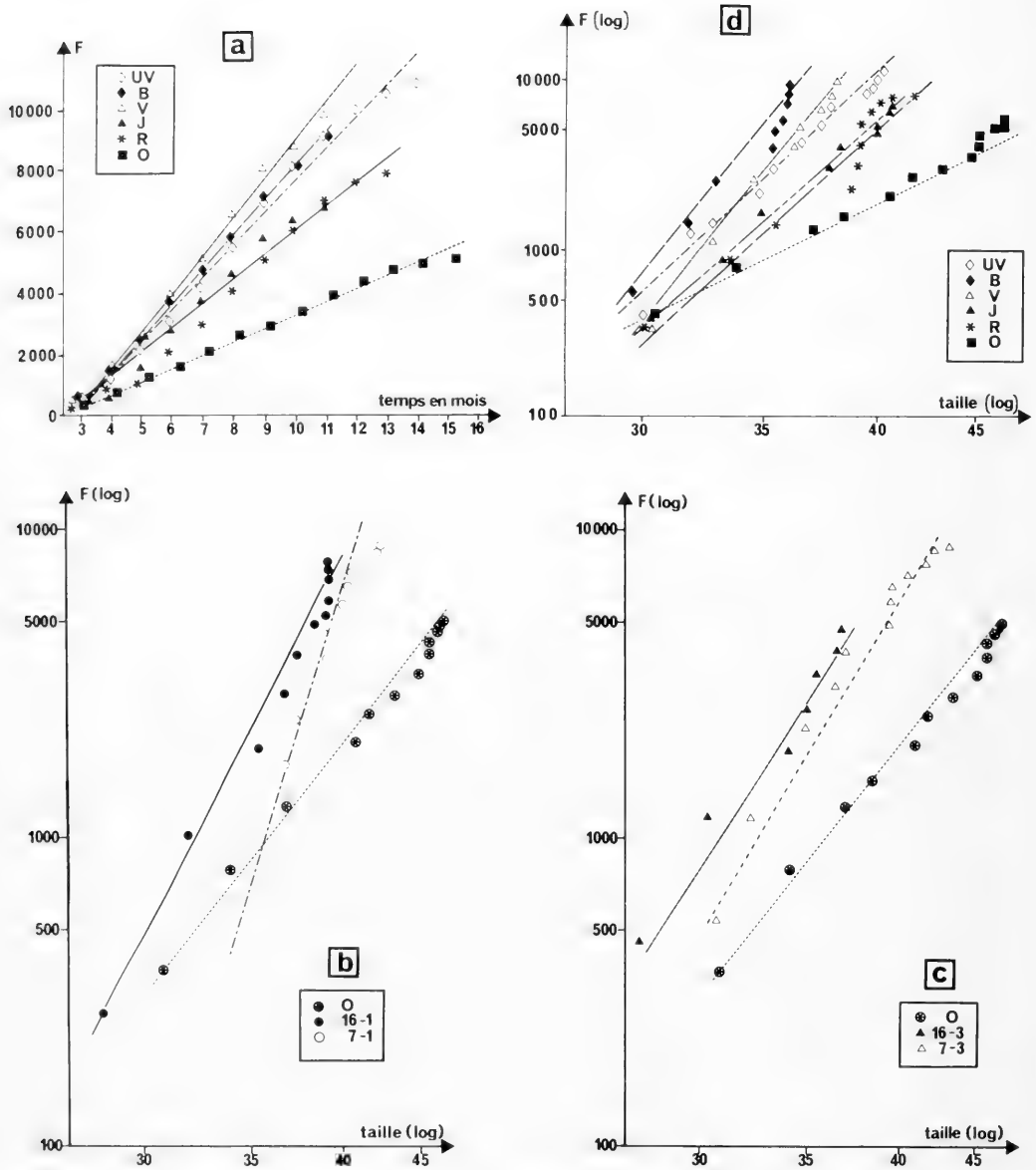

FIG. 3. Analyse de la fécondité cumulée (F). 3a: en fonction de l'âge des animaux, cas des animaux élevés sous les lumières colorées et à l'obscurité; 3b, c et d: en fonction de la taille (T) après transformaton log. log. des données; influence de la photophase en forte (3b) et faible (3c) intensité lumineuse; influence des lumières colorées (3d).

de l'embryogenèse et à la fréquence des anomalies observées (embryons doubles ou multiples); précisons que les oeufs ont été incubés dans les conditions exactes où les parents ont été élevés.

CONCLUSIONS ET DISCUSSION

Le potentiel reproducteur des limnées soumises à différentes conditions lumineuses sera analysé en envisageant la durée de la

TABLEAU 2. Fertilité des oeufs, temps moyen d'éclosion (T.M.E.) et pourcentages d'anomalies (embryons doubles et multiples) en fonction des différentes conditions lumineuses (incubation des oeufs dans les conditions d'origine). Pour les groupes: idem Tableau 1.

Groupes expérimentaux et témoins	Fertilité		TME (jours)	Anomalies	
	Nombre d'oeufs	% éclosion		Nombre d'oeufs	%
16-1	1466	83,6	22	1606	0,68
16-2	1865	92,1	21	5825	0,21
16-3	1125	86,2	22	4328	0,15
16-4	1204	86,7	22	7982	0,11
7-1	1605	80,9	21	3829	0,18
7-2	1911	91,6	21	7216	0,18
7-3	1155	86,3	22	8087	0,05
Obscurité	989	66,5	22	2277	0,66
U.V.	2286	97,1	25	5615	0,19
Bleu	3305	85	20	5944	0,15
Vert	2525	91,2	19	7424	0,45
Jaune	3490	97,7	22	7932	8,76
Rouge	3153	92,2	19	3518	0,17

période de ponte puis la fécondité; enfin, nous tenterons de réaliser une synthèse des effets de la lumière sur la croissance et la fécondité.

Le fécondité peut être étudiée selon deux méthodes; d'abord en envisageant l'évolution des données mensuelles (f) puis celle des données cumulées. L'étude de la fécondité cumulée (F) peut être effectuée en fonction de l'âge des animaux ou en fonction de leur taille. Dans le premier cas plusieurs méthodes d'appréciation peuvent être envisagées, soit après un temps donné (5 à 6 mois par exemple), soit à la mort des témoins, soit à la mort des animaux expérimentaux. Selon nous, la comparaison du potentiel reproducteur des animaux expérimentaux et témoins ne doit être effectuée qu'après leur mort pour prendre en compte, entre autre, le problème de la longévité. Dans le second cas la fécondité cumulée (F) peut être exprimée en fonction de la taille (T) sous la forme $F = aT^z$ selon la formule proposée par Levina (1973) où z est la pente de la droite log (F) = z log (T) + log (a).

Période de ponte

L'âge des limnées lors des premières pontes (Tableau 1) varie seulement en fonction de la longueur de la photophase: un retard de trois semaines est observé dans le cas de la photopase courte (LD 7:17). Cette conclusion est en accord avec celle de Bohlken et al. (1978) chez *Lymnaea stagnalis* mais est différente de celle de de Witt & Sloan (1960) chez *Physa pomilia*. Dans les conditions extrêmes d'intensité lumineuse (16-3, 7-3) la longeur de la photophase ne semble plus intervenir.

La durée de la période de ponte, qui varie entre 7 et 11 mois sous jours longs, est nettement plus longue aussi bien sous jours courts (12 ou 13 mois) qu'à l'obscurité (13 mois). Relativement au témoin lumière-blanche respectif toutes les possibilités sont observées dans le cas des lumières colorées: raccourcissement de la période de ponte (lumière jaune: 2 mois), allongement faible (lumière bleue et verte: 1 mois) ou important (lumière U.V.: 4 mois et rouge: 5 mois).

Remarquons que, dans tous les cas, les animaux pondent jusqu'à leur mort; les différences notées ci-dessus sont donc dues à l'influence de la photophase sur leur longévité comme nous l'avons montré par ailleurs (Seugé, 1980; Seugé & Bluzat, 1982b).

Influence de la photophase

L'évolution de la fécondité mensuelle (f) se traduit par une courbe en cloche (Fig. 1a, b et c); un maximum d'environ 1100 oeufs par

mois est atteint (16-1) et en règle générale la fécondité des animaux élevés en jours longs (LD 16:8) est supérieure à celle des animaux élevés en jours courts.

Au contraire l'examen de la fécondité cumulée (F) fait ressortir que, dans tous les cas, la production d'oeufs est, au total, plus importante en courte photophase (LD 7:17) qu'en longue photophase (Tableau 1): (F) est augmentée de 9.6% (16-1, 7-1), de 33.1% (16-2, 7-2), de 89.5% (16-3, 7-3). Pour l'essentiel, cette augmentation est due à celle du nombre de pontes (P) par animal liée à la plus grande longévité des animaux.

Par ailleurs, la Fig. 3 (b et c) permet de constater que, pour une taille donnée, les animaux élevés sous courte photophase pondent moins que les autres; cet effet ne tend à s'effacer que dans la série 7-1 où la valeur anormalement élevée da la pente z (12.9) traduit nettement que l'équilibre mis en évidence entre la croissance et la fécondité (Geraerts, 1976a, b) n'existe plus dans ce cas (Seugé, 1980).

Notre méthode d'étude, se basant essentiellement sur la fécondité cumulée, peut sans doute expliquer nos divergences avec les auteurs ayant abordé ce problème (Van der Steen, 1967; Bohlken et al., 1978; Dogterom, 1980 et Joosse, 1980).

Influence de l'intensité lumineuse

Alors qu'aucun effet n'est enregistré en jour court (LD 7:17), la fécondité cumulée parait directement liée à l'intensité lumineuse en jour long (LD 16:8): −15% (16-1, 16-2), −40% (16-1, 16-4) et −41% (16-1, 16-3). La comparaison (Tableau 1) des séries 16-1, 16-2, 16-3 et 16-4 indique que la diminution de la fécondité est due à la réduction du nombre de pontes (P) émises par animal liée à une longévité plus faible.

La confrontation des résultats des séries 16-3, 7-3 et 0 (Fig. 3c) montre qu'une intensité lumineuse aussi faible que 10 lux est perçue très différemment de l'obscurité. Cette conclusion rejoint celle de Van der Steen (1967) qui démontre, pour sa part, que les limnées sont sensibles à une intensité lumineuse de 1 lux.

Influence de l'obscurité

La fécondité mensuelle est caractérisée par des valeurs demeurant inférieures à 500 oeufs par animal pendant toute la période de ponte. La fécondité cumulée des animaux 0 est toujours faible: environ −42% en moyenne relativement aux animaux élevés sous courte photophase. Cette diminution est due spécifiquement à un nombre plus réduit de pontes (P) puisque, dans ce cas, une différence de longévité ne peut être invoquée. De plus les oeufs pondus et incubés ont une fertilité significativement abaissée (Tableau 2).

L'influence de l'obscurité sur la fécondité des Gastéropodes a déjà été envisagée; elle semble très variable: elle est sans effet chez *Australorbis glabratus* (Deschiens & Byan, 1956) et chez *Bulinus truncatus* (Deschiens, 1957, et Gaud, 1958) mais a une action dépressive bien marquée chez *Bulinus contortus* (Deschiens & Byan, 1956) et chez *Lymnaea stagnalis* (Van der Steen, 1967). Un arrêt complet de la ponte n'a, malgré tout, jamais été observé; ce fait peut être expliqué par le travail de Roubos (1975) qui démontre que chez des limnées aveuglées l'hormone d'ovulation continue d'être secrétée mais que son rythme d'élaboration est supprimé.

Influence des lumières colorées

Dans l'ignorance des mécanismes récepteurs en jeu, nous n'avons pas tenu compte, lors de cette première investigation, du rendement énergétique de chacune des gammes de radiations utilisées (Seliger & McElroy, 1965). Nous nous sommes limités à établir des comparaisons avec des témoins élevés sous lumière blanche (LD 16:8), à des intensités lumineuses de même ordre que celles réalisées pour les lumières colorées.

L'étude de la fécondité mensuelle (Fig. 2a,b) montre que le pic de fécondité maximale correspond rigoureusement à celui du témoin dans le cas de la lumière jaune; ce pic est plus tardif pour les animaux élevés sous lumières U.V. et rouge. Dans le cas des lumières bleue et verte la courbe est atypique. Le niveau des moyennes mensuelles maximales (jusqu'à plus de 1400 oeufs par limnée dans les cas U.V. et vert) souligne que nos conditions d'élevage ne gênent en rien l'expression de la fécondité des animaux.

L'étude de la fécondité cumulée en fonction de l'âge (Figs. 2c,d, 3a) permet de conclure qu'à l'exception du groupe élevé sous lumière jaune la production d'oeufs est plus forte sous les lumières colorées que celle du témoin respectif: +36% (bleu), +47% (vert), +62% (U.V.) et +77% (rouge); la faible élévation de

température observée dans un seul cas (lumière rouge: eau = 21°C) ne peut expliquer ces résultats. Dans deux cas (lumière jaune et lumière verte) le nombre moyen d'oeufs par ponts (Nm), soit diminué de 16% (16-1, J) soit augmenté de 16% (16-2, V), rend compte des variations de la fécondité. Dans les trois autres cas, la fécondité plus élevée est liée, au contraire, à un nombre plus important de pontes émises par animal (P) qui, lui-même, peut être expliqué tantôt par une longévité plus importante (U.V. et rouge) tantôt par un effet spécifique (bleu).

La Fig. 3d, fécondité en fonction de la taille, fait ressortir d'une part, l'effet spécifique de la lumière bleue (fécondité plus importante pour une taille donnée), d'autre part, que toutes les lumières colorées sont perçues par les limnées (les diverses valeurs de z étant largement supérieures à celle qui caractérise l'obscurité).

Dans la mesure où nous avons établi précédemment, que sous longue photophase, une diminution de l'intensité lumineuse se traduit par une baisse de la fécondité les résultats obtenus dans les élevages sous lumières colorées soulignent l'existence d'un effet spécifique de ces gammes de radiations. Précisons par ailleurs, que, si la fertilité des oeufs n'est pas atteinte, la durée de leur embryogenèse est modifiée (Tableau 2) dans trois cas; nos résultats relatifs à la fertilité sont en contradiction avec ceux de Yung (1878) qui signale même l'impossibilité d'obtenir un développement sous lumière verte. Dans l'ensemble de ces expériences seul l'élevage sous lumière jaune a provoqué un fréquence anormalement élevée (8.76%) d'anomalies de l'embryogenèse.

A notre connaissance deux auteurs seulement ont étudié l'influence de la couleur de la lumière sur la fécondité des Gastéropodes: Gorf (1963) chez *Viviparus viviparus* et Van der Steen (1967) chez *Lymnaea stagnalis*. D'après le premier auteur les lumières orange et rouge stimulent la ponte alors que, pour le second, l'élevage sous les lumières verte et rouge n'entraîne aucune modification de la fécondité mais, selon nous, le temps d'observation extrêmement court (3 jours) explique facilement sa conclusion.

Nous disposons par ailleurs de quelques informations anciennes sur le comportement de *Lymnaea* sous des lumières colorées: pour Graber (1884: Franc, 1968: 498, 499) cet animal perçoit le bleu mais pas le rouge; selon Liche (1934a,b) la limnée distingue le bleu et le rouge; enfin d'après Carmichael (1933: Franc, 1958: 499) les Pulmonés ne perçoivent pas la lumière U.V., sauf peut être *Limax flavus*.

"Potentiel limnée"

Dans le but d'intégrer les effets des conditions d'éclairement sur la croissance (Seugé & Bluzat, 1982b) et sur la fécondité (présents résultats), nous avons tenté de déterminer le rendement biologique des limnées à la fin de leur vie, ce qui permet d'établir des comparaisons entre les différents groupes considérés. Dans ce but, nous calculons le produit du volume moyen atteint par la coquille au terme de l'expérience (V) par la fécondité cumulée (F): P.L. = V × F.

Les valeurs du P.L. (Tableau 1) montrent que, dans nos expériences, les courtes photophases (LD 7:17) sont de ce point de vue de meilleures conditions de vie que les longues photophases (LD 16:8): l'augmentation enregistrée est de 53,5% (1200 lux), 111,8% (180 lux) et 300% (10 lux). Une relation entre la valeur du P.L. et celle de l'intensité lumineuse n'a pu être établie que dans le cas des élevages sous longue photophase; il augmente alors de 36% entre 10 et 180 lux et de 26,5% entre 180 et 1200 lux. Les limnées élevées à l'obscurité, malgré leur faible fécondité, ont un rendement biologique (5.3×10^7) intermédiaire entre celui des animaux maintenus sous longue photophase (2.5×10^7) et celui des animaux élevés sous courte photophase (7.5×10^7). Ces résultats nous conduisent à conclure que l'élevage à l'obscurité ne peut être rapproché d'un élevage sous faible intensité lumineuse ni en photophase longue, ni en photophase courte.

Dans le cas des lumières colorées, nous constatons, par rapport au témoin respectif, une augmentation des valeurs du P.L.: de 12% (bleu), 41% (vert), 112% (U.V.) et 158% (rouge); un cas fait exception, celui de la lumière jaune où la valeur du P.L. est plus faible que celle de son témoin (−14%). Ces variations sont imputables soit à un effet dépresseur sur la fécondité (jaune) soit à une augmentation de la croissance et de la fécondité (U.V., vert et rouge), soit enfin à la résultante de deux effets antagonistes (bleu).

Différents articles montrent qu'en règle générale les Invertébrés sont sensibles aux conditions lumineuses (Charles, 1966; Gardiner, 1972; Lickey et al., 1976; Stoll et al., 1976). Chez les Insectes, celles-ci ont une

influence déterminante sur le cycle biologique (Saunders, 1976) mais les travaux les plus récents n'ont pas encore pu élucider la totalité des mécanismes en jeu.

Notre étude souligne l'importance de ces facteurs sur la longévité, la croissance (Seugé & Bluzat, 1982b) et la fécondité (présents résultats) du Mollusque *Lymnaea stagnalis* élevé au laboratoire ce qui peut contribuer à faciliter la compréhension du comportement de cette espèce dans son milieu naturel.

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EFFECTS OF LIGHT CONDITIONS ON THE REPRODUCTIVE POTENTIAL OF *LYMNAEA STAGNALIS* (GASTROPODA: PULMONATA)

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ABSTRACT

The fecundity of the snail *Lymnaea stagnalis* reared under short and long photophases, various intensities, five coloured lights and darkness was studied. The overall fecundity of snails reared under short photophase (LD 7:17) was always greater (9.6% to 89.5%) than that of snails reared under long photophase (LD 16:8). This difference could be explained by an increased longevity which led to an increased number of egg masses per snail. The fecundity was not related to the light intensity during short days whereas it was proportional to this factor during long days.

The overall fecundity was only reduced (−13.4%) under the yellow light and was increased under the others: +35.7% (blue), +47.4% (green), +61.8% (U.V.) and +77% (red) when each experimental group was compared to its control. The yellow light excepted, the number of egg-masses laid per snail was always enhanced and the laying period was increased significantly under U.V. and red lights.

Our results show that the five coloured lights were discerned by the snails. Rearing in continuous darkness induced a decrease of the fecundity due essentially to a specific effect on the number of egg-masses laid per snail.

The "*Lymnaea* Yield": P.L. (shell volume × overall fecundity) was higher when the snails were reared during short days. The P.L. was proportional to the light intensities studied only in the snails reared during long days and it reached a paradoxical value in the snails reared in darkness. The P.L. was reduced under the yellow light and in the four other cases of coloured light it was increased; this points out a specific effect of these coloured lights.

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DONNÉES ANALYTIQUES SUR LES CONCRÉTIONS DU TISSU CONJONCTIF DE QUELQUES GASTÉROPODES D'EAU DOUCE

Micheline Martoja[1] et Michel Truchet[2]

RÉSUMÉ

Les concrétions du tissu conjonctif de cinq espèces de Gastéropodes Prosobranches et Pulmonés d'eau douce ont été analysées par spectrographie des rayons X, émission ionique secondaire et spectrométrie Raman, sur coupes histologiques de 6 à 10 μm d'épaisseur. Le carbonate de calcium a été identifié chez *Valvata cristata* et *Planorbarius corneus*, le phosphate de calcium chez *Viviparus viviparus* et *Bithynia tentaculata*; de petits cristaux à carbonate de calcium et des sphérocristaux à phosphate coexistent chez *Lymnaea peregra*. La composition peut être simple (calcite pure chez *Valvata*) ou complexe (quatre sels et six éléments décelés chez *Lymnaea*). Elle est sans rapport avec la position systématique des espèces.

Mots-clés: Gastéropodes; conjonctif; concrétions; calcium; microanalyse.

INTRODUCTION

Depuis leur découverte par Barfurth (1881), les sphérocristaux du tissu conjonctif des Gastéropodes, qui peuvent être groupés autour des artères ou dispersés dans le manteau, la masse viscérale et le pied, sont considérés comme étant formés de carbonate de calcium. A cet égard, il est classique de les opposer à ceux de la glande digestive qui sont constitués de phosphate de calcium (voir Manigault, 1960). La présence de carbonate de calcium a été confirmée depuis lors (Kapur & Gibson, 1968; Timmermans, 1969; Richardot & Wautier, 1972; Sen Gupta, 1977), mais les méthodes d'analyse plus précises montrent que leur composition élémentaire peut être complexe (Tompa & Watabe, 1976; Sminia et al., 1977; M. Martoja et al., 1980). D'autre part, le tissu conjonctif fait parfois fonction de rein d'accumulation et ses concrétions sont alors de nature purique. L'exemple de *Pomatias* pourrait n'être pas unique; il en irait de même des cellules interstitielles de la glande digestive d'*Helix* (Abolins-Krogis, 1960) et du tissu conjonctif péri-rénal de *Viviparus* (Andrews, 1979).

Il nous a donc paru intéressant de reprendre l'analyse de ces sphérocristaux par des procédés physiques récemment adaptés à l'étude de coupes de tissus animaux, Comme exemple, nous avons choisi des Gastéropodes d'eau douce. C'est, en effet, chez ceux-ci que les concrétions du tissu conjonctif sont les plus spectaculaires, ainsi que l'a signalé Cuénot (1899).

MATÉRIEL ET MÉTHODES

La durée des analyses spectrales à la microsonde Raman et la disponibilité encore très limitée de l'appareil ne nous ont pas permis d'examiner un grand nombre d'animaux. La recherche de variations affectant les bioaccumulations n'a donc pas été abordée. Nous avons préféré multiplier les espèces et notre étude a porté sur trois Prosobranches Mésogasteropodes et deux Pulmonés Basommatophores récoltés et fixés dans les conditions suivantes: *Viviparus viviparus* (L.) (Viviparoidea), 1 individu fixé au formol, récolté en septembre dans le canal du Nivernais; *Valvata cristata* Müll. (Valvatoidea), 2 individus fixés l'un au formol, l'autre par le mélange de Carnoy, récoltés fin octobre dans le Léman; *Bithynia tentaculata* (L.) (Rissooidea), 2 individus fixés par le mélange de Carnoy récoltés l'un en juin dans la rivière Saône, l'autre fin octobre dans le Léman; *Lymnaea (Radix) peregra* (Müll.) (Lymnaeoidea), 2 individus fixés par le mélange de Carnoy, récoltés dans le Léman, l'un en juillet, l'autre en octobre; *Planorbarius (Coretus) corneus* (L.) (Planorboidea), 2 individus fixés l'un par le mélange de Carnoy,

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l'autre par le glutaraldéhyde, récoltés en septembre dans une mare de l'Ile de France. (Nomenclature et orthographe conformes à celles qui figurent en Macan, 1969.)

Les animaux ont été fixés sitôt après leur capture, puis inclus à la paraffine et débités en coupes de 6 à 10 μm d'épaisseur. Les sphérocristaux ont été repérés sur des coupes colorées par le rouge solide-picro-indigocarmin, voisines de celles qui ont été analysées par procédés physiques. Dans certains cas, nous avons fait appel à des méthodes histochimiques: la coloration par la laque d'alizarine (Dahl et McGee-Russell) et la méthode de substitution par l'argent (Von Kossa) ont été employées pour la mise en évidence des accumulations calciques. Les méthodes au ferricyanure ferrique (Schmorl) et à l'argentométhénamine (Gomori) ont été utilisées pour la recherche des déchets puriques (voir Pearse, 1972, pour l'exposé de ces méthodes).

Des examens ultrastructuraux ont été pratiqués sur des poumons de Planorbe fixés au glutaraldéhyde (2% tampon phosphate pH 7,4) et post-osmiés (1% tampon phosphate pH 7,4); les coupes ont été contrastées à l'uranium-plomb.

Trois procédés d'analyse physique ont été appliqués:

1. *Analyse par spectrographie des rayons X*

Les coupes ont été analysées par spectrographie dispersive en longueur d'onde, avec une microsonde CAMECA MS 46, dans les conditions suivantes: tension d'accélération, 15 kV; intensité de la sonde, 50 nA; cristaux K.A.P. (Potassium acid phtalate) et P.E.T. (pentacrythritol).

2. *Analyse élémentaire par émission ionique secondaire*

Utilisant la pulvérisation cathodique et la spectrométrie de masse, la méthode permet de caractériser et de localiser les éléments et leurs isotopes à l'échelle de la microscopie photonique (Castaing & Slodzian, 1962; Truchet, 1975). Dans un appareil CAMECA SMI 300, équipé d'un filtre électrostatique, les échantillons ont été bombardés à oxygène O_2^+, sous 5,5 kV et 7,5 μA avec une forte défocalisation (30/10). Le diaphragme de la lentille était de 200 μm, le diaphragme de champ de 3700 μm, la bande passante de 10V et le convertisseur réglé pour un grossissement de 115 environ. La résolution spatiale était de 1 μm environ et la résolution en masse de 300. L'analyse spectrale a porté sur l'émission positive de 1 à 240. Les images ont été faites sur les alcalins, Na et K, et sur les alcalino-terreux, Mg, Ca, Sr et Ba.

3. *Analyse moléculaire par microspectrométrie Raman*

Constitué d'un laser, d'un microscope optique et d'un monochromateur (Delhaye & Dhamelincourt, 1975), l'appreil ultilise l'effet Raman. Il permet de déterminer la nature moléculaire de tout composé suffisamment concentré dans des volumes de quelques μm^3. Nous avons employé l'appareil JOBIN-YVON MOLE 77, équipé d'un laser à argon ionisé. Les raies 457,9 nm, 488 nm et 514 nm ont servi de radiations excitatrices. Dans tous les cas, l'analyse a été faite avec un objectif 100 de grande ouverture numérique afin de standardiser les conditions de collecte du flux et de recueillir le maximum de lumière. Ce mode de collection dit "rétro-Raman à grand angle solide" ne permet pas d'effectuer des mesures de polarisation. Le diaphragme de champ a servi à délimiter le volume analysé et à réduire la fluorescence parasite. La largeur des fentes du monochromateur, les réglages du compteur de photons et de l'enregistreur, les vitesses de défilement, adaptés à chaque cas, sont précisés dans les légendes des spectres. Les figures 1, 2 et 3 représentent les spectres Raman de trois échantillons de référence.

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Figures 1 à 3, 5 à 8 et 10 et 11 (spectres Raman): L'axe des *abscisses* représente le nombre d'onde (fréquence), compté en cm^{-1} depuis la raie excitatrice. L'axe des ordonnées représente l'intensité de la raie et du fond de lumière parasite, exprimée en nombre de photons par seconde (cps).

RÉSULTATS

1° *Viviparus viviparus*

Le tissu conjonctif qui entoure le complexe réno-péricardique renferme de gros sphérocristaux isolés (Fig. 4A). Selon Cuénot (1899), ils seraient formés de carbonate de

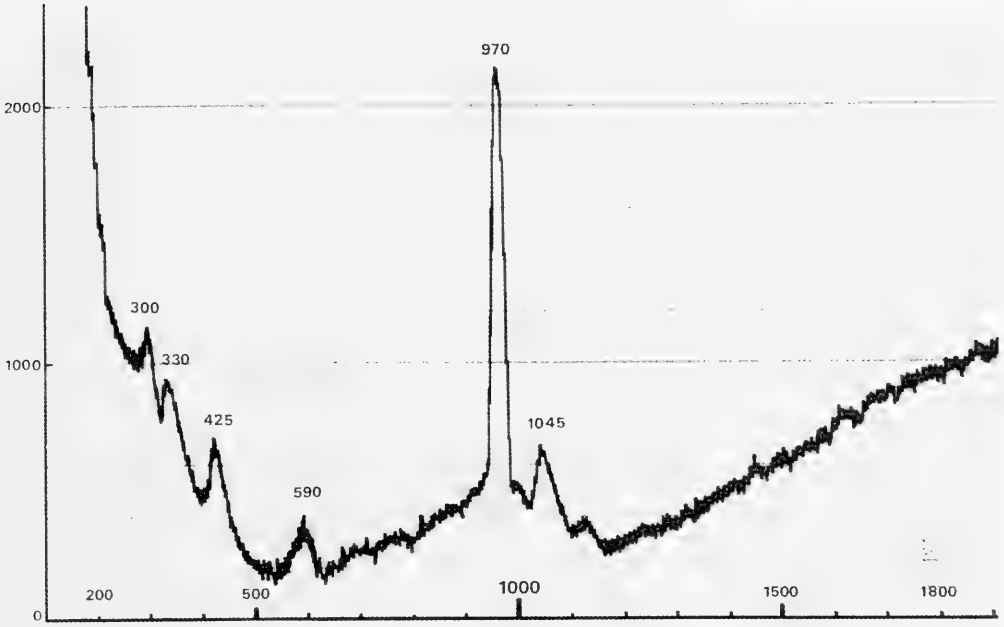

FIG. 1. Phosphate de calcium (spectre Raman): produit pur. Excitation: 514.5 nm; 600 mW (filtrée). Fentes: 750 μm. Comptage: 10^4 cps, 4s. Enregistrement: 100 mV; 20 cm^{-1}/mn et 100 cm^{-1}/cm (sans diaphragme).

calcium mais, d'après analyse histochimique, Andrews (1979) estime qu'ils sont constitués de calcium et d'acide urique ou d'urates.

Les deux éléments dominants décelables par spectrographie des rayons X en sont le calcium (jusqu'à 4000 chocs/sec.) et le phosphore (jusqu'à 2500 chocs/sec.); il s'y ajoute des traces de magnésium.

Analysés aux longueurs d'onde 514,5 et 457,9 nm, les sphérocristaux n'ont montré entre 850 et 1800 cm^{-1}, qu'un pic centré sur 965–970 cm^{-1}, correspondant à la vibration de valence symétrique P-O du phosphate de calcium (Fig. 5). Par sa largeur, la raie indique un état cristallin médiocre: le phosphate serait donc à l'état d'une poudre de fins cristaux. Rien dans la région 1000–1100 ne permet de soupçonner la présence de carbonate. De même, la recherche de composés puriques dans la région 575–700 est restée négative. Ces derniers, s'ils sont présents ne peuvent donc être que peu concentrés. La fluorescence est assez forte; elle atteint son minimum, 2500 coups/secondes, après une heure d'irradiation environ. Centrée vers le rouge, elle est très étale et demeure importante à 457,9 nm. Il n'est donc pas exclu que les sphérocristaux referment d'autres composés moins concentrés.

Les divergences entre nos résultats et ceux d'Andrews (1979) nous ont conduits à rechercher les déchets puriques par voie histochimique chez d'autres individus fixés par le formol ou le mélange de Carnoy. Cette détection n'a jamais donné de réaction franchement positive. Tous les animaux ayant été autopsiés à la fin de l'été, une décharge périodique pourrait être à envisager. Au contraire, dans tous les cas, le calcium a pu être révélé par ce même type de méthodes.

2° *Valvata cristata*

Le céphalopodium et la masse viscérale contiennent une énorme quantité de petits sphérocristaux souvent groupés. Leur répartition, beaucoup plus vaste que dans les autres espèces peut même s'étendre aux masses musculaires. Leur composition ne semble pas avoir été étudiée jusqu'à présent.

L'analyse en a été faite à 514,5 et 488 nm (Fig. 6). Après 15 minutes d'irradiation, le fond de fluorescence n'est plus que de 350 coups/sec. et tombe en moins d'une heure au taux le plus bas jamais observé sur coupe histologique: 80–100 coups/sec., pour 750 mW au laser. Dans ces conditions, chaque

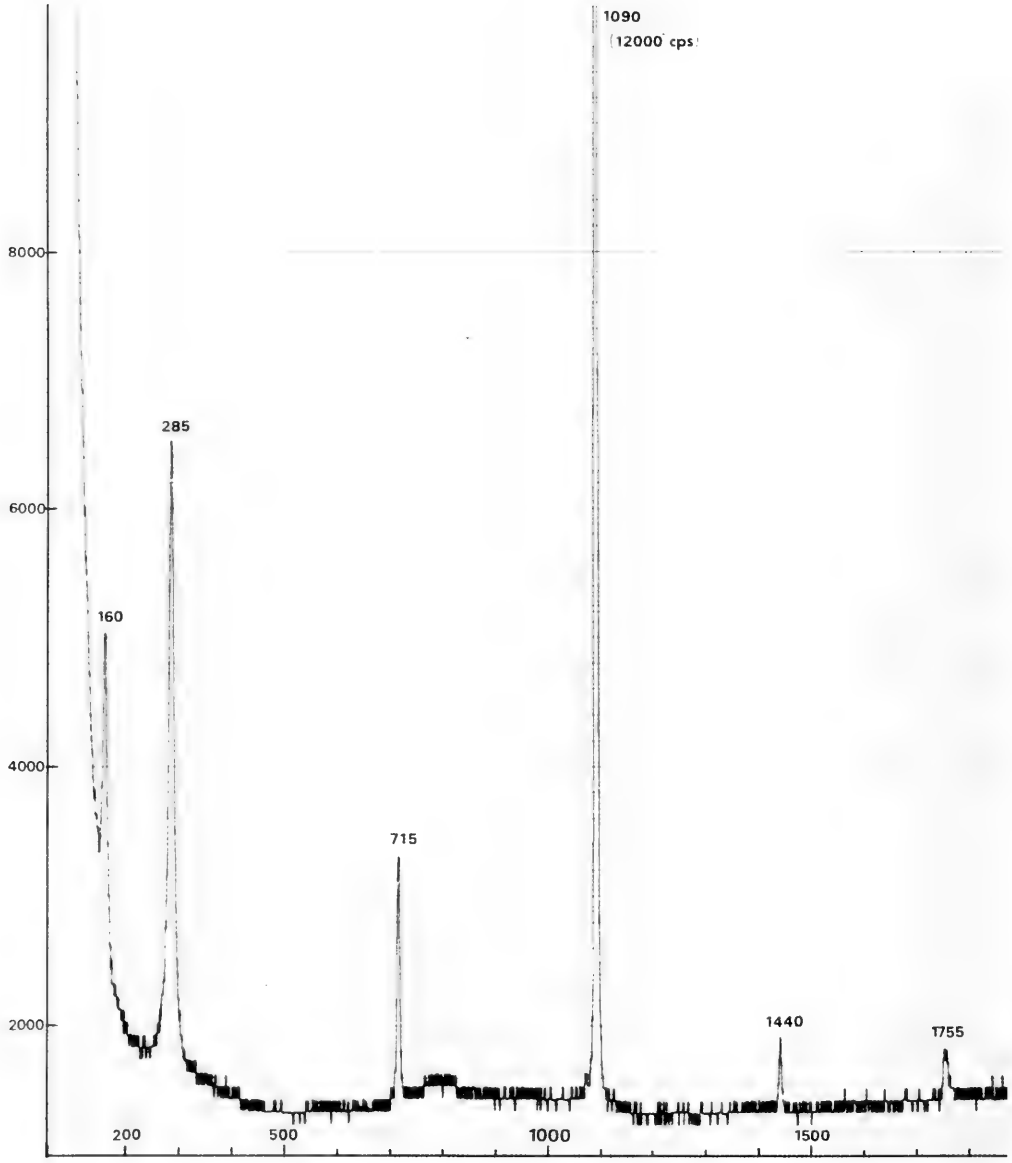

FIG. 2. Carbonate de calcium (spectre Raman): produit pur cristallisé en *Calcite.* Excitation: 514.5 nm; 600 mW (filtrée). Fentes: 750 μm. Comptage: 10^4 cps, 1 s. Enregistrement: 100 mV; 50 cm^{-1}/mn et 100 cm^{-1}/cm (sans diaphragme).

concrétion analysée fournit un spectre identique à celui d'un échantillon témoin de calcite, sans aucune autre raie. L'enregistrement effectué entre 2800 et 3400 cm^{-1}, dans la région des liaisons C-H non spécifiques, démontre l'absence de matière organique. Ces concrétions sont donc formées de calcite pure.

L'accumulation de calcite est sujette à des variations individuelles. En effet, dans le second exemplaire récolté le même jour au même point, les sphérocristaux se présen-

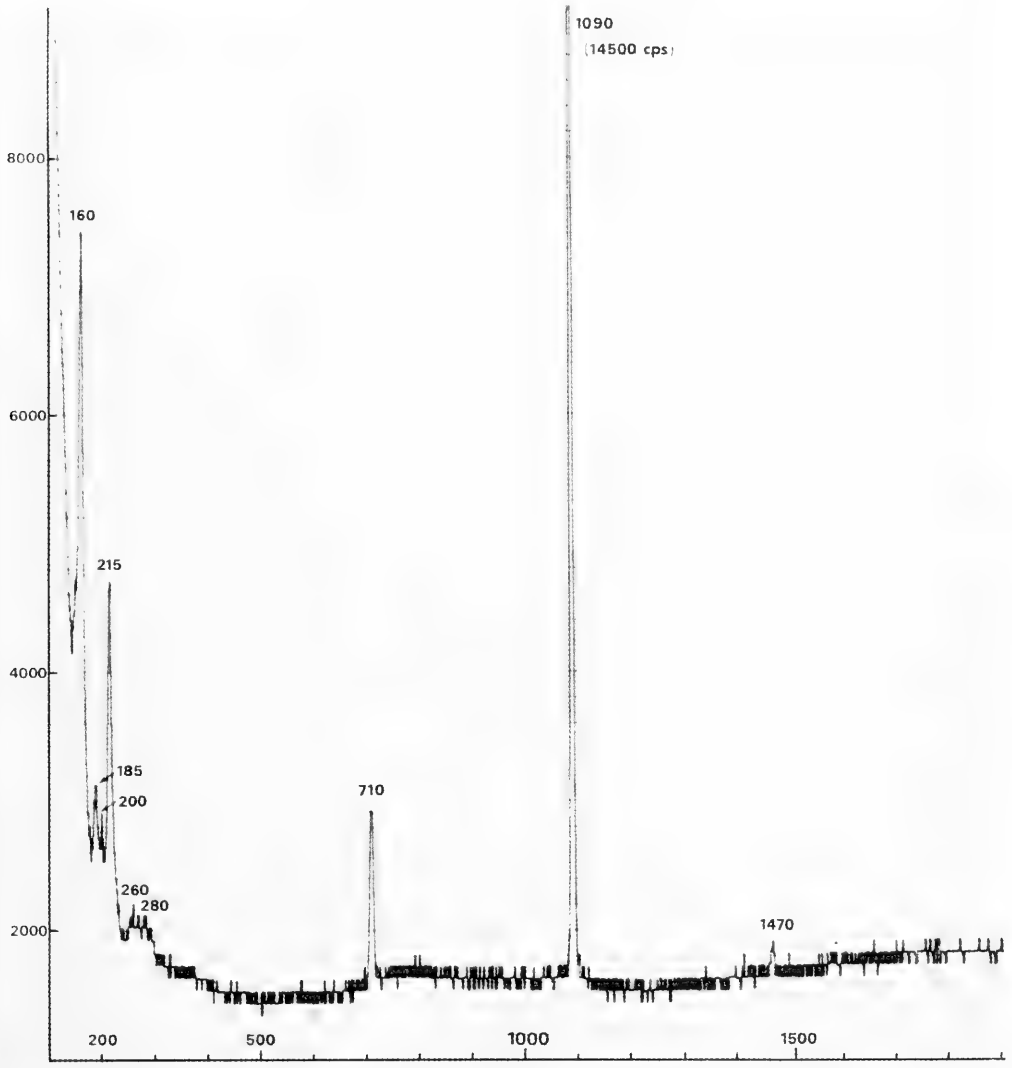

FIG. 3. Carbonate de calcium (spectre Raman): produit pur cristallisé en *Aragonite*. Conditions d'enregistrement identiques à celles de la calcite.

taient sous forme de "fantômes" d'ailleurs bien visibles; le calcium n'a pu y être mis en évidence par histochimie. Aucune raie n'a été obtenue sur le spectre Raman, ce qui indique que les concrétions sont vides ou qu'elles ne renferment qu'un mélange de substances trop peu concentrées pour fournir un signal.

3° *Bithynia tentaculata*

Nous avons observé dans le pied, des amas de tout petits cristaux sous-jacents au tégument et de sphérocristaux plus gros, à l'intérieur de la masse musculo-conjonctive (Fig. 4b). Ces derniers avaient été vus par Garnault (1887) qui les considérait comme homologues de ceux de *Pomatias elegans*, mais constitués de carbonate de calcium.

Dans les deux cas, la spectrographie des rayons X a décelé du calcium et du phosphore. Dans les sphérocristaux les teneurs ponctuelles atteignent 3000 chocs/sec. pour le calcium et 1500 chocs/sec. pour le phosphore.

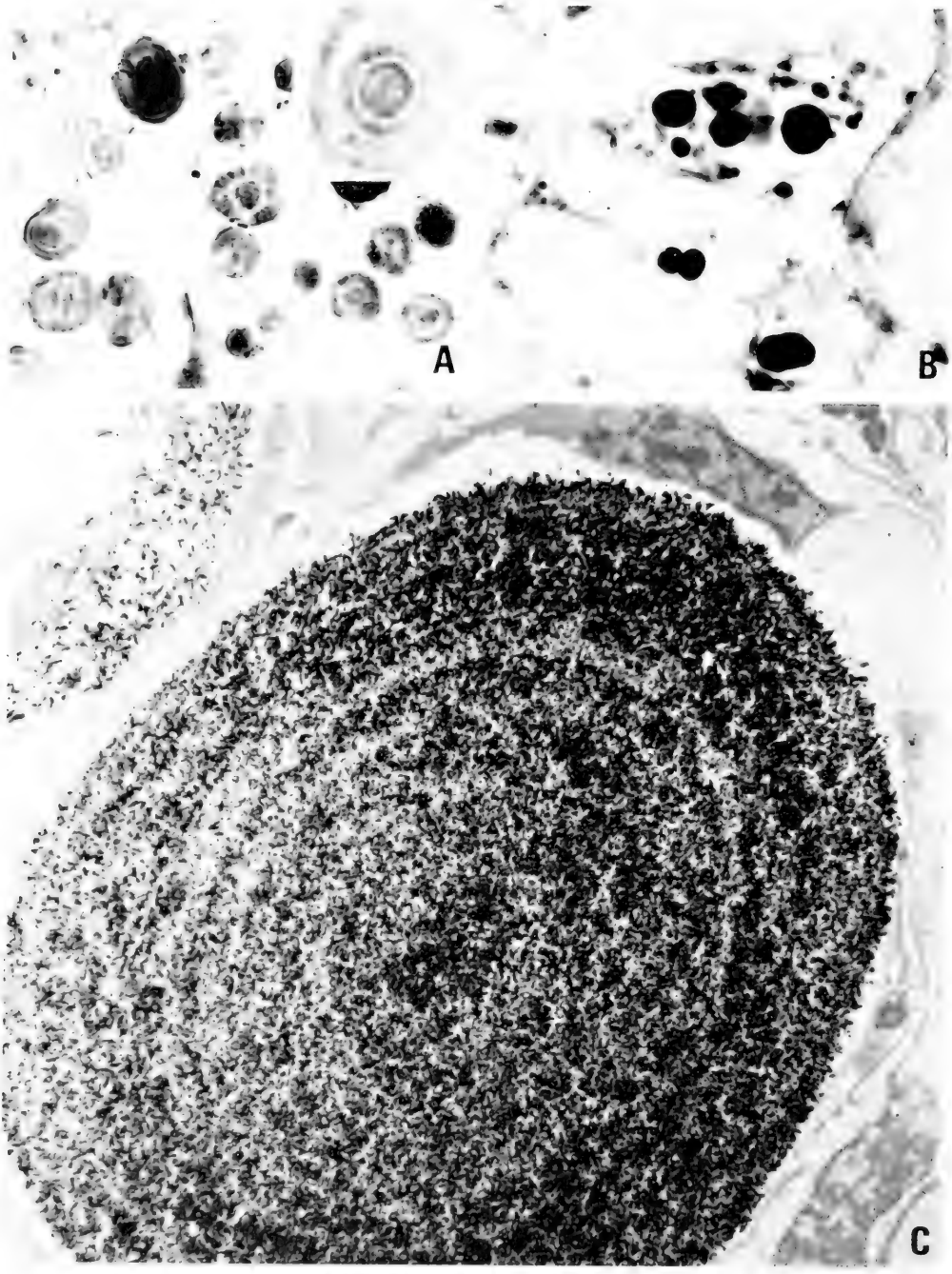

FIG. 4. Aspect morphologique des sphérocristaux. A. *Viviparus viviparus* (formol; réaction au ferricyanure ferrique; ×230). En cartouche, détail d'un sphérocristal (×550). B. *Bithynia tentaculata* (Carnoy; coloration par la laque d'alizarine; ×550). C. *Planorbarius corneus* (glutaraldéhyde-osmium; uranium-plomb; ×2600). En haut à gauche, apparaît un sphérocristal en partie vidé contrastant avec celui qui occupe le reste du cliché.

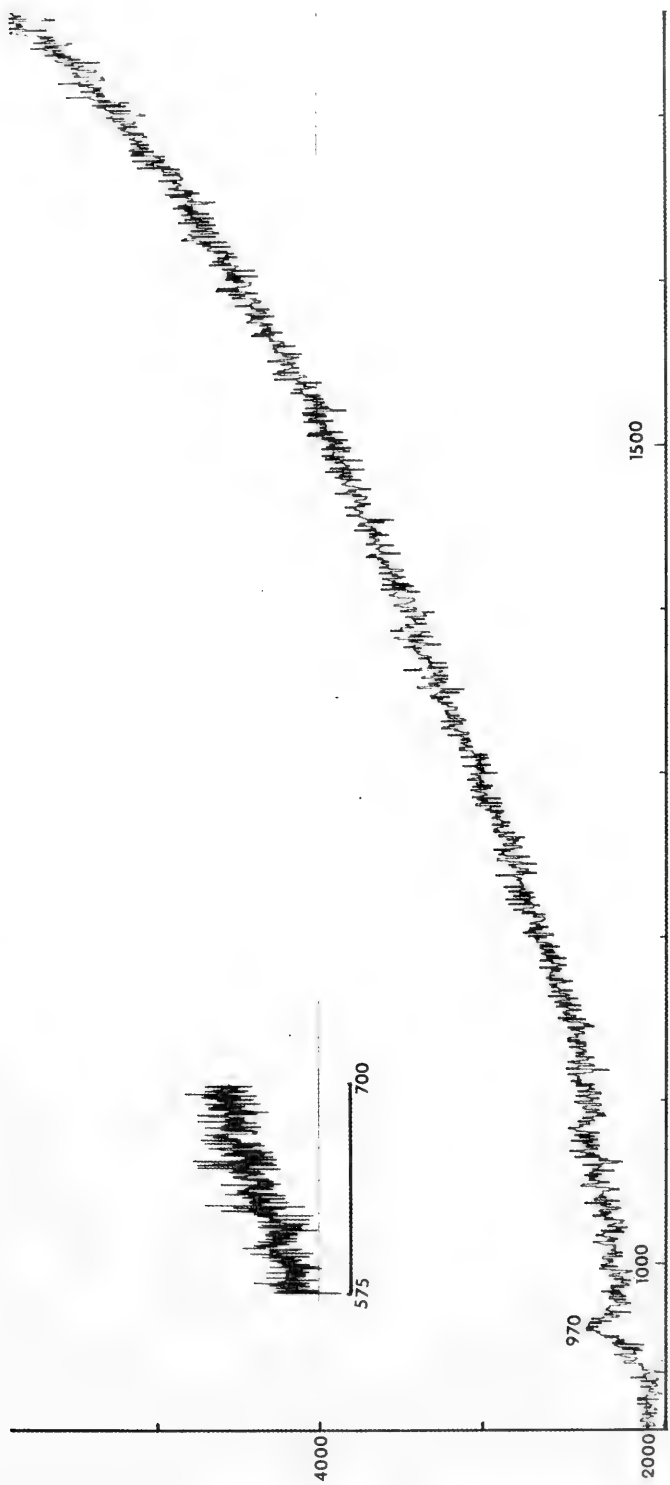

FIG. 5. *Viviparus viviparus* (spectre Raman): sphérocristaux du tissu conjonctif péri-rénal. Excitation: 457.9 nm; 120 mW (filtrée). Fentes: 600 μm. Comptage: 10^4 cps, 4 s. Enregistrement: 500 mV; 10 cm^{-1}/mn et 20 cm^{-1}/cm (diaphragme fermé). En cartouche: Région 575 à 700 cm^{-1} montrant l'absence de déchets puriques, caractérises en spectrométrie Raman par leurs raies intenses à 625 et à 650 cm^{-1}.

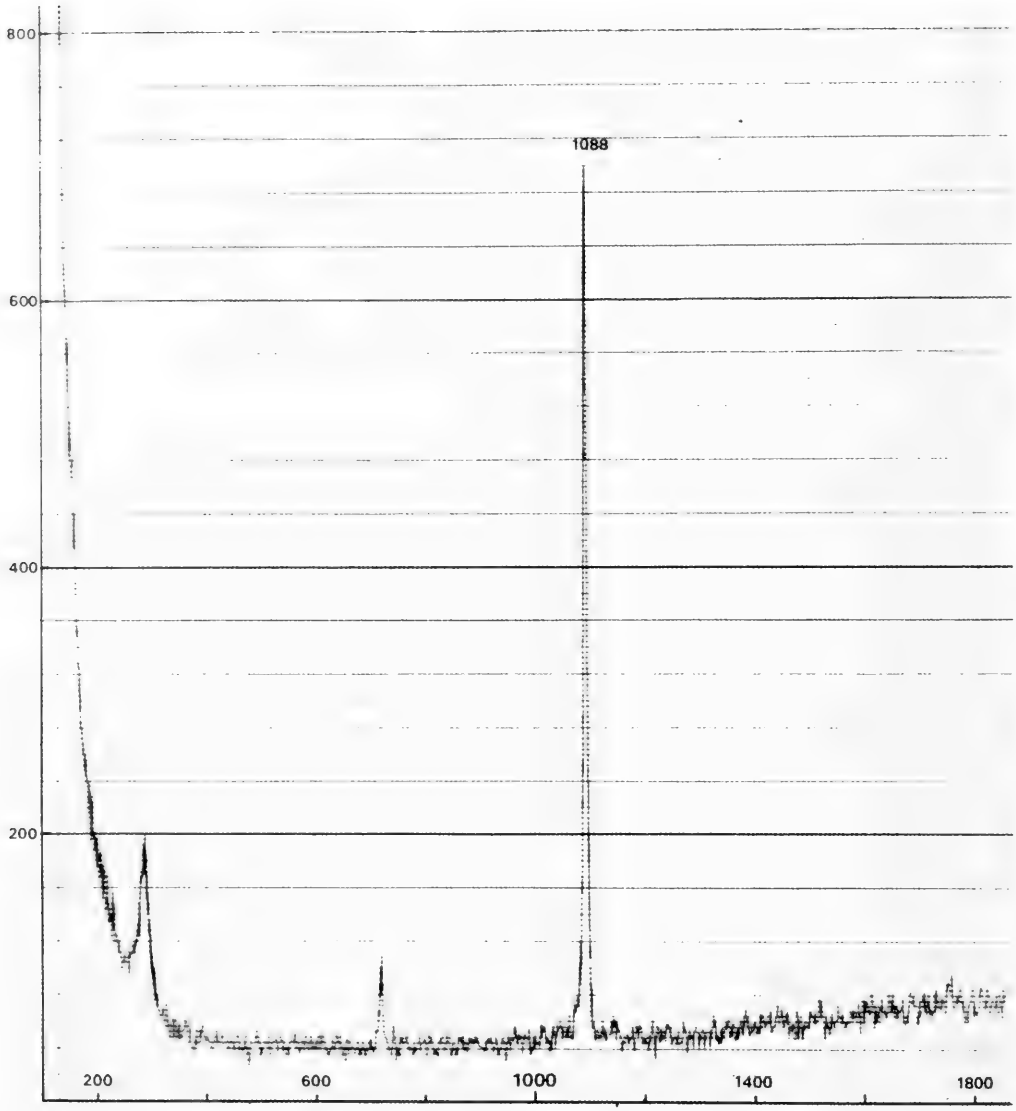

FIG. 6. *Valvata cristata* (spectre Raman): amas de sphérocristaux. Excitation: 488 nm; 600 mW (filtrée). Fentes: 700 μm. Comptage: 10^3 cps, 1 s. Enregistrement: 100 mV; 50 cm^{-1}/mn et 100 cm^{-1}/cm (diaphragme fermé).

L'analyse Raman s'est montrée difficile en raison de la fragilité du matériel et d'une fluorescence importante. Les radiations 457,9 nm et 488 nm, absorbées, n'ont pu être utilisées. L'irradiation à 514.5 nm a dû être modérée: à 900 cm^{-1}, le fond de fluorescence reste de 1500 coups/sec., pour une puissance au laser de 150 mW seulement. Dans ces conditions, le rapport pic/bruit de fond est mauvais. La région explorée va de 900 cm^{-1} à 1100 pour les amas de petits cristaux et de 500 à 1300 pour les sphérocristaux. Les petits cristaux ne donnent qu'un faible signal vers 960–970 cm^{-1} et les sphérocristaux, un spectre un peu mieux résolu où apparaissent trois signaux à 965, 1070 et 1140 cm^{-1} (Fig. 7, A et B).

Le pic situé à 970 cm^{-1} qui se retrouve

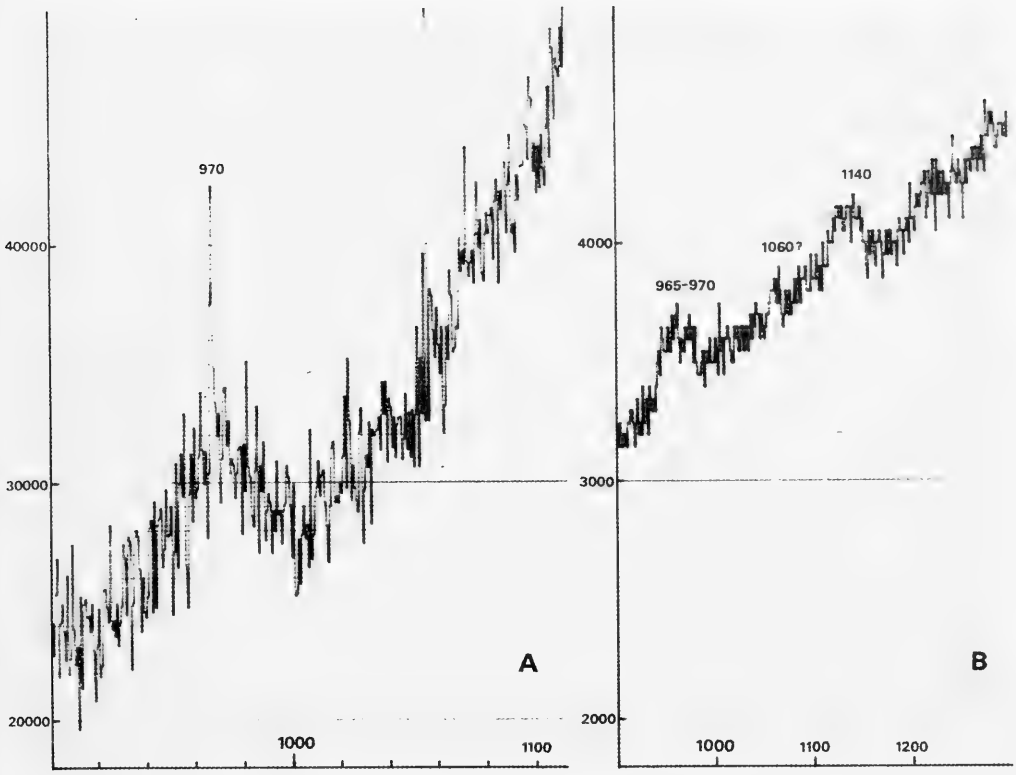

FIG. 7. *Bithynia tentaculata* (spectre Raman): A. Petits cristaux sous-tégumentaires. B. Sphérocristaux de la zone musculo-conjonctive du pied. Excitation: 514.5 nm; 150 mW. Fentes: 600 μm. Comptage: A: 10^4 cps, 1 s.; B: 10^3 cps, 4 s. Enregistrement: A: 500 mV, 20 cm^{-1}/mn et 20 cm^{-1}/cm. B: 500 mV, 20 cm^{-1}/mn et 50 cm^{-1}/cm.

dans les deux types d'accumulation, correspond à la raie principale du phosphate de calcium; comme chez *Viviparus,* son étalement montre que les cristaux sont de petite taille. Les raies 1140 et 1070 sont difficiles à interpréter. La réponse à 1140, déjà rencontrée sur d'autres échantillons riches en phosphate de calcium, pourrait traduire la présence de la trame de cristallisation ou de sites de nucléation et correspondre à une vibration de valence, C-O par exemple. La raie 1070 pourrait être due à un nitrate ou à un carbonate de calcium, bien qu'elle ne corresponde exactement ni à l'un (1054) ni à l'autre (1088). Toutefois, c'est la présence de carbonate qui est la plus probable puisque, sur des spectres d'os, le pic de la calcite qui accompagne le phosphate est décalé vers 1080 (R. Martoja et al., 1981). Il est possible, en effet, comme le suggère le pic 1140, qu'un autre composé soit lié à ces sels et qu'une interaction moléculaire provoque un léger déplacement des modes caractéristiques, même pour les vibrations de valence. De tels déplacements ont déjà été observés pour les phosphates (Daudon et al., 1980).

L'absence de tout signal dans la région des 600 cm^{-1} permet d'affirmer que les déchets puriques ne représentent pas les composants essentiels des sphérocristaux.

Les sphérocristaux peuvent être vidés de leurs sels minéraux. Ainsi, chez l'animal autopsié en juin, la teneur ponctuelle en calcium déterminée par spectrographie des rayons X n'excédait pas 30 chocs/sec. alors que les sphérocristaux restaient visibles à l'examen histologique sous forme de "fantômes."

Les conclusions de Garnault (1887), obtenues au moyen de méthodes microchimi-

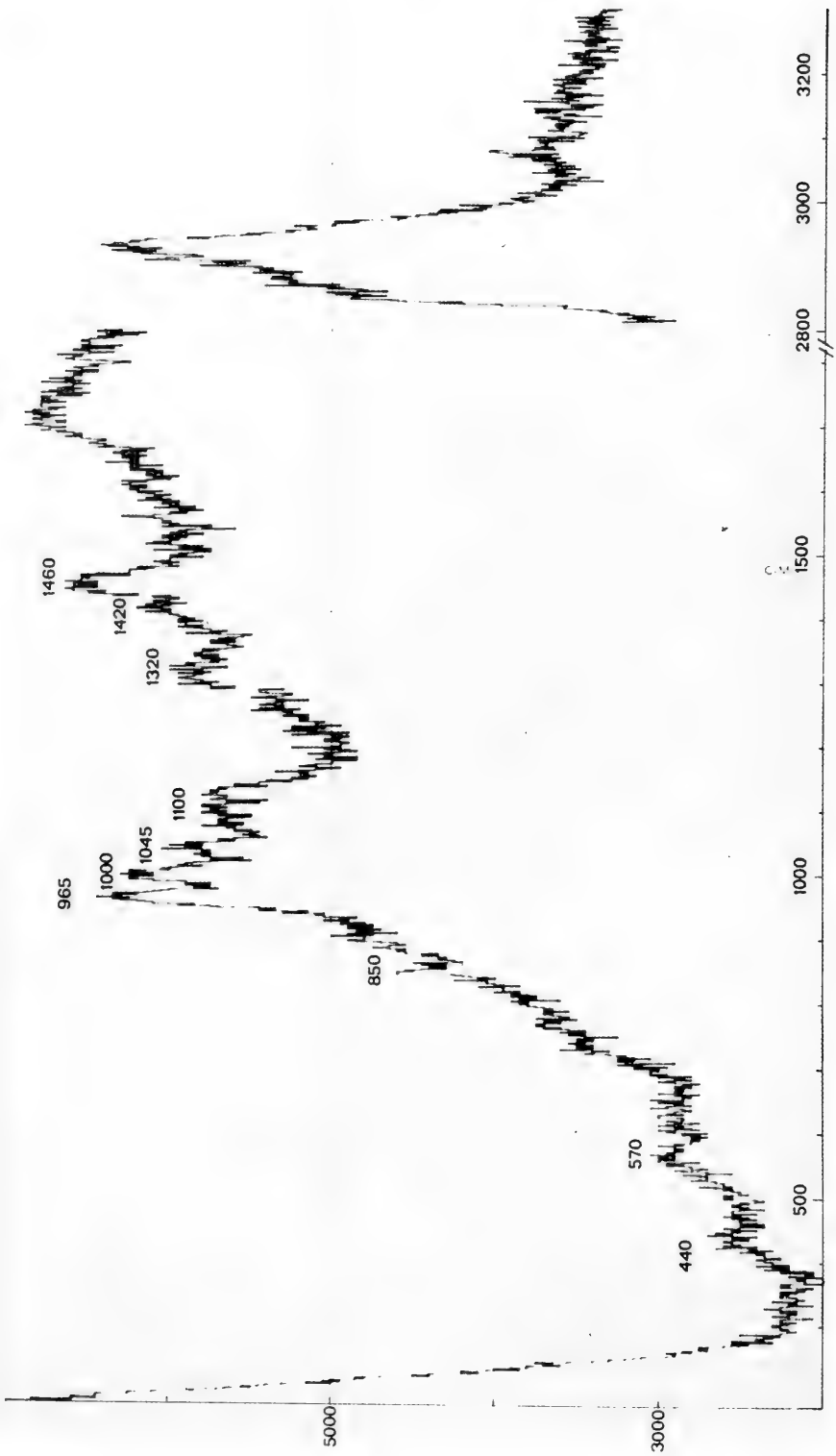

FIG. 8. *Lymnaea peregera* (spectre Raman): sphérocristaux de la zone musculo-conjonctive du pied. Excitation: 415.5 nm; 700 mW (filtrée). Fentes: 600 μm. Comptage: 10^4 cps, 4 s. Enregistrement: 100 mV, 20 cm^{-1}/mn et 50 cm^{-1}/cm (diaphragme fermé).

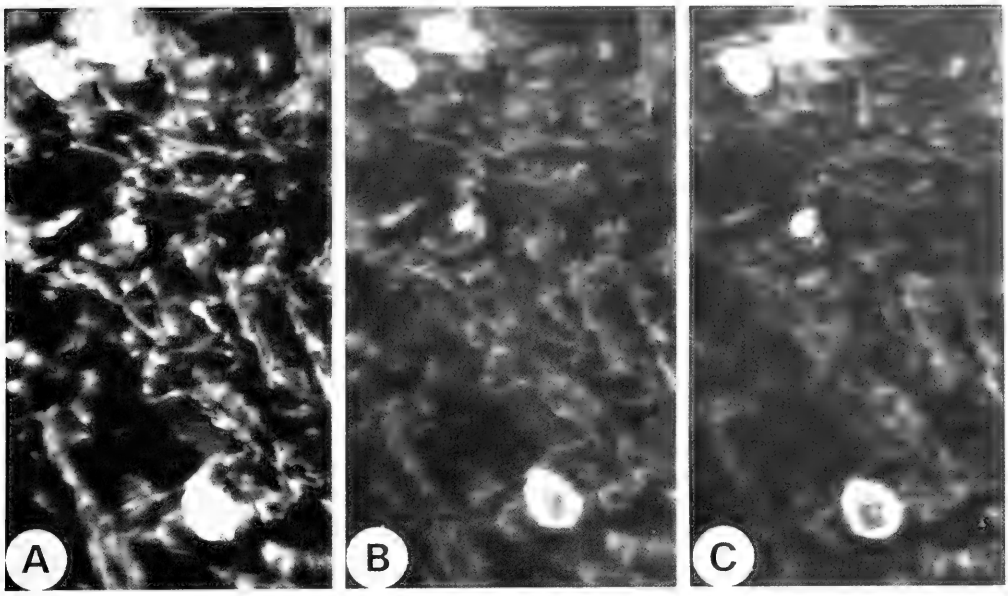

FIG. 9. *Lymnaea peregra* (images d'émission ionique): sphérocristaux de la zone musculo-conjonctive du pied. A. Calcium (Ca). B. Strontium (Sr). C. Baryum (Ba). Noter la forte émission de calcium par les 3 sphérocristaux, et les légères différences de localisation entre Sr et Ba au sein des deux sphérocristaux situés en haut de la figure. Intensités d'émission ionique (en Ampères: A) et temps de pose (en secondes: s): $^{40}Ca^{+}$: $1{,}5.10^{-14}$ A; 20 s. $^{88}Sr^{+}$: IO^{-16} A; 15 mn (900 s). $^{138}Ba^{+}$: 4.10^{-17} A; 15 mm (900 s).

ques très rudimentaires, ne sont donc pas confirmées. Le phosphate de calcium est le constituant dominant des sphérocristaux.

4° *Lymnaea peregra*

Nous avons analysé les concrétions de la zone musculo-conjonctive du pied où nous avons retrouvé les sphérocristaux et les mini-sphérules en poussière que Prenant (1924) avait signalées chez *Lymnaea stagnalis.* Dans cette dernière espèce, les sphérocristaux de la masse viscérale ont fait l'objet d'une étude récente (Sminia et al., 1977).

Par spectrographie des rayons X, nous n'avons détecté dans les mini-sphérules que du calcium (1000 chocs/sec.) et du phosphore (40 à 120 chocs/sec.) alors que les sphérocristaux ont montré la composition suivante: Ca, 4000 à 8000 chocs/sec.; P, 400 à 1000 chocs/sec.; S, 20 chocs/sec.; Mg, 10 chocs/sec.; Ba, 5 chocs/sec.

En raison d'une fluorescence importante, les sphérocristaux n'ont pu être analysés qu'après une longue irradiation et sous une illumination de forte intensité (700 nW). De 200 à 1800 cm^{-1}, douze pics apparaissent sur le spectre (Fig. 8). On peut attribuer sans difficulté les signaux 965 au phosphate de calcium, 1460 à l'oxalate de calcium, 1045 au nitrate de calcium et 1000 au sulfate de calcium. Les pics 1320 et 1420, déjà observés chez d'autres Invertébrés (Ballan-Dufrançais et al., 1979), comme les pics 440, 850 et 1725, pourraient être dûs à la trame organique, abondante si l'on juge par l'intensité de la région 2900 cm^{-1} qui est celle des hydrocarbures non spécifiques. Rien ne signale l'existence de déchets puriques dans la zone des 600–650 cm^{-1}.

L'analyseur ionique décèle les quatre alcalino-terreux, Mg, Ca, Sr et Ba, mais les plus fortes intensités d'émission restent celles du calcium (Fig. 9, A, B, C). Le strontium et le baryum se rencontrent d'ailleurs partout où le calcium est abondant, en particulier dans les zones pigmentées du tégument. A noter que le strontium, trop peu concentré, échappe à la microsonde alors que l'analyseur ionique le met facilement en évidence. Quoi qu'il en soit, les deux méthodes confirment la complexité des sphérocristaux qu'indiquent les nombreuses raies Raman.

Malgré une forte fluorescence, l'analyse

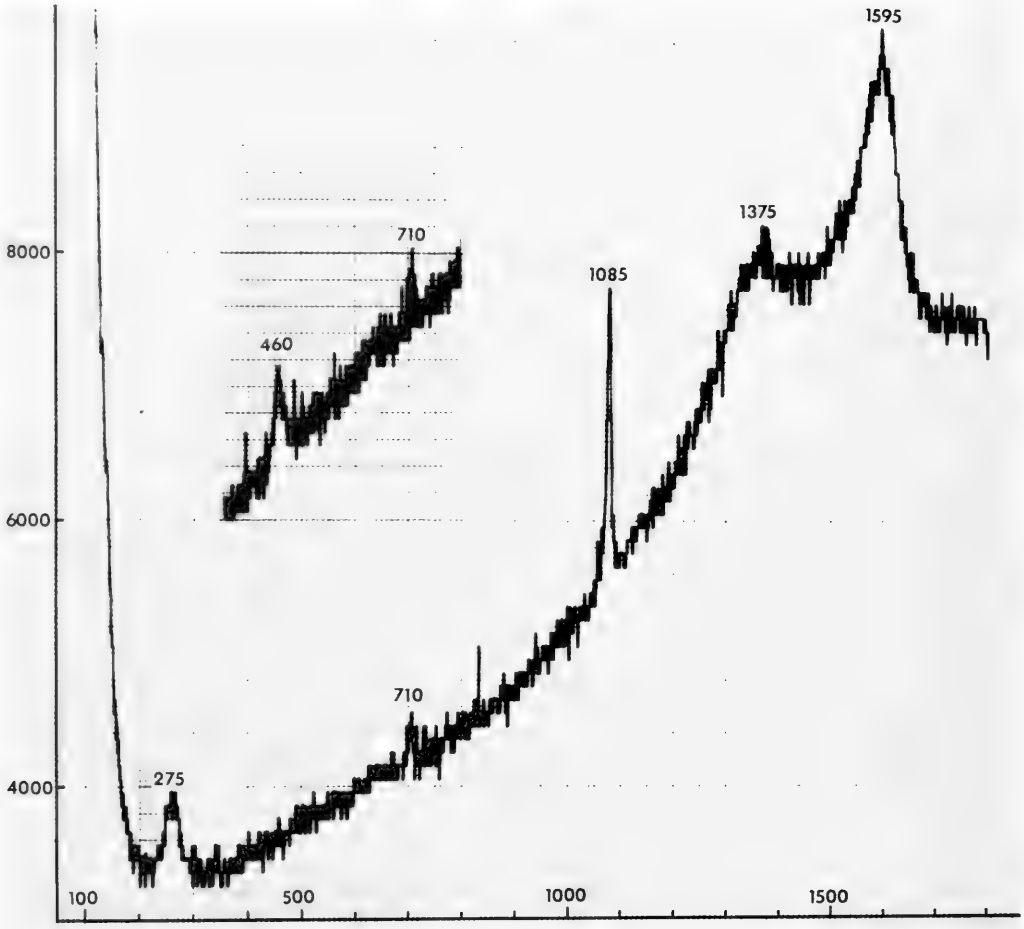

FIG. 10. *Lymnaea peregra* (spectre Raman): petits cristaux de la zone musculo-conjonctive du pied. Excitation: 488 nm; 260 mW (filtrée). Fentes: 750 μm. Comptage: 10^5 cps, 1,5 s. Enregistrement: 100 mW; 50 cm^{-1}/mn et 100 cm^{-1}/cm (diaphragme fermé). En cartouche: Région 350 à 700 cm^{-1} d'un autre point montrant un pic à 460 susceptible d'être attribué à la silice.

d'amas de petits cristaux a été réalisée à 488 et 457,9 nm. Dans les deux cas, les pics suivants sont apparus; 275, 1080, 1375 et 1595 cm^{-1}. Sur plusieurs spectres, un pic a pu être caractérisé à 710 cm^{-1} et, en un seul point, un pic bien défini s'est manifesté à 460 cm^{-1}. Les réponses à 275, 710 et 1080 cm^{-1} caractérisent le carbonate de calcium sous forme de calcite. Par leurs positions en fréquence, leurs formes et leurs intensités relatives, les pics à 1375 et 1595 cm^{-1} correspondent au graphite sous une forme assez élaborée (Fig. 10). Enfin, le pic à 460 cm^{-1} pourrait traduire la présence de silice. Aucun autre sel de calcium ou d'un autre alcalino-terreux, aucun déchet purique ne peut être identifié. En particulier, aucun phosphate n'apparaît sur les spectres bien que la microsonde détecte une certaine quantité de phosphore. La lumière diffusée par les phosphates étant beaucoup plus faible que celle des carbonates, leur présence n'est pas exclue, mais il ne peut s'agir que de traces.

La structure des petits cristaux est donc plus simple que celle des sphérocristaux: ils sont formés essentiellement de calcite et de graphite, ce dernier pouvant être responsable de la couleur jaune-brun des amas cristallins.

Comme dans les espèces précédentes, les sphérocristaux sont susceptibles de se vider de leur contenu minéral. Dans l'individu autopsié en juillet, l'analyse n'a fourni qu'un très faible signal vers 970 cm^{-1} indiquant la présence de phosphate de calcium et quelques signaux vers 2900 cm^{-1} correspondant à la trame organique. Ces sphéro-

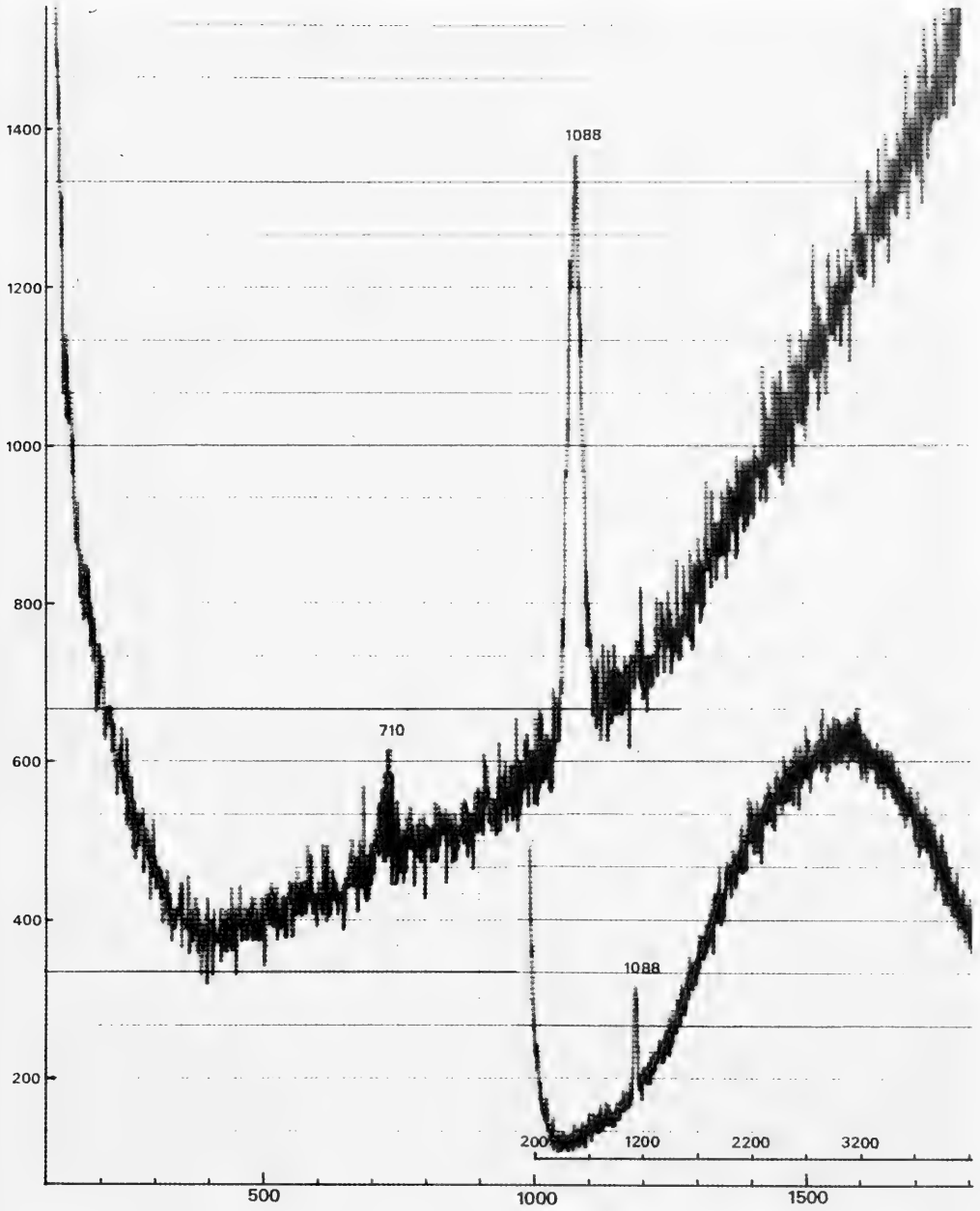

FIG. 11. *Planorbarius corneus* (spectre Raman): sphérocristaux de la paroi du poumon. Excitation: 514.5 nm; 350 mW (filtrée). Fentes: 600 μm. Comptage: 10^4 cps, 0,6 s. (diaphragme fermé). En cartouche: Autre concrétion, région de 150 à 4200 cm^{-1}, montrant le maximum de fluorescence (4000 cps) à 610 nm, ou 3100 cm^{-1}, dans l'orange.

cristaux doivent garder une composition très complexe, mais aucune des substances présentes n'est assez concentrée pour fournir un signal détectable en Raman simple. A l'examen histologique, ils présentent un aspect éclaté différent des fantômes rencontrés dans les autres espèces. Nous n'avons pas observé de variations au niveau des amas de petits cristaux.

L'originalité de *Lymnaea peregra* réside en

une ségrégation des sites de précipitation des phosphates et des carbonates. Dans les deux cas, le présence de déchets puriques est à écarter. Notre résultat global concorde avec celui de Sminia et al. (1977) pour *L. stagnalis.* Utilisant des méthodes chimiques, ces auteurs ont pu caractériser les ions calcium, magnésium, carbonate et phosphate que nous avons nous aussi identifiés. Alors qu'ils les situent dans un seul type de bioaccumulation complexe, l'analyse *in situ* nous a permis de les rapporter à deux catégories distinctes de concrétions, ce qui confirme les observations de Prenant (1924).

L'existence de graphite dans les petits cristaux échappe actuellement à toute interprétation. Le seul cas analogue relaté à ce jour est celui d'un Poisson (Delhaye et al., 1979).

5° *Planorbarius corneus*

L'abondance exceptionnelle de "carbonate calcique" dans la paroi du poumon du Planorbe a été mentionnée par Cuénot (1899). Chez un autre Planorbidae, *Helisoma duryi eudiscus,* le carbonate de calcium a été identifié par méthode histochimique dans les concrétions du pied (Kapur & Gibson, 1968).

L'analyse des sphérocristaux de la paroi du poumon par spectrographie des rayons X a décelé du calcium (2800 à 3000 chocs/sec.), du potassium (10 chocs/sec.) et du soufre (5 chocs/sec.).

La spectrométrie Raman a été effectuée à 514,5 nm; la fluorescence, centrée sur le rouge-orange, est peu importante. A l'exception d'un sphérocristal qui n'a donné aucune réponse, la raie 1088, caractéristique du carbonate de calcium apparaît constamment et la raie 710 assez souvent (Fig. 11). La vibration de réseau n'a été observée qu'une fois à 282. Aucune autre raie ne se démarque du bruit de fond. En particulier, la région 2800–3600 cm^{-1} qui corespond à la matière organique est vide. Cependant, un éclat de calcite pure de même taille que les sphérocristaux a donné un signal 20 fois plus intense à 1088 dans des conditions d'enregistrement identique.

Conformément aux données bibliographiques, les sphérocristaux du Planorbe contiennent du carbonate de calcium. Dans l'un, la calcite a pu être identifiée. Dans les autres, compte tenu des intensités des pics 1088 et 710, la raie de la calcite aurait dû être observée: son absence indique qu'il pourrait s'agir d'aragonite dont la raie vers 210 est moins intense. Contrairement à *Valvata,* le carbonate de calcium n'est pas à l'état pur et la comparaison avec l'échantillon de référence suggère une concentration de 5%. En l'absence de raie, il n'a pas été possible de déterminer la nature des autres constituants mais, d'après l'allure générale du spectre, ils doivent être nombreux et peu concentrés. La microsonde décèle d'ailleurs les éléments K et S. Enfin, la variabilité observée dans les autres espèces existe ici à l'intérieur même de l'individu (Fig. 4C) puisque se côtoient des sphérocristaux vides, d'autres contenant de la calcite et d'autres probablement de l'aragonite.

DISCUSSION—CONCLUSION

Nos résultats ne nous permettent pas d'intervenir dans le débat concernant le rôle physiologique des concrétions; nous renvoyons donc aux mémoires de Curtis & Cowden (1979) et de Richardot (1979) pour l'exposé du problème et la bibliographie. Nous confirmons seulement l'existence d'une variabilité individuelle et d'une diversité des formes minéralogiques d'un même sel. La variabilité individuelle pourrait être due à des conditions alimentaires propres à chaque animal, l'inanition ayant pour effet de vider les sphérules de leur contenu calcique (Vovelle & Grasset, 1979; De With & Sminia, 1980). La diversité des formes minéralogiques observées chez le Planorbe corrobore les résultats de Richardot & Wautier (1972) qui ont montré que le carbonate de calcium amorphe, l'aragonite et la calcite se rencontraient chez *Ferrissia wautieri.*

Nous n'avons pas observé de différences entre les Pulmonés et les Prosobranches. Or, si le rein des premiers conserve la structure fondamentale de l'organe rénal des Gastéropodes (Bouillon & Delhaye, 1970), les trois Prosobranches, *Bithynia* (Lilly, 1953), *Valvata* (Cleland, 1954) et *Viviparus* (Andrews, 1979) se singularisent par un rein modifié qui ne semble pas pouvoir assumer les fonctions d'un rein normal, Il serait donc logique que le tissu conjonctif de ces Prosobranches fasse office de rein d'accumulation et qu'il s'y dépose des composés puriques, comme l'a pensé Andrews (1979). Si nous n'y avons décelé que des sels de calcium, nous n'excluons pas l'idée qu'il s'y adjoigne des déchets puriques, à d'autres moments du cycle annuel. On sait que chez *Pomatias,* les

TABLEAU 1. Résumé des principaux resultats.

		Nombre de types de concrétions	Region examinée	Aspect des concrétions	Principal sel de calcium identifié
Prosobranches	*Viviparus*	1	conjonctif péri-rénal	sphérocristaux	phosphate
	Valvata	1	ensemble du conjonctif	petits sphérocristaux	carbonate
	Bithynia	2	conjonctif du pied	petits cristaux	phosphate
				sphérocristaux	phosphate
Pulmonés	*Lymnaea*	2	conjonctif du pied	petits cristaux	carbonate
				sphérocristaux	phosphate
	Planorbarius	1	conjonctif péri-pulmonaire	sphérocristaux	carbonate

composés puriques coexistent avec de la calcite et du phosphate de calcium amorphe (Martoja, 1974) et que, chez les Gastéropodes, il y a une "multiplicité des facteurs internes et externes qui influent sur l'excrétion azotée" (Daguzan, 1980).

La composition élémentaire des bioaccumulation calciques n'est liée ni à l'habitat puisque tous nos animaux sont dulcicoles, ni au rythme saisonnier puisque tous ont été autopsiés la même époque, ni à la position systématique puisque le carbonate et le phosphate de calcium ont été trouvés indifféremment dans les deux sous-classes. Le cas extrême est représenté par la Limnée, capable d'accumuler à la fois le carbonate et le phosphate dans deux types distincts de concrétions. A l'heure actuelle, nous ne sommes pas en mesure d'interpréter ces résultats.

La présence de phosphate de calcium dans la glande digestive a été reconnue très tôt, par les zoologistes du siècle dernier. Récemment, ce composé a été identifié dans les concrétions du rein de Gastéropodes et de Bivalves (Martoja, 1975; Doyle et al., 1978; Hignette, 1979). Nos résultats présents permettent d'ajouter à cette liste, le tissu conjonctif de certains Gastéropodes dulcicoles.

En conclusion, le principe de la spécificité minéralogique valable pour la coquille, ne s'applique pas aux sphérocristaux du tissu conjonctif. D'autre part, il n'y a pas lieu d'opposer de façon systématique les dépôts calcaires de la glande digestive ou du rein à ceux du tissu conjonctif, Celui-ci contient certes souvent du carbonate de calcium mais des sphérocristaux de phosphate de calcium auquel sont associés éventuellement d'autres sels, peuvent aussi s'y former.

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ANALYTICAL DATA ON CONNECTIVE TISSUE CONCRETIONS OF SEVERAL FRESH-WATER GASTROPODS

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SUMMARY

Concretions within the connective tissue of five species of fresh-water prosobranch and pulmonate gastropods have been investigated by X-ray spectrometry, secondary ion emission analysis and Raman spectrometry on paraffin wax sections cut between 6 and 10 μm. Calcium carbonate has been characterized in *Valvata cristata* and *Planorobarius corneus*, calcium phosphate in *Viviparus viviparus* and *Bithynia tentaculata*; small carbonate crystals and phosphate spherocrystals exist together in *Lymnaea peregra.* The composition may be simple (pure calcite in *Valvata*) or complex (4 salts and 6 elements found in *Lymnaea*). It has no relation to the systematic position of the species.

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BIOCHEMICAL GENETICS OF THE SNAIL GENUS *PHYSA*: A COMPARISON OF POPULATIONS OF TWO SPECIES

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ABSTRACT

Vertical starch gel electrophoresis was employed to compare two population samples each of *Physa gyrina* and *Physa anatina* collected near Urbana, Illinois. Eleven enzyme systems were examined and gene products of 14 presumptive loci were resolved. Six of the loci (aspartate aminotransferase, Aat-A; two esterases, Est-1 and Est-2; glucosephosphate isomerase, Gpi-A; glycerol-3-phosphate dehydrogenase, G-3-pdh-A; and phosphoglucomutase, Pgm-A) were polymorphic. The Aat-A, G-3-pdh-A, and Pgm-A gene products exhibited species-specific electrophoretic patterns. Average heterozygosity values ranged between 5.1 and 6.7%. Intraspecific genetic distances were $D = 0.012$ or less. The genetic distance between *P. anatina* and *P. gyrina* was $D = 0.45$.

Key words: Physidae; *Physa*; allozymes; electrophoresis; polymorphism; heterozygosity.

INTRODUCTION

Since the first demonstration by Hubby & Lewontin (1966) and Harris (1966) that genetic polymorphism at structural loci can be easily detected by gel electrophoresis, there has been an explosion of information about genic polymorphism in natural populations. Many of the investigations of genetic variability of aquatic macroinvertebrates have been conducted with snails (Wium-Anderson, 1973; Coker & Kuma, 1974; Narang, 1974; Narang & Narang, 1974, 1976a, 1976b; Ukoli, 1974; Michelson & Dubois, 1975; Nickerson, 1975; Nyman, 1975; Wu & Burch, 1975; Ishay et al., 1976; Logvinenko et al., 1976; Monteiro & Narang, 1976; Chambers, 1977, 1978, 1980; Nyman & Skoog, 1977; Selander et al., 1978; Te, 1978; Davis, 1979; Wurzinger, 1979; Wurzinger & Saliba, 1979; Dillon & Davis, 1980). In most studies utilizing aquatic snails prior to 1970, allozymes were separated with paper, cellulose acetate, or polyacrylamide media. In these earlier studies, banding patterns were recorded and compared with no attempts to ascertain the genetic control of the gene products observed. Most of the recent studies have dealt with species of the genera *Bulinus* or *Biomphalaria* as they serve as intermediate hosts for schistosomes and electrophoretic methods can aid in identifying strain and species within these genera.

The Physidae are a speciose family of freshwater snails that have been used in various areas of environmental research such as model ecosystem studies of pesticide degradation and accumulation (Metcalf et al., 1971), toxicity testing (Patrick et al., 1968), and water quality assessment (Tucker & Ettinger, 1975). The chaotic taxonomy of the Physidae has recently been treated by Te (1975, 1978) and his work included some electrophoretic analyses. However, several aspects of the population genetics of this important family have yet to be investigated. The major objectives of this study are to: (1) adapt known techniques of vertical starch gel electrophoresis for use on *Physa,* (2) examine gene expression in several enzyme systems in representative species of *Physa,* and (3) examine genetic divergence among populations of *Physa* at the intraspecific and interspecific levels.

MATERIALS AND METHODS

Snails were collected with dipnets and by hand. Live specimens were transported to the laboratory at ambient temperature, then frozen and stored at −20°C. Electrophoretic examinations were completed within three weeks of the initial freezing. Voucher specimens from each locality were deposited in the collection of the Illinois Natural History Survey

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(INHS—JS series). The following listing of populations sampled includes the number of individuals examined from each site in parentheses.

Physa anatina Lea. I. Busey Woods Pond, 0.16 km NE of Urbana, Champaign Co., IL, T19N, R9E, NW ¼, NE ¼, NW ¼, Sect. 8 of Thomasboro 7½ min. quad., (14) [JS 1002]; II. Pond No. 16 of the Illinois Natural History Survey, 0.8 km S of Champaign at the Natural Resources Annex, Champaign Co., IL, T19N, R8E, SE ¼, NE ¼, SE ¼, Sect. 24 of Urbana 7½ min. quad (10) [JS 1003].

Physa gyrina Say. I. Busey Woods Pond, same locality as for *P. anatina* I, (26) [JS 1001]; II. Boneyard Creek, in Champaign at Second Street, Champaign Co., IL, T19N, R9E, SW ¼, SW ¼, SW ¼, Sect. 7 of Urbana 7½ min. quad., (28) [JS 1004].

All soft body parts were dissected from each specimen, mixed with an equal volume of 0.1 M Tris-HCl at pH 7.0, mechanically homogenized, and centrifuged at approximately 480 *g* at 4°C for 15 mins. The supernatant fractions of the whole body extracts were subjected to electrophoresis at 4°C using 14% starch gels (lot #303; Electrostarch Co., Madison, WI 53701). Enzyme systems examined and their histochemical staining conditions are listed in Table 1. Electrophoretic buffers used include a 0.25 M sodium borate buffer at pH 8.6 (Sackler, 1966), a 0.41 M sodium citrate buffer at pH 7.0 or 8.0 (Brewer, 1970), a Tris-citrate buffer at pH 7.0 (Whitt, 1970), and an EDTA-borate-Tris buffer at pH 8.6 (Whitt et al., 1973; Wilson et al., 1973). Electrophoretic conditions were those of Buth & Burr (1978) using vertical starch gel apparatus.

Gene products were scored as those of autosomal loci with codominant alleles. Electromorphs of common electrophoretic mobility are assumed to represent homologous allelic products of a given locus. A gene locus was designated as the "A-locus" for a particular enzyme in cases of presumptive single gene control of such enzymes. Multilocus systems were lettered (or numbered in the case of non-specific esterases) based on the mobility of their gene products from cathode to anode. Locus homologies with taxa outside the genus *Physa* are not reflected in our genetic nomenclature and should not be inferred without additional study. Allelic terminology is based on the electrophoretic mobility of the allelic products relative to the origin as used by Selander & Kaufman (1975) and Selander et al. (1978). The reference allele at each locus (=100) was chosen as the most common allele in the population of *Physa gyrina* from the Busey Woods Pond locality. Cathodally migrating allelic products are assigned negative values.

Heterozygosity estimates are based on a direct count of heterozygotes (Selander et al., 1978) and the calculation of average heterozygosity (Nei, 1978).

RESULTS

The electrophoretic examination of 11 enzyme systems allowed the resolution of the gene products of 14 presumptive loci. Six of these loci were polymorphic within or among the populations of *P. anatina* and *P. gyrina*. The allele frequencies at these polymorphic loci are given in Table 2; the genotypic distri-

TABLE 1. Enzyme systems examined and their electrophoretic requirements. See text for buffer references.

Enzyme	Enzyme commission number	Locus	Electrophoretic buffer	Staining reference
Acid phosphatase	3.1.3.2	Acph-A	Tris-citrate	Shaw & Prasad (1970)
Alkaline phosphatase	3.1.3.1	Akph-A	Tris-citrate	Shaw & Prasad (1970)
Aspartate aminotransferase	2.6.1.1	Aat-A	Citrate, pH8	Shaw & Prasad (1970)
Esterase	—	Est-1,2	EBT	Brewer (1970)
Glucosephosphate isomerase	5.3.1.9	Gpi-A	EBT	Buth & Murphy (1980)
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G-3-pdh-A,B	Citrate, pH8	Shaw & Prasad (1970)
Leucine aminopeptidase	3.4.1.1	Lap-A	Citrate, pH7	Brewer (1970)
L-iditol dehydrogenase	1.1.1.14	Iddh-A	Borate	Shaw & Prasad (1970)
Malate dehydrogenase	1.1.1.37	Mdh-A	Tris-citrate	Shaw & Prasad (1970)
Phosphoglucomutase	2.7.5.1	Pgm-A	Citrate, pH8	Buth & Murphy (1980)
Superoxide dismutase	1.15.1.1	Sod-A,B	EBT	Johnson et al. (1970)

TABLE 2. Allele frequencies at six polymorphic loci in four populations of *Physa*.

		P. anatina		*P. gyrina*	
Locus	Allele	Busey Woods	INHS Pond	Busey Woods	Boneyard Creek
Aat-A	100	—	—	1.00	1.00
	156	1.00	1.00	—	—
Est-1	–100	0.11	0.25	1.00	1.00
	–162	0.89	0.75	—	—
Est-2	100	—	—	0.63	0.61
	116	—	—	0.37	0.25
	132	0.54	0.15	—	0.14
	154	0.46	0.80	—	—
	170	—	0.05	—	—
Gpi-A	89	—	—	—	0.09
	100	—	—	0.77	0.77
	111	1.00	1.00	0.23	0.14
G-3-pdh-A	100	—	—	1.00	1.00
	156	1.00	1.00	—	—
Pgm-A	83	1.00	1.00	—	—
	100	—	—	1.00	1.00

butions at the three intraspecifically polymorphic loci are provided in Table 3. The small sample sizes in this preliminary study precludes accurate Chi-square tests of Hardy-Weinberg expectations. Given the distributions resolved in this study and the potential hermaphroditic mode of reproduction in *Physa*, we recommend much larger sample sizes for populations in future studies.

Genetic variability, as ascertained via several measures of heterozygosity, in these populations is compared in Table 4. *Physa anatina* and *P. gyrina* show no marked differences in these measures with both species exhibiting approximately 5% heterozygosity.

Genetic differentiation within and between the species was quantified by calculating Nei's (1972) coefficients of genetic similarity (I) and genetic distance (D) between all pairs of populations (Table 5). Intraspecific genetic distances were $D = 0.012$ or less. The genetic distance between *P. anatina* and *P. gyrina* is $D = 0.45$.

DISCUSSION

The use of whole-body homogenates in the study of gastropod enzymes has yielded complex patterns in genera other than *Physa*, presumably due to the expression of multiple loci (Nyman & Skoog, 1977). Relatively few multi-locus systems were resolved in this study. Gene duplication is believed to have played a negligible role in the evolution of the genus *Physa* (White, 1978), although gene expression differences may be of great utility in ascertaining relationships among pulmonate genera as have been used in studies of vertebrate polyploids, e.g. Ferris & Whitt (1978) and Buth (1979).

The enzyme systems examined in this study were divided into four groups based on gene expression and allelic variability. The groups are discussed below.

Single locus—Monomorphic: The five enzymes in this category include acid and alkaline phosphatases, leucine aminopeptidase, L-iditol (sorbitol) dehydrogenase (Fig. 1A), and malate dehydrogenase (Fig. 1B). A considerable mobility difference observed between the gene products of the Acph-A and Akph-A loci supports the genetic recognition of these as separate systems although they share a common substrate. Several other areas of enzymatic activity appeared with the LAP stain although these may be esterases rather than additional LAP loci. Multiple LAP loci have been reported in other gastropods, e.g. *Rumina decollata* (Selander & Hudson, 1976), although the zymograms for these species are unavailable for comparison. The single locus expression of MDH in *Physa* may be unusual. In vertebrates, MDH is at least a two-locus system in a mitochondrial-cytosol

TABLE 3. Genotypic distributions at the three intraspecifically polymorphic loci in the four populations of *Physa*. The allelic designations are those used in Table 2.

1. Est-1	−100/−100	−100/−162	−162/−162						
P. anatina I	0	3	11						
P. anatina II	1	3	6						
P. gyrina I	26	0	0						
P. gyrina II	28	0	0						
2. Est-2	100/100	100/116	116/116	116/132	100/132	132/132	132/154	154/154	132/170
P. anatina I	0	0	0	0	0	4	7	3	0
P. anatina II	0	0	0	0	0	0	2	7	1
P. gyrina I	12	9	5	0	0	0	0	0	0
P. gyrina II	13	1	6	1	7	0	0	0	0
3. Gpi-A	89/89	89/100	100/100	100/111	111/111				
P. anatina I	0	0	0	0	14				
P. anatina II	0	0	0	0	10				
P. gyrina I	0	0	15	10	1				
P. gyrina II	1	3	16	8	0				

TABLE 4. Genetic variability in four populations of *Physa*. Calculations are based on the gene products of 14 loci.

Taxon	Locality	Sample size	Proportion of heterozygotes observed	Proportion of heterozygotes expected*	Average heterozygosity (Nei, 1978) ± S.E.	Effective number of alleles
P. anatina	Busey Woods	14	0.051	0.050	0.051 ± 0.039	1.088
P. anatina	INHS Pond	10	0.043	0.051	0.053 ± 0.036	1.079
P. gyrina	Busey Woods	26	0.052	0.059	0.060 ± 0.041	1.102
P. gyrina	Boneyard Creek	28	0.051	0.066	0.067 ± 0.047	1.130

*"Hardy-Weinberg heterozygosity," sample size not considered.

TABLE 5. Comparison of Nei's (1972) genetic similarity coefficients (*I*; above diagonal) and genetic distance coefficients (*D*; below diagonal) between populations. Standard errors of the distance coefficients are given in parentheses. Calculations are based on the gene products of 14 loci.

	P. anatina I	*P. anatina* II	*P. gyrina* I	*P. gyrina* II
P. anatina I (Busey Woods)	—	0.988	0.630	0.631
P. anatina II (INHS Pond)	0.012 (±0.022)	—	0.641	0.638
P. gyrina I (Busey Woods)	0.462 (±0.157)	0.445 (±0.153)	—	0.998
P. gyrina II (Boneyard Creek)	0.460 (±0.156)	0.449 (±0.154)	0.002 (±0.009)	—

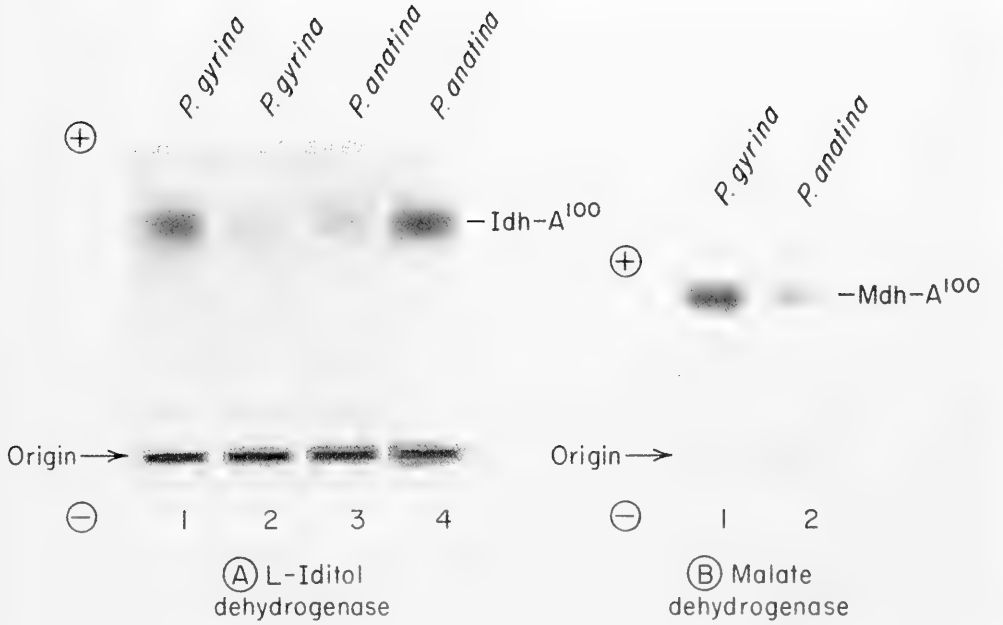

FIG. 1. Zymograms of monomorphic L-iditol (A) and malate dehydrogenase (B) expression in *P. anatina* and *P. gyrina*.

relationship (e.g. Rainboth & Whitt, 1974). Two MDH loci have been reported in other gastropods, e.g. *Campeloma decisa* (Selander et al., 1978). The absence of a second MDH system in *Physa* may be due to its weaker expression and/or restriction in expression to a proportionately small organ.

Single locus—Polymorphic: The aspartate aminotransferase, glucosephosphate isomerase (Fig. 2A), and phosphoglucomutase (Fig. 2B) systems are included in this group. Species-specific allelic differences in the AAT system have been of taxonomic utility in *Goniobasis* (Chambers, 1978) and serve to distinguish *P. anatina* and *P. gyrina.* AAT, formerly "GOT" (glutamate oxalacetate transaminase), might be expected to be a multilocus system in a mitochondrial-cytosol relationship comparable to MDH as is the case in vertebrates. In *Physa*, faint cathodally-migrating gene products of what may be a second AAT locus were inconsistently resolved. Multiple AAT ("GOT") loci have been reported from other gastropods, e.g. *Rumina decollata* (Selander & Hudson, 1976). The GPI polymorphism in *P. gyrina* yields three-electromorph presumptive heterozygotes. The formation of a single heteropolymer in this situation suggests a dimeric structure of GPI in *Physa* as is the case in vertebrates. We have resolved a single PGM system in *Physa* although as many as five loci have been reported in other gastropods (Selander & Hudson, 1976). Our resolution of PGM products is less than optimal with the production of numerous equidistant anodal subbands appearing in all specimens (Fig. 2B).

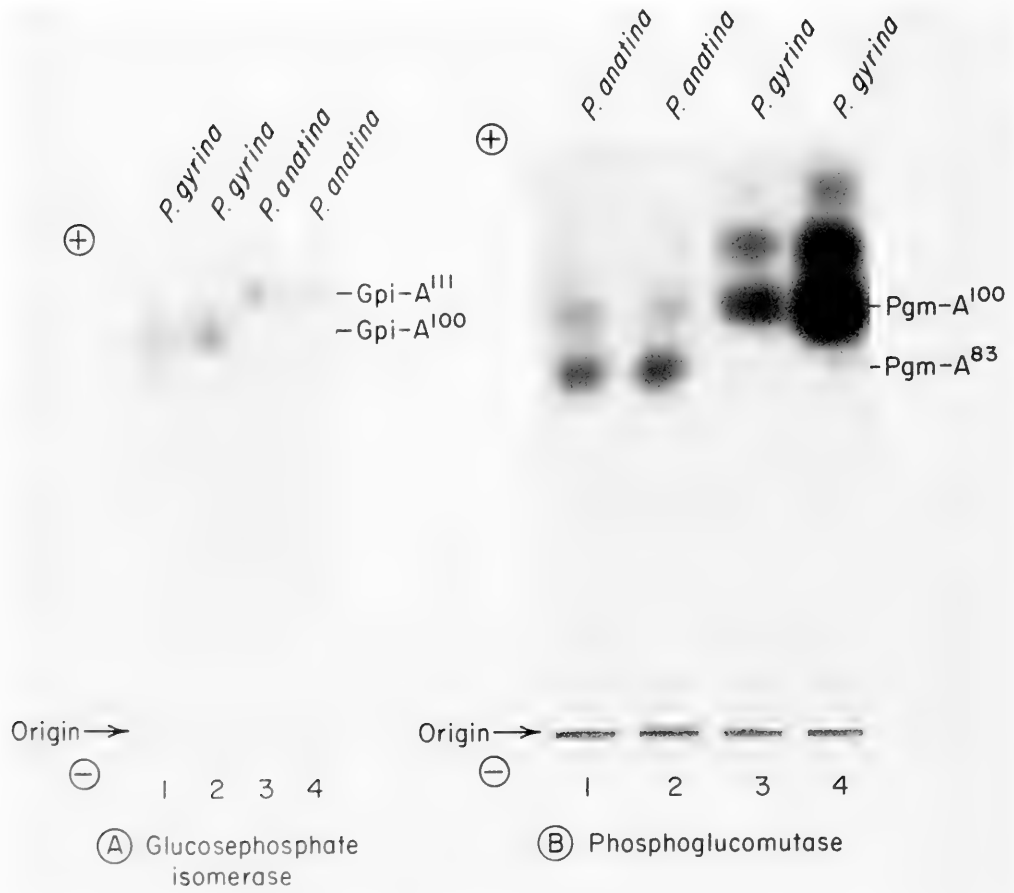

FIG. 2. Zymograms of polymorphic glucosephosphate isomerase (A), and phosphoglucomutase expression (B) in *P. anatina* and *P. gyrina.* All individuals in Fig. 2 are in the homozygous state.

Multiple locus—Monomorphic: The gene products of two superoxide dismutase loci (SOD) plus a presumptive interlocus heterodimer were resolved from all specimens. Multiple SOD loci, formerly "IPO" (indophenol oxidase), have been reported from other gastropods, e.g. *Rumina decollata* (Selander & Hudson, 1976).

Multiple locus—Polymorphic: Two G-3-PDH loci are expressed in *Physa.* The G-3-pdh-B locus is monomorphic in both *P. anatina* and *P. gyrina* whereas a species-specific allelic difference is observed at the G-3-pdh-A locus. An interlocus heteropolymer is formed (Fig. 3), suggesting a dimeric structure for G-3-PDH as is the case in vertebrates. The G-3-PDH system has received little attention in previous gastropod electrophoretic studies. The low staining cost and clarity of its resolution make this enzyme system particularly desirable for use in future studies. Two very polymorphic esterase systems (one cathodal and one anodal) were clearly resolved in *Physa.* A third apparently polymorphic esterase locus, with extremely rapid anodal migration of its products (Est-3), was not consistently resolved. Esterases have been extensively

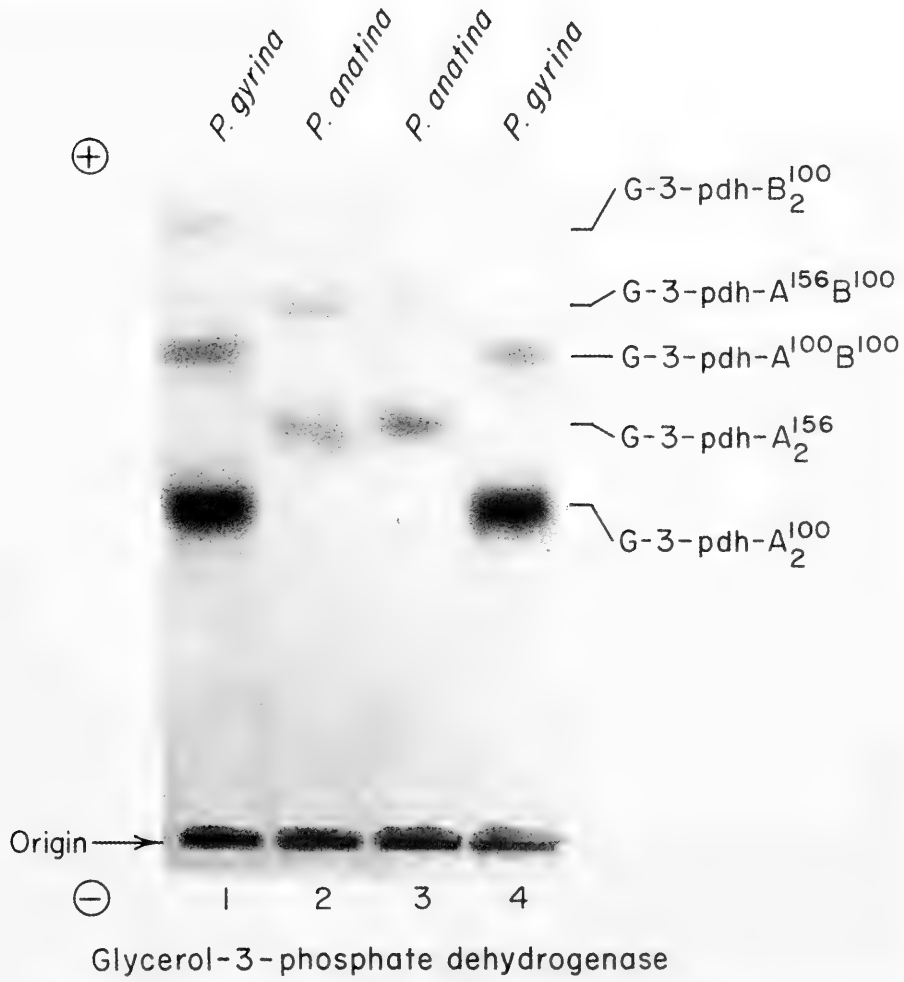

FIG. 3. Zymogram of the multilocus glycerol-3-phosphate dehydrogenase system in *P. anatina* and *P. gyrina.* The subunit composition of each electromorph is indicated.

studied in gastropods. Their genetic control has not always been easily elucidated (e.g. Nyman & Skogg, 1977), however, some investigators have resolved a considerable number of esterase loci, e.g. twelve in *Rumina decollata* (Selander & Hudson, 1976). All esterase heterozygotes in this study have two-electromorph expression, suggesting the monomeric composition of the enzyme.

Our examination of geographically proximate populations of *Physa* has provided no evidence for substantial local intraspecific differentiation in these species. This, however, does not preclude regional differentiation elsewhere in the ranges of these widespread forms. The substantial levels of polymorphism observed in *Physa* should allow the resolution of such regional restrictions in gene flow if they exist. The genetic differentiation between these subgenerically distinct species of *Physa* is substantial yet not absolute. Thus, electrophoretic characteristics may be of taxonomic utility at several levels within the genus: populations, species, subgenera.

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GENETIC AND MORPHOLOGICAL DIVERGENCE AMONG NOMINAL SPECIES OF NORTH AMERICAN *ANODONTA* (BIVALVIA: UNIONIDAE)

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ABSTRACT

Stomach morphology of six species of the unionid genus *Anodonta* is illustrated, and a phenogram based on percent similarity of stomach morphological character-states is presented for these and two additional species. The eight species can be divided into four groups based on stomach structure. The first and second groups seem closely related, and contain species in the subgenus *Utterbackia (A. imbecilis, A. couperiana, A. peggyae)* and the subgenus *Anodonta,* s.s. *(A. cygnea)*; the third group is composed of some of the members of the subgenus *Pyganodon (A. cataracta cataracta, A. grandis, A. gibbosa),* and a fourth group is formed by a single species, *A. implicata.* Support for the separation of *A. implicata* from the rest of the subgenus *Pyganodon* comes from electrophoretic data. This proposed separation, the close relationship in stomach structure between the American subgenus *Utterbackia* and the European subgenus *Anodonta,* s.s. and the proposed European affinity of *A. c. fragilis* from Nova Scotia are all contrary to present taxonomic classifications. These data indicate that an integrated study of the entire genus based on shell, soft-part, and electrophoretic characters is in order.

Key words: *Anodonta*; stomach morphology; electrophoresis; taxonomy; Unionidae.

INTRODUCTION

Bivalves of the family Unionidae are characterized by a high degree of phenotypic plasticity in shell shape and soft-part morphology. Early taxonomists relied almost exclusively on conchological characters to describe species, with the result that geographically widespread, variable species are burdened with extensive synonymies. Characters of the soft anatomy used in modern classification include marsupial structure and morphology of the gills and characteristics of the siphons, mantle margin, and mode of reproduction (i.e. hermaphroditic, dioecious). However, these soft-part characters still are used almost entirely at taxonomic levels above that of the species, whereas conchological characters such as umbonal sculpture, dentition type, nacre color, and shell shape are used to differentiate among species within a genus. Allozyme electrophoretic methods, as well as immunoelectrophoresis, are important taxonomic tools for discriminating among species (Davis & Fuller, 1981; Davis et al., 1981), but even these methods pale in the face of the high genetic similarity that can occur among some conchologically defined species of the apparently recently radiating genus *Elliptio* (Davis et al., 1981). It is evident that an integrated approach that uses soft-part and conchological characteristics as well as electrophoretic techniques should be used to resolve the prevailing taxonomic confusion about the Unionidae.

Details of stomach structure have been used successfully in the past for determination of taxonomic affinities within the Bivalvia (Purchon, 1956, 1957, 1958, 1960, 1968; Graham, 1949; Dinamani, 1967). Relationships between and patterns of variation among various structural components of the stomach, however, have generally been used to differentiate among rather large taxonomic groupings, such as orders or families. To my knowledge, bivalve stomach structure has not been examined in order to determine relationships within a genus, probably in part because Graham (1949) and Purchon (1956 et seq.) indicated a fundamental similarity of structure throughout the class, so that consistent differences would be expected to occur only among higher taxa. For example, with respect to the Unionacea, Purchon (1958) mentioned that ". . . the internal structure of the stomach is of high stability . . ." and

found little difference between *Anodonta cygnea* and *Velesunio ambiguus* (Philippi, 1847), taxa from different families (Unionidae and Hyriidae) within the Unionacea.

Species of the circumboreal genus *Anodonta* were chosen in this study for several reasons. The genus seems to have undergone a moderate radiation yielding 14 species on the North American subcontinent (see Johnson, 1970; Burch, 1975). While conchologically defined species are not beset by extensive synonymies indicative of the confusion prevalent among some other genera, it has become increasingly apparent that the *Anodonta* complex needs careful revision. As the name implies, *Anodonta* lacks dentition, and taxonomic characters used to discriminate among species include umbonal sculpture, glochidial shell structure, and reproductive mode. The inadequacy of this system became clear because of recent debate about the taxonomic validity of *A. peggyae,* which had been included in *A. imbecilis*; uncertainty as to the status of several nominal species such as *A. hallenbeckii* Lea, 1858, *A. henryana* Lea, 1857, and *A. corpulenta* Cooper, 1834, and proliferation of subspecies of the phenotypically diverse *A. grandis.*

Six species of *Anodonta* that occur in the eastern United States (*A. cataracta cataracta, A. couperiana, A. gibbosa, A. imbecilis, A. implicata,* and *A. peggyae*) were chosen for this study in an attempt to determine whether stomach characteristics could be used to differentiate among unionid species. In addition, stomach morphology of *A. grandis* and *A. cygnea* was studied for comparative purposes.

METHODS

The classification and collection localities of the taxa studied are presented in Table 1. Voucher specimens have been deposited at the Academy of Natural Sciences of Philadelphia (ANSP).

TABLE 1. Classification and collection localities of the species of *Anodonta* included in this study.

Family Unionidae (Fleming, 1828) Ortmann, 1911	
Subfamily Anodontinae (Swainson, 1840) Ortmann, 1910	
Genus *Anodonta* Lamarck, 1799	
Subgenus *Anodonta,* s.s.	
A. cygnea (Linnaeus, 1758) ANSP 355545	Waterwingebied 1 km E of Loenen, Noord Holland, The Netherlands
Subgenus *Pyganodon* Crosse & Fischer, 1894	
A. cataracta Say, 1817 ANSP 355544	Norwich Creek, 8 km E of Wye Mills, Talbot Co., Maryland
A. cataracta fragilis (*sensu* Clarke & Rick, Clarke & Rick, 1963) No vouchers	First Lake O' Law, ca. 25 km NW of Baddeck, Victoria Co., Nova Scotia, Canada
A. grandis Say, 1829 No vouchers (shells broken)	Lake Champlain, 5 km W of Colchester, Chittenden Co., Vermont
A. gibbosa Say, 1824 ANSP 355542	Ocmulgee River, 10 km SW of Jacksonville, Ben Hill Co., Georgia
A. implicata Say, 1829 ANSP 355543	Norwich Creek, 8 km E of Wye Mills, Talbot Co., Maryland
Subgenus *Utterbackia* F. C. Baker, 1927	
A. couperiana Lea, 1840 ANSP 355539	Lake Osborne, Lantana, Palm Beach Co., Florida
A. peggyae Johnson, 1965 ANSP 355541	Withlacoochee River, 1.5 km N of Lacoochee, Pasco Co., Florida
A. imbecilis Say, 1829 ANSP 355540	Lake Osborne, Lantana, Palm Beach Co., Florida

Stomach structure was studied in preserved specimens that had been relaxed with sodium nembutal, fixed in 10% formalin, and preserved in 70% ethyl alcohol. The stomach was exposed by removing the surrounding muscle fibers and digestive diverticula. The stomach then was opened by first removing the entire dorsal region with a circular cut beginning and ending at the oesophagus, and then making a second cut around the base of the stomach to expose the stomach floor. The gastric shield was removed. This method has the advantage of imparting clarity to the rather complex anatomy of the stomach, a clarity commonly lacking in previous studies that often distorted the shape of the stomach for purposes of illustration. The dorsal region of the stomach exhibited only minor variation among the species studied, and consequently only the stomach floor has been illustrated. A minimum of four individuals of each species was examined in order to determine the extent of intraspecific variation, and the illustrations represent a compilation of features from these individuals. Intraspecific variation did occur, but it was never sufficient to cause confusion among species.

The terminology of stomach structures used by Owen (1955), Purchon (1958), and Dinamani (1967) has been followed in this paper.

Degree of resemblance among species was determined on the basis of levels of similarity of nine structural components of the stomach, including shape of the minor typhlosole, shapes of the style sac and midgut, and the relationship between the minor typhlosole and the right sorting pouch. Each character was assigned a set of character states (Table 2) according to which each species was classified (Table 3). It is important to point out, however, that the level of resemblance be-

TABLE 2. Stomach characters analysed to determine relationships among species of *Anodonta*.

1. Shape and relationship of the minor typhlosole to the midgut
    - A. Hooded
    - B. Open
2. Shape of the style sac
    - A. Rounded, with continuous raised rim
    - B. Rounded, with partially raised rim
    - C. Elongate, with partially raised rim
3. Shape and relationship of the major typhlosole fold
    - A. Regularly curved
    - B. Regularly curved, terminal concavity
    - C. Sinusoidal
4. Curvature of the major typhlosole fold
    - A. Relatively straight
    - B. Curved
    - C. Wavy
5. Shape of the sorting pouch
    - A. Slightly curved
    - B. Highly curved
    - C. Hooked
6. Position of the junction between the sorting pouch and the minor typhlosole fold
    - A. Dorsal
    - B. Medial
    - C. Distal
7. Curvature of the minor typhlosole fold
    - A. Uniformly curved
    - B. Non-uniformly curved
    - C. Straight sections
8. Distance separating the minor typhlosole fold and the anterior part of the minor typhlosole
    - A. Considerable
    - B. Slight
9. Curvature of the terminal portion of the minor typhlosole
    - A. Slightly curved
    - B. Highly curved
    - C. Hooked

TABLE 3. Character states (see Table 2) for the various species of *Anodonta* examined. Levels of similarity between species pairs presented in Table 4 are based on these character states.

Species	Character and character state								
	1	2	3	4	5	6	7	8	9
A. c. cataracta	A	A	B	A	A	A	A	B	A
A. c. fragilis	A	B	A	B	A	B	B	A	A
A. grandis	A	A	A	B	A	A	A	B	A
A. implicata	B	C	A	C	C	C	C	A	C
A. gibbosa	A	A	C	A	A	A	B	B	B
A. couperiana	B	A	A	A	B	B	C	A	A
A. peggyae	B	B	C	B	B	B	C	A	C
A. imbecilis	B	B	A	B	B	B	B	A	C
A. cygnea	A	B	A	C	A	A	B	A	A

tween species pairs is not calculated only from the number of characters in common between each species pair. Rather, each character was examined separately among representatives of each species pair, assigned a rating of "identical," "similar," or "different," and given a value of 2, 1, or 0, respectively. The values of all nine features were then added and divided by 18 (9 × 2) in order to ascertain level of resemblance between species pairs. By this method, species could share no characters in common in Table 3, but still exhibit some degree of similarity. As examples, *Anodonta c. cataracta* and *A. c. fragilis* share three identical characters, four similar characters, and two dissimilar characters = $3 \times 2 + 4 \times 1 + 2 \times 0 = 10/18 = .55$. *A. imbecilis* and *A. cygnea* share three identical characters, four similar and two different characters = $3 \times 2 + 4 \times 1 + 2 \times 0 = 10/18 = .55$. This particular approach was adopted because it accounts for intraspecific variability better than does a method that assigns discrete character states to each feature. There nevertheless exists overall accordance between the number of character states in common and levels of similarity, especially among closely related species.

The relationships among species and species groups were calculated from the percent

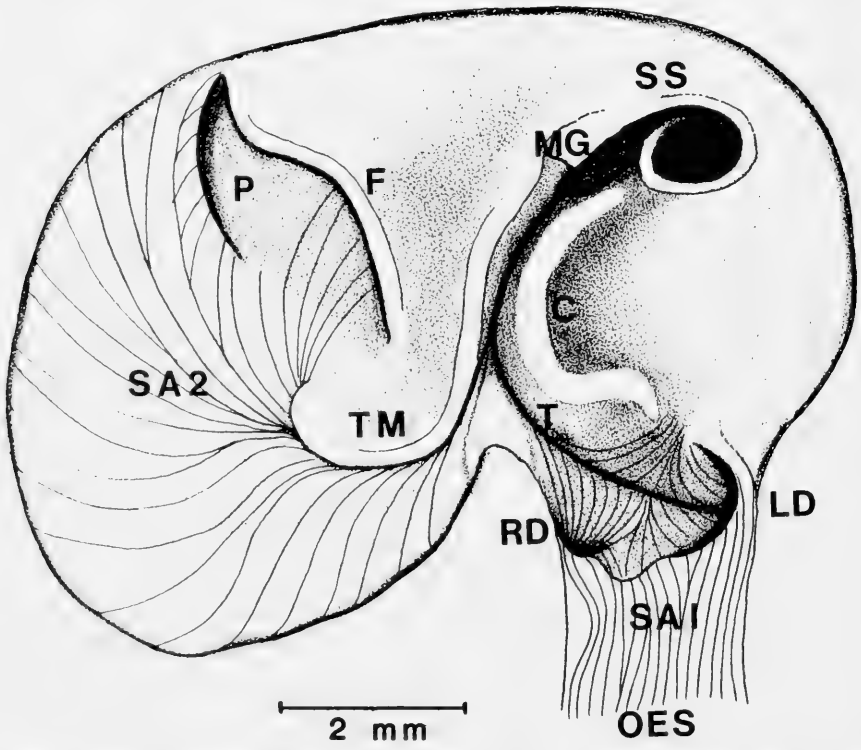

FIG. 1. Stomach floor of *Anodonta cataracta*. C—conical mound, F—minor typhlosole fold, LD—left duct to the digestive diverticula, MG—midgut, OES—oesophagus, P—right sorting area pouch, RD—right duct to the digestive diverticula, SA1—sorting area immediately inside the oesophageal opening, SA2—right sorting area, SS—style sac, T—major typhlosole, TM—minor typhlosole.

similarity of stomach features between species pairs. Several "rounds" of comparison were set up in which individual species or species groups were compared to each other. In cases where species groups were involved, the features were averaged. This averaging technique results in the level of similarity of 0.51 in comparisons between *Anodonta implicata* and the *A. cygnea-couperiana-imbecilis-peggyae* "group," for instance, while comparisons between *A. implicata* and individual members of this group can result in higher or lower levels of resemblance.

Horizontal starch gel electrophoresis was performed on three populations of *Anodonta implicata,* three populations of *A. cataracta fragilis,* two populations of *A. c. cataracta,* and one population of *A. gibbosa* according to the methods described by Ayala et al. (1973) which were modified for unionids by Davis et al. (1981). Fourteen loci were examined and scored according to the methods of Ayala et al. (1973). Nei's (1972) genetic distances were computer generated; these distances then were used in a cluster analysis (with unweighted arithmetic averages) routine available in the multivariate statistical program NT-SYS (Rohlf et al., 1974). This routine was used to generate a phenogram depicting the relationships among the taxa examined.

RESULTS

The general anodontine stomach groundplan can be described as follows. The short, wide oesophagus (OES; all structures labeled in Fig. 1) is longitudinally grooved and enters the anterodorsal region of the stomach. Immediately inside the oesophageal opening lies a small sorting area (SA1), also described by Purchon (1958) for *Anodonta cygnea*, which is considerably smaller than that indicated for *Lamellidens corianus* (Lea, 1836) by Dinamani (1967). The posterior section of the

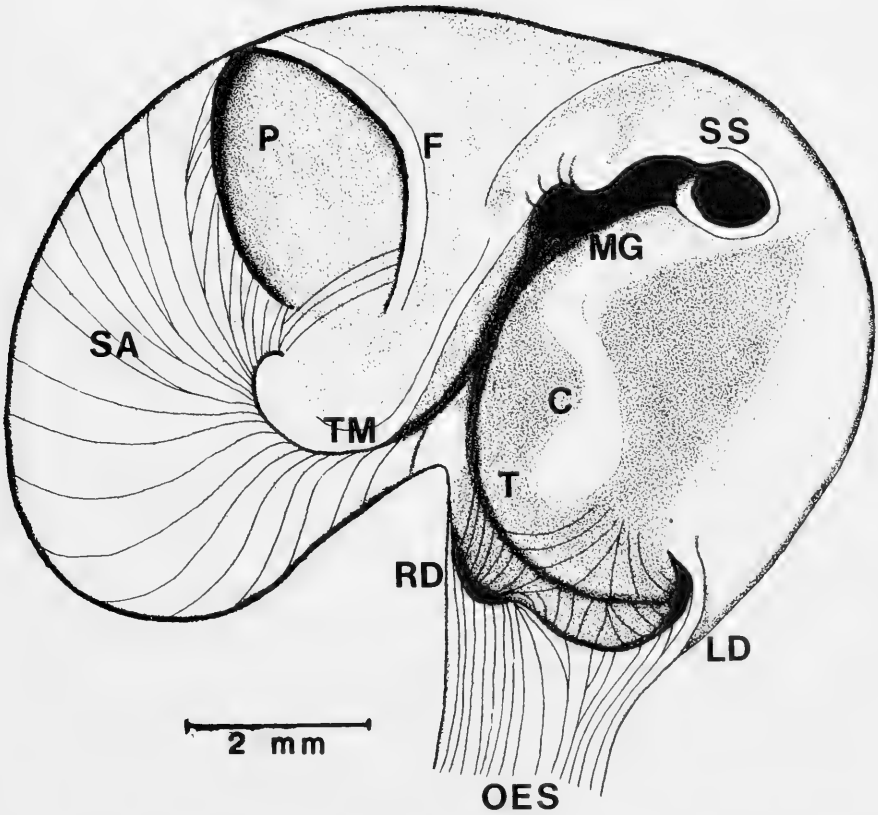

FIG. 2. Stomach floor of *Anodonta gibbosa.* For explanation of lettering see caption to Fig. 1.

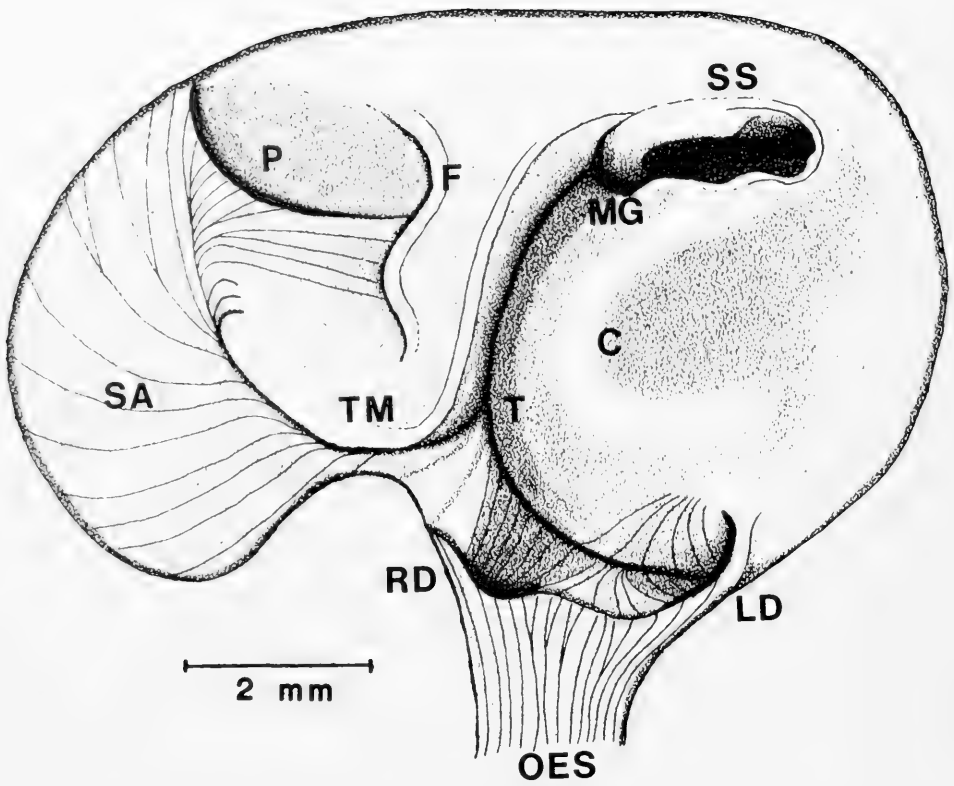

FIG. 3. Stomach floor of *Anodonta imbecilis.* For explanation of lettering see caption to Fig. 1.

stomach is folded inward so that a broad, featureless shelf (not illustrated) overlies the style sac and the right sorting pouch (P); the posterior section of this shelf connects to the roof of the stomach.

The major typhlosole (T) connects the common aperture of the style sac (SS) and the midgut (MG) to the ducts of the digestive diverticula (LD, RD), which lie in the anteroventral region of the stomach. As already described by Purchon (1958) and Dinamani (1967), all specimens of *Anodonta* dissected in this study displayed a prominent "conical mound" (C) on the stomach floor between the style sac and the digestive diverticula openings. Purchon (1958) proposed that the conical mound forms a division between the anterior and posterior section of the stomach; it could also function to keep the gastric shield in place.

The minor typhlosole (TM) runs adjacent to the major typhlosole for a short distance, but then turns sharply to the right and terminates in the right sorting area (SA2). This sorting area is composed of two sections, one above the other, the lowermost connected to the uppermost by a prominent pouch (P). To the left of this pouch a fold (F) defines the border of the sorting area.

The general structure of the anodontine stomach corresponds to Type IV of Purchon (1958) and Type IIIC of Dinamani (1967).

Figs. 1–6 illustrate the stomachs of *Anodonta cataracta cataracta, A. gibbosa, A. imbecilis, A. couperiana, A. peggyae,* and *A. implicata.* Table 4 contains levels of resemblance among these species, as well as *A. cygnea, A. grandis* and *A. c. fragilis.* Fig. 7 depicts relationships among eight of the species examined: *A. c. fragilis* was not included because of remaining uncertainty about its taxonomic status, which will probably remain unclear until topotypical *A. fragilis* from Newfoundland can be examined.

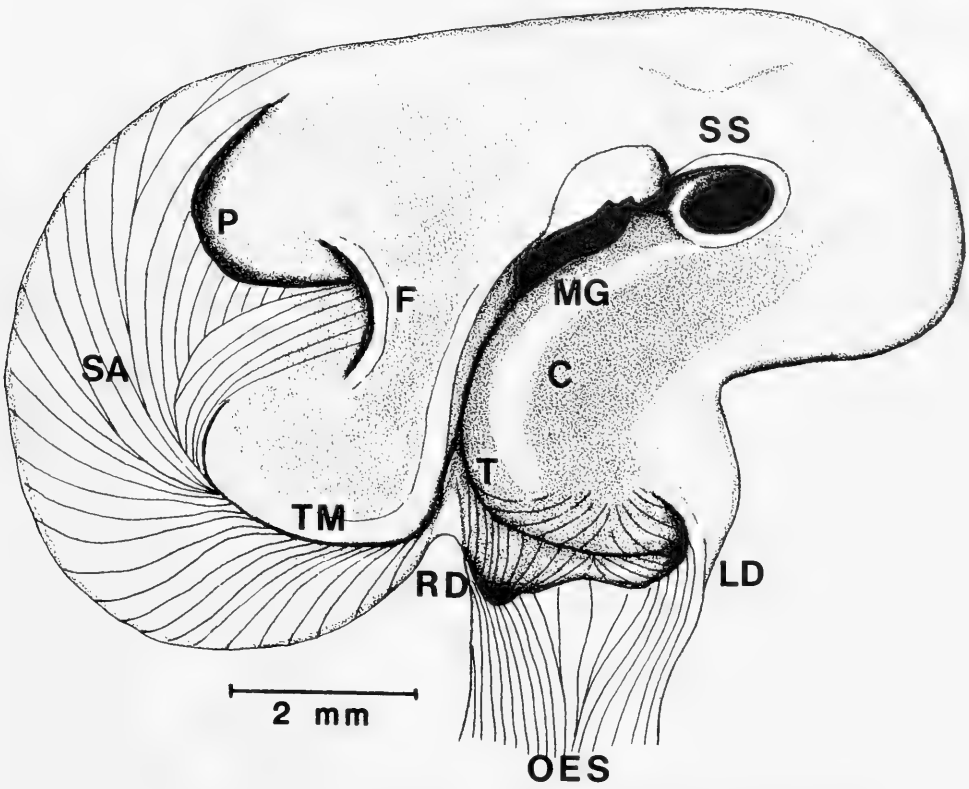

FIG. 4. Stomach floor of *Anodonta couperiana.* For explanation of lettering see caption to Fig. 1.

TABLE 4. Levels of similarity of stomach morphology among species of *Anodonta* examined.

	A. cygnea	*A. peggyae*	*A. imbecilis*	*A. couperiana*	*A. c. cataracta*	*A. implicata*	*A. gibbosa*	*A. grandis*	*A. c. fragilis*
A. cygnea	1.0	.61	.56	.50	.44	.33	.39	.39	.72
A. peggyae		1.0	.86	.50	.44	.67	.56	.50	.44
A. imbecilis			1.0	.78	.39	.61	.56	.56	.61
A. couperiana				1.0	.61	.44	.44	.67	.44
A. c. cataracta					1.0	.39	.83	.89	.55
A. implicata						1.0	.39	.39	.22
A. gibbosa							1.0	.72	.50
A. grandis								1.0	.55
A. c. fragilis									1.0

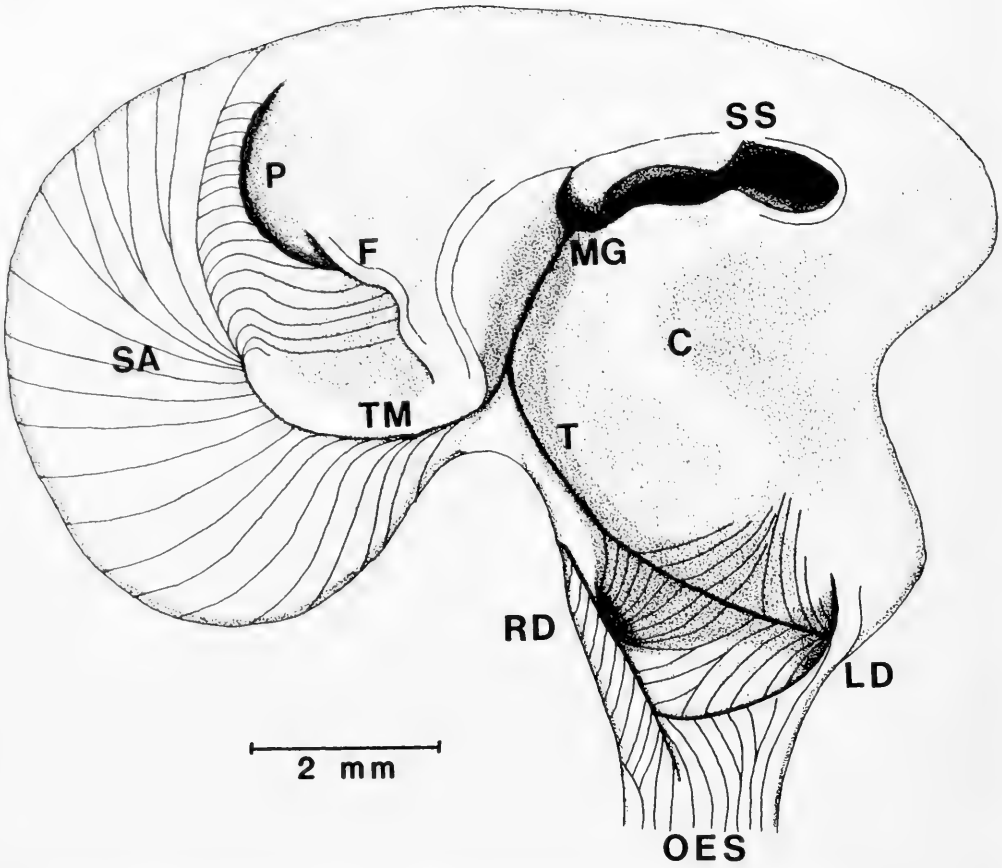

FIG. 5. Stomach floor of *Anodonta peggyae*. For explanation of lettering see caption to Fig. 1.

TABLE 5. Matrix of average Nei distances (above the diagonal) and identities (below the diagonal) between taxa of *Anodonta*.

	A. implicata	*A. gibbosa*	*A. c. cataracta*	*A. c. fragilis*
A. implicata	—	1.323	.997	1.012
A. gibbosa	.267	—	.497	.373
A. c. cataracta	.370	.608	—	.502
A. c. fragilis	.363	.689	.611	—

TABLE 6. Levels of heterozygosity (H) and polymorphism (P) for some representative species of *Anodonta*.

	Heterozygosity			Polymorphism		
Species	H	SD	Range	P	SD	Range
A. c. cataracta	0.028	0.006	0.022–0.035	0.113	0.039	0.071–0.142
A. gibbosa	0.106	—	—	0.285	—	—
A. implicata	0.061	0.007	0.056–0.069	0.357	0.000	0.357
A. c. fragilis	0.081	0.004	0.078–0.085	0.237	0.041	0.214–0.286

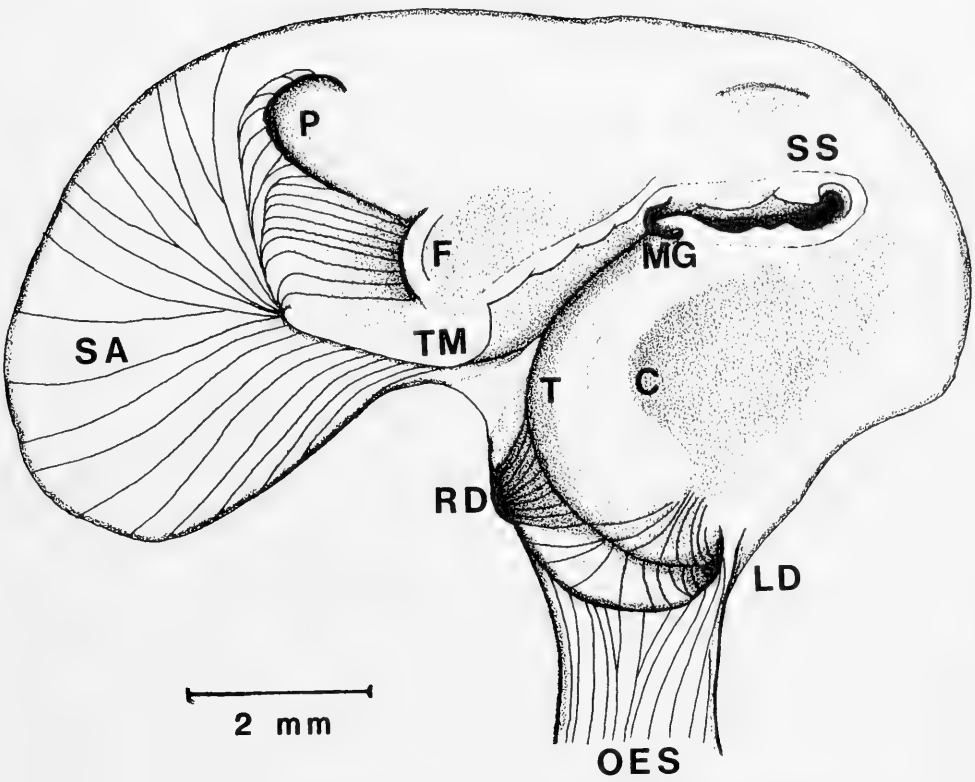

FIG. 6. Stomach floor of *Anodonta implicata.* For explanation of lettering see caption to Fig. 1.

Genetic distances and identities (Nei, 1972) (Table 5) and values for average observed heterozygosity (H) and polymorphism (P) are listed for the four taxa electrophoresed (Table 6). As noted by Davis et al. (1981), heterozygosity and polymorphism are quite low. Heterozygosity values range from about 3% for *Anodonta cataracta cataracta* to about 10% for *A. gibbosa,* and the most polymorphic species is *A. implicata* with about 36%. Table 7 presents allele frequencies and Table 8 compares distribution of alleles among the four taxa; other than *A. implicata,* few other taxa exhibit more than one allele per locus, even among those loci found to be "fast evolving" among other unionids (Davis et al., 1981). Fig. 8 depicts molecular genetic relationships among the taxa examined.

DISCUSSION

The species of *Anodonta* examined in this study can be divided into four groups based on stomach structure (Fig. 7). The first group includes species in the subgenus *Utterbackia (A. imbecilis, A. peggyae, A. couperiana),* the second contains *A. cygnea,* the third is composed of the "*cataracta*" group *(A. c. cataracta, A. grandis, A. gibbosa)* and the fourth is composed of a single species, *A. implicata.*

This study confirms the separation of *Anodonta peggyae* and *A. imbecilis*; nevertheless, the similarity level of 0.86 indicates a rather close relationships between the two species, which might have diverged rather recently; Heard (1975) mentioned that foot muscle proteins of *A. imbecilis* from Michigan and *A. peggyae* from Florida are similar at the 75% level.

Anodonta cygnea resembles *A. peggyae* most closely of all the species to which it was compared (0.61), with resemblance decreasing through *A. imbecilis* (0.56) and *A. couperiana* (0.50), and seems only distantly related to *A. grandis* and *A. c. cataracta.* Previous authors have hesitated to construct a taxonomic connection between *A. cygnea*

TABLE 7. Allele frequencies among the species of *Anodonta* examined. The exact locality data for each population are presented in the Appendix.

		Species and population								
		A. implicata			*A. c. fragilis*			*A. c. cataracta*		*A. gibbosa*
Enzyme	Allele	1	2	3	1	2	3	1	2	1
Gpi	20				.50	.50	.50			
	19									.50
	18	1.0	1.0	1.0				.50	.50	
	15				.50	.50	.50			
	14							.50	.50	.50
Pgm I	24							1.0	1.0	
	23	.06								
	21									.67
	20	.94	1.0	1.0	1.0	1.0	1.0			
	18									.33
Pgm II	32									1.0
	31				1.0	1.0	1.0			
	29	1.0	1.0	1.0				1.0	1.0	
Lap	30	.50	.50	.50						
	28				1.0	1.0	1.0			1.0
	27	.50	.50	.50				1.0	1.0	
Mdh 1	20									.50
	14							1.0	1.0	.50
	13	1.0	1.0	1.0	1.0	1.0	1.0			
Mdh II	–8		.05	.06						
	–11	1.0	.95	.94	1.0	1.0	1.0	1.0	1.0	1.0
Hex	35	1.0	1.0	1.0						
	31				1.0	1.0	1.0	1.0	1.0	1.0
Mpi	23			.05	.05	.15				
	22	.97	1.0	1.0						
	21							.48	.37	
	20				.95	.95	.85			.34
	18	.03						.52	.63	
	17									.16
6Pgd	4	1.0	1.0	1.0						
	3				1.0	1.0	1.0	1.0	1.0	1.0
Oct	10	.94	.50	.46						1.0
	9				1.0	1.0	1.0	1.0	1.0	
	8	.06	.50	.54						
Aat	15	.31	.30	.34						
	14	.69	.70	.66	1.0	1.0	1.0	1.0	1.0	1.0
Sod	24	.31	.15	.47						
	20	.69	.85	.53						
	13				1.0	1.0	1.0	1.0	1.0	1.0
G3pdh	13	1.0	1.0	1.0						
	10				1.0	1.0	1.0	1.0	1.0	1.0
αgpdh	31									1.0
	30				1.0	1.0	1.0	1.0	1.0	
	28	1.0	1.0	1.0						

TABLE 8. Distribution of alleles per locus for species of *Anodonta* and *Elliptio.** Acc = *Anodonta c. cataracta*, Acf = *A. c. fragilis*, Aim = *A. implicata*, Agb = *A. gibbosa*.

Enzyme	Locus	Acc	Acf	Aim	Agb	*Elliptio*
GPI+	I	2	2	1	2	5
PGM+	I	1	1	2	2	4
	II	1	2	1	1	5
LAP+	I	1	1	2	1	23
MDH	I	2	2	1	2	3
	II	1	1	2	1	2
HK+	I	1	1	1	1	5
MPI	I	2	2	2	2	3
6PGD	I	1	1	1	1	3
OCT	I	1	1	2	1	3
AAT	I	1	1	2	1	1
SOD	I	1	1	2	1	1
G3PDH	I	1	1	1	1	1
GPDH	I	1	1	1	1	1

*Distribution of alleles per locus for species of *Elliptio* studied by Davis et al. (1981).
+Fast-evolving loci according to Davis et al. (1981).

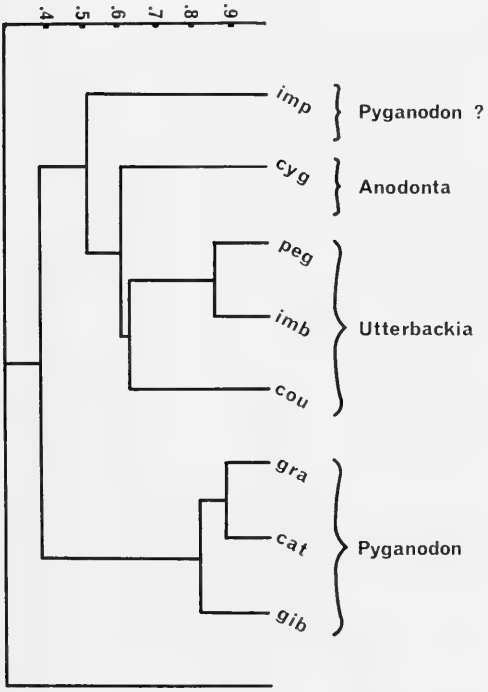

FIG. 7. Phenogram (based on Table 4) depicting levels of similarity (1.0 = most similar) in stomach morphology between *Anodonta implicata, A. cygnea, A.peggyae, A. imbecilis, A. couperiana, A. grandis, A. c. cataracta,* and *A. gibbosa.*

and the American *Utterbackia*: similarities in reproductive mode (i.e. hermaphroditism) and umbonal characteristics were considered unreliable means of classification because convergence (rather than parallelism) could have given rise to the similarities. Similarity in stomach structure, however, revives the possibility of a common ancestry for *A. cygnea* and *Utterbackia.*

Anodonta cataracta cataracta, A. grandis and *A. gibbosa* are all rather closely related. It is important to point out that the specimens of *A. grandis* examined were collected in Vermont and thus could have hybridized to a certain extent with *A. c. cataracta*; Ortmann (1914) pointed out that conchological intergrades between the species exist where their geographic ranges merge. While hybridization could have resulted in some blending of stomach characteristics to create a high degree of similarity, the fact that these species hybridize in the first place points to a close genetic relationship. Also interesting is the close relationship between *A. c. cataracta* and *A. gibbosa*, which was first pointed out by Frierson (1912), who thought *A. gibbosa* to be a subspecies of *A. c. cataracta.*

Because of substantial differences in stomach structure between the groups discussed above and that of *A. implicata*, this species was placed in a separate group. Of the

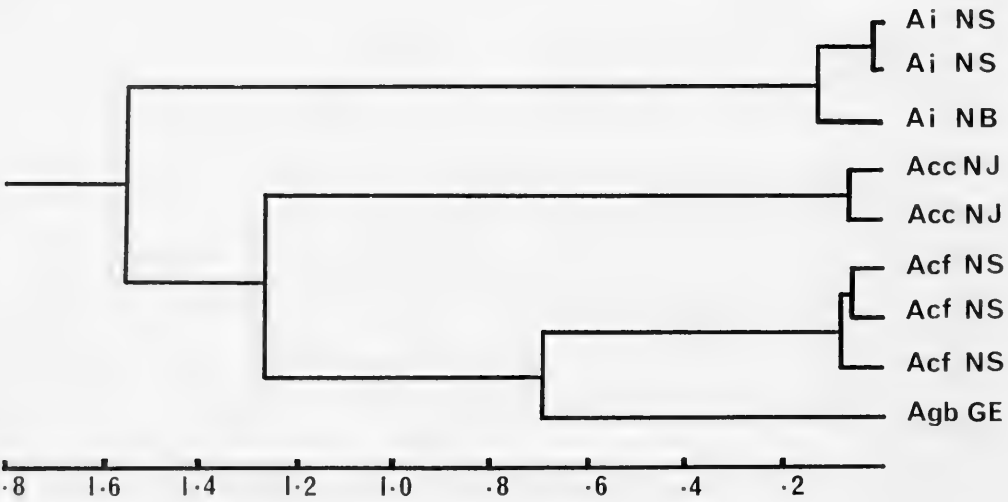

FIG. 8. Phenogram (based on Nei distances) depicting levels of similarity (0 = most similar) between *Anodonta implicata* (Ai), *A. c. cataracta* (Acc), *A. c. fragilis* (Acf) and *A. gibbosa* (Agb), Collection locations: NS = Nova Scotia, NB = New Brunswick, NJ = New Jersey, GE = Georgia.

anodontine stomachs here examined, that of *A. implicata* is the most complex. In addition, *A. implicata* stomachs have three unique character states (Table 3): an elongate, relatively complex style sac and midgut opening, unique placement of the sorting pouch in relation to the minor typhlosole, and a hook-shaped sorting pouch (Fig. 6). Electrophoretic data support separation of *A. implicata* from *Pyganodon* (Table 5). The level of genetic identity between *A. implicata* and *A. c. cataracta* (0.370) is much lower than that observed between *A. c. cataracta* and *A. gibbosa* (0.608), and the distribution of alleles among loci of *A. implicata* differs radically from that of *A. c. cataracta* and *A. gibbosa* (Table 7). Also, recent immunoelectrophoretic studies (Davis & Fuller, 1981) show considerable differences between *A. implicata* and *A. c. cataracta*; in fact, *A. implicata* seems more closely related to *Alasmidonta undulata* (Say, 1816) and *Lasmigona costata* (Rafinesque, 1820) by multivariate analysis of immunoelectrophoretic distances than to all congeners. These differences separate *A. implicata* from the subgenus *Pyganodon*, and stomach morphology places the species close to, but not within, the subgenera *Anodonta* and *Utterbackia* (Fig. 7).

An interesting correlate to this study involves comparison between *Anodonta cataracta cataracta* and *A. c. fragilis* from Nova Scotia. Clarke & Rick (1963) mentioned that *A. fragilis* was described from Newfoundland and that this species intergrades with *A. c. cataracta* on Nova Scotia, establishing the concept *A. c. fragilis.* Examination of *A. c. fragilis* stomachs reveals similarities to those of *A. cygnea* and *A. imbecilis* in the region of the right sorting area pouch and the midgut, and similarities to that of *A. c. cataracta* in the region of the minor typhlosole. This Nova Scotian anodontine thus could represent a taxon with European affinities which survived Wisconsinan glaciation of eastern Canada; Clarke (1966) found a close conchological relationship between *A. kennerlyi* Lea, 1860 (California to British Columbia and Alberta) and both *A. cygnea* and *A. c. fragilis,* indicating that such anodontines with European affinities could be more widespread than previously supposed. Electrophoretic results reveal that *A. c. fragilis* resembles *A. c. cataracta* at a level similar to that of the species relationship between *A. c. cataracta* and *A. gibbosa* (I = 0.611), and exhibits both higher heterozygosity and polymorphism than *A. c. cataracta* (Table 6). An electrophoretic examination of *A. fragilis* from the type locality and a careful study of the possible phenomenon of hybridization among species in the subgenus *Pyganodon (A. c. cataracta* × *A. fragilis, A. c. cataracta* × *A. grandis*) are clearly indicated.

The species groupings presented above are based on an examination of a single organ and some initial electrophoretic data, and, while this permits a certain amount of speculation about taxonomic affinities, it does not permit taxonomic revisions. Nevertheless, while most relationships fall within accepted boundaries, the hypothesized relationships between American *Utterbackia* and *A. cygnea* and the placement of *A. implicata* in a group separate from the rest of *Pyganodon* should constitute sufficient encouragement for a synthetic study of the entire genus based on shell, soft-part, and electrophoretic characters. Furthermore, these results should encourage use of stomach morphology at the species level to determine relationships among other problematical groups within the Unionidae.

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APPENDIX

Collection localities of the populations and species examined electrophoretically.

A. *Anodonta implicata*

Population 1. French Lake at Sunbury-Oromocto State Park, ca.10 km S of Oromocto, Sunbury Co., New Brunswick, Canada.
Population 2. Sydney River, Blachette Lake, ca. 6 km SW of Sydney, Cape Breton Co., Nova Scotia, Canada.
Population 3. Shubenacadie Grand Lake, Grand Lake, Halifax Co., Nova Scotia, Canada.

B. *Anodonta cataracta fragilis*

Population 1. First Lake O' Law, ca. 25 km NW of Baddeck, Victoria Co., Nova Scotia, Canada.
Population 2. Shaw Lake, ca. 6 km NNE of Arichat, Isle Madame, Richmond Co., Nova Scotia, Canada.
Population 3. Newville Lake, Halfway River East, Cumberland Co., Nova Scotia, Canada.

C. *Anodonta cataracta cataracta*

Population 1. Swartswood Lake, Delaware River Drainage, Sussex Co., New Jersey.
Population 2. Concord Pond, Nanticoke River Drainage, Sussex Co., Delaware.

D. *Anodonta gibbosa*

Population 1. Ocmulgee River at Ben Hill/Coffee Counties Public Boat Ramp, Ben Hill Co., Georgia.

MALACOLOGIA, 1983, 23(2): 375–396

THE BIOLOGY AND FUNCTIONAL MORPHOLOGY OF THE TWISTED ARK *TRISIDOS SEMITORTA* (BIVALVIA: ARCACEA) WITH A DISCUSSION ON SHELL "TORSION" IN THE GENUS

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ABSTRACT

The biology and functional morphology of the twisted ark *Trisidos semitorta* (Lamarck) are described. The adult occupies clean sands, with the anterior end buried and the anterior sagittal plane vertical to the surface. Because of posterior shell twisting, the posterior face of the right valve lies beneath that of the left which projects above the sand surface. The posterior sagittal plane of the shell thus lies parallel with the sand-water interface. The mode of life of the juvenile is also described for the first time. It lies within the inner surface of empty bivalve shells, firmly attached by a byssus. Juveniles of both *T. semitorta* and *T. tortuosa* (Linnaeus) are less twisted than adults, the two species being similar when young. The adult *Trisidos* can be derived from an ancestor in which anatomical modifications adapting it to a byssally attached, nestling mode of life have been retained, indeed enhanced, in the transition from juvenile to adult to permit colonization of sands with fast-flowing currents above. Only the posterior region of the shell is twisted about the dorsoventral axis of ligament and byssus. In the free-living adult the byssus is absent, but the growth processes begun in the juvenile are continued into adult life. Shell twisting results from the contraction of asymmetrically developed posterior pedal retractor muscles, the left being larger and lying behind the diminutive right. This is analogous to the phenomenon of torsion in the Gastropoda, but the end result is different and the term twisting, and not torsion as used by McGhee (1978), more aptly defines the process of *Trisidos*.

Key words: *Trisidos*; anatomy; twisting; posterior pedal retractors; evolution.

INTRODUCTION

Members of the arcid genus *Trisidos* are unusual in being twisted. More particularly, it would seem as though the anterior end of the shell had been held stable and the posterior end twisted through approximately 90°. This phenomenon has been studied most recently by McGhee (1978) and Tevesz & Carter (1979) in species, e.g. *T. tortuosa,* in which twisting is most obvious. The first study is a largely theoretical account of the pattern of torsion (the term being used synonymously with twisting by the former author) within the Arcidae and extinct Bakevelliidae. The latter account derives *Trisidos* from a *Barbatia*-like ancestor but is of insufficient detail to confirm or deny this hypothesis. The anatomical description is superficial.

Most authors describe twisting in *Trisidos,* but neither demonstrates *how* it occurs in this unusual group of animals. An understanding of *Trisidos* may cast light on the mechanism of twisting in the Bakevelliidae, the only other group of twisted bivalves (McGhee, 1978).

Little is known of the biology of *Trisidos*. The adult is known to inhabit soft deposits into which it can reburrow, but the mode of life of the juvenile is unknown.

This study was initiated when juveniles of *T. semitorta* were found. Juvenile adaptations cast light on how shell twisting in the adult is achieved and the claim by Tevesz & Carter (1979) that the *Trisidos* ancestor was a *Barbatia*-like nestler.

MATERIALS AND METHODS

Adult and juvenile specimens of *Trisidos semitorta* were obtained with an Agassiz trawl from about 8 m of water in the NE territorial waters of Hong Kong (Mirs Bay), some 3 km to the ESE of the village of Sha Tau in the People's Republic of China (grid reference 225,994 from 1:100,000.L681, 1970).

The sea bed comprises a coarse sand and is covered by empty shells (occupied by the juvenile *T. semitorta*) of the bivalves *Anadara antiquata* (Linnaeus) and *Tapes dorsatus* (Lamarck). Also present is the burrowing ark *Cucullaea concamerata* (Martini) (Morton,

1981) and the shell boring gastrochaenid *Cucurbitula cymbium* (Spengler) (Morton, 1982). The samples were hosed down in a 5 mm sieve and returned to the departmental sea water aquarium. Large specimens were separately placed in shallow tanks with a thick bed of sand and circulating sea water.

The ciliary currents of the organs of the mantle cavity and the stomach were elucidated using carmine in sea water. Two small specimens were fixed in Bouin's fluid and serially sectioned at 6 μm. The sections were stained in either Ehrlich's haematoxylin and eosin or Mallory's triple stain.

Specimens of *Trisidos tortuosa* in the collections of the British Museum (Natural History) have been examined as follows:

11 adult (dried valves) (BM (N.H.) reg. no. 1953.1.23.377) from Singapore. R. Winckworth collection. Acc. No. 1838.

6 juveniles (dried valves) (no reg. no.) from Karachi, India. F. W. Townsend collection. Acc. No. 1831.

1 adult (alcohol preserved) (Reg. no. 81.11010) from Port Collis. 11 fathoms 'Alert' collection.

The latter specimen was opened to confirm details of the posterior pedal retractor muscles previously noted for *T. semitorta*.

ABBREVIATIONS USED IN THE FIGURES

A	A cell layer of the style sac
AA	anterior adductor muscle or scar
AA(Q)	"Quick" component of the anterior adductor muscle
AA(S)	"Slow" component of the anterior adductor muscle
AN	anus
AP	anal papilla
APP	anterior pedal protractor muscle
APR	anterior pedal retractor muscle
ASO	abdominal sense organ
AU	auricle
B	B cell layer of the style sac (the major typhlosole)
B_1	B_1 cell layer of the stye sac (the minor typhlosole)
BG	byssal gland
BY	byssus
BYG	byssal groove
C	C cell layer of the style sac
CA	ctenidial axis
CFC	coarse frontal cilia
CR	"chitinous" rod
CS	crystalline style
CSM	conjoined style sac and mid-gut
CSS	crystalline style sac
D	D cell layer of the style sac
DD	digestive diverticula
DDD	duct to digestive diverticula
DH	dorsal hood
EA	exhalant aperture
F	foot
FC	food sorting caecum
FFC	fine frontal cilia
FGC	filament gland cell
FO	fold in the stomach wall
FS	fragmentation spherules
G	gonad
GO	gonoduct
GP	gonopore
GS	gastric shield
H	heart
HG	hind-gut
IA	inhalant aperture
ID	inner demibranch
IG	intestinal groove
ILP	inner labial palp
IME	inner mantle epithelium
IMF	inner mantle fold
K	kidney
LC	lateral cilia
LEC	left ctenidium
LFC	latero-frontal cilia
LP	left pouch
MG	mid-gut
MI	mantle isthmus
MMF	middle mantle fold
MT	minor typhlosole
N	nerve
NF	nerve fibers
NU	nucleus
O	oesophagus
OC	*Oulastrea crispata*
OD	outer demibranch
OFM	oblique fibres of mantle
OLP	outer labial palp
OME	outer mantle epithelium
OMF	outer mantle fold
P	periostracum
PA	posterior adductor muscle or scar
PA(Q)	"quick" component of the posterior adductor muscle
PA(S)	"Slow" component of the posterior adductor muscle
PE	pericardium
PEG	pedal gland
PG	pallial glands
PO	*Polydora* sp.
PPR(L)	left posterior pedal retractor muscle
PPR(R)	right posterior pedal retractor muscle

PRM	pallial retractor muscle
R	ridge entering the dorsal hood
RA	renal aperture
RE	rectum
RPA	reno-pericardial aperture
SA	sorting area of the stomach
SC	secretory cell
SEC	sensory cell nucleus
T	major typhlosole
TF	transverse fibres
TFM	transverse fibres of mantle
V	ventricle

TAXONOMY

Trisidos Röding, 1798 is a genus of the family Arcidae (see Newell, 1969 for taxonomic details), the latter being divided into two subfamilies—the Arcinae Lamarck, 1809 and the Anadarinae Reinhart, 1935. The former are generally considered to be either powerfully attached nestlers, e.g. *Barbatia* Gray, 1842 and *Arca* Linnaeus, 1758, or borers, e.g. *Litharca* Gray, 1842, while the latter are typically either burrowing and abyssate, e.g. *Anadara* Gray, 1847, *Scapharca* Gray, 1847 or but weakly byssally attached, e.g. *Bathyarca* Kobelt, 1891 and *Bentharca* Verrill & Bush, 1898. Despite this disparity in habitat, Newell (1969) places the burrowing, abyssate *Trisidos* in the Arcinae—a suggestion that will be discussed here.

The genus *Trisidos* is relatively modern (Eocene) and has an Indo-Pacific distribution. The type-species is *T. semitorta* Lamarck, 1819. According to Oyama (1974), there are three other species of the genus: *T. torta* (Mörch), *T. kiyonoi* (Makiyama) and *T. tortuosa* (Linnaeus). Oyama considers *T. yongei* Iredale to be a junior synonym of *T. tortuosa.* The form of *Trisidos* is very variable and some of these species may be in doubt, the genus warranting careful taxonomic revision.

BIOLOGY

Juvenile specimens of *Trisidos semitorta* are byssally attached and in Hong Kong waters occupy the inner surfaces of empty, large bivalve shells (Fig. 1). The byssus is relatively large and the animal is securely attached. It has been recorded from *Tapes dorsatus, Anadara antiquata* and adults of its own species. This habit undoubtedly ensures protection both from predators and from the rapid water movement that must occur over the well-aerated sands the adult inhabits. As the bivalve grows, the host shell must become more restrictive until a time is reached when the juvenile detaches. At this time the byssus is lost and the adult assumes a burrowing mode of life. *Cucullaea concamerata* occurs in the same habitat and is also adapted for fast current speeds (Morton, 1981). Observations on adult *T. semitorta* have shown that, as with *T. yongei* (Tevesz & Carter, 1979), reburrowing can occur though this process is slow, taking many days to complete.

Typically, the shell of *T. semitorta* is eroded posteriorly and colonized by other organisms, mostly on the left valve (Figs. 2, 3A). Colonizing species include the scleractinian coral *Oulastrea crispata,* the boring polychaete worm *Polydora* sp. and small *Lithophaga malaccana* (Reeve). Thus, the often extensively damaged posterior surface of the left valve projects above the sand surface while the remainder of the shell is buried. Makiyama (1931) has figured *Arca* (= *Trisidos*) *kiyonoi* in its natural position in the sand.

In Hong Kong occasional adult specimens of *T. semitorta* have been collected intertidally from sand flats in Hoi Sing Wan (Starfish Bay), Tolo Harbour and more frequently by Agassiz trawl off this and other beaches.

Tevesz & Carter (1979) report a similar habitat for *T. yongei,* i.e. a muddy, fine to medium sand subject to the effects of tidal currents and wave action and containing abundant fragmental shell material.

FUNCTIONAL MORPHOLOGY

The shell

The aragonitic shell of *Trisidos tortuosa,* as in other members of the Arcacea (Taylor, Kennedy & Hall, 1969), comprises a crossed lamellar outer layer and a complex crossed lamellar inner layer though, unusually, the pallial myostraca is prismatic in the umbonal regions only.

Both *T. semitorta* and *T. tortuosa* are antero-posteriorly elongate, with a long multivincular ligament. The hinge plate is narrow with a continuous row of taxodont teeth. These are represented only by minute projections under the central part of the hinge. Laterally, however, the teeth are relatively large and function in valve alignment and in preventing shear.

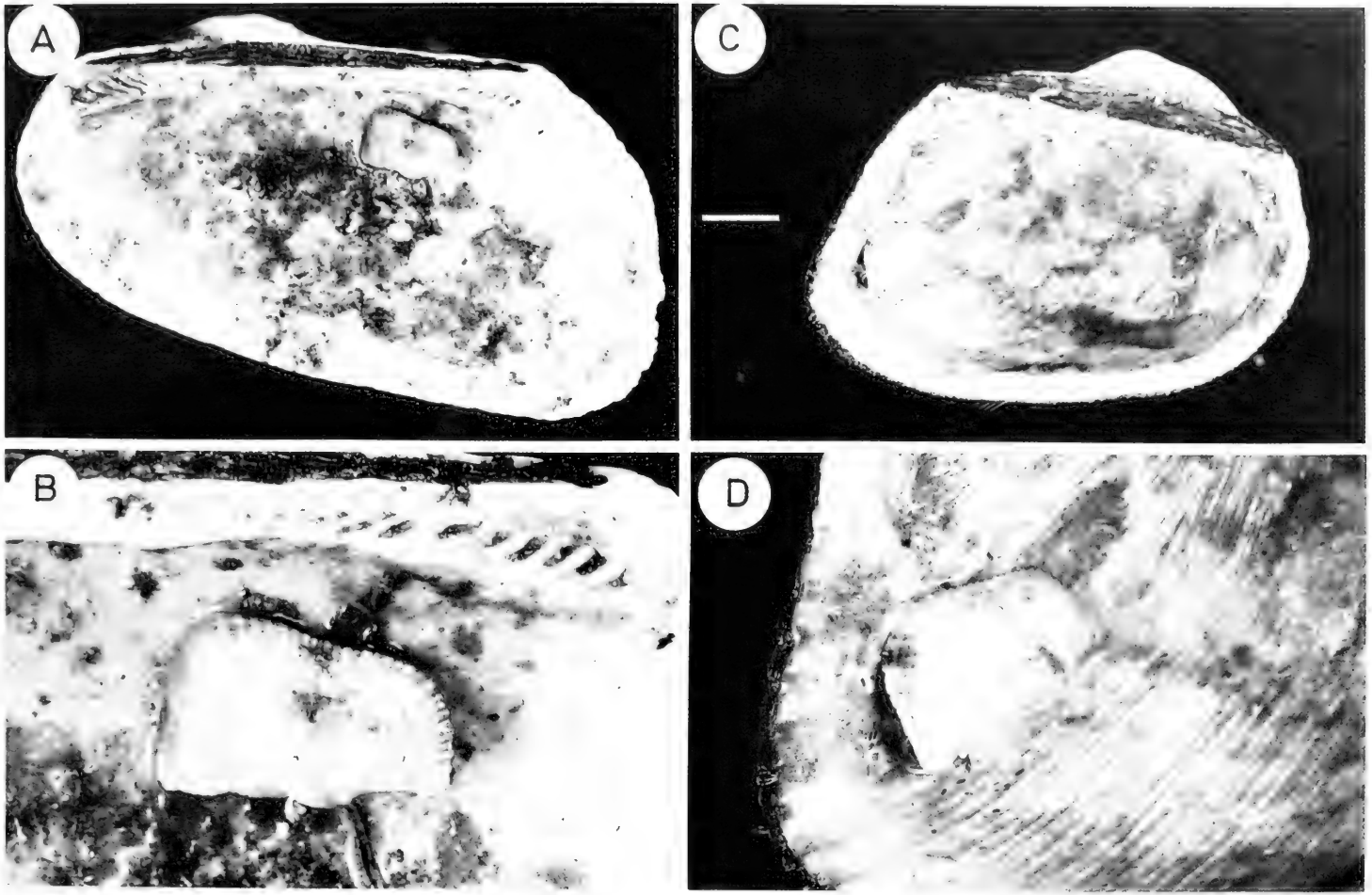

FIG. 1. *Trisidos semitorta*. Low (A, C) and high (B, D) power photographs of two juvenile specimens attached to the internal surface of shell valves of (A, B) adult *T. semitorta* and (C, D) *Anadara antiquata*. Scale = 1 cm.

It appears that the posterior end has been twisted clockwise, the posterior part of the sagittal plane turned through approximately 45° in *T. semitorta* and almost 90° in *T. tortuosa.* The animal, though lying approximately vertically disposed in the sand with the anterior dorsoventral axis of the shell at right angles to the sediment-water interface, has its posterior margin lying approximately flush with the sand. The right valve, located underneath the left, is buried. In both, but more noticeably in *T. tortuosa,* the posterior edge of the left valve projects beyond the margin of the right valve. The animal is slightly tilted in the sand, anterior end down, so that only the posterior face of the left valve is seen in life.

The anterior end is not (except coincidentally) involved in the twisting process and thus the anterior region of the hinge plate remains vertically aligned whereas the hinge plate is twisted posteriorly and the posterior teeth interlock in a different plane to those anterior. Because of the twisting, the posterior adductor acts at a different angle to that of the anterior and may augment the function of the hinge teeth in preventing shear (McGhee, 1978). The dorso-ventral axis of the shell, through the ligament and byssus, constitutes the fixed pivot point around which posterior twisting occurs. Tevesz & Carter (1979) considered the hinge axis alone to be the pivotal point while McGhee (1978) considered it the byssus. The ligament is straight, though much larger in *T. semitorta* than *T. tortuosa. T. tortuosa* is more delicate than *T. semitorta.* In *T. semitorta* the shell is delicately ribbed, though this is often masked by the thick, fibrous periostracum and by heavy erosion of the left valve posteriorly. *T. semitorta* also possesses a weak postero-ventral sulcus extending from the umbo. This is most noticeable in the left valve. In *T. tortuosa*, the sulcus is much more sharply defined and angles the left valve so that the posterior face lies at right angles both to the remainder of the valve and to the sediment-water interface. As a result of the twisting process, the ventral margin of both species is sinusoidally curved. There is no trace of a byssal notch or ventral indentation, though Newell (1969) considers this characteristic of the subfamily. In *T. tortuosa*, but not *T. semitorta*, both posterior and anterior halves of the shell are laterally compressed, emphasizing the angularity of the shell.

Juveniles of *T. semitorta* are byssally attached, though whether this is also true of *T. tortuosa* is unknown but probable. Fig. 5

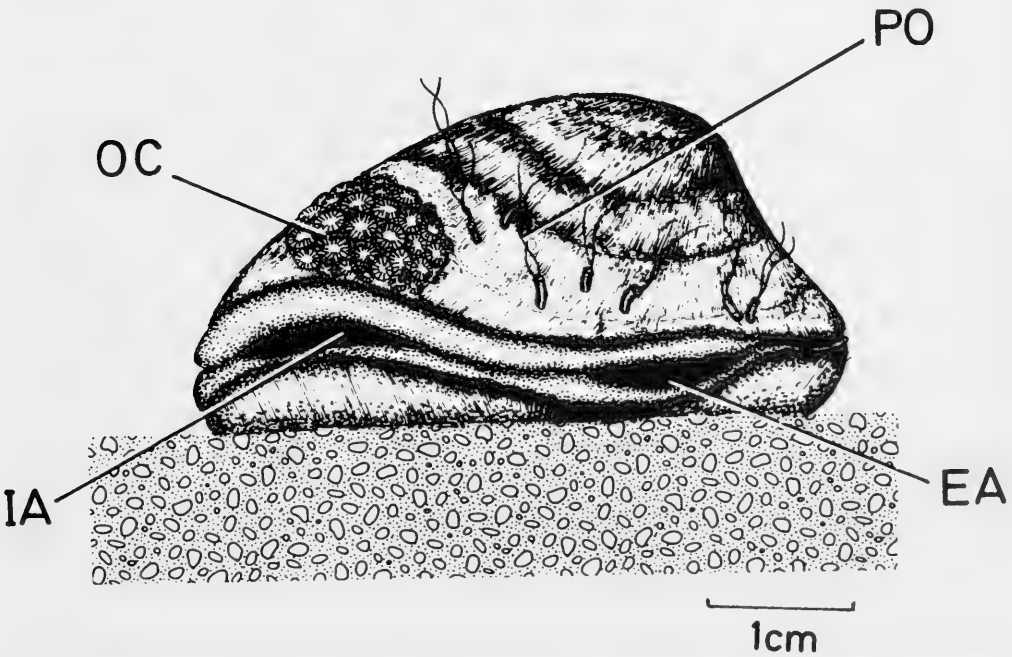

FIG. 2. *Trisidos semitorta.* A posterior view of the animal in a natural position in the sand with the inhalant and exhalant apertures open. For abbreviations see p. 376.

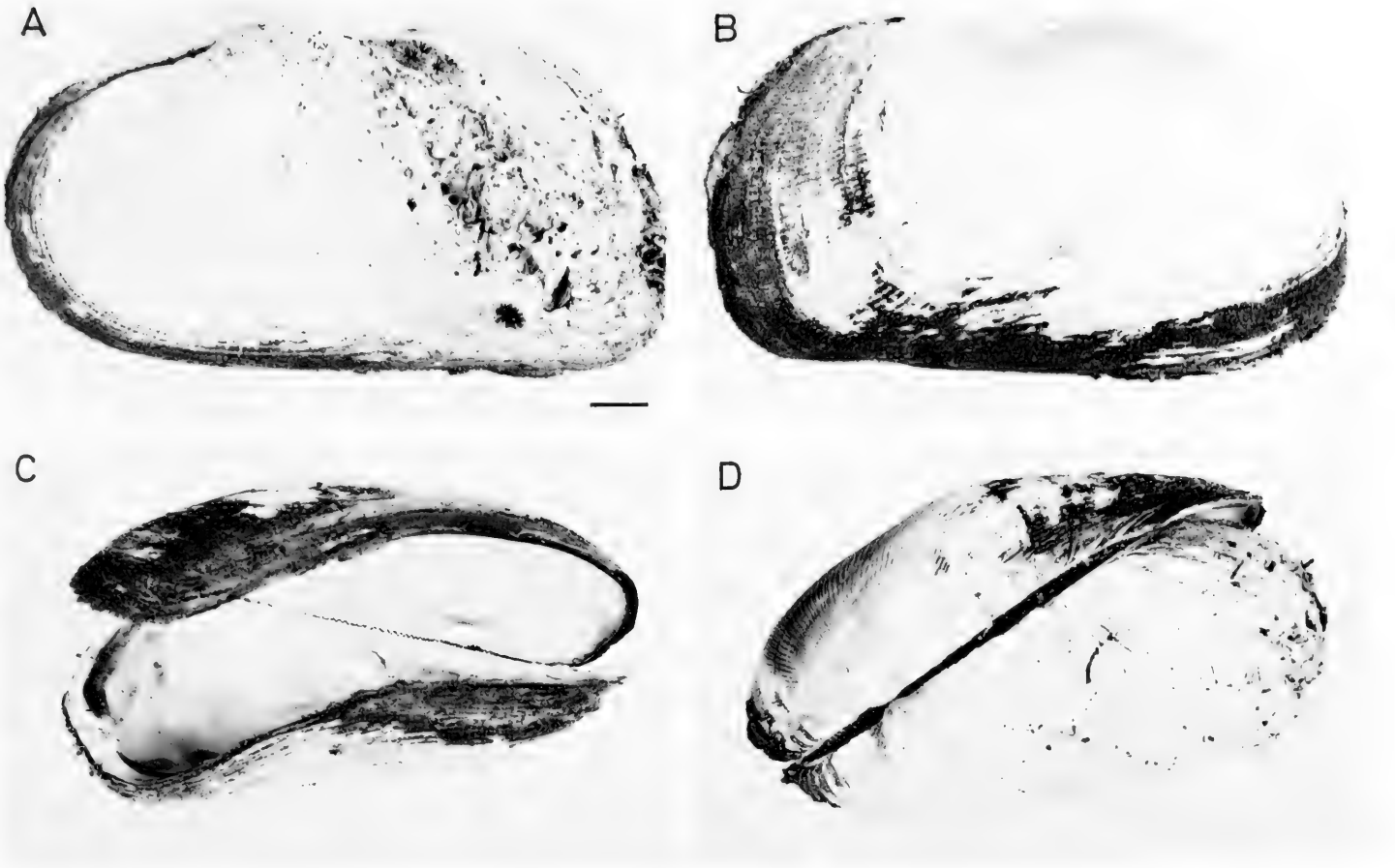

FIG. 3. *Trisidos semitorta.* The shell viewed from A, the left; B, the right; C, the ventral and D, the dorsal aspects. Scale = 1 cm.

shows dorsal and ventral views of a juvenile specimen of *T. tortuosa* 11 mm long and an adult 73 mm long. It can be seen that the juvenile is less twisted than the adult, the twisting process progressively influencing shell form with age and growth. Although no newly settled individuals have been seen, growth probably proceeds from an equilateral body plan. Neither juvenile *T. semitorta* nor *T. tortuosa* possess a byssal notch, though the former at least is byssally attached when young and possesses a ventral byssal indentation (McGhee, 1978). This results in a slight heteromyarian form with an inflated posterior region relative to the anterior, the inequilaterality being enhanced by the byssal indentation. Figs. 1 and 6A of a young specimen of *T. semitorta*, 13 mm long, demonstrate the low degree of twisting. The shell, somewhat inequivalve, has the characteristics of a nestling bivalve with relatively reduced anterior and inflated posterior shell slopes. The greatest shell width is dorsal to the dorso-ventral axis of the shell so that contraction of the byssal retractor muscles effectively serves to pull the animal down into the inner surface of large bivalve shells. The inflated posterior region of the shell increases the size of the apertures to the water above, thereby enhancing exchange and is typical of nestling species, e.g. *Philobrya* (Limopsacea), *Neogaimardia* (Cyamiacea) and *Trapezium* (Arcticacea) (Morton, 1978; 1979a, b). The similarity between juveniles of *T. tortuosa* and *T. semitorta* is evidence for similar life styles (Fig. 6). Conversely, the adults are dissimilar, though both exhibit twisting. Clearly, the constraints of the juvenile niche are responsible for a uniformity of body form that subsequently, in adult freedom, achieves individual character.

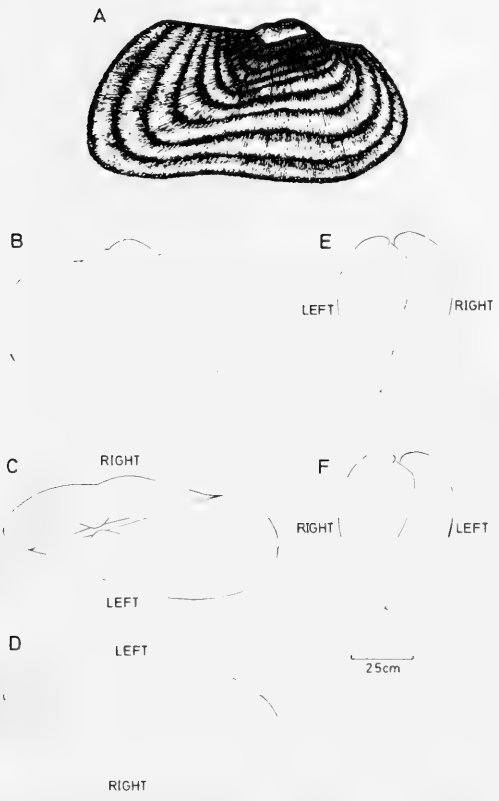

FIG. 4. *Trisidos semitorta.* The adult shell viewed from various aspects. A, the right valve; B, the left valve; C, dorsal aspect; D, ventral aspect; E, posterior aspect; F, anterior aspect.

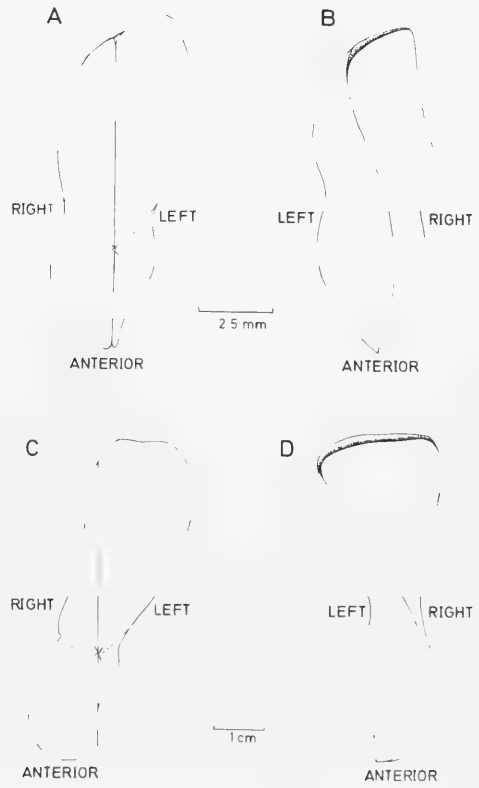

FIG. 5. *Trisidos tortuosa.* A and B, dorsal and ventral views of a juvenile; C and D, dorsal and ventral views of an adult.

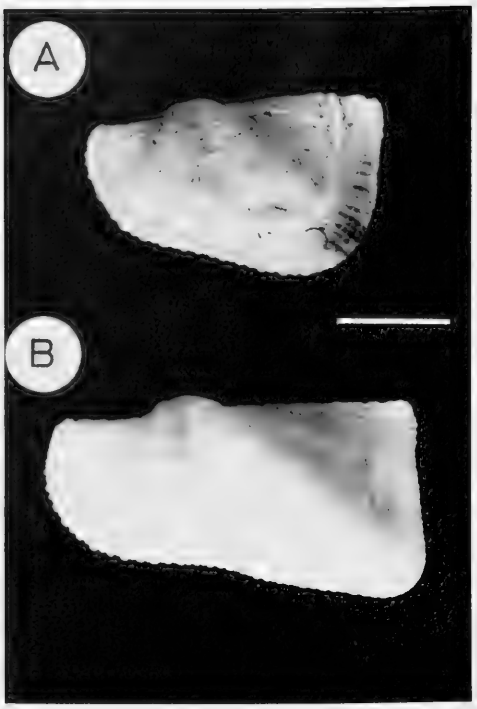

FIG. 6. The left shell valves of juvenile specimens of A, *Trisidos semitorta* and B, *T. tortuosa.* Scale = 5 mm.

The musculature

Anterior and posterior adductor muscles are present (Figs. 11 and 17), the former being smaller and more dorsal than the latter. Inequality of the adult adductors represents a continuation of the juvenile condition. Both adductors are divided into slow (AA(S); PA(S)) and quick (AA(Q); PA(Q)) components of approximately equal size.

Ventral to the anterior adductor, a pair of anterior pedal protractor muscles (APP) extend into the visceral mass. Similarly, from the postero-dorsal edge of the anterior adductor is attached a pair of anterior pedal retractor muscles (APR). Left and right anterior pedal retractors and protractors are of equal size.

How twisting in *Trisidos* is achieved has never been elucidated. Anterior to the posterior adductor muscle of *T. semitorta* is a pair of posterior pedal retractor muscles (PPR). Figs. 7A and 8 show that the left muscle (PPR(L)) is large, with a wide area of attachment and passes into the visceral mass and foot to largely assume responsibility for posterior retraction of the foot both on the left and right sides. The right posterior pedal retractor (PPR(R)) is small, with a small attachment area and is located anterior to the left retractor and its muscle blocks extend only a small way into the visceral mass.

The situation in *T. tortuosa* is similar (Fig. 7B) but exaggerated, i.e. the right posterior pedal retractor is minute in comparison with the left and as such is unusual, seen only in *Trisidos* (as far as is known), and results in the posterior twisting of the shell.

The mantle

The mantle is very thick and fleshy, tinted brown, with no mantle fusion. Posteriorly, the left and right mantle lobes are apposed so that inhalant and exhalant apertures are formed. When the animal is lying in sand (Fig. 2, IA, EA), these are clearly visible. The large foot can protrude mid-ventrally from between the mantle lobes to effect digging.

In transverse section (Fig. 9), the mantle epithelia are widely separate and the enclosed haemocoel divisible into two components. Beneath the inner epithelium (IME) is an extensive haemocoel crossed by a few obliquely oriented muscle fibres (OFM) that presumably maintain the turgidity of the haemocoel, perhaps ensuring it is not overfilled with blood. This cavity contains numerous amoebocytes. Similar oblique muscle fibres occur in the spacious haemocoel in the mantle of the anomalodesmatan *Pholadomya candida* (Morton, 1980). Beneath this region is a further haemocoel abutting the outer mantle epithelium (OME) and crossed by many transverse fibres (TFM). Clearly, this haemocoel can expand very little.

Throughout the mantle occur large numbers of cells (SC), termed secretory cells, each apparently discharging at an epithelium and containing granules staining bright red in Ehrlich's haematoxylin and eosin and either red or green in Mallory's triple stain. These cells, a maximum of some 25 μm in diameter, are also found throughout the body, being particularly common in those epithelia in contact with the mantle cavity and in the gut epithelia.

The mantle margin (Fig. 10) comprises three folds (Yonge, 1957), the inner (IMF) being the largest and the middle fold (MMF) extremely reduced. Discharging onto the general outer surface of the inner fold are many

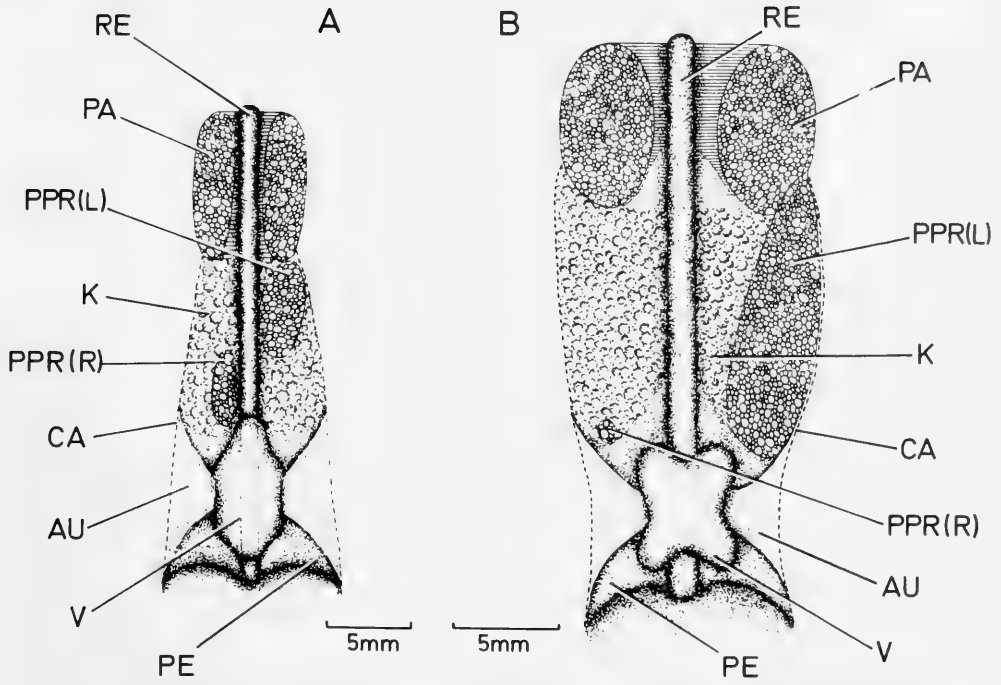

FIG. 7. Dorsal views of the pericardium of A, *Trisidos semitorta*; B, *T. tortuosa*. For abbreviations see p. 376.

subepithelial gland cells (PG), staining blue/green in Mallory's triple stain and pale red in Ehrlich's haematoxylin and eosin. A mucin probably is produced. Most of the branches of the pallial retractor muscle (PRM) penetrate the inner fold. Beneath the surfaces of the outer fold (OMF), which is subdivided into two sub-folds, occur large numbers of the subepithelial secretory cells (SC) noted above. The periostracum (P) is thin.

The ciliary currents of the mantle

Waste material landing on the surface of the mantle is rejected posteriorly. On each lobe (Fig. 11), a major rejection tract commences ventral to the anterior adductor muscle, extends postero-ventrally and then turns in a postero-dorsal direction so that unwanted material is eventually discharged via the exhalant aperture. This is achieved largely by ciliary means, as in other arcids (Lim, 1966) and mytilids (Morton, 1973). This major rejection tract is fed from the dorsal and ventral areas of the mantle by, respectively, down-

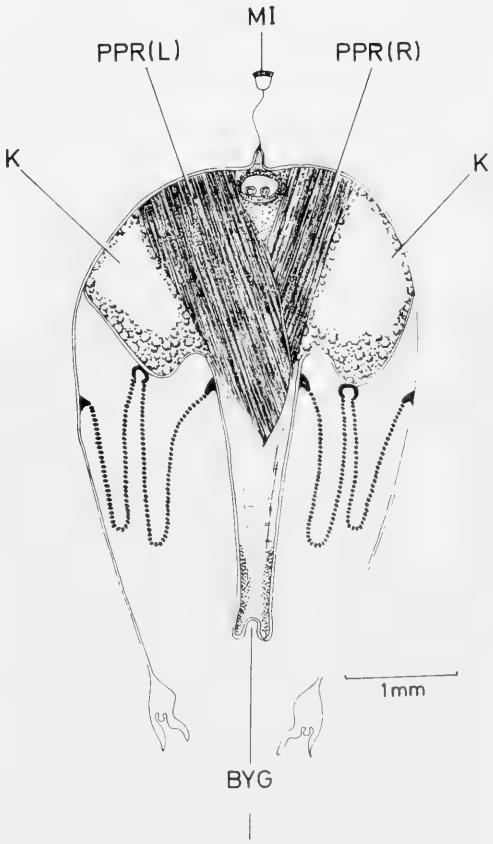

FIG. 8. *Trisidos semitorta*. Transverse section through the posterior pedal retractor muscle. For abbreviations see p. 376.

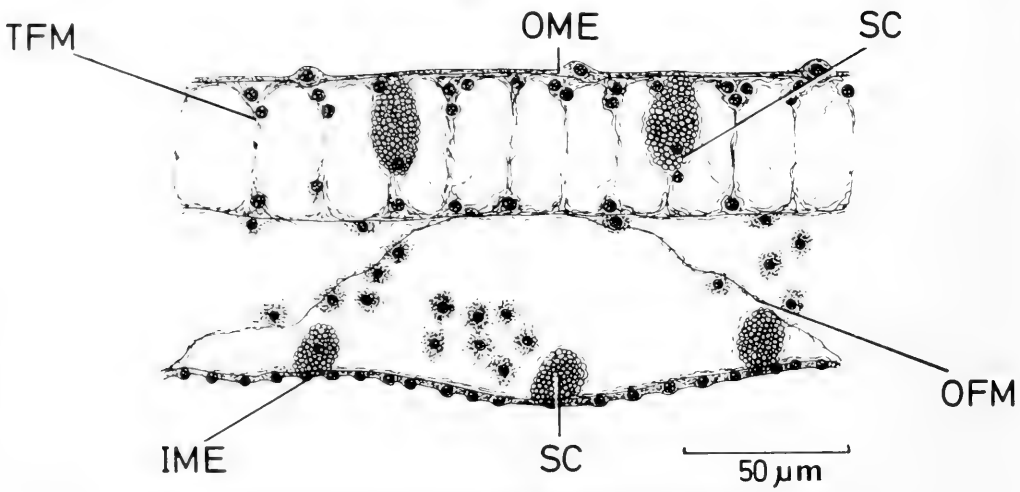

FIG. 9. *Trisidos semitorta*. Section through the general mantle. For abbreviations see p. 376.

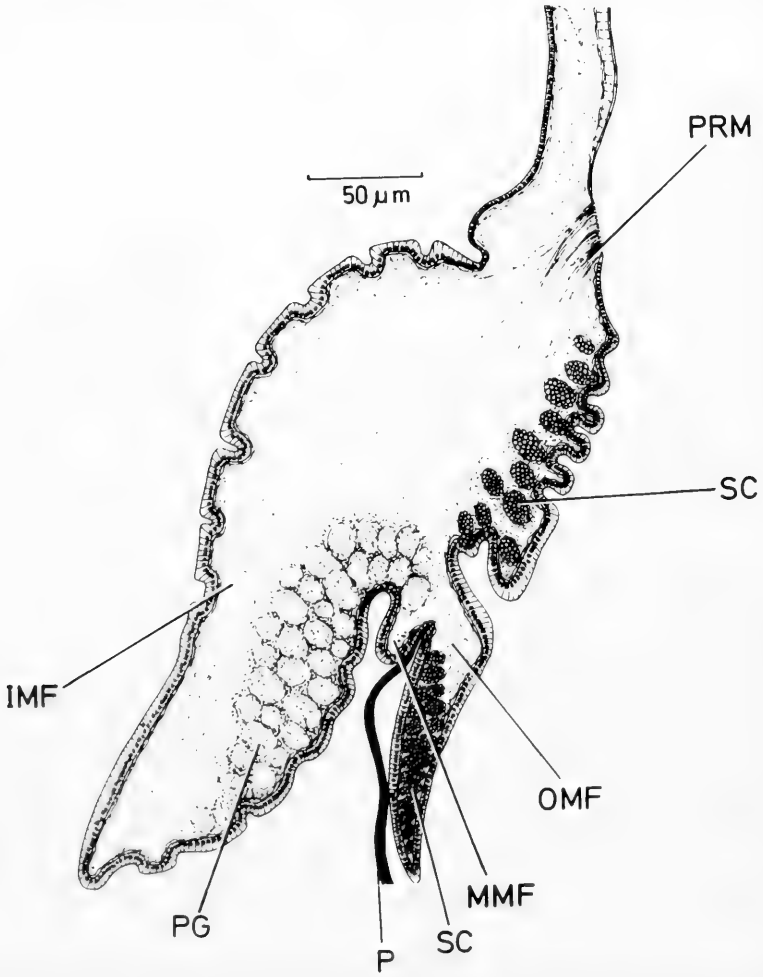

FIG. 10. *Trisidos semitorta*. Transverse section through the ventral mantle margin. For abbreviations see p. 376.

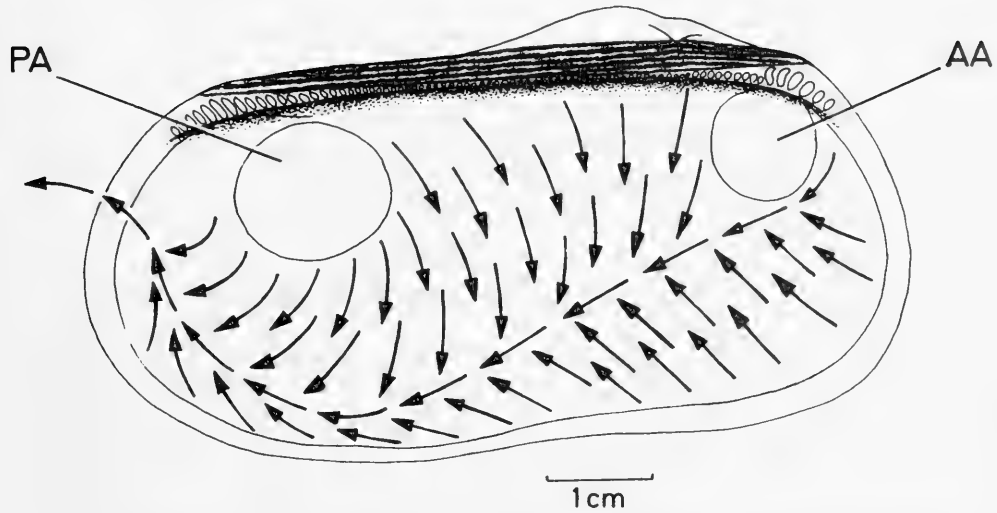

FIG. 11. *Trisidos semitorta.* The ciliary rejection currents of the left mantle lobe. For abbreviations see p. 376.

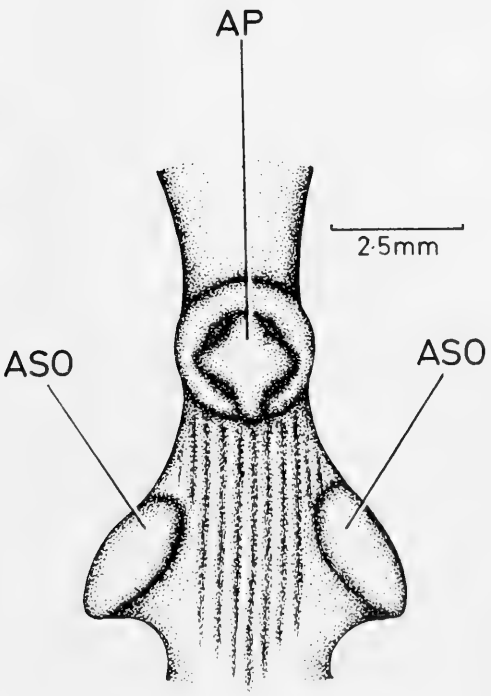

FIG. 12. *Trisidos semitorta.* Surface view of the anus and abdominal sense organs located beneath the posterior adductor muscle. For abbreviations see p. 376.

wardly and upwardly beating cilia. The ciliary currents are extremely strong.

The abdominal sense organs

Members of the Arcacea typically possess a pair of large abdominal sense organs (Heath, 1941) (Fig. 12, ASO) located close to the anus with its anal papilla (AP) and in close proximity to the postero-ventral face of the posterior adductor muscle. Heath (1941) has shown that in *T. tortuosa*, along with the very great asymmetry of the valves, the right abdominal sense organ is markedly larger than that of the left. This is not so in the less twisted *T. semitorta*.

In section (Fig. 13) large numbers of secretory cells (SC) (earlier described) are present in the sense organs, apparently being discharged from the epithelium. The epithelium comprises a very regular row of vertically aligned cells with long (8 μm) nuclei (SEC), located just beneath the outer cell membrane. Beneath occur large numbers of round nuclei (NU), some 4 μm in diameter and forming a layer 16 μm thick, and beneath this again is a zone 4 μm thick comprising vertically aligned, fine fibrils (NF) overlying a layer (4 μm) of horizontally aligned nervous tissue (N). It would seem that the fibrils arise from the nerve and extend upwards towards the vertically aligned apical nuclei, but this con-

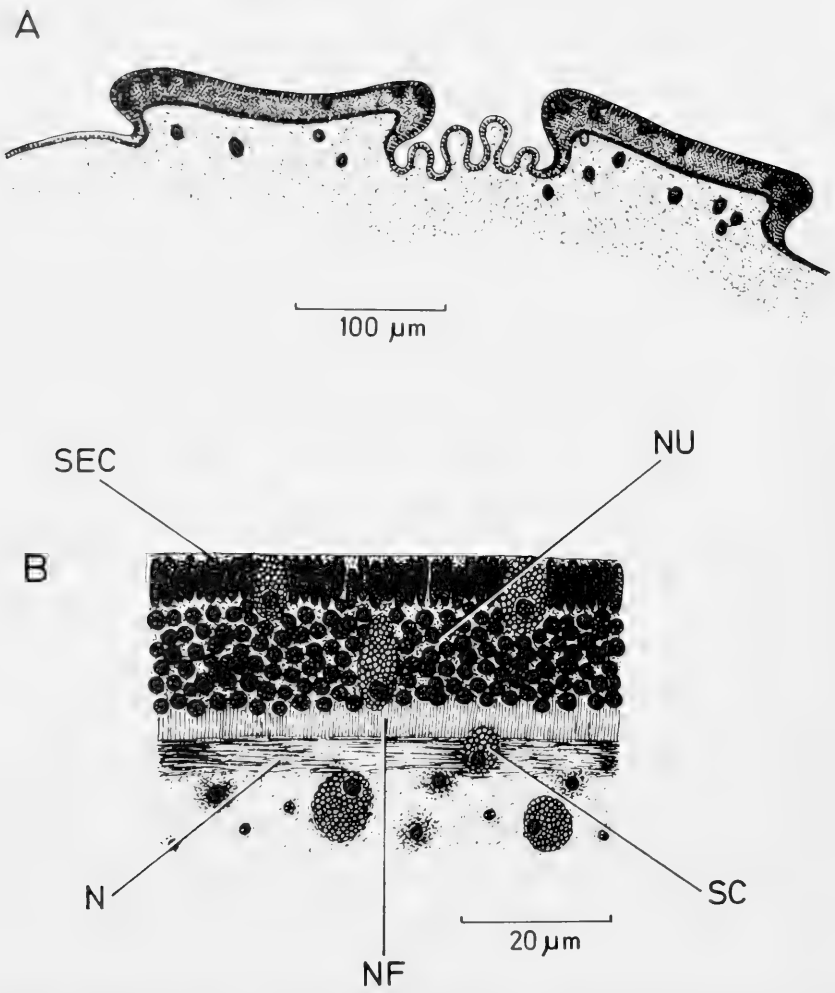

FIG. 13. *Trisidos semitorta*. Low (A) and high (B) power sections through the abdominal sense organ(s). For abbreviations see p. 376.

nection is obscured by the mass of intervening nuclei.

The ctenidia

The ctenidia of *T. semitorta* (Fig. 14) comprise two equal demibranchs: left and right ctenidia are similarly equal, i.e. valve inequality does not affect gill dimensions. Gill ciliation is of Type B(1a) (Atkins, 1937b), typical of the Arcacea and Limopsacea (Atkins, 1937a; Lim, 1966; Morton, 1978). Acceptance tracts are located in the ctenidial axis and in the junctions between the ascending lamella of the inner (ID) and outer (OD) demibranchs with the visceral mass and mantle, respectively. The ventral marginal grooves pass large particles posteriorly to be rejected from between the posterior borders of the mantle along with pseudofaeces collected by the visceral mass and mantle. The posterior extremities of the ctenidia are supported by a thick, muscular, suspensory membrane which gives this region great mobility. The ctenidia, with acceptance and rejection tracts separately located, act as primary sorting mechanisms, facilitated by the ciliation of the gill filament.

In transverse section (Fig. 15), each filament has an apical crown of some six cells,

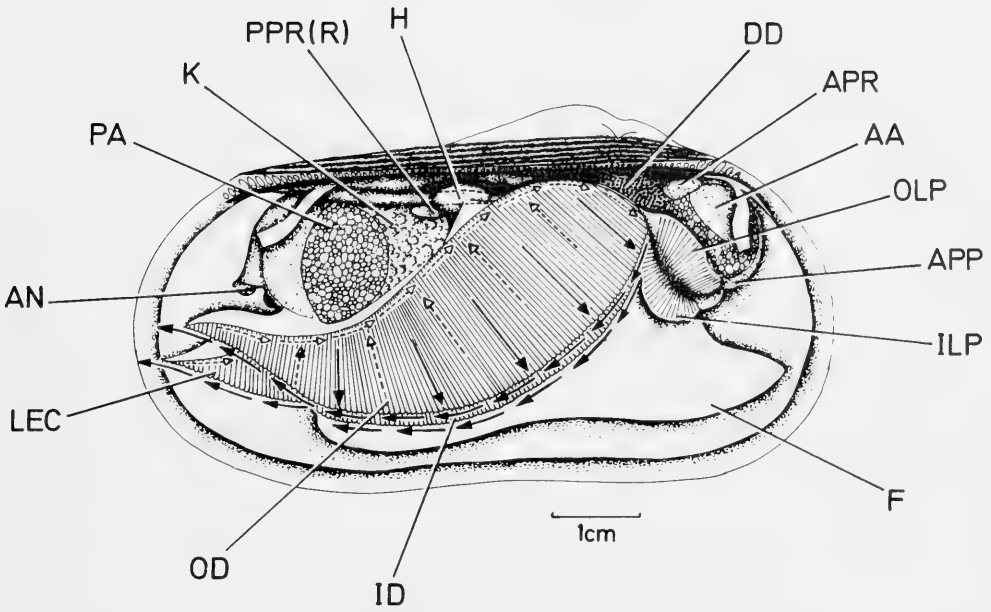

FIG. 14. *Trisidos semitorta.* The organs of the mantle cavity as seen from the right side after removal of the right shell valve and mantle lobe. For abbreviations see p. 376.

each ciliated. The cilia are arranged in three vertical rows. A central row of coarse frontal cilia (CFC) 5–6 μm long, is flanked by rows of fine frontal cilia (FFC) 3–4 μm long. Lateral to the fine frontal ciliated cells is another cell with a long (8–10 μm), stiff cilium designated the latero-frontal cilium (LFC) by Atkins (1937a). Lateral again is a secretory cell (FGC), probably producing mucus, and a series of, typically, three cells possessing long (12 μm) lateral cilia (LC) responsible for creating the flow of water through the ctenidium and another large secretory cell (FGC), again probably producing mucus. The apex of the filament is supported by "chitinous" rods (CR); and the base of the filament enclosing the filament blood vessel is long, thin and composed of narrow cells, the two sides cross-linked by transverse fibres (TF).

The labial palps

The labial palps (Fig. 14, ILP, OLP) are located on the postero-ventral face of the anterior adductor muscle. Only the tips of the demibranchs extend between the palps. The ctenidial-labial palp junction is of category 3 (Stasek, 1963), typical of the Arcacea. The palps of *T. semitorta* have a parallel series of ridges and grooves on their inner surfaces oriented at approximately right angles to the oral groove.

Very fine particles quickly pass over the crests of the palps towards the mouth (Fig. 16). Large particles pass into the depths of the troughs between ridges and are transported outwards towards the palp margin where they fall off onto either the visceral mass or the mantle and are thence removed. On the oral surfaces of the crests, the ciliary currents beat downwards whereas on the aboral surface they generally beat upwards, out of the troughs. On this surface are a number of laterally directed resorting currents. Resorting currents also exist on the crests of each ridge and these re-subject particles of intermediate size to either the acceptance or the rejection currents. In this process, apposition or parting of the crests ensures that virtually all or very little material is accepted or rejected.

The foot and byssus

The foot of the adult (Figs. 14 and 17, F) is antero-posteriorly elongate with a rather small digging "toe." There is a long, ventral byssal groove (BYG) but no byssus, though adult *T.*

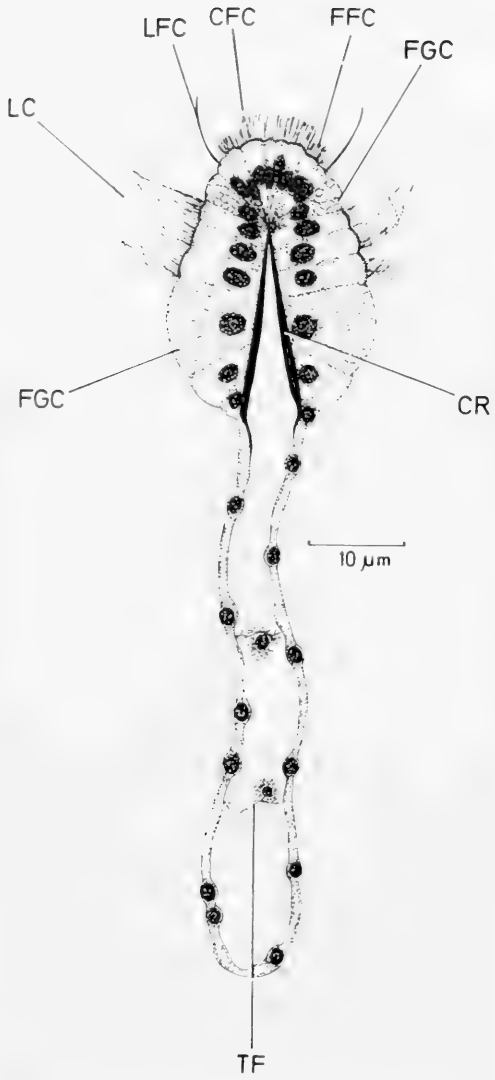

FIG. 15. *Trisidos semitorta*. A transverse section through a single ctenidial filament. For abbreviations see p. 376.

tortuosa possesses a long, thin byssus (McGhee, 1978; Tevesz & Carter, 1979).

Sections of juvenile *T. semitorta* (Fig. 18) having a stout byssus (BY) show the byssal roots radiating deeply into the visceral mass. The epithelium of the byssal groove is surrounded by dense numbers of subepithelial cells of the byssal gland (BG) which stain bright red in both Ehrlich's haematoxylin and eosin and Mallory's triple stain.

Ventrally the foot contains another extensive sub-epithelial gland (PEG) that is not involved in secretion of the byssus but which may be responsible for the mucus copiously produced here. The cells are basophilic and stain red in Mallory's triple stain.

The ciliary currents of the visceral mass

The ciliary currents of the visceral mass (Fig. 14) complement those of the mantle. Thus a major rejection tract on each side of the body commences ventral to the anterior adductor muscle and extends to the postero-ventral edge of the visceral mass. Cilia on the visceral mass beat towards it. There are few currents supplying it from the foot. Waste material arriving at the posterior edge of the visceral mass falls off, largely onto the right mantle lobe.

The alimentary canal

The mouth, located on the ventral face of the anterior adductor muscle, opens to the oesophagus which passes dorsally to merge with the stomach. In transverse section (Fig. 19A) the oesophagus of a small juvenile specimen comprises a tube some 160 μm in diameter composed of a columnar epithelium, approximately 60–80 μm tall with cilia 10 μm long. It is thrown into four longitudinal folds, though this number may increase in the adult, as in *T. tortuosa* (Heath, 1941, pl. 10, fig. 4).

From the postero-ventral wall of the stomach the conjoined style sac and mid-gut extends vertically down into the visceral mass. Transverse sections (Fig. 19B) show that the greater part of the style sac is lined by a columnar epithelium termed the A cell layer (A) consisting of cells 30 μm tall with a nucleus 8 μm in diameter and a thick border of cilia 10 μm long. In the Arcacea major and minor typhlosoles largely serve to separate the mid-gut and style sac. In section they comprise thin, elongate cells some 100 μm tall, possessing a centrally located, similarly elongate (6 μm) nucleus and with a fringe of cilia 8 μm long. Internal to each typhlosole (the B (major) and B_1 (minor) cell layers) is a C cell layer (C), forming part of the epithelial lining of the mid-gut. The cells are approximately 65 μm tall each with a fringe of stiff, bristle-like cilia, 4 μm long, that characterize this region (Henschen, 1904; Kato & Kubomura, 1954; Morton, 1969). The remainder of the mid-gut epithelium comprises the D cell layer (D) of cuboid cells 16 μm tall and with cilia 8 μm long.

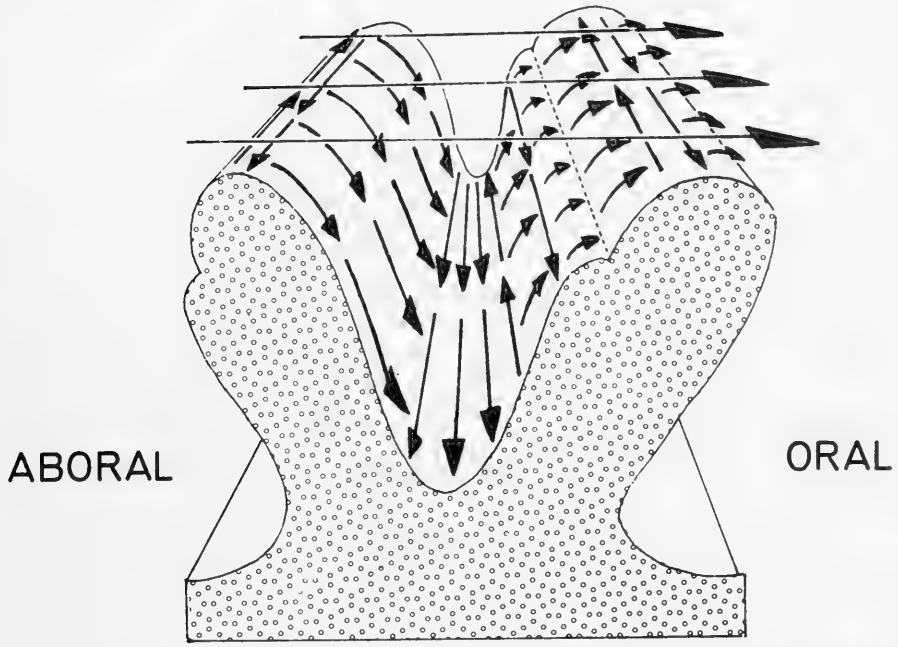

FIG. 16. *Trisidos semitorta.* The ciliary currents of two ridges and a groove of the labial palps.

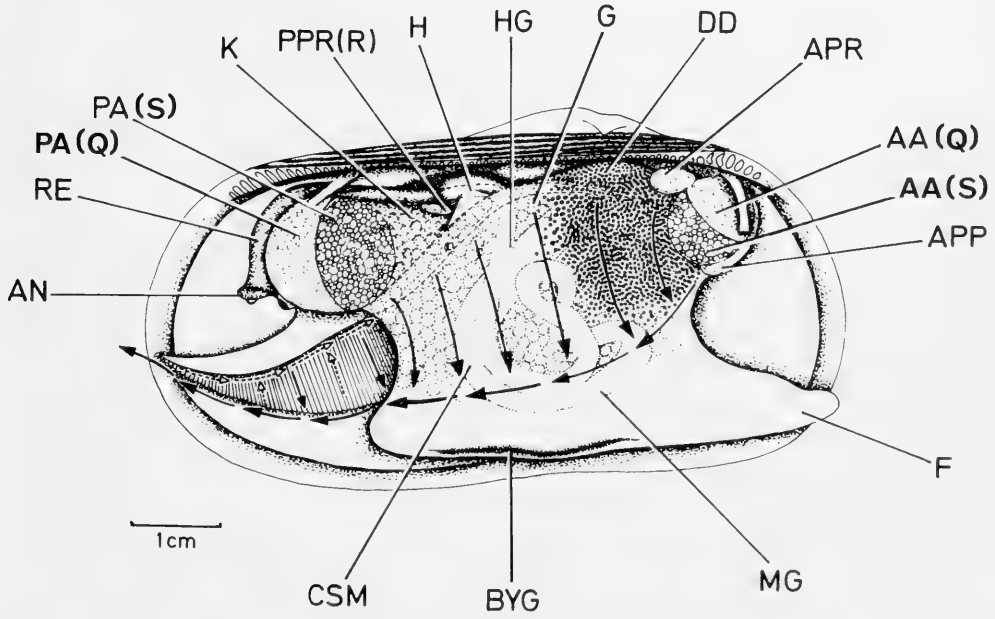

FIG. 17. *Trisidos semitorta.* The structure and ciliary currents of the visceral mass as seen from the right side after removal of the right shell valve, mantle lobe and ctenidium. For abbreviations see p. 376.

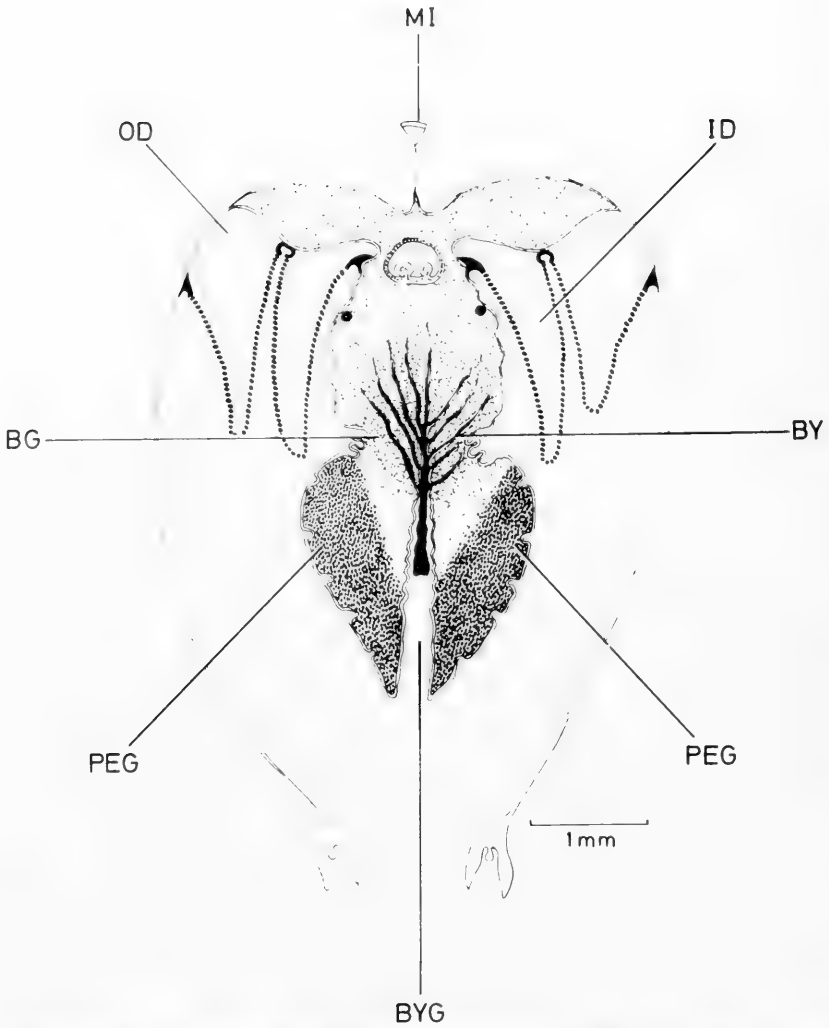

FIG. 18. *Trisidos semitorta*. A transverse section through the visceral mass of a juvenile in the region of the byssus. For abbreviations see p. 376.

In the ventral region of the visceral mass (Fig. 17), the mid-gut (MG) is separate from the style sac and coils before passing dorsally as the hind-gut (HG). In transverse section (Fig. 19C) the mid-gut is a circular tube 200 μm in diameter with a single typhlosole comprising cells 80 μm tall with cilia 14 μm long. The remainder of the mid-gut epithelium comprises a columnar epithelium 40 μm tall similarly ciliated. The hind-gut gives rise to the rectum. In section (Fig. 19D) the rectum comprises a tube 140 μm in diameter and comprising cells 20 μm tall with cilia 6 μm long. In the rectum the typhlosole divides into two ventral, longitudinal ridges. The rectum (Fig. 17, RE) penetrates the ventricle of the heart (H), passes between the posterior pedal retractor muscles (PPR) and thus also between the kidneys (K) and over the posterior adductor muscle (PA) to terminate on the posteroventral face of this muscle at an anus (AN) with a distinctive anal papilla.

The stomach

The large stomach (Fig. 20) lies ventral to the anterior region of the hinge plate and is of Type III (Purchon, 1957). The terminology

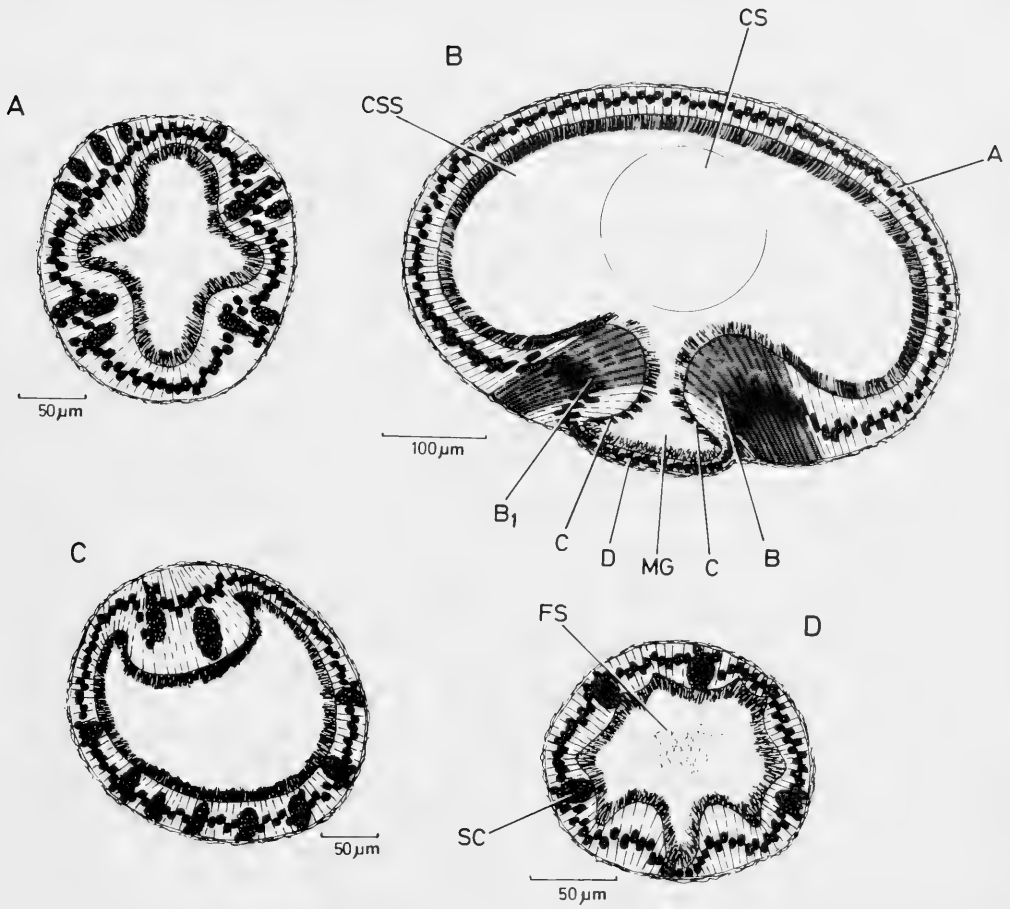

FIG. 19. *Trisidos semitorta*. Transverse sections through (A), the oesophagus; (B), the conjoined style sac and mid gut; (C), the mid gut; (D), the rectum. For abbreviations see p. 376.

used in this description follows that of Purchon. The minor typhlosole terminates soon after emerging into the stomach. The major typhlosole extends across the floor of the stomach from right to left to terminate in a capacious food sorting caecum (FC). The food sorting caecum extends dorsal to the entrance of the oesophagus (O). The major typhlosole has on its right side the intestinal groove (IG) which transports waste material into the mid-gut. In the food sorting caecum is a very large sorting area (SA). Between each of the adjacent ridges of the sorting area is an aperture which leads into a component part of the digestive diverticula (DDD). A ridge dorsal to the row of sorting ridges carries material into the caecum. There is, in addition to the intestinal groove, a further ridge (R) carrying recycled material to the dorsal hood (DH) from where it is probably returned to the head of the style (CS) rotating against the gastric shield (GS). The latter is very small in relation to the size of the stomach and is located on the postero-dorsal wall. It sends spurs into the dorsal hood and the left pouch (LP). From the left pouch a series of ducts opens into the digestive diverticula. Particles settling on the surface of the major typhlosole are swept towards the left pouch and the ridge leading to the dorsal hood.

The pericardium and associated organs

The heart (Figs. 7 and 21) comprises a single ventricle (V) and paired lateral auricles (AU). From the posterior wall of the pericardium (Fig. 21, PE), paired reno-pericardial apertures (RPA) open into the paired kidneys

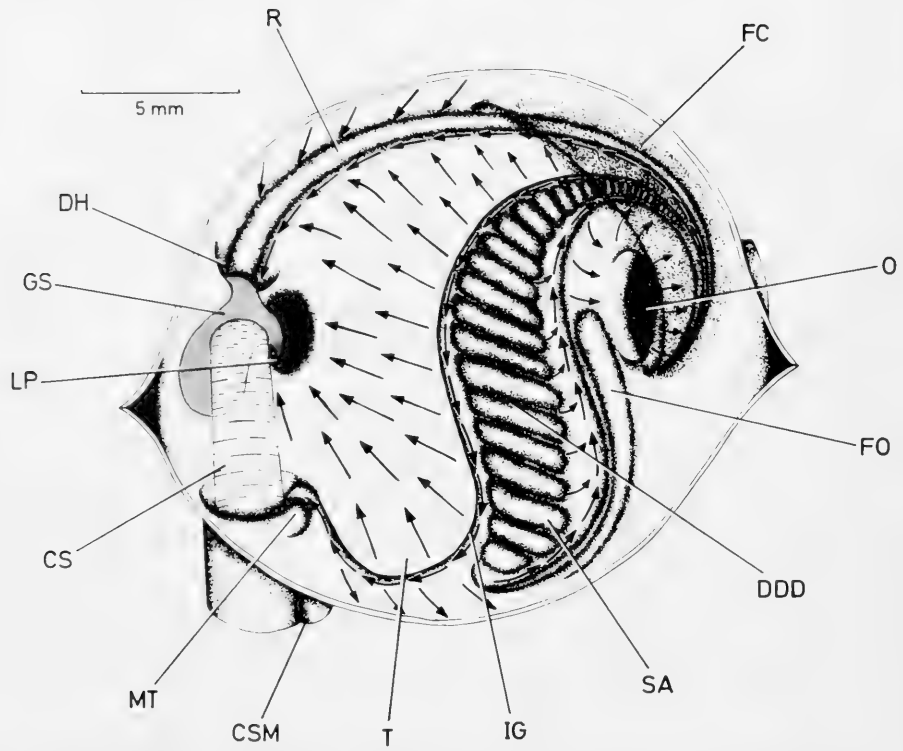

FIG. 20. *Trisidos semitorta.* The internal structure and ciliary currents of the stomach after opening by an incision in the right wall. For abbreviations see p. 376.

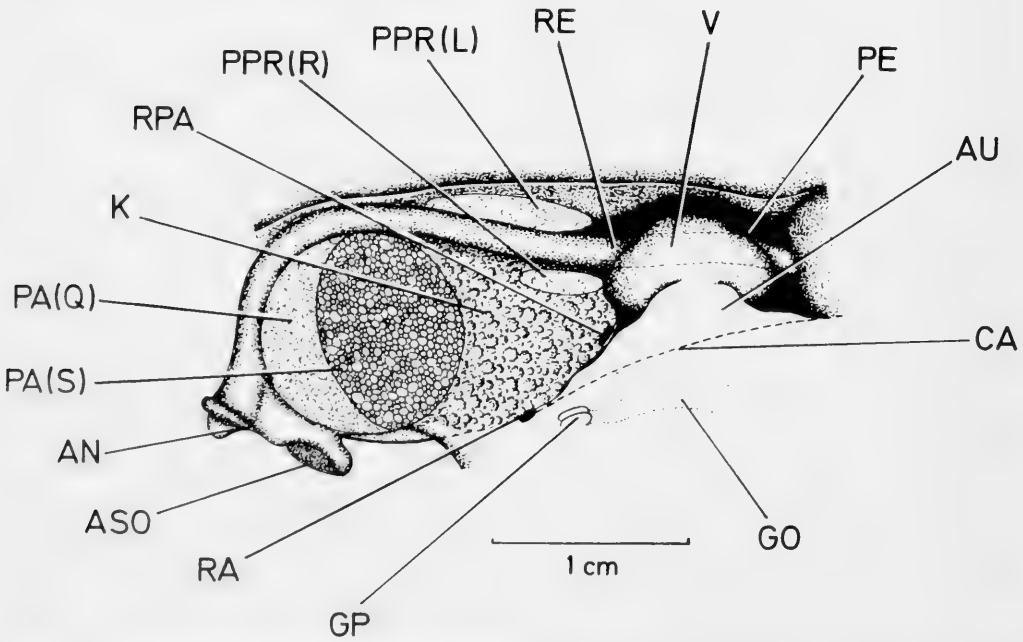

FIG. 21. *Trisidos semitorta.* The organs of the pericardium as seen from the right side. For abbreviations see p. 376.

(K). The kidneys lie posterior to the pericardium and lateral to the posterior pedal retractor muscles (Fig. 8). In section the kidneys comprise a ventral series of tubules, but dorsally there are few tubule cells as described for *T. tortuosa* (Health, 1941, pl. 11, fig. 3). The renal apertures (RA) discharge into the suprabranchial chamber.

Close to the renal aperture is the gonopore (GP), which has thick fleshy lips. In *T. tortuosa* gonopore and renal aperture are joined (Heath, 1941).

DISCUSSION

The shell of *Trisidos,* especially *T. tortuosa*, has been discussed previously (Makiyama, 1931; McGhee, 1978; Tevesz & Carter, 1979). *Trisidos* is unusual in being the only known extant twisted bivalve genus. In most morphological respects, *Trisidos semitorta* is a typical ark. Thus, the ctenidia and labial palps are little modified, the general disposition of the mantle and organs of the mantle cavity and visceral mass are typically arcacean. The ciliary rejection currents of the mantle cavity, whilst also arcacean, are modified to enable the adult, partially buried in soft deposits, to remove large amounts of sediment that may enter the largely open mantle cavity. The shell is eroded posteriorly on the left valve only, the posterior end lying flush with the sediment-water interface and the right valve being wholly buried. The anterior end lies vertical to the surface. The structure and position of the posterior abdominal sense organs suggests a photoreceptive function, there being no sensory cilia suitable for monitoring water flow. *T. kiyonoi* and *T. tortuosa* live in the same way as *T. semitorta* (Makiyama, 1931; Tevesz & Carter, 1979). Twisting does not seem to have any effect upon the distribution or size of the organs of the mantle cavity. Thus in *T. semitorta* left and right ctenidia, mantle lobes and labial palps are of approximately the same size in marked contrast to the left and right inequality seen in the tangentially coiled Chamidae and Cleidothaeridae (Yonge, 1967; Morton, 1974) and to a lesser extent in the markedly inequivalve *Claudiconcha japonica* (Morton, 1977). In *T. tortuosa*, however, left and right abdominal organs are of notably different size (Heath, 1941)—this is not so in *T. semitorta*. Also, the left and right posterior pedal retractor muscles are of different sizes; this influences slightly the size of left and right kidneys.

Tevesz & Carter (1979) suggest that *Trisidos* is more probably evolved from a morphologically less specialized representative of the Arcinae similar to the modern *Barbatia*. They argue that because of its relatively efficient ligament (Thomas, 1976) and streamlined shape a *Barbatia*-like ancestor was preadapted for the evolution of a shallow burrowing life habit. Superficially it would seem more reasonable to derive the burrowing, abyssate *Trisidos* from a shallow burrowing limopsacean (Limopsidae and Glycymerididae), but Thomas (1976) has shown that the duplivincular ligament of these is inherently weak, arguing for morphological conservatism and not conducive to evolutionary diversification.

Purchon (1957) describes stomachs of representatives of the Arcidae and Glycymerididae, and it is clear that a *Barbatia-Trisidos* link is supported. The stomach of *T. semitorta* is similar to that of both *Anadara* and *Arca* and different from that of representatives of the Glycymerididae. It is, however, difficult, at first, looking only at the adult, to understand why *Trisidos* could not have evolved from a burrowing arcoid lineage, by posterior elongation, twisting and, in *T. tortuosa,* some degree of lateral flattening. There appears no reason for not deriving *Trisidos* from, say, an anadarine ancestor, especially as *T. semitorta* more closely fits the definition of the Anadarinae (Newell, 1969) than the Arcinae. However, the views of Newell (1969) and Tevesz & Carter (1979) are borne out by this research. Juvenile *T. semitorta* are byssally attached to the inside of empty bivalve shells; subsequently, attachment and the byssus are lost. The byssus functions in the classical manner as a means of securing post-larvae in a position suitable for the growth of the juvenile. Juvenile *T. tortuosa* and *T. semitorta* are not so twisted when young, twisting being a progressive condition.

The terms "torsion" and "twisted" require consideration. Traditionally *Trisidos* is referred to as the "twisted" ark, but McGhee (1978) replaced this with "torted." Generally, gastropods are torted, the mantle cavity moving from a posterior to an anterior position in larval development which is related to an "asymmetry in the development of the retractor muscles" (Garstang, 1929). In primitive prosobranchs (Crofts, 1955) and tectibranchs (Saunders & Poole, 1910), torsion is initiated by the contraction of a single asymmetrical, precociously developed larval cephalopedal retractor muscle. The term "twisted" has been

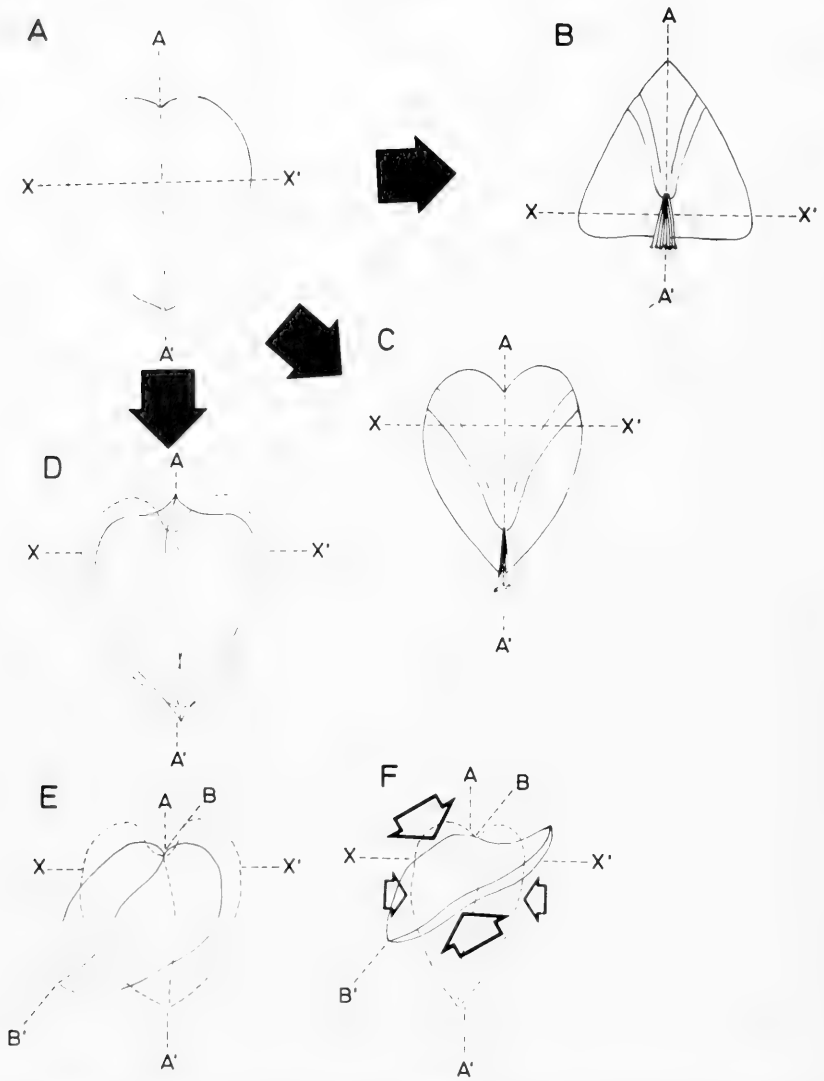

FIG. 22. A, Transverse section through the shell of an isomyarian, equilateral bivalve. B, Transverse section through the shell of a heteromyarian, equilateral bivalve, with byssus. C, Transverse section through an equilateral, partly heteromyarian, nestling bivalve. D, Transverse sections through the anterior (dotted lines) and posterior (solid lines) shell of a juvenile *T. semitorta*. E, The same through an adult *T. semitorta*. F, The same through an adult *T. tortuosa*. (Large open arrows indicate lateral compression in F). (A-A_1, the dorso-ventral axis of the shell; B-B_1, the dorso-ventral axis through the posterior region of the shell of *Trisidos*; X-X_1, the region of shell exhibiting the greatest shell width).

applied to *Trisidos* though, hitherto, it was not known how this was achieved. The process of twisting in *Trisidos* has obvious similarities and major differences with torsion of the larval gastropod. Thus, twisting results from the contraction of unequal, asymmetrically aligned posterior pedal retractor muscles. These can be regarded as "cephalopedal" retractors because, though posteriorly located, they would in the primitive bivalve have withdrawn the head-foot. The difference is that twisting occurs *laterally* about the sagittal plane of the mantle shell and not by altering the mantle cavity from a posterior to an anterior position. Moreover, it would seem that twisting in *Trisidos* is a post-larval and not a

larval feature. It is contended that the term torsion should be used only with reference to that process characteristic of the larval gastropod. In *Trisidos* the term twisted should be used, as it more appropriately defines the situation and distinguishes a process that is interesting though of restricted phylogenetic importance.

From an isomyarian, infaunal, abyssate (except in the larva) ancestor (Fig. 22A), evolution in the byssally attached adult bivalves has proceeded in a number of directions. In the various heteromyarian bivalve lineages, e.g. Mytilacea and Dreissenacea (Yonge & Campbell, 1968) (Fig. 22B), the byssus acts as the point about which the reorganization of the body occurs. In epibyssate heteromyarian bivalves, the greatest shell width lies ventral to the mid-point of the dorsoventral axis of the shell. This ensures stability on wave-tossed, exposed beaches or in fast-flowing fresh waters (Morton, 1969). There are also heteromyarian byssally nestling species. In the nestling bivalve (Fig. 22C), the greatest shell width usually lies dorsal to the mid-point of the dorso-ventral axis of the shell. The narrow ventral region of the shell enables the shell to tightly fit into crevices. From Fig. 22D it can be seen that the juvenile *T. semitorta* is fundamentally a nestler, its shell form matching that of others (Fig. 22C). In the adult (Fig. 22E and F), the anterior end retains its typical nestling form. There is a morphological compromise which, perfectly suitable for neither juvenile nor adult, permits maintenance of both. Both forms are essential if juvenile and adult are to survive in fast-moving waters, but twisting optimises success for both. It should be noted that adult *T. semitorta* possesses no byssus and that the twisting process seen in the free adult is a continuation of a processs *begun* in the post-larva. The twisted shell allows *Trisidos* to lie in the sediment in a way that minimizes the effect of fast currents and scour. Tevesz & Carter (1979) suggested that in very twisted *T. yongei* lateral compression may reduce the ventilation efficiency of the mantle cavity. While this cannot be as acute in the more rounded *T. semitorta*, species of *Trisidos* occupy well-aerated sands, possibly facing into the current, so that the problems of ventilation are reduced. Even in *T. tortuosa,* the anterior region of the mantle cavity is still relatively unaffected by twisting and the mantle cavity functions in the usual manner.

ACKNOWLEDGEMENTS

I am grateful to Solene Morris of the British Museum (Natural History) for the loan of specimens of *Trisidos tortuosa,* to Dr. R. S. S. Wu of the Agriculture and Fisheries Research Station, Aberdeen, Hong Kong for the provision of boat facilities to collect *T. semitorta* and to Mr. H. C. Leung of the University of Hong Kong for histological assistance.

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MALACOLOGIA, 1983, 23(2): 397–426

PHYLOGENETIC RELATIONSHIPS IN THE CEPHALOPOD FAMILY CRANCHIIDAE (OEGOPSIDA)

Nancy A. Voss[1] and Robert S. Voss[2]

ABSTRACT

Fourteen qualitative morphological characters of squids of the oegopsid family Cranchiidae are described, and the distribution of their states among thirteen genera tabulated. Primitive and derived conditions for each character are inferred on the basis of outgroup comparisons and analyses of ontogenetic transformation series. Application of a Wagner Tree algorithm and Character Compatibility Analysis to the resulting data matrix yields nearly identical reconstructions of cranchiid phylogeny. Hypotheses of monophyly for the traditional subfamilies Cranchiinae and Taoniinae as well as for two of the three groupings of taoniin genera proposed by N. Voss (1980) are shown to be well corroborated, and refinements of previous ideas about cranchiid relationships are also proposed. Little homoplasy is evident in most of the characters of the study, but the anatomical position of digestive duct appendages appears to possess considerable evolutionary lability. Sources of new data for phylogenetic tests are suggested, and the need for additional research on teuthoid comparative morphology is emphasized.

Key words: Mollusca; Cephalopoda; Cranchiidae; phylogenetic inference; Wagner Tree; Character Compatibility.

INTRODUCTION

Phylogenetic studies of living Cephalopoda are long overdue, but until recently have hardly been possible because of the uncertain taxonomy of many groups and the absence of sufficient comparative anatomical data on which to base reliable estimates of evolutionary relationships (G. Voss, 1977a). Past taxonomic studies have usually emphasized external morphology with only incidental treatment, if any, of internal structures, and the systematic potential of many organ systems has, therefore, seldom been explored. This is unfortunate because it seems desirable that classifications be based on as broad a suite of biological attributes as possible. Additionally, the fossil record of cephalopods, as it relates to the genealogy of most contemporary taxa, is inadequate (Donovan, 1977).

The large and morphologically diverse pelagic squid family Cranchiidae was recently revised by N. Voss (1980). Thirteen valid genera were recognized and were arranged into two subfamilies, the Cranchiinae with three constituent genera (*Cranchia, Liocranchia* and *Leachia*), and the Taoniinae with ten (*Helicocranchia, Bathothauma, Sandalops, Liguriella, Taonius, Galiteuthis, Mesonychoteuthis, Egea, Megalocranchia* and *Teuthowenia*). Hypotheses of natural generic groupings within the Taoniinae were presented and the taxonomic distribution of a large number of morphological characters was tabulated. The present paper subjects data gathered by N. Voss (1980) on cranchiid comparative morphology to quantitative phylogenetic analysis in order to derive maximally-corroborated hypotheses of relationships for these squids. It is our intention by so doing to test ideas about cranchiid classification presented in the 1980 paper, to argue the utility of much broader surveys of teuthoid morphology than have hitherto been undertaken, and to demonstrate the application of explicitly phylogenetic procedures to systematic studies of contemporary cephalopods.

MATERIALS AND METHODS

The material examined during this study is from the extensive cranchiid collection amassed at the University of Miami over a period of several years from numerous loaning institutions and from the general cephalopod collection of Miami's invertebrate museum, supplemented by the collections of the

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[2]Museum of Zoology and Division of Biological Sciences, University of Michigan, Ann Arbor, Michigan 48109, U.S.A.

U.S. National Museum. Specimens illustrated in the text belong to the following institutions: Australian Museum (AM), Dominion Museum, New Zealand (DMNZ), Institut Océanographique, Monaco (IOM), Institut für Seefischerei und Zoologisches Museum der Universität Hamburg (ZMH), Newfoundland Biological Station (NBS), Scripps Institution of Oceanography (SIO), South African Museum (SAM), United States National Museum of Natural History (USNM) and the University of Miami Rosenstiel School of Marine and Atmospheric Science (UMML).

1. Character Analysis

This study analyzes the historical information content of 14 qualitative morphological characters and samples variation in anatomical features associated with reproduction, locomotion, feeding, digestion, excretion, structural support and concealment from predators; aspects of both larval and adult morphology are represented. Characters here treated were selected from among those discussed by N. Voss (1980) on the basis of their within-genus constancy and because the variants of the morphological expressions they represent could be coded as discrete states with minimal ambiguity. Character constancy within taxa of low rank seems desirable because such constancy may reflect evolutionary conservatism (Farris, 1966), and choosing characters with easily-described states minimizes the possibility of misclassifying the objects of study. Less rigorous criteria for character choice would have admitted a larger number of characters for phylogenetic analysis but probably at the risk of introducing more homoplasy to the data. The single exception, in this study, to our requirement that character expressions be constant within genera is discussed in the analysis of Character 11, below.

Our arguments for determinations of cranchiid character polarities are presented individually, character by character, but fall broadly into two categories based on the criteria that we used to estimate relative primitiveness. Recent reviews and discussions of methods of polarity estimation are provided by Stevens (1980) and Watrous & Wheeler (1981).

Outgroup comparisons: Of two or more alternative morphological conditions observed among cranchiid squids, the one that also occurs among other teuthoid cephalopods is here hypothesized to be primitive. When two or more states of a cranchiid character whose polarity was in question were encountered among other teuthoids, however, then polarity estimation required comparisons within yet narrower limits of cranchiid relationships. Choice of a more restricted outgroup was also dictated by the impracticality of tabulating character state distributions for all of the 24 families and 66 genera of living, non-cranchiid teuthoids (see G. Voss, 1977b) when original dissections were necessary to determine anatomical features rarely described or figured in the literature. In the absence of any well-corroborated estimate of teuthoid phylogeny from which an appropriate cranchiid sister group might have been chosen, we restricted our attention, when necessary, to comparisons with only seven other oegopsid families: Thysanoteuthidae, Cycloteuthidae, Chiroteuthidae, Grimalditeuthidae, Mastigoteuthidae, Joubiniteuthidae, and Promachoteuthidae. These families share, with cranchiids, 1) a funnel-locking apparatus other than a simple ridge-and-groove, and 2) ventral connectives between the buccal membranes and arms IV (Young & Roper, 1969a, b; Roper *et al.,* 1969). Whether these traits are really synapomorphies that would indicate a close relationship of the seven families to the Cranchiidae, however, is not yet known. Young & Roper (1968) believed that a simple ridge-and-groove funnel locking apparatus is the primitive oegopsid condition, but they also thought that the other types of locking (or fusion) arrangements may have been derived independently. Furthermore, because a ventral attachment of the buccal connectives to arms IV also occurs in the myopsids as well as the majority of the oegopsids, this character likewise provides but weak justification for our choice of cranchiid outgroups. Nevertheless, some manageable basis for comparisons had to be established in order to implement our analyses, and the two characters cited above are among the few available on which to base such a selection.

Ontogenetic precedence: In the absence of unambiguous results from the outgroup comparisons, relative primitiveness is estimated on the basis of developmental data; the ontogenetically antecedent character state is hypothesized to be primitive while ontogenetically subsequent alternative expressions are hypothesized to be derived. In effect, we assume that evolutionary novelties are, more often than not, developmental modifications of phylogenetically ancestral conditions. Examples of neoteny and paedo-

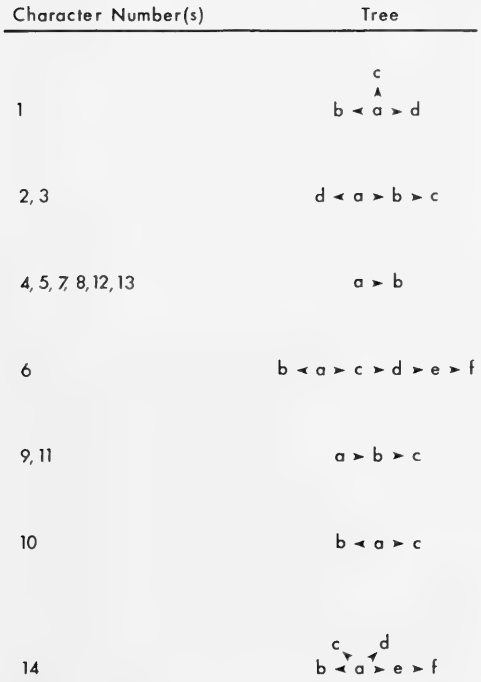

FIG. 1. Tree diagrams illustrating the estimates of polarity of states of characters described in the text. Hypotheses of polarity are presented in the right-hand column, and the characters whose states are believed to have evolved in the sequences illustrated are listed to the left.

genesis would provide obvious exceptions to this generalization; two cases of apparent paedomorphosis in cranchiid phylogeny are discussed below.

The (two or more) derived conditions of multistate characters were arranged, whenever possible, as geometrical or topological series (e.g., Character 6, Fig. 1) that could reasonably be expected to represent the sequential order of appearance of advanced states from the plesiomorph under a gradualistic model of phyletic change. Where no such series could be discerned (e.g., Characters 1 and 14, Fig. 1), all non-primitive states were regarded as independently derived by default. Multistate characters were then subjected to an additive binary recoding procedure (see Farris *et al.*, 1970) that reduces transformation series (morphoclines) with t states to t-1 binary (two-state) factors while preserving all of the phyletic information contained in the original character state tree topology. Table 1 presents the distribution of states of the original, unfactored characters among the cranchiid genera, Fig. 1 provides diagrams of character state trees, and Table 2 is the data matrix that results from application of binary recoding to the character state distribution of Table 1 given the character state tree topologies of Fig. 1. Binary factors of multistate characters are labelled with the name of the character state that is the apomorph for the transformation represented by the factor. Thus, binary factor 6d of Table 2 represents the character state transition (c→d) of Character 6; cranchiid genera with a score of (1) for binary factor 6d exhibit either state (d) of Character 6 or one of the two other states derived from (d) in the state tree for Character 6 (Fig. 1).

2. Inferring Tree Topologies

Numerous quantitative methods have been proposed to construct hierarchical arrangements of organisms, but only a few are directly pertinent to the problem of deriving well-corroborated hypotheses of phylogeny. Phenetic clustering algorithms, typically applied to matrices of overall similarity measures, do not address phylogenetic inference *per se* and are not employed here; Colless (1970) has argued that phenograms sometimes provide reasonable estimates of phylogeny, but the set of assumptions under which they may be presumed to do so seems to us unnecessarily onerous. Of explicitly phylogenetic methods we have chosen two that operationalize, at least in part, the analytic procedures of Hennig (1966).

The Wagner Tree method (Kluge & Farris, 1969; Farris, 1970) implements a heuristic procedure for discovering the most parsimonious hypothesis of phylogeny for a study collection of organisms and a set of cladistic characters. A most parsimonious phylogeny is defined to be that tree topology that requires the least number of convergent or reversed evolutionary events in order to derive the character state distributions observed among the extant organisms of the study from the hypothesized morphology of the common ancestor. Unlike earlier parsimony approaches (for example, Camin & Sokal, 1965), the Wagner method does not assume that evolution is irreversible, and for this reason we regard it as the more biologically reasonable. Caveats regarding uncritical use of the Wagner Tree method, however, have recently been offered by researchers (e.g.,

TABLE 1. Primary data matrix. Columns represent cranchiid genera; rows represent characters as numbered and described in the text. The entry for a given column × row is the character state label appropriate to the corresponding genus and character. Abbreviations of taxa for this and the subsequent tables and figures: *Cra, Cranchia; Lio, Liocranchia; Lea, Leachia; Hel, Helicocranchia; Bat, Bathothauma; San, Sandalops; Lig, Liguriella; Tao, Taonius; Gal, Galiteuthis; Mes, Mesonychoteuthis; Ege, Egea; Meg, Megalocranchia; Teu, Teuthowenia.*

Character number	Taxa												
	Cra	*Lio*	*Lea*	*Hel*	*Bat*	*San*	*Lig*	*Tao*	*Gal*	*Mes*	*Ege*	*Meg*	*Teu*
1	d	d	d	c	c	c	a	a	a	a	b	b	a
2	a	b	a	a	d	a	b	c	c	c	c	c	c
3	a	a	a	d	d	a	a	b	b	b	c	c	c
4	b	b	b	a	a	a	a	a	a	a	a	a	a
5	a	a	b	b	b	b	b	b	b	b	a	a	b
6	b	b	b	c	c	d	d	e	e	e	e	e	f
7	a	a	a	b	b	b	b	b	b	b	b	b	b
8	b	b	b	a	a	a	a	a	a	a	b	b	b
9	a	a	a	a	a	a	a	b	c	c	a	a	a
10	a	a	a	a	b	b	b	a	a	a	a	c	a
11	c	c	a	c	c	c	c	b	b/c	c	b	a	b
12	b	b	b	a	a	a	a	a	a	a	a	a	a
13	a	a	b	b	b	b	b	b	b	b	b	b	b
14	b	b	d	e	f	a	e	e	e	e	c	c	e

Colless, 1980, but see also Mickevich & Farris, 1981) who report that applications of the algorithm to some data yield phylogenetic reconstructions that are not uniquely most parsimonious; other, equally or more parsimonious hypotheses of evolutionary relationships may exist, and consideration of plausible alternative methods of phylogenetic inference are therefore of interest.

The method of Character Compatibility Analysis (Estabrook *et al.*, 1977; Meacham, 1980) rests on the concept of the compatibility of cladistic characters (see also Estabrook, 1972). Two cladistic characters are said to be compatible if there exists at least one hypothesis of phylogeny for the organisms of the study collection that both can support. Thus, if two characters are incompatible, then both cannot support historical truth; at least one (and perhaps both) has undergone homoplasy in the course of the evolution of the study collection. All characters that support true statements of phylogenetic relationships, however, must be mutually compatible, while characters that do not support historical truth may or may not be pairwise compatible with each other and/or with true characters. Given a study collection of organisms and a set of cladistic characters, the compatibility of all character pairs can be analyzed and groups (cliques) of mutually compatible characters identified. For any clique of mutually compatible characters there exists at least one phylogeny that all member characters can support, and that tree supported by the largest clique is sometimes chosen as the best estimate of true evolutionary history. In this study, Character Compatibility Analysis was used to develop alternative hypotheses of phylogeny to be tested against the results of Wagner analyses.

Minimum tree lengths and estimates of hypothetical ancestral phenotypes were calculated using the parsimony-optimizing procedure proposed by Farris (1970) subject to the constraint that the most recent common cranchiid ancestor exhibit the primitive morphology determined *a priori* by the methods of individual character analysis described above.

The computer programs we used to execute the Wagner analyses were written by J. S. Farris; the program for Character Compatibility Analysis was written by K. L. Fiala and G. F. Estabrook. Analyses were performed on the Michigan Terminal System at the University of Michigan.

RESULTS

1. Character Descriptions and Analyses

Character 1. *Funnel-mantle fusion cartilages:*

TABLE 2. Factored data matrix. Columns are labelled as in Table 1. For an explanation of factor labels and table entries, see the Methods section of the text.

Factor label	Taxa												
	Cra	*Lio*	*Lea*	*Hel*	*Bat*	*San*	*Lig*	*Tao*	*Gal*	*Mes*	*Ege*	*Meg*	*Teu*
1b	0	0	0	0	0	0	0	0	0	0	1	1	0
1c	0	0	0	1	1	1	0	0	0	0	0	0	0
1d	1	1	1	0	0	0	0	0	0	0	0	0	0
2b	0	1	0	0	0	0	1	1	1	1	1	1	1
2c	0	0	0	0	0	0	0	1	1	1	1	1	1
2d	0	0	0	0	1	0	0	0	0	0	0	0	0
3b	0	0	0	0	0	0	0	1	1	1	1	1	1
3c	0	0	0	0	0	0	0	0	0	0	1	1	1
3d	0	0	0	1	1	0	0	0	0	0	0	0	0
4b	1	1	1	0	0	0	0	0	0	0	0	0	0
5b	0	0	1	1	1	1	1	1	1	1	0	0	1
6b	1	1	1	0	0	0	0	0	0	0	0	0	0
6c	0	0	0	1	1	1	1	1	1	1	1	1	1
6d	0	0	0	0	0	1	1	1	1	1	1	1	1
6e	0	0	0	0	0	0	0	1	1	1	1	1	1
6f	0	0	0	0	0	0	0	0	0	0	0	0	1
7b	0	0	0	1	1	1	1	1	1	1	1	1	1
8b	1	1	1	0	0	0	0	0	0	0	1	1	1
9b	0	0	0	0	0	0	0	1	1	1	0	0	0
9c	0	0	0	0	0	0	0	0	1	1	0	0	0
10b	0	0	0	0	1	1	1	0	0	0	0	0	0
10c	0	0	0	0	0	0	0	0	0	0	0	1	0
11b	1	1	0	1	1	1	1	1	1	1	1	0	1
11c	1	1	0	1	1	1	1	0	0/1	1	0	0	0
12b	1	1	1	0	0	0	0	0	0	0	0	0	0
13b	0	0	1	1	1	1	1	1	1	1	1	1	1
14b	1	1	0	0	0	0	0	0	0	0	0	0	0
14c	0	0	0	0	0	0	0	0	0	0	1	1	0
14d	0	0	1	0	0	0	0	0	0	0	0	0	0
14e	0	0	0	1	1	0	1	1	1	1	0	0	1
14f	0	0	0	0	1	0	0	0	0	0	0	0	0

(a) stout, roughly oval, subtriangular or spindle-shaped;
(b) elongate, triangular;
(c) narrow, straight;
(d) fused into ventral cartilaginous strips.

In the Cranchiidae, the mantle is fused to the funnel at its posterolateral corners along two acutely diverging lines. These lines of fusion, found only in the cranchiids, replace the diverse forms of funnel-mantle locking cartilages present in all other teuthoids. In all members of the Cranchiinae, external cartilaginous strips, located on the ventral surface of the mantle, extend along one (the dorsalmost: *Leachia*) or both (*Cranchia, Liocranchia*) of the paired internal lines of funnel-mantle fusion and probably serve to strengthen the attachments (Fig. 2.4). The strips may run for partial or full length of, or beyond, the lines of fusion. In *Cranchia,* these external strips are short, of coequal length and smooth except for a multipointed apical tubercle, while in *Liocranchia* they are relatively long, of coequal or unequal length, and tuberculate for their full extent. In *Leachia*, the strips are always tuberculate and vary, among the species of that genus, from about 10 to 50 percent of the mantle length.

Outside of the cranchiids, fusion of the funnel and mantle occurs in only two other teuthoid genera: *Symplectoteuthis* (Ommastrephidae) and *Grimalditeuthis* of the monotypic family Grimalditeuthidae. In *Symplectoteuthis,* only the posterior portion of the funnel-mantle locking cartilages are fused, while in *Grimalditeuthis* there is complete fusion of the cartilages. In the Taoniinae, only remnants, termed funnel-mantle fusion cartilages, remain of the typical, separate locking

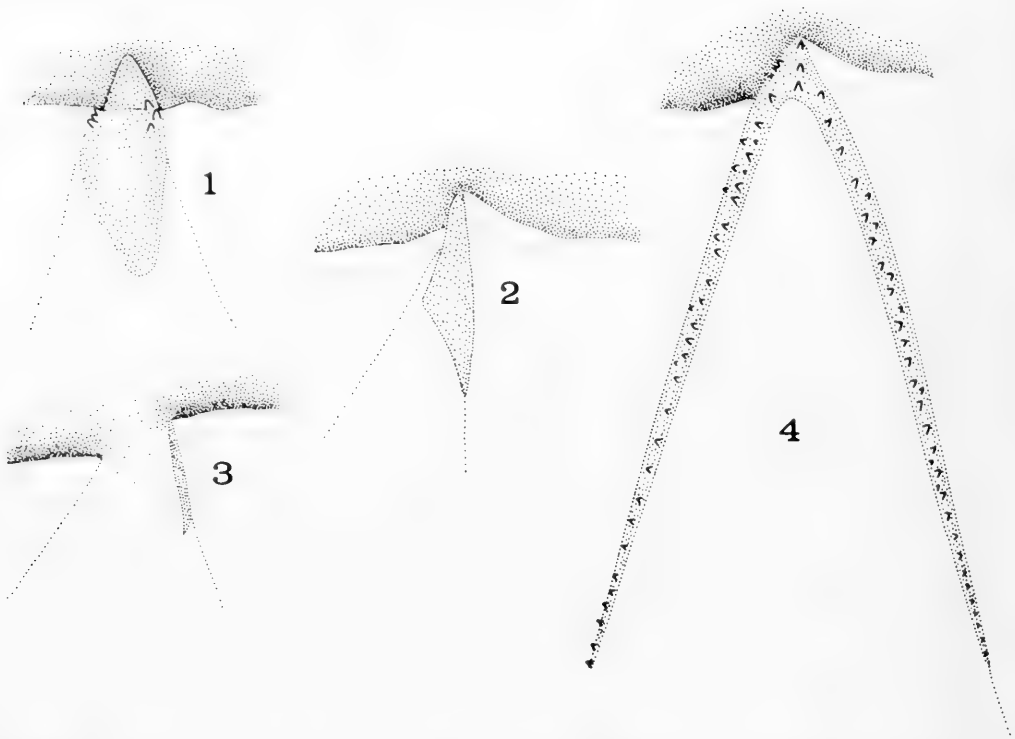

FIG. 2. Funnel-mantle fusion cartilage, left: (1) *Galiteuthis glacialis,* Elt 1112 (USNM), adult, 395 mm mantle length; (2) *Egea inermis,* WH 467-71 (ZMH), adult, 260 mm mantle length; (3) *Bathothauma lyromma,* AD 329-79 (ZMH), subadult, 190 mm mantle length; (4) *Liocranchia reinhardtii*, CI 71-98 (USNM), subadult, 160 mm mantle length. Dotted lines trace internal lines of funnel-mantle fusion; full extent of fusion lines not shown (see Character 1).

cartilages of non-cranchiid teuthoids. Funnel-mantle fusion cartilages cannot be identified as separate elements in the Cranchiinae, but it seems probable that such cartilages were the points of origin from which the ventral cartilaginous strips evolved.

The funnel-mantle fusion cartilages and the derived strips are the only known instances of cartilaginous elements present externally at the two funnel-mantle junctions. Among other teuthoids, with the exception of *Symplectoteuthis* and *Grimalditeuthis,* the locking apparatus consists of two separate, complimentary, internal elements—one on the mantle and the other on the funnel. In the majority of oegopsid families, the locking apparatus is a simple, straight groove-and-ridge arrangement that was hypothesized to be primitive for the order by Young & Roper (1968). In two families, Ommastrephidae and Thysanoteuthidae, the locking apparatus is ⊥- or ⊣-shaped; in the remaining six families, which together with the Thysanoteuthidae comprise the outgroup, the funnel locking apparatus is round, oval, or subtriangular in shape.

The form of the funnel-mantle fusion cartilages varies within the Taoniinae. In *Helicocranchia* and *Bathothauma,* where the external funnel-mantle fusion area is markedly broad, the cartilage is straight, very slender and barely discernible (Fig. 2.3). In *Sandalops,* where the external fusion area is not broad but narrow as in the other taoniins, the cartilage is also straight, but distinct, and is shorter and wider than in *Helicocranchia* and *Bathothauma.* In all three genera, the cartilage (coded as "narrow, straight," above) follows the dorsalmost of the two internal lines of funnel-mantle fusion. The cartilage in *Egea* and *Megalocranchia* is elongate and triangular, with the longest side following the dorsalmost internal line of fusion (Fig. 2.2). In the remaining five taoniin genera, *Liguriella, Taonius, Galiteuthis, Mesonychoteuthis* and

Teuthowenia, the cartilage is stouter, varies considerably in shape, and is positioned more apically with respect to the internal lines of fusion, the long axis of the cartilage sometimes tending to follow the ventralmost line (Fig. 2.1). In this group of five genera, the cartilage also bears tubercles on the anterior end. These are present only in the young of *Mesonychoteuthis* and of some species of *Taonius,* but are present in both young and adults of *Liguriella, Teuthowenia* and most species of *Galiteuthis.* Because the more apical position and generally stouter outline of the fusion cartilage in these five genera most closely approaches the orientation and shape of the funnel-mantle locking cartilages among members of the outgroup, the character state to which we have assigned these squids is judged to be primitive for the extant cranchiids, and the remaining four states are hypothesized to have been independently derived.

Character 2. *Posterior end of gladius:*

(a) conus present in larva and adult;
(b) conus present in larva, lost or obscured in pseudoconus of adult;
(c) conus lacking, pseudoconus present in larva and adult;
(d) conus and pseudoconus absent.

Re-evaluation of the shape of the posterior end of the gladius of adults and a careful examination of the gladius in the larvae of all of the cranchiid genera have revealed anatomical differences in addition to those described earlier (N. Voss, 1980: Table 1). Voss described the character states: a) short conus; b) medium to long conus; and c) conus lacking, but did not distinguish between the two types of "conus" that occur within the family: 1) a "true" conus that exhibits no evidence of fusion or convergence of the edges of the gladial vanes along the ventral midline, and 2) a pseudoconus formed by the infolding of the posterolateral margins of the gladial vanes that converge along the midventral line, with or without subsequent fusion (Fig. 3). The definition of pseudoconus has been expanded here from that of McSweeny (1978) in order to include the instances of ontogenetically subsequent fusion that follow infolding in some cranchiids (N. Voss, 1980), and in some families of the outgroup and of other

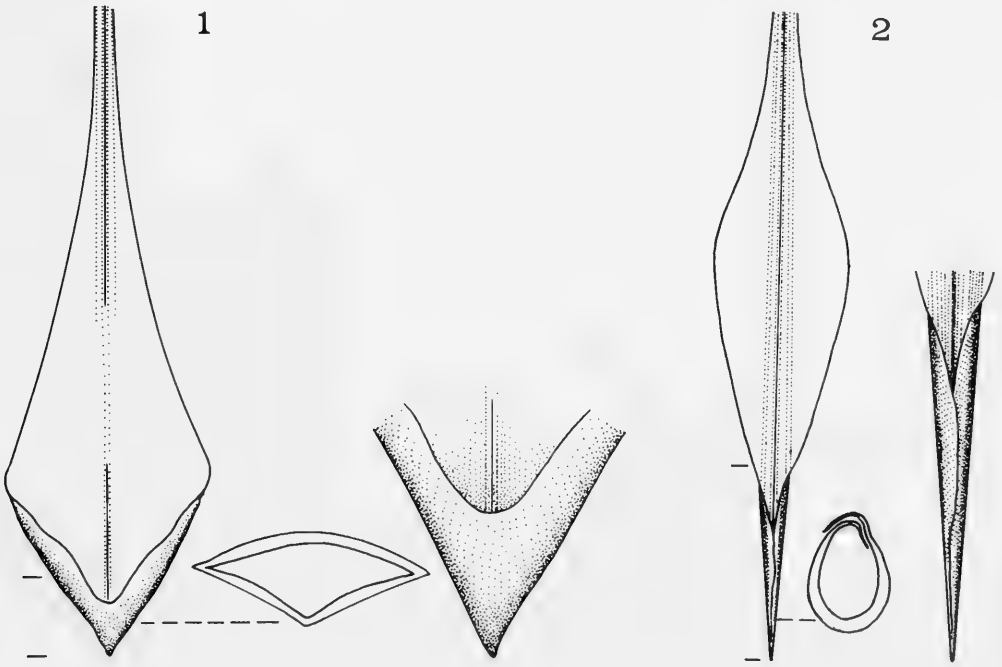

FIG. 3. Posterior end of gladius, ventral view, showing: (1) conus with enlarged cross-section and detail of *Sandalops* sp. B, WH 443-71 (ZMH), adult, 144 mm gladial length; (2) pseudoconus with enlarged cross-section and detail of *Teuthowenia megalops*, B 6 (NBS), adult, 254 mm gladial length (see Character 2).

oegopsids. In all observed cases, a fusion line is distinguishable. Among extant teuthoids, the conus portion of the gladius, presumably a vestige of the ancestral phragmacone, may be very small, or is sometimes found only in the young, or may be entirely absent (Naef, 1921/1923). All three conditions, in addition to the formation of a pseudoconus, are encountered among cranchiids.

Among members of the outgroup, a gladius with a narrow, usually elongate pseudoconus is typical of the Chiroteuthidae, Grimalditeuthidae, Mastigoteuthidae and Joubiniteuthidae. Specimens of three of the four nominal cycloteuthid species were examined; the gladius has what appears to be a true conus in one species, a pseudoconus in a second species and neither a conus nor a pseudoconus in a third species. In the little known Promachoteuthidae, a gladius with what appears to be a weakly-formed conus is found in an unnamed species (R. Toll, personal communication). Thysanoteuthids have a weakly formed conus in the young stages (R. Toll, personal communication) but lack both conus and pseudoconus in the adults. A pseudoconus is also found in three other oegopsid families that do not belong to the outgroup: Lepidoteuthidae, Brachioteuthidae and Batoteuthidae.

Even though a pseudoconus is the commoner structure in the outgroup, we believe that the presence, in larva and adult, of a small conus displaying no evidence of midventral fusion or convergence of the lateral margins of the vanes is primitive for cranchiids. We would support this judgment by the observation that, where both conus and pseudoconus are sequentially exhibited in the ontogeny of extant cranchiids, it is the conus that is invariably precedent and the pseudoconus that is developmentally subsequent. The absence of both conus and pseudoconus, a condition found only in *Bathothauma*, we judge to have been derived independently from the primitive state. This judgment is based on the unique modification of the posterior end of the gladius in *Bathothauma* in which the vanes are transformed into a transverse bar that gradually expands laterally to shovel-shaped ends on which the fins insert (Fig. 4.4). An elongation of the posterior end of the conus in *Leachia* and *Helicocranchia* serves to extend support for the

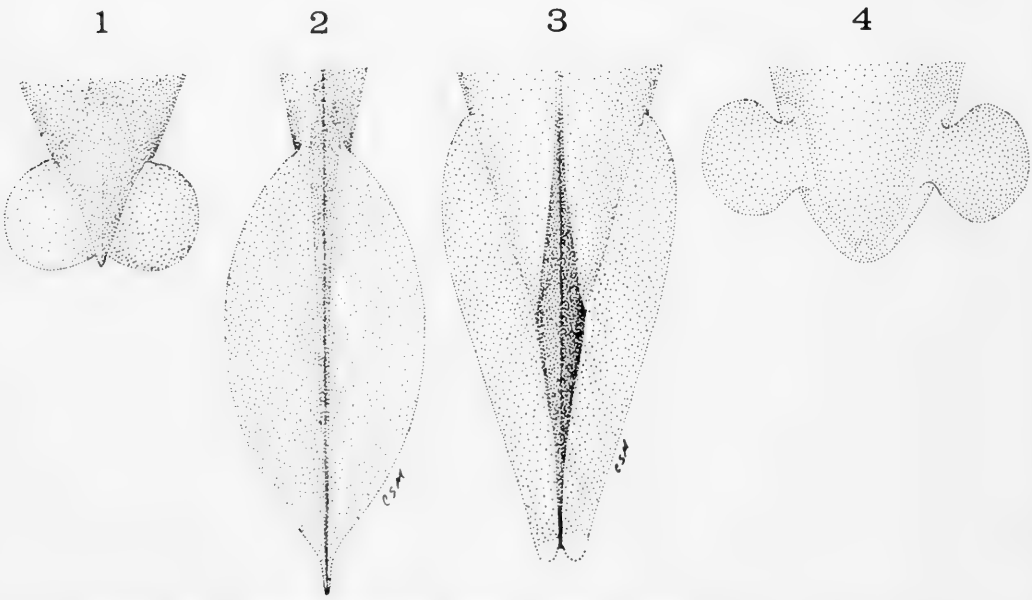

FIG. 4. Posterior end of mantle, dorsal view, showing variation in shape of fins: (1) *Sandalops* sp. C, Cl 71-6-26 (USNM), adult, 102 mm mantle length; (2) *Galiteuthis glacialis,* Elt 1323 (USNM), adult, 333 mm mantle length; (3) *Teuthowenia megalops,* B 17 (NBS), adult, 352 mm mantle length; (4) *Bathothauma lyromma,* O 4713 (UMML), subadult, 165 mm mantle length (1–3 from N. Voss, 1980) (see Character 3).

fins; it is a solid structure and does not appear to be homologous with the hollow pseudoconus.

Character 3. *Shape of fins:*

(a) elliptical, oval or circular, terminal;
(b) lanceolate or stout, ovate, terminal;
(c) lanceolate or long-narrow, terminal-lateral;
(d) small, paddle-shaped, subterminal.

Ontogenetically, the fins develop from the shell fold (Naef, 1921/1923). In the early larva of all cranchiids, the fins are small, separate and paddle-shaped. They then typically become longer and rounded with growth, later become contiguous, and finally elongate to varying degrees to accompany the elongation of the posterior end of the gladius.

While growth of the oegopsid fin is typically anterior, fin growth among cranchiids is typically posterior. The fins are terminal, elliptical, oval or circular in all genera in which the conus is present in the larvae (Fig. 4.1), with the exception of *Helicocranchia.* With ontogenetic disappearance of the conus, the fins are extended posteriorly on the developing pseudoconus and assume a generally lanceolate form. They remain terminal in *Taonius, Galiteuthis* and *Mesonychoteuthis* (Fig. 4.2), while in *Egea, Megalocranchia* and *Teuthowenia,* they simultaneously grow anteriorly on the mantle to become terminal-lateral (Fig. 4.3). The musculature of the fins is usually poorly developed except in *Mesonychoteuthis* in which the fins become stout and ovate in shape, and very muscular medially.

The form of the fins in the outgroup varies considerably. The typical shape is elliptical, oval or circular, with the marked exception of thysanoteuthids in which the fins are rhomboid. The fins may be subterminal, terminal, terminal-lateral or extended to nearly the full length of the body. The pseudoconus often projects beyond the posterior margins of the fins as a slender to needle-like tail of varying length; this structure may bear a supplementary, or auxiliary finlike structure.

On the basis of its ontogenetic precedence, the state of "elliptical, oval or circular, terminal" fins is considered to be primitive for extant adult cranchiids. The common developmental trend in the family toward posterior elongation of the fins with support afforded by a lengthening pseudoconus, together with the subsequent occurrence (in three genera) of anterior growth to form terminal-lateral fins, is interpreted to reflect the evolutionary sequence of appearance of these conditions in cranchiid phylogeny. The retention, into adulthood, of the larval state of small, paddle-shaped fins in *Helicocranchia* and *Bathothauma* (Fig. 4.4) is interpreted to represent a neotenous condition independently derived from the primitive state.

Character 4. *Funnel-head fusion:*

(a) funnel not fused to head laterally;
(b) funnel fused to head laterally.

In the Cranchiidae, lateral fusion of the funnel to the head occurs only in *Cranchia, Liocranchia* and *Leachia.* The funnel is free laterally in all members of the Taoniinae. Among other teuthoids, additional instances of the fused state are found in the Bathyteuthidae and in the sole member of the Joubiniteuthidae; the Ommastrephidae, and some members of the Chiroteuthidae and Mastigoteuthidae also display varying degrees of lateral fusion. In the majority of teuthoids, however, including members of the remaining four outgroup families, the funnel is free laterally. The free, unfused condition is tentatively interpreted as primitive for the family Cranchiidae, and the fused condition as derived.

Though the functional significance of the varying degrees of lateral fusion is not known, it presumably relates to the role that the funnel plays in locomotion in the different groups.

Character 5. *Funnel valve:*

(a) present;
(b) absent.

A valve with a free anterior margin is found on the inner, dorsal surface of the anterior part of the funnel in all Cephalopoda except for the Octopoda and some genera of the Teuthoidea: *Valbyteuthis* (Chiroteuthidae) and nine of the thirteen cranchiid genera. Among cranchiids, a funnel valve is found only in *Cranchia, Liocranchia, Egea* and *Megalocranchia.* Considering its near universal occurrence among all other oegopsids, it is inferred that a funnel valve was likely found in the most recent common ancestor of the extant cranchiids and, therefore, would best be considered primitive for the family.

Though it is commonly believed (Naef, 1921/1923) that the valve functions to prevent water from entering the funnel when the mantle is being expanded, Zuev (1967) as-

sociated the absence of a valve with the loss of the ability to swim headfirst (forward movement).

Character 6. *Ocular photophores:*

(a) unknown, extinct;
(b) four or more, small, simple photophores;
(c) one, large, complex photophore;
(d) one large plus one small, contiguous, complex photophore;
(e) one large plus one small, non-contiguous, complex photophore;
(f) one large plus two small, non-contiguous complex photophores.

Ocular photophores, found also in many other families of teuthoids, occur in all members of the Cranchiidae (Fig. 5). Three changes have here been made in the character state coding employed by N. Voss (1980: Table 1). Firstly, newly-acquired taoniin specimens show that the first small, non-contiguous photophore grades from "short" to "long-narrow," without the distinct break that was formerly thought to occur in the group. As a result, the states originally described as "one large plus one small, short non-contiguous photophore" and "one large plus one long, narrow non-contiguous photophore" have been united to read "one large plus one small, non-contiguous, complex photophore." Secondly, an additinal new state "one large plus two small, non-contiguous, complex photophores" is coded for the unique condition exhibited by *Teuthowenia* (described in a footnote in the original table). Thirdly, more detailed study of photophore morphology has resulted in the insertion of "simple" and "complex" to express important differences subsequently observed.

Between the Cranchiinae and the Taoniinae, there are differences in the appearance, structural morphology, photogenic material and ontogeny of the ocular photophores. In the Cranchiinae, the organs are small, round to oval in shape, and relatively simple in structure (Fig. 5.5), comprised of apparently ectodermal invaginations that retain their connections with the ectodermal epithelium (Chun, 1910); consequently, the cup of photogenic tissue has direct contact to the exterior. By contrast, taoniin photophores are markedly dissimilar in size, one of them is usually crescent- or sickle-shaped, and all are more complex in structure than the corresponding organs among cranchiins. The photogenic tissue in taoniins is embedded below the surface of the photophore in a narrow band along one margin, with the emitted light spread over the wide surface of the organ by means of a thick layer of light guides (Dilly & Herring, 1974; Dilly & Nixon, 1976; Herring, 1977). Studying the ocular photophore in *Bathothauma,* Dilly & Herring (1974) found that the photogenic tissue contained paracrystalline material. Herring (1977) later reported the same material in the ocular organs of *Egea* and *Megalocranchia* (correct generic identifications for Herring's *Phasmatopsis lucifer* and *P. oceanica* respectively) and considered that it probably occurs in all taoniins, in contrast to the cranchiins in which it does not occur.

Larvae of the majority of cranchiid species, including representatives of every genus, were examined. In all members of the Cranchiinae, the ocular photophores first appear as separate organs in their approximate final adult position. They make their appearance in the developing young in groups or singly over varying periods of time until the definitive adult pattern is attained. This is not the case in the Taoniinae. In all of the taoniins, a single, poorly-defined patch first appears on the narrow, posteroventral end of the oval, stalked eye of the larva. With growth, the photophore becomes better defined and enlarges to conform approximately to the ventral surface of the eye. This is the only photophore that develops in *Helicocranchia* and *Bathothauma* (Fig. 5.1), but in the other genera (Fig. 5.2–5.3) a second and, in *Teuthowenia* (Fig. 5.4), a third small organ forms as the eye enlarges and gradually becomes sessile and near-hemispherical in shape. In the larvae of *Taonius, Galiteuthis, Mesonychoteuthis* and *Teuthowenia* (Fig. 5.6–5.9), the initial photophore patch extends from the undersurface to along the edge of the narrow, posteroventral end of the eye. Along this edge a thickening and a break occur in the patch to form the second organ which then gradually separates and assumes the final position. The third organ in *Teuthowenia* splits off from the inner end of the second organ as it, in turn, separates from the first.

In *Sandalops, Liguriella, Egea* and *Megalocranchia,* the narrow, posteroventral end of the oval larval eye is extended by a pronounced cone-shaped rostrum, or ocular appendage, that J. Young (1970) found (in *Bathothauma*) to be filled with loose connec-

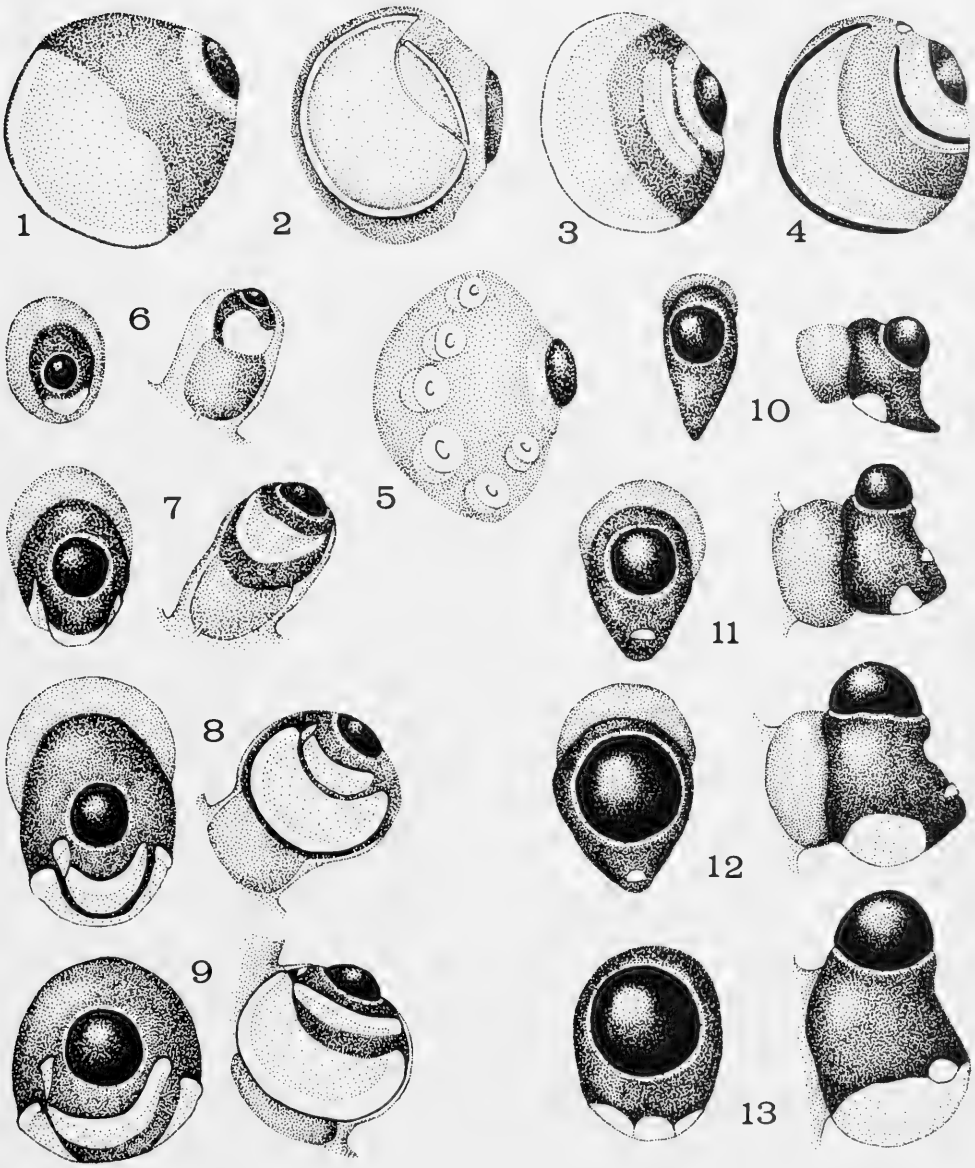

FIG. 5. Eye, left, showing variation in shape of ocular photophores: (1) *Bathothauma lyromma,* O 4713 (UMML), subadult, 165 mm mantle length; (2) *Liguriella podophtalma,* WH 417-I-71 (ZMH), subadult, 243 mm mantle length; (3) *Galiteuthis glacialis,* Elt 1323 (USNM), adult, 333 mm mantle length; (4) *Teuthowenia* sp. B, WH 417-71 (ZMH), subadult, 154 mm mantle length; (5) *Leachia atlantica,* IOM 1880, adult, 105 mm mantle length. (6–9) *Teuthowenia* sp. B, ontogenetic series showing development of ocular photophores, anterolateral and ventral views of left eye: (6) Elt 1776 (USNM), 7 mm mantle length; (7) Elt 2270 (USNM), 27 mm mantle length; (8) DMNZ, 60 mm mantle length; (9) SAM A31421, 81 mm mantle length. (10–13) *Sandalops* sp. C, ontogenetic series showing development of ocular photophores, anterior and ventrolateral views of left eye: (10) F II (USNM), 27 mm mantle length; (11) Elt 31-24A (USNM), 26 mm mantle length; (12) F IV (USNM), 33 mm mantle length; (13) F IV (USNM), 38 mm mantle length (2 from Voss, 1980) (see Character 6).

tive tissue. R. Young (1975b) described a somewhat different development of the photophores in *Sandalops* (Fig. 5.10–5.13). The first, large organ appeared as a patch on the underside of the rostrum, but did not extend to the edge of the apex. With growth, the rostrum progressively shortened until only a remnant remained, on top of which a second ocular photophore appeared. With the disappearance of the rostrum, the second organ assumed a contiguous position with the first. An examination of the larvae of *Liguriella, Egea* and *Megalocranchia* demonstrated a similar type of development, but in the latter two genera, the second organ subsequently separates from the first as the eye becomes sessile, while in *Liguriella* and *Sandalops* the two photophores remain contiguous. N. Voss (1974) did not have an adequate series of larvae with intact eyes to show the details of development of the ocular photophores in *Egea*. It appears that the separate development of the two photophores in the above four taoniin genera is a result of the penetration and subsequent division of the tissue of the photophore patch at an early stage by the development of a pronounced ocular rostrum.

The majority of the ocular photophores found among the other teuthoids are of the complex type, with the surface layer of light guides diffusing the light from a photogenic core, similar to that found in the taoniins (Herring, 1977). This is supported by our investigations. In the outgroup, ocular photophores are found in the Chiroteuthidae (majority of species), Mastigoteuthidae (one out of numerous species) and the Cycloteuthidae (two out of four nominal species). They are not found in the Promachoteuthidae. In Grimalditeuthidae, Joubiniteuthidae, the juvenile state of Thysanoteuthidae and a few members of the Chiroteuthidae, there is a broad, usually thick, highly reflective, gold band surrounding the lens and often extending around the ventral surface of the eye. Whether this band contains luminous tissue in any of the groups was not determined. In the Chiroteuthidae, ocular photophores may occur as long bands, small round organs arranged in rows, or bands of what appear to be incompletely separated round organs. The situation suggested to Herring (1977) that the separate round organs coalesce to form the long bands. Instead, the opposite may occur as in the taoniins, where several small organs are derived, at least ontogenetically, from a single large one. Naef (1921/1923) suggested that the primitive form for ocular photophores in cephalopods "may be a diffuse luminescence of the whole skin of the eyeball."

The marked differences in the structure and ontogeny of the ocular photophores between the cranchiins and the taoniins suggest separate lines of development. A common ancestral state cannot be confidently identified from among the conditions exhibited by extant cranchiids, and is therefore presumed to be extinct. The ontogenetic findings reported here suggest the existence of a single evolutionary trend towards photophore fragmentation in the Taoniinae; the character state "one large, complex photophore" is therefore judged to be the most primitive for this morphocline.

The ocular photophores of cranchiids appear to function, at least in part, as a ventral camouflage mechanism of advantage to the animal in avoiding predators (R. Young, 1975b).

Character 7. *Hectocotylus:*

(a) present;
(b) absent.

One or more arms of the males of many cephalopods are modified for courtship and copulation. The modification, commonly termed hectocotylization, may be symmetrical, equally affecting both arms of a pair, or asymmetrical, affecting only one arm or a pair of arms unequally. There is great diversity in the modification. It may involve the whole arm or only part of the arm and affect any or all of its features—suckers, sucker pedestals, protective membranes, general surface and overall shape and size.

The word "hectocotylus" was originally used for the autotomous third (right or left) arm found in certain families of pelagic incirrate octopods—Tremoctopodidae, Ocythoidae, Argonautidae and Alloposidae. The arm is used for insemination. During mating it detaches from the male and remains within the mantle cavity of the female, carrying with it the spermatophore of the male. In most other incirrate octopods, a lesser modification for insemination is found in which the terminal portion of the same third (right or left) arm is transformed into a discrete organ, called a ligula, which remains attached. This organ is not found in cirrate octopods (G. Voss, personal communication). Steenstrup (1857) considered that the autotomous structure found in *Tremoctopus* and the other pelagic

incirrate octopods mentioned above is but an elaborate modification of the sessile structure found in most of the remaining incirrates. This is supported by the ontogeny of the structure in *Tremoctopus* described by Thomas (1977). Instances of lesser symmetrical modification, such as enlarged suckers, are scattered throughout the octopods.

The Sepioidea and Teuthoidea display a wider diversity of both asymmetrical and symmetrical modifications of the male arms. The arms most strongly affected in these two groups are the first and fourth pairs, and, when the modification is asymmetrical, it always involves one of these pairs. In the Sepioidea, the modification is primarily asymmetrical and is found in most of the member families. In the Teuthoidea, asymmetrical modification occurs in both families of myopsids but is only known to occur in six of the twenty-three families of oegopsids—Enoploteuthidae, Lycoteuthidae, Architeuthidae, Ommastrephidae, Thysanoteuthidae and Cranchiidae. Various types of symmetrical modification of one or more arm pairs frequently occurs. The nature of the symmetrical modifications suggests a holding and caressing function. The asymmetrical modification occurs earlier in the ontogeny of the animal than do the symmetrical modifications which occur at varying later periods, some appearing just prior to maturity.

The word "hectocotylus" is commonly used for the single asymmetrically modified arm in the sepioids and teuthoids, which is known in some (and presumed in the remainder) to be used to transfer the spermatophores to the female either by directly grasping the spermatophores or by acting as a bridge. Robson (1926) doubted that the modified arm was used in the same way in the octopods as it is in the sepioids and teuthoids, and suggested that the so-called hectocotylus of the latter two orders be termed the nuptial arm. In the sepioids and teuthoids, there is no structure formed that may be termed an "organ" that is common to all members similar to that found in the octopods. Indeed, in some groups many of the details of the asymmetrically modified arm are so bizarre that it is difficult to imagine their function in handling spermatophores, and some appear to have developed for holding or tactile purposes, perhaps giving the arm a dual purpose. This is not inconsistent with the observed use of the modified arm in *Octopus* (Robson, 1926). In sepioids and teuthoids it is difficult to observe the exact use of the arm because copulation occurs so rapidly. Spermatophores, however, have been observed on the modified arm during courtship in *Sepioteuthis* (Arnold, 1965), and transferred to the female by the modified arm during copulation in several species of *Loligo* (Drew, 1911; McGowan, 1954; Hamabe & Shimizu, 1957; Arnold, 1962). Thus it appears that the primary function of the asymmetrically modified arm of the male in these two orders is similar to that in octopods and therefore can be correctly called the hectocotylus, and asymmetrical modification of both arms of a pair can be referred to as hectocotylization. The occurrence and diversity of structure of the hectocotylus in the sepioids and teuthoids suggests that it is polyphyletically derived (Naef, 1921/1923). N. Voss (1980) called the symmetrical modification of arm pairs or all of the arms of the male "secondary sexual modification" to distinguish their known or presumed use of holding or caressing from the primary use of the hectocotylus.

The occurrence, position and general form of the hectocotylus are generally correlated with taxon membership (Steenstrup, 1857), and are usually constant within families. In the females of a number of groups, there are different structures for the reception of the spermatophores, sperm reservoirs or sperm that correspond to the particular arrangement of the hectocotylus and method of transfer of the spermatophores (Hoyle, 1907). On the grounds of its usually constant occurrence within a family, we have judged the presence of a hectocotylus to be primitive, and its absence to be derived in the Cranchiidae. In the cranchiids, a hectocotylus is only found in the three genera of the Cranchiinae (N. Voss, 1980; Figs. 1b, 2c, 3b). It occurs on the fourth (right or left) arm and is similar in appearance in all species. There is no special structure in the females of either subfamily for the reception of the spermatophores. Throughout the family, spermatophores appear to be transferred directly to the exterior dorsal surface of the mantle; sperm reservoirs have been found embedded in the mantle walls (occasionally in head and arms) and in various stages of emergence into the mantle cavity of mature females in *Liocranchia, Leachia, Helicocranchia, Bathothauma, Sandalops, Galiteuthis, Megalocranchia* and *Teuthowenia* (N. Voss, unpublished notes).

The symmetrical or secondary modifications of the arms of the males, which are compared in Table 2 of N. Voss (1980), are too

variable for use in this study. The modifications, however, are usually similar within a genus. They are more numerous in occurrence and variable in form in the Taoniinae than in the Cranchiinae.

Character 8. *Brachial end-organs:*

(a) absent;
(b) present.

The brachial end-organ is a leaf or spoon-shaped organ found on the distal ends of arm pairs in near-mature and mature females of some cranchiid genera (N. Voss, 1980; Fig. 3e). It occurs in all species of the Cranchiinae, and in all species of the taoniin genera *Egea, Megalocranchia* and *Teuthowenia.* Typically the end-organ appears when the female squid nears maturity and has descended into the deeper waters; at that time, the trabeculate protective membrane on both sides of the affected arms expands and becomes darkly pigmented. This process is accompanied by a reduction and eventual loss of the suckers, and the oral surface of the affected portion usually becomes rugose or spongy; the pedestals of the affected suckers may be lost or greatly modified. The end-organ varies in proportional size and extent of occurrence on the arms in the different species. Among the cranchiins, the organ occurs only on arms III in *Liocranchia* and in all of the species of *Leachia,* except for *L. danae*, and occurs on all of the arms in *L. danae* and in *Cranchia*; among the taoniins, it appears on arms III in *Egea* and in some species of *Megalocranchia* (rarely on arms II), on arms I, II and III in the remaining species of *Megalocranchia,* and on all of the arms in *Teuthowenia.* The organ ranges in size from about 5 to 30% of the arm length, and is approximately the same size on the different arms in a species, except in *Cranchia* where it is markedly disproportionately developed. It tends to be proportionally larger in the cranchiins than in the taoniins.

The brachial end-organ is reported to occur only in the Cranchiidae; the collections of the U.S. National Museum, however, contain two large teuthoids, a male and a female as yet unidentified to family, which both display long, similar-appearing organs on the ends of arms IV. In these specimens (kindly shown us by C. F. E. Roper), only the dorsal protective membrane of the arm is modified to form the organ, not the entire oral surface as among cranchiids. The mature stage is not known for many members of the outgroup, and it is possible that some will eventually be found to have brachial end-organs. At this time, however, the presence of these peculiar structures seems best regarded as a derived condition for extant cranchiids since its absence is conspicuously more widespread among other teuthoids.

The brachial end-organ appears to be a photophore of unique structure that probably functions as a sexual attractant (R. Young, 1975a).

Character 9. *Clubs*:

(a) without hooks;
(b) with hooklike teeth on large suckers;
(c) with hooks.

Hooks are found on the tentacular clubs in only four of the twenty-five families of teuthoids—the Cranchiidae, and three other families that are not presently thought to share recent common ancestry with cranchiids, Gonatidae, Enoploteuthidae and Onychoteuthidae. Hooks are not present on the clubs in any of the members of the outgroup. Among gonatids, hooks occur on the clubs in only one of the two genera where the adult morphologies of the clubs are known. In the large family Enoploteuthidae, hooks are found on the clubs of all species except for those of the genus *Pterygioteuthis*. In all onychoteuthid species, the clubs, where known, bear hooks. Of the thirteen genera belonging to the Cranchiidae, only two, *Galiteuthis* and *Mesonychoteuthis,* have hook-bearing clubs.

In all of the families in which they occur, the hooks are absent in the larvae and first appear in the early or midjuvenile stages. They are formed from typical suckers (Fig. 6.1) of the median one or two rows on the manus, and their appearance is often accompanied by a reduction or loss of the suckers of the marginal rows. The hooks develop by gradual enlargement of a median tooth on the distal margin of the sucker ring (Figs. 6.3–6.8). As the median tooth enlarges, incorporating the lateral teeth, the ring aperture is greatly reduced; in the process, the outer margin of the sucker is transformed into a hood for the hook. Among cranchiids, an intermediate stage between sucker and hook is found in the members of the genus *Taonius* (Fig. 6.2); in the postlarval animal, the suckers of the two median rows of the manus elongate and become greaty enlarged, with the distal margin of the sucker ring drawn out into one or two large, central, hooklike teeth. The aperture of the ring, however, is not reduced, and the

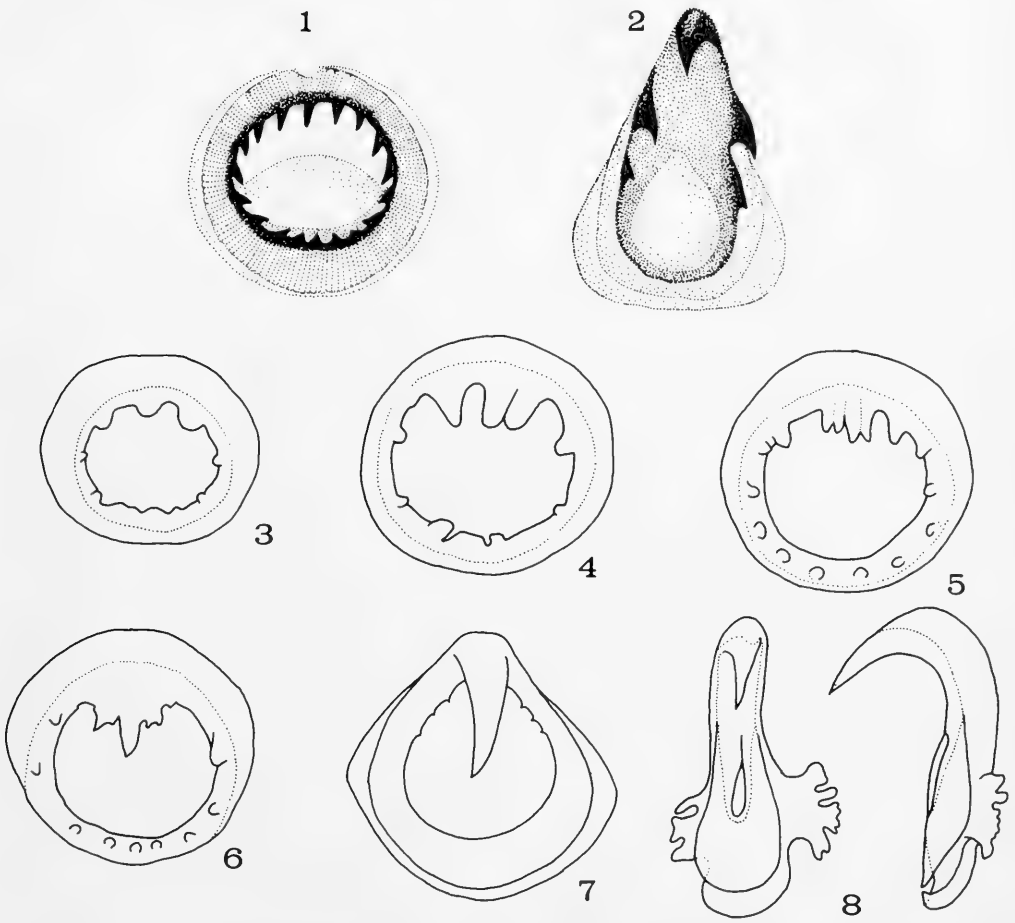

FIG. 6. Largest sucker from tentacular club of: (1) *Teuthowenia megalops,* WH 712-73 (ZMH), subadult, 187 mm mantle length; (2) *Taonius pavo,* O 4812 (UMML), adult, 540 mm mantle length. (3–8) *Galiteuthis glacialis*, ontogenetic series showing modification of ring from largest tentacular sucker to form hook: (3) Elt 697 (USNM), 29 mm mantle length; (4) SC 24–62 (USNM), 38 mm mantle length; (5) Elt 935 (USNM), 55 mm mantle length; (6) Elt 949, (USNM), 58 mm mantle length; (7) Elt 943 (USNM), 73 mm mantle length; (8) Elt H371 (USNM), 297 mm mantle length (3–8 redrawn from McSweeny, 1978) (see Character 9).

structure presumably can still function as a sucker in these species.

As the morphological sequence from sucker, to hooklike sucker, to hook appears to reflect increasing functional specialization, so also do the clubs on which the different structures are found; there is a progressive definition of a carpal sucker cluster, reduction of the suckers of the marginal rows of the manus, and reduction of the dactylus and the dorsal keel; the end result of this transformation series is a simpler and more efficient club for capturing and holding soft-bodied animals (Naef, 1921/1923). Ontogenetic evidence and out-group comparisons combine to suggest that the ancestral state for the cranchiids is the club without hooks (i.e., solely with typical suckers); clubs with hooklike teeth on the suckers, as in *Taonius,* and clubs with well developed hooks, as in *Galiteuthis* and *Mesonychoteuthis,* appear to be successively derived conditions.

Character 10. *Digestive gland*:

(a) stout, spindle-shaped;
(b) elongate, spindle-shaped;
(c) rounded, with a large photophore.

The digestive gland in oegopsids is usually stout and spindle- or ovoid-shaped, and lies at

an acute angle to, or parallel with, the longitudinal axis of the body. In the Cranchiidae, the digestive gland is spindle-shaped in all genera with the exception of the later growth stages of *Megalocranchia* species, and is suspended at a right angle to the longitudinal body axis. From dissections and from the literature it appears that this unusual position of the digestive gland, while common in oegopsid larvae, is found in the adults of only two other teuthoid families, both members of the outgroup—the Grimalditeuthidae, and in some species of the Chiroteuthidae. The elongation of the spindle shape of the gland, as found in the young and subadult of *Liguriella* (adult unknown) and in all growth stages of *Bathothauma* and *Sandalops*, appears derived from the stout, spindle shape that we hypothesize to be the primitive state for the cranchiids.

A large, rounded digestive gland with an associated compound photophore overlying the ink sac characterizes all members of the genus *Megalocranchia.* In the larva of *Megalocranchia,* the gland is typically stout and spindle-shaped, with the photophore first appearing in the late larva. With growth, the gland gradually becomes rounded and the photophore proportionally enlarges to cover the entire ventral surface. In the outgroup, a photophore is also found on the ink sac in a number of species of the Chiroteuthidae and in two of the four nominal species of the Cycloteuthidae. Nevertheless, the ontogenetic derivation of the condition found in *Megalocranchia* from the commoner photophore-less condition of the digestive gland seen among all other cranchiids would appear to argue that the presence of a photophore on the gland is an independently derived condition.

R. Young (1975b, 1977) suggests that the spindle shape and vertical orientation of the opaque digestive gland, by reducing the ventral countershading problem of the animal, and the photophore on the large, rounded digestive gland in *Megalocranchia,* by its countershading luminescence, are devices for ventral camouflage.

Character 11. *Digestive duct appendages*:

(a) on ducts;
(b) on ducts and digestive gland;
(c) on digestive gland.

From the researches of Bidder (1966, 1976) and Schipp & von Boletzky (1975, 1976), among others, it appears that the structure and function of the digestive duct appendages, which are formed from the digestive ducts, differ between the Octopoda, Sepioidea and Teuthoidea, and can be related to the different position of the organ in each group. In octopods, the appendages are found on the posteroventral surface of the digestive gland and lie within its connective tissue envelope, while in the sepioids and teuthoids, the appendages are found outside of the envelope of the digestive gland and are covered by renal epithelium. Among sepioids, the digestive duct appendages always occur as grapelike follicles on the digestive ducts and are in close topical relationship with "renal" epithelium; by contrast, the position and gross morphology of the appendages are variable in the teuthoids, often within families and sometimes within genera. Greater variation is found among cranchiids than among members of the outgroup.

In the Cranchiidae, digestive duct appendages may occur on the ducts, on the ducts and digestive gland, or on the digestive gland alone. The state "on the ducts" is a correction of the state mistakenly described in Table 1 of N. Voss (1980) as "on posterior end of ducts or on caecum." The generic definitions given in the text and a re-examination of the specimens support this correction. The appendages appear in the form of two large, compound lobes on the posterior portion of the united duct in *Leachia,* and in the form of small clusters of follicles on the posterior portion of the separate ducts in *Megalocranchia.* In *Taonius, Egea, Teuthowenia* and two species of *Galiteuthis* the appendages are in the form of two large, compound lobes on the posterodorsal surface of the digestive gland at the exit of the digestive ducts and in the form of small clusters of follicles on the entire length of the ducts. In the remaining seven genera, *Cranchia, Liocranchia, Helicocranchia, Bathothauma, Sandalops, Liguriella, Mesonychoteuthis* and four species of *Galiteuthis,* the appendages occur as two large, compound lobes in the same position on the digestive gland as in the preceding group.

In the outgroup, the appendages appear as small clusters of follicles on the entire length of the ducts in Thysanoteuthidae, Cycloteuthidae (one species), Mastigoteuthidae (two species), Promachoteuthidae and Joubiniteuthidae. They appear as a thick coating of spongy tissue on the major or entire length of

the ducts in Chiroteuthidae (one species), Grimalditeuthidae and Cycloteuthidae (one species). In the remaining five species of chiroteuthids and five species of mastigoteuthids examined, the appendages occur as medium to large, compound lobes on portions of the ducts. Thus, it would appear that the position of the digestive duct appendages on the ducts is most parsimoniously regarded as ancestral for extant cranchiids, and that the presence of these appendages on the digestive gland is likely a derived condition.

The investigations of Schipp & von Boletzky (1975, 1976) suggest that the digestive duct appendages in the sepioids play a role in excretion and nutrient absorption as well as osmoregulation and urine formation. Our present lack of knowledge of the fine structure of the appendages in the teuthoids, however, precludes meaningful speculation on the functional significance of the differences in their position and gross morphology.

Character 12. *Caecum*:

(a) smaller than stomach;
(b) larger than stomach.

The relative size of the caecum and the stomach varies within the oegopsids. In the Cranchiidae, the caecum is larger than the stomach in the three genera of the Cranchiinae and is smaller than the stomach in the ten genera of the Taoniinae. An examination of as many members as possible of the outgroup revealed that the caecum is larger than the stomach in the Cycloteuthidae and the Promachoteuthidae, larger than or approximately the same size as the stomach in the Mastigoteuthidae, and is smaller than the stomach in the Thysanoteuthidae, Chiroteuthidae, Grimalditeuthidae and Joubiniteuthidae. Considering that the caecum is smaller than the stomach in the majority of the outgroup members, we are inclined to regard that state as primitive for the extant cranchiids.

The functional significance of the relative size differences of the caecum and the stomach is not known but might reflect differences in feeding habits (see Bidder, 1966).

Character 13. *Eyes of larvae*:

(a) sessile;
(b) stalked.

Stalked eyes are found in the larvae of all cranchiids with the exception of *Cranchia* and *Liocranchia*. The length of the larval eye stalks and the period of their persistence varies considerably among the ontogenies of the different genera. Though markedly protruding eyes are found in the larvae of some teuthoids, for example, in the Octopodoteuthidae, Thysanoteuthidae and at least one of the four nominal species of Cycloteuthidae, there is no known occurrence of stalked eyes in cephalopod larvae outside of the Cranchiidae. The absence of stalked eyes in the larvae of all other known cephalopods (and of two genera of the Cranchiidae, and the variability of the character within the remaining members of the family) would indicate that sessile eyes may be considered primitive for cranchiids. N. Voss (1980), however, referred to the loss of the character of stalked eyes in *Cranchia* and *Liocranchia*. The present, broader analysis of this character, suggests that the contrary is true, i.e. that sessile eyes are retained in these two genera as an unmodified ancestral state and that the presence of stalked eyes in the remaining cranchiid genera is likely derived.

Clarke *et al.* (1979) support the suggestion made by J. Young (1970) that the eye stalks of cranchiids may contain ammonium to provide buoyancy, but eye stalks may be of additional advantage to the larva by providing greater mobility to the eyes, thereby affording broader vision (J. Young, 1970; R. Young, 1975b; Weihs & Moser, 1981). The loss of the eye stalks with growth can be related to the vertical distribution of the animal (R. Young, 1975a,b). The length of time that the larvae spend in the shallower waters appears to correspond with the varying persistence of eye stalks in the different species, but does not necessarily correspond with the degree of development of the stalks.

Character 14. *Dorsal pad of funnel organ*:

(a) one median papilla plus two lateral flaps;
(b) one median flap plus two lateral flaps;
(c) two lateral flaps;
(d) one median papilla plus two to six markedly flattened, lateral papillae;
(e) one median papilla plus two round or elliptical, lateral papillae;
(f) two lateral papillae.

The funnel organ, comprised of one or more pads of mucus-secreting epithelium, is found on the inner surface of the funnel in all cephalopods. Usually located in the middle

part of the funnel, the organ is sometimes confined to the dorsal surface, as in nautiloids, some octopods, and *Vampyroteuthis,* or may be found on both the dorsal and ventral surfaces, as in the majority of cephalopods. In octopods, the organ is generally W-shaped, but the lateralmost of the vertical bars are sometimes separate, or the organ may take the form of two modified V-shaped pads. In sepioids and teuthoids, the funnel organ is typically three parted—an inverted V- or U-shaped dorsal pad and two paired, usually oval and elliptical-shaped, ventral pads. Variations in the size, outline and surface sculpture of these two basic forms of pads is considerable, especially among oegopsids. Variation is greater in some families than in others, and is displayed to the highest degree among cranchiids.

The sculpture of the dorsal member of the funnel organ has received the most taxonomic attention. In the Cranchiidae, the dorsal pad always has sculpture on the lateral arms. The pad may exhibit one median papilla plus two lateral flaps (*Sandalops*), one median flap plus two lateral flaps (*Cranchia* and *Liocranchia*), two lateral flaps (*Egea* and *Megalocranchia*), one median papilla plus two to six markedly flattened, lateral papillae (*Leachia*), one median papilla plus two round or elliptical, lateral papillae (*Helicocranchia, Liguriella, Taonius, Galiteuthis, Mesonychoteuthis* and *Teuthowenia*), or two lateral papillae (*Bathothauma*) (Fig. 7).

The lateral flaps that occur in *Sandalops, Cranchia, Liocranchia, Egea* and *Megalocranchia* are all longitudinally (i.e. anteroposteriorly) oriented. In *Leachia,* the flattened, lateral papillae are also longitudinally oriented, and when the lateral papillae are multiple on a side, they form along a single anteroposterior line and are sometimes connected by a basal ridge, all suggesting that the papillae have developed from a longitudinal flap. There is a trend in *Leachia* toward multiple lateral papillae; the number of papillae may vary within a species or an individual, where sometimes there is a single papilla on one side and two on the other. In the majority of the members of the outgrop, the dorsal pad is unsculptured except for a median papilla. Several members have a longitudinal ridge,

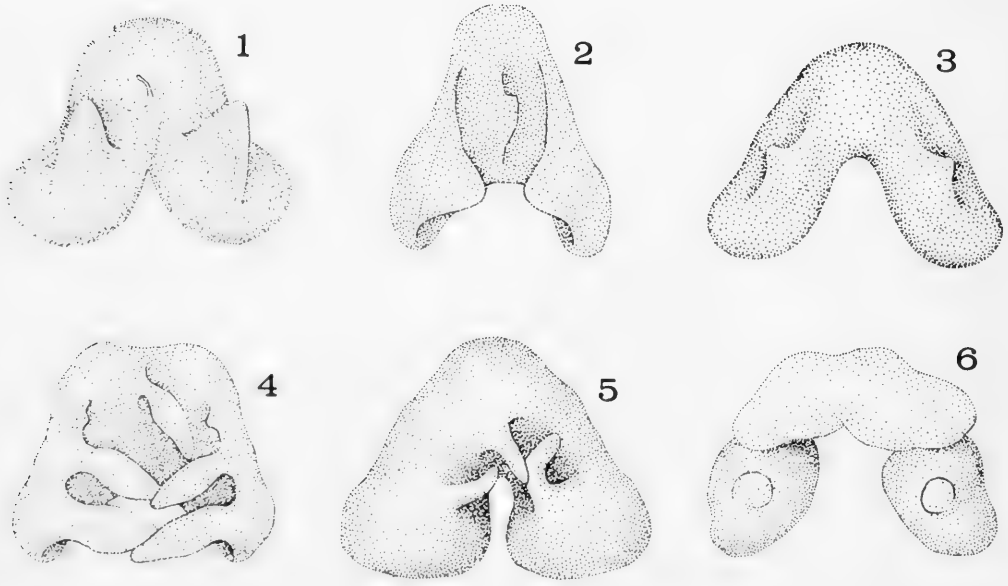

FIG. 7. Dorsal pad of funnel organ: (1) *Sandalops* sp. C, CI 71-6-26 (USNM), adult, 102 mm mantle length; (2) *Cranchia scabra,* WH 439-II-71 (ZMH), subadult, 122 mm mantle length; (3) *Egea inermis,* WH 471-II-71 (ZMH), subadult, 207 mm mantle length; (4) *Leachia danae,* MV 65-I-53 (SIO), subadult, 167 mm mantle length; (5) *Galiteuthis glacialis,* Elt 1323 (USNM), subadult, 333 mm mantle length; (6) *Bathothauma* sp. D, C 108662 (AM), subadult, 187 mm mantle length (1, 3 from N. Voss, 1980; 5 redrawn from McSweeny, 1978) (see Character 14).

developed to various extents, either on the lateral or median sections of the pad or on both.

The occurrence of a median papilla in the majority of the cranchiids, and of a longitudinally oriented flap, or its apparent modifications, on each lateral arm in nearly half of the family, is similar to the occurrence in the outgroup of a median papilla in most of the members, and of a longitudinal ridge on the lateral arm when lateral sculpture is present. This suggests that a dorsal pad with one median papilla and two lateral flaps as found now only in *Sandalops*, might be considered primitive to the cranchiids. The remaining character states appear to be independently derived except for "(f) two lateral papillae," which is hypothesized to have been derived from "(e) one median papilla plus two round or elliptical, lateral papillae." Nesis (1974), in his analysis of the sculpture of the dorsal pad in the Taoniinae, concluded that the state "one median papilla and two lateral papillae" was basic to the subfamily and that the flaps were derived. His conclusions resulted from analysis of the distribution of character states only in the taoniins, however; he did not study the family as a whole nor compare the character as it occurs in other families considered to be allied. Our conclusions appear supported by a broader comparative approach.

Though the function of the funnel organ and the significance of its variations are not known, it has been considered that the mucus produced is used for keeping the funnel and perhaps mantle cavity clean of debris. An alternate or additional function is suggested by the observations of Hall (1956), Nicol (1964) and M. R. Clarke (as reported by Dilly & Nixon, 1976) that the mucus might serve as a carrier for the ink produced by the ink sac and expelled through the funnel by the animal when irritated.

2. A Phylogenetic Hypothesis

Application of the Wagner method to the binary data matrix of Table 2 yields the reconstruction of cranchiid evolutionary history illustrated in Fig. 8. In Fig. 8, internal nodes (branching points) represent hypothetical ancestors and are labelled with capital letters; external nodes (branch tips) represent extant cranchiids and are labelled with the first three

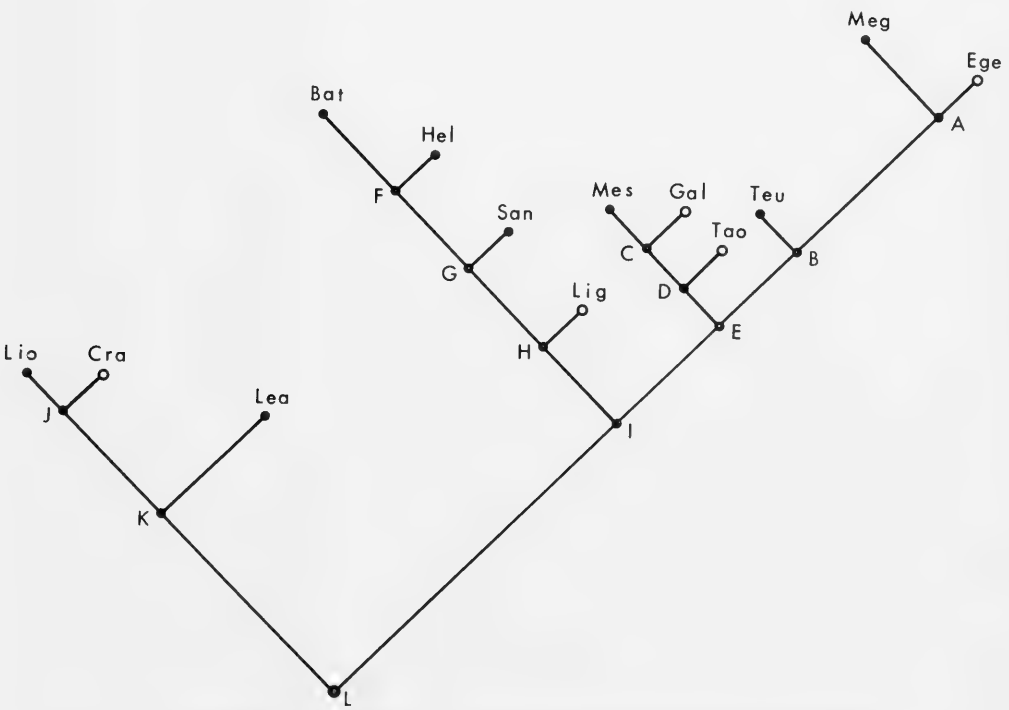

FIG. 8. Wagner reconstruction of cranchiid phylogeny. See text for explanation.

letters of the generic name; lines connecting the nodes represent phyletic lineages and are drawn proportional to the estimated amounts of morphological evolution (number of character state transitions) that separate extant cranchiids from their hypothetical ancestors or hypothetical ancestors from one another. Some extant cranchiids are indistinguishable from their most recent shared ancestors *with respect to the characters employed in this study*; the external nodes representing these forms (e.g., *Liguriella*) are drawn as open circles and have been removed an arbitrary one branch length unit from their most recent ancestors. Phenotypes of hypothetical ancestors are provided in Table 3.

The Wagner Tree hypothesizes a basal separation of the Cranchiidae into two phyletic lineages that correspond in membership to the traditional subfamilies Cranchiinae and Taoniinae, the Cranchiinae containing *Cranchia, Liocranchia* and *Leachia,* and the Taoniinae comprised by the remaining ten genera. Within the taoniin clade, three major generic assemblages can be discerned, two of which, the group *Megalocranchia* + *Egea* + *Teuthowenia* and the group *Taonius* + *Galiteuthis* + *Mesonychoteuthis*, are further hypothesized to have shared a common ancestor more recently than either did with members of a third group consisting of *Sandalops* + *Liguriella* + *Helicocranchia* + *Bathothauma*. All relationships are fully resolved in this estimate of cranchiid phylogeny, and the topology of the tree requires a minimum of 45 character state transitions in order to derive observed phenotypes of extant cranchiids from the morphology of the common ancestor estimated in the preceding section; the consistency index (Kluge & Farris, 1969) for the Wagner Tree is .69, indicating a remarkably good fit of hypothesis to data.

Because it cannot be known with certainty, however, that the Wagner Tree is actually the most parsimonious of all possible reconstructions of cranchiid relationships, the binary data matrix of Table 2 was subjected to Compatibility Analysis in order to develop testable alternatives. The 31 binary factors of our data form 21 cliques of mutually compatible members, and each of these cliques supports one (or more) estimate(s) of cranchiid evolution that is (are) not supported by any other clique. The compatibility matrix for the binary factors is presented in Table 4, character memberships of the ten largest cliques are provided in Table 5, and the cladograms supported by the two largest cliques are drawn in Fig. 9.

TABLE 3. Reconstructed phenotypes of hypothetical cranchiid ancestors. Columns represent the hypothetical ancestors labelled with capital letters in Fig. 8. Character numbers and character state labels are the same as those in Table 1 and described in the text.

Character number	Ancestors											
	A	B	C	D	E	F	G	H	I	J	K	L
1	b	a	a	a	a	c	c	a	a	d	d	a
2	c	c	c	c	c	a	a	b	b	a	a	a
3	c	c	b	b	b	d	a	a	a	a	a	a
4	a	a	a	a	a	a	a	a	a	b	b	a
5	a	b	b	b	b	b	b	b	b	a	a	a
6	e	e	e	e	e	c	d	d	d	b	b	a
7	b	b	b	b	b	b	b	b	b	a	a	a
8	b	b	a	a	a	a	a	a	a	b	b	a
9	a	a	c	b	a	a	a	a	a	a	a	a
10	a	a	a	a	a	b	b	b	a	a	a	a
11	b	b	b	b	b	c	c	c	b	c	a	a
12	a	a	a	a	a	a	a	a	a	b	b	a
13	b	b	b	b	b	b	b	b	b	a	a	a
14	c	e	e	e	e	e	e	e	e	b	a	a

Cliques I and II, whose trees are drawn in Fig. 9, share 21 binary factors (1b, 1d, 2c, 2d, 3b, 3c, 3d, 4b, 6b, 6c, 6e, 6f, 7b, 9b, 9c, 10c, 12b, 14b, 14c, 14d, 14f), and this large set of characters determines those cladistic patterns common to both compatibility trees and to the results of Wagner analysis. Disagreement between the two trees of Fig. 9 reflects underlying differences in clique memberships and concerns only the relationships of *Sandalops* and *Liguriella* within the Taoniinae. Clique I differs from clique II by the inclusion of binary factor 1c which asserts that *Bathothauma, Helicocranchia* and *Sandalops* comprise a monophyletic group, but leaves the relationships of *Liguriella* unresolved. Clique II omits factor 1c but includes factor 6d whose effect is to remove *Sandalops* and *Liguriella,* but not *Helicocranchia* or *Bathothauma,* to a monophyletic group with the remaining six taoniin genera; the relationships of *Sandalops* and *Liguriella* within the latter group are unresolved, however.

The trees supported by cliques I and II both include trichotomies because binary characters that might fully resolve the relationships of *Liguriella* and/or *Sandalops* do not support other aspects of the cladograms drawn in Fig. 9. In order to test the compatibility results

TABLE 4. Compatibility matrix for the binary factors whose distributions are provided in Table 2. Because the matrix is symmetrical, only the lower half is illustrated. Rows and columns are binary factors; an entry of (0) for a given row and column signifies that the corresponding pair of binary factors is not compatible, an entry of (1) that the pair of factors is compatible.

Binary factor label	1b	1c	1d	2b	2c	2d	3b	3c	3d	4b	5b	6b	6c	6d	6e	6f	7b	8b	9b	9c	10b	10c	11b	11c	12b	13b	14b	14c	14d	14e
1c	1																													
1d	1	1																												
2b	1	1	0																											
2c	1	1	1	1																										
2d	1	1	1	1	1																									
3b	1	1	1	1	1	1																								
3c	1	1	1	1	1	1	1																							
3d	1	1	1	1	1	1	1	1																						
4b	1	1	1	0	1	1	1	1	1																					
5b	1	1	0	0	0	1	0	0	1	0																				
6b	1	1	1	0	1	1	1	1	1	1	0																			
6c	1	1	1	0	1	1	1	1	1	1	0	1																		
6d	1	0	1	0	1	1	1	1	1	1	0	1	1																	
6e	1	1	1	1	1	1	1	1	1	1	0	1	1	1																
6f	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1															
7b	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1														
8b	1	1	1	0	0	1	0	1	1	1	0	1	0	0	0	1	0													
9b	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1												
9c	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1											
10b	1	0	1	0	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1										
10c	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1									
11b	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	1	1	1	1								
11c	1	1	0	0	0	1	0	1	1	0	0	0	0	0	0	1	0	0	0	0	1	1	1							
12b	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	0						
13b	1	1	0	0	1	1	1	1	1	0	1	0	1	1	1	1	1	0	1	1	1	1	0	0	0					
14b	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1				
14c	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1			
14d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
14e	1	0	1	0	0	1	0	0	1	1	1	1	1	0	0	1	1	0	1	1	0	1	1	0	1	1	1	1	1	
14f	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Binary factor label

TABLE 5. Memberships for the ten largest cliques of mutually compatible binary factors.

Clique number	Membership
I	1b, 1c, 1d, 2c, 2d, 3b, 3c, 3d, 4b, 6b, 6c, 6e, 6f, 7b, 9b, 9c, 10c, 12b, 14b, 14c, 14d, 14f.
II	1b, 1d, 2c, 2d, 3b, 3c, 3d, 4b, 6b, 6c, 6d, 6e, 6f, 7b, 9b, 9c, 10c, 12b, 14b, 14c, 14d, 14f.
III	1b, 1d, 2c, 2d, 3b, 3c, 4b, 6b, 6c, 6e, 6f, 7b, 9b, 9c, 10b, 10c, 12b, 14b, 14c, 14d, 14f.
IV	1b, 1c, 2c, 2d, 3b, 3c, 3d, 6c, 6e, 6f, 7b, 9b, 9c, 10c, 13b, 14b, 14c, 14d, 14f.
V	1b, 2c, 2d, 3b, 3c, 3d, 6c, 6d, 6e, 6f, 7b, 9b, 9c, 10c, 13b, 14b, 14c, 14d, 14f.
VI	1b, 1d, 2d, 3d, 4b, 6b, 6c, 6f, 7b, 9b, 9c, 10c, 12b, 14b, 14c, 14d, 14e, 14f.
VII	1b, 1c, 1d, 2d, 3c, 3d, 4b, 6b, 6f, 8b, 9b, 9c, 10c, 12b, 14b, 14c, 14d, 14f.
VIII	1b, 2c, 2d, 3b, 3c, 6c, 6e, 6f, 7b, 9b, 9c, 10b, 10c, 13b, 14b, 14c, 14d, 14f.
IX	1b, 1d, 2d, 3c, 4b, 6b, 6f, 8b, 9b, 9c, 10b, 10c, 12b, 14b, 14c, 14d, 14f.
X	1b, 1c, 2b, 2c, 2d, 3b, 3c, 3d, 6e, 6f, 9b, 9c, 10c, 14c, 14d, 14f.

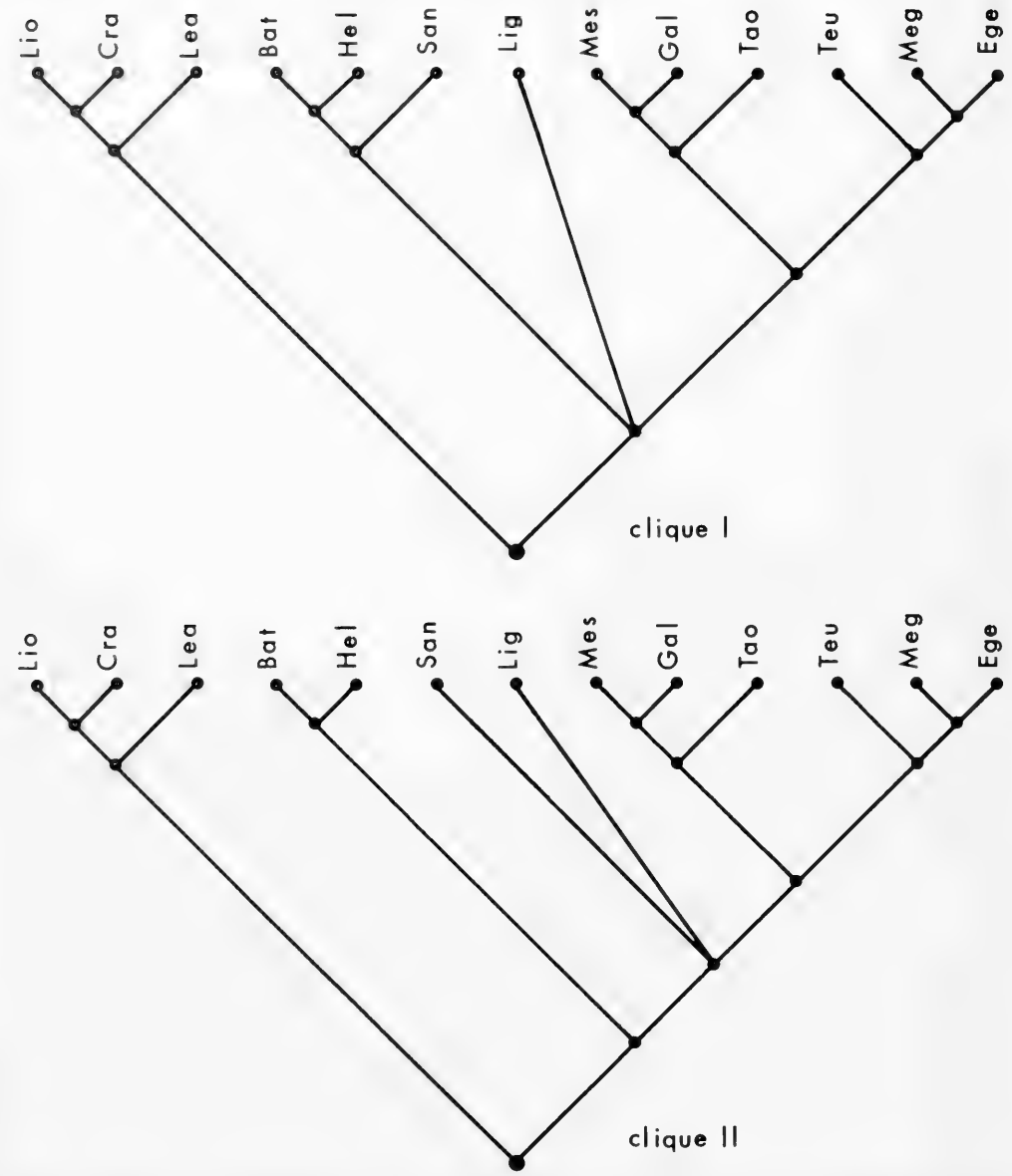

FIG. 9. Cladograms corresponding to the estimates of cranchiid evolution supported by cliques I and II (see Table 5). Branch lengths are arbitrary and do not represent any estimated parameter of phylogeny.

against those of the Wagner analysis, however, it is convenient to resolve such trichotomies fully. To each trichotomy in a cladogram there correspond three completely bifurcating alternative interpretations (Nelson & Platnick, 1980), and the six alternatives that result from so interpreting the ambiguities of Fig. 9 are shown in Fig. 10. Only that portion of the taoniin lineage descended from the cranchiid ancestor but ancestral to the monophyletic group *Megalocranchia* + *Egea* + *Teuthowenia* + *Taonius* + *Galiteuthis* + *Mesonychoteuthis* is depicted for each variant; the unillustrated portions of the cladograms of Fig. 10 are identical to those elicited in all preceding analyses. We note that one of

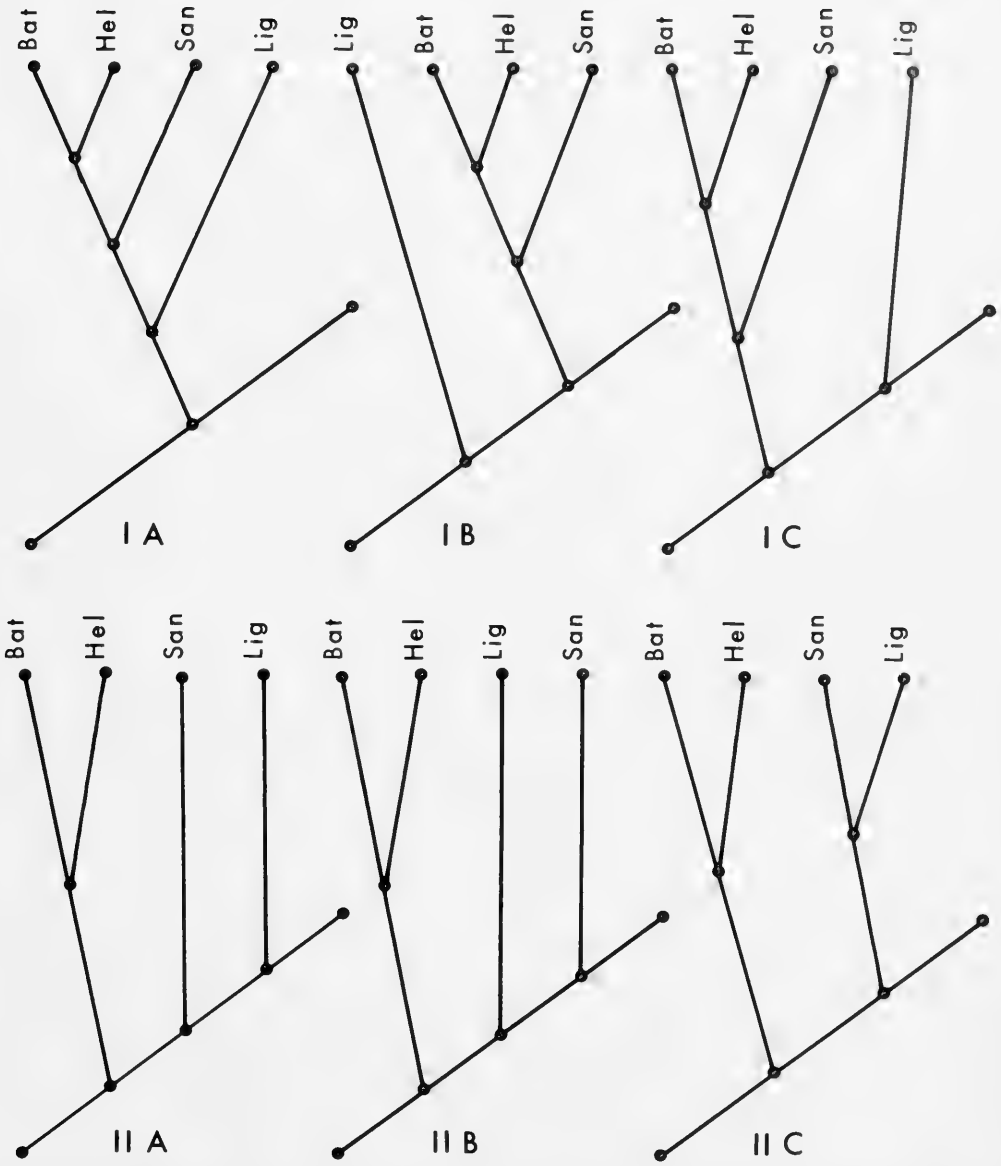

FIG. 10. Three bifurcating interpretations for each of the trichotomous branchings in the cladograms of Fig. 9. See text for explanation.

the bifurcating interpretations of the tree determined by clique I is identical with the Wagner Tree.

A modified version (see Materials and Methods) of the parsimony-optimizing procedure of Farris (1970) was used to fit observed character state distributions (Table 1) to the six variants in order to determine which of them provides the most parsimonious interpretation of cranchiid phylogeny. The Wagner Tree (Fig. 8 and 1A of Fig. 10) with 45 required character state transitions proved most parsimonious, followed by trees IC, IIA and IIC with 46 necessary transitions apiece, and trees IB and IIB with 47 transitions each. The character state transition in which two of

these hypotheses differ are illustrated in Fig. 11. As can be seen, while both hypotheses 'explain' the same observed phenotypes for the four genera diagrammed, they differ in the simplicity with which they do so.

DISCUSSION

In the absence of fossil cranchiids and of *a priori* knowledge of character conservatism among these squids, the principle of parsimony appears to us the only defensible criterion with which to test alternative hypotheses of cranchiid phylogeny. However, because more than 300 billion different bifurcating tree diagrams could be drawn to unite our 13 terminal taxa (Felsenstein, 1978) exhaustive testing by any criterion is clearly impractical. The purpose of applying operational phylogenetic techniques, as those employed here, is simply to reduce this vast array of possibles to a much smaller set of well-corroborated alternatives among which the true phylogeny has a reasonably high likelihood of being included. The close congruence revealed above between the results of Wagner and of Character Compatibility analyses lends credence to the possibility that the tree diagram of Fig. 8 represents, if not historical truth exactly, then at least an estimate sufficiently close that an examination of the details of the reconstruction will not be far wrong. Although Pfeffer (1912) and Nesis (1974) previously discussed phylogenetic relationships among cranchiids, the inadequate materials available to them resulted in such taxonomic confusion as to effectively preclude meaningful comparisons of their conclusions with our results; the reader is referred to N. Voss (1980) for a discussion of their generic assignments.

A basal division of the Cranchiidae into the traditional subfamilies Cranchiinae and Taoniinae is supported by five characters (1, 4, 6, 7, 12), and character state transitions separating the cranchiin and taoniin ancestors (Table 3) account for nearly 30% of all of the morphological evolution estimated to have occurred in the course of cranchiid phylogeny. Apparently unique synapomorphies uniting the three cranchiin genera are the cartilaginous strengthenings along one or both of the paired ventral lines of funnel-mantle fusion (1d), the lateral fusion of the funnel to the head (4b), the possession of four or more small and simple ocular photophores (6b) and of a caecum larger than the stomach (12b). Unique synapomorphies to support a hy-

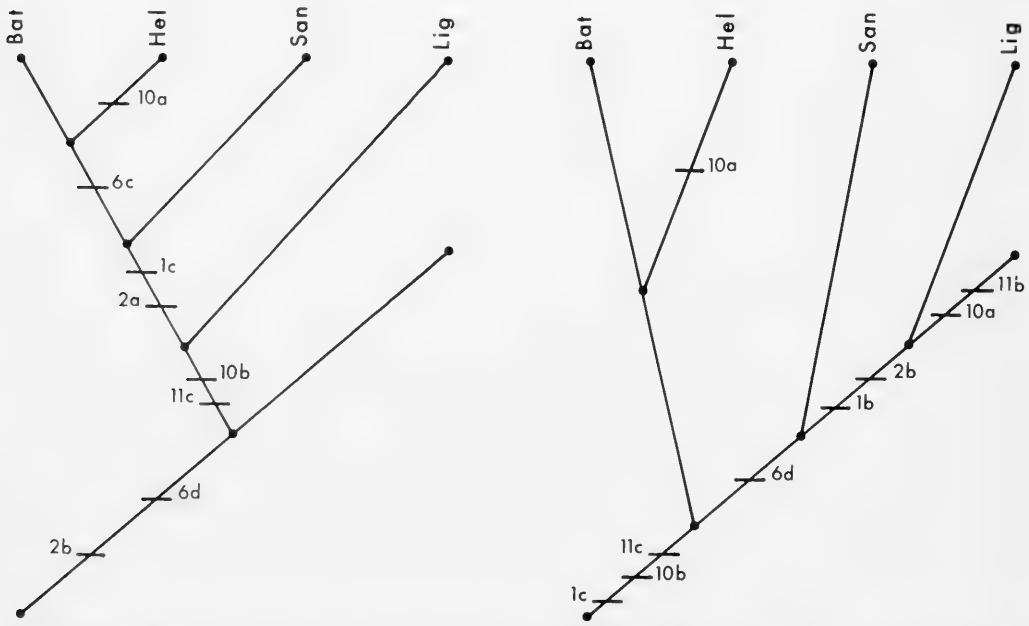

FIG. 11. Two alternative estimates of relationships for four cranchiid genera. The labelled slashes across branches of the tree signify the evolution of the corresponding character state from a locally more plesiomorphic condition. Only those character state transitions in which the two hypotheses differ are illustrated.

pothesis of taoniin monophyly are the loss of the hectocotylus (7b) and the possession of ocular photophores of complex construction (6c–f). Other derived conditions that may have characterized the cranchiin or taoniin ancestor are the results of non-unique character state transitions (i.e. those replicated or reversed elsewhere) that do not, therefore, support the cranchiin-taoniin dichotomy *per se.*

Within the Cranchiinae, the genera *Cranchia* and *Liocranchia* form a monophyletic group that is supported by a derived morphology of the funnel organ (14b) as well as by the position of the digestive duct appendages on the digestive gland alone (11c); the latter state, however, is shared with some taoniin genera as well (see below). *Leachia* appears well separated from the other two cranchiins in the characters discussed above and in the morphology of funnel-mantle fusion (see discussion of Character 1) and shares with the Taoniinae the derived absence of a funnel valve (5b; except *Egea* and *Megalocranchia*) and the presence of stalked larval eyes (13b). These last two anomalous traits may either represent convergent evolution of the derived conditions in question, or we may be mistaken in assuming sessile larval eyes and the presence of a funnel valve to be primitive for the family. If it is our estimation of polarities that is in error, then a funnel valve and sessile larval eyes are synapomorphies for *Cranchia* and *Liocranchia*.

The major monophyletic clusters identifiable within the Taoniinae include the generic groups recognized by N. Voss (1980), but substantial refinements of earlier hypotheses of taoniin interrelationships are also represented in Fig. 8. The *Megalocranchia* group (*Megalocranchia* + *Egea* + *Teuthowenia*) and the *Taonius* group (*Taonius* + *Galiteuthis* + *Mesonychoteuthis*) together comprise a monophyletic unit defined by shared, derived aspects of gladius morphology (2c), fin shape (3b,c) and ocular photophore arrangement (6e,f) that appears well separated from its putative (see below) sister group, the *Sandalops* assemblage (*Sandalops* + *Liguriella* + *Bathothauma* + *Helicocranchia*).

Monophyly of the *Megalocranchia* group is supported by shared possession of elongated, terminal-lateral fins (3c) and by the presence of brachial end-organs on the arms of mature females (8b). Brachial end-organs, however, also occur among cranchiin squids and seem best regarded as another instance of convergent evolution. To argue otherwise, for example that cranchiins and the *Megalocranchia* group form a monophyletic assemblage by virtue of a unique derivation of brachial end-organs, would necessarily invoke homoplasy in so many other characters (e.g. 2, 3, 6, 7) as to be extravagantly unparsimonious. Within the *Megalocranchia* group the genera *Megalocranchia* and *Egea* form a morphologically distinctive pair as noted by N. Voss (1980: 406).

Members of the *Taonius* group uniquely share the derived presence of hooks or of hooklike teeth on the larger suckers of the clubs (9b,c). *Taonius, Galiteuthis* and *Mesonychoteuthis* are also united by having lanceolate or stout, ovate, terminal fins (3b), a character state derived for the Taoniinae as a whole but a plesiomorph within the monophyletic assemblage that includes *Megalocranchia* and its allies. *Mesonychoteuthis* and *Galiteuthis* both exhibit clubs with well-developed hooks (9c), a uniquely derived condition not shared with *Taonius.* Four species of *Galiteuthis* share, with *Mesonychoteuthis,* the (not uniquely) derived position of digestive duct appendages on the digestive gland alone (11c), but two other species of *Galiteuthis* share with *Taonius* the more plesiomorphic position of appendages on both the gland and the digestive ducts (11b). All of the analyses reported here were repeated, using either 11b or 11c to characterize *Galiteuthis,* with identical results: the cladistic position of the genus was unaffected by the substitution. The distinctiveness of neither *Mesonychoteuthis* nor *Galiteuthis* is compromised by the interspecific variation in Character 11 observed within the latter; the two genera are well defined with respect to other morphological features discussed by N. Voss (1980: 392–396).

All of the relationships discussed above are common to the results of both Wagner and Character Compatibility analyses and appear adequately supported by the comparative morphological evidence at our disposal. Regrettably, the same cannot be said of any arrangement of the genera *Bathothauma, Helicocranchia, Sandalops* and *Liguriella* along the phyletic line descended from the cranchiid ancestor but ancestral to the *Taonius* and *Megalocranchia* groups. While the hypothesis that the four genera of the *Sandalops* assemblage form a monophyletic cluster is slightly more sparing of character state transitions than any of the other five alternatives treated here (Fig. 10), we would

point out that no unique synapomorphy can be adduced in support of this arrangement. Instead, members of the *Sandalops* group are united by character states that are shared by other cranchiids as well (4a, 5b, 7b, 8a, 9a, 11c, 12a, 13b) and evidence for their near affinity is therefore largely by phenetic similarity.

By contrast, derived resemblances in ocular photophores (6d and derivatives) argue that *Sandalops* and *Liguriella* form a monophyletic unit with the *Taonius* and *Megalocranchia* groups that does not include *Bathothauma* or *Helicocranchia* (tree II of Fig. 9) while the shared, derived possession of narrow, straight funnel-mantle fusion cartilages supports the inclusion of *Bathothauma, Helicocranchia* and *Sandalops*, but not *Liguriella,* in a different monophyletic arrangement (tree I of Fig. 9). The most parsimonious arrangement (Fig. 8 and IA of Fig. 10) is supported weakly by assuming the presence of digestive duct appendages on the digestive gland alone (11c) to be a local synapomorphy; the condition is shared with *Mesonychoteuthis,* one group of *Galiteuthis* species, *Cranchia* and *Liocranchia,* however, and the hypothesis that the *Sandalops* assemblage is monophyletic should be regarded as a best guess among alternatives but slightly less parsimonious; only the discovery of new characters seems likely to satisfactorily resolve the phyletic structure of this problem group. *Bathothauma* and *Helicocranchia* share morphologies of the fins (3d) and ocular photophores (6c) that are unique among adult cranchiids though widespread in the larval stages of other genera; as adult features of *Bathothauma* and *Helicocranchia,* the traits appear to represent derived, neotenous conditions.

Cranchiid adaptive radiation appears to have involved, to a significant degree, the evolution of differing schedules of ontogenetic descent in the water column (N. Voss, unpublished notes), and several of the monophyletic groups discussed in the preceding paragraphs may be characterized by the ecological distribution of the growth stages of their member taxa. Thus, the three cranchiin genera on the one hand and the genera of the *Megalocranchia* group on the other represent apparently independent clades whose larvae (with the single known exception of *Liocranchia valdiviae*) nevertheless resemble one another ecologically by remaining in the upper waters for longer periods in their development than do larvae of other cranchiid groups. This ecological resemblance, either convergently evolved or inherited unmodified from the cranchiid ancestor, might account for the peculiar similarities between the groups in possession of brachial end-organs and (between *Liocranchia-Cranchia* and *Egea-Megalocranchia*) in the presence of a valve in the funnel. However, as we know so little of the adaptive significance of either anatomical trait, their causal relationships (if any) to ontogenetic lingering in the upper waters are unclear.

The *Taonius* group, in contrast, consists of cranchiids that typically descend to mid and deep water at a much earlier immature stage than do members of the Cranchiinae or of the *Megalocranchia* group. It may be noted in passing that the phenomena of early ontogenetic descent displayed by the *Taonius* group might be related to the extended geographic distribution of the member genera. Though circumglobal distribution in tropical and subtropical waters is typical for most of the cranchiid genera, the ranges of the *Taonius* assemblage extend into subpolar and polar regions, with *Mesonychoteuthis* restricted primarily to Antarctic waters. Geographic range extension into cold waters is not unique to this group, however, for *Teuthowenia,* and, to a lesser extent, *Liguriella* and *Bathothauma* are also found in subpolar waters, but in a more limited pattern of distribution.

The *Sandalops* group is not easily defined in ecological terms and the lack of morphological cohesiveness remarked earlier for these squids may be a reflection of the apparent absence of any ecological distinctiveness.

Functional correlations appear to have contributed little, if at all, to the hierarchic pattern of character state distributions revealed in the preceding analyses: the phylogeny is supported by morphological features associated with such a diversity of biological activities (see discussions of Characters 1, 3, 4, 7, 9, 12) that we think it unlikely that our estimate of relationships reflects divergence in only a single co-adapted anatomical complex or functional role. Of the characters treated here, only two seem obviously associated in a close functional sense: the form of the posterior end of the gladius (Character 2) and the shape of the fins (Character 3). As discussed previously in the analyses of these characters, changes in the shape of the posterior end of the gladius are usually accompanied by changes of fin

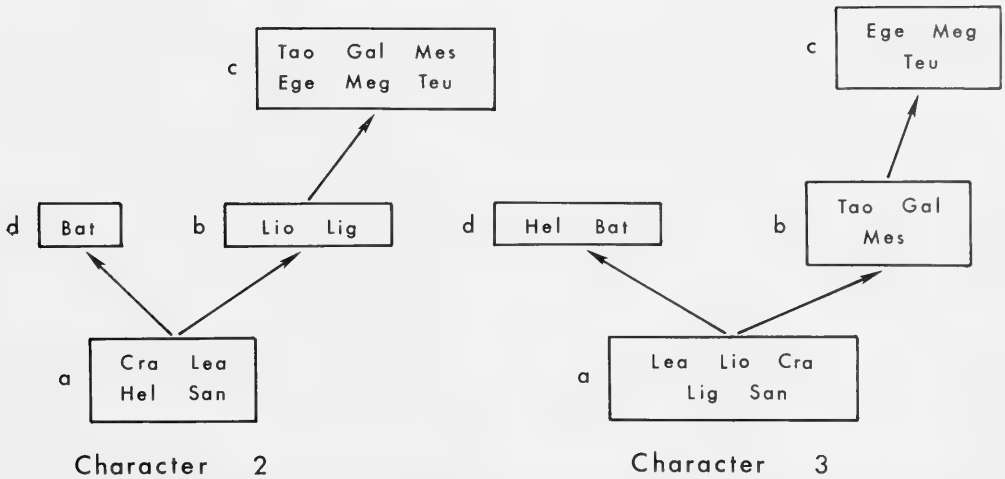

FIG. 12. Enlarged tree diagrams for Characters 2 and 3. Lower case letters label the states of Characters 2 and 3, drawn as boxes containing the cranchiid genera that exhibit the appropriate morphological condition; arrows indicate polarities hypothesized in the text. The two characters are seen to constitute different (but compatible) partial estimates of cranchiid relationships.

shape, perhaps for reasons of structural support. Nevertheless, as can be seen from the enlarged character state trees in Fig. 12, the relationship between fins and gladius is evidently not wholly deterministic, and the two characters each contribute some phyletic information not contained in the other. We observe that Character 2 does not, in fact, support our hypothesis of phylogeny while Character 3 does.

The fact that not all of the characters we studied are pairwise compatible (Table 4) is sufficient demonstration of the existence of homoplasy in the course of cranchiid evolution. If the tree topology of Fig. 8 and the reconstructed ancestral phenotypes of Table 3 be accepted as reasonable estimations, then the minimal amount of homoplasy in each character commensurable with those estimates is easily determined and may provide an approximate measure of conservatism that might inform the choice and weighting of characters in subsequent systematic studies (see also Farris, 1969).

Characters 1, 3, 4, 7, 9, and 12 support the estimate of Fig. 8; if that estimate is taken to be correct, then these characters, in addition to being mutually compatible, are also true characters: they have undergone no homoplasy in the course of cranchiid evolution. Characters 2, 5, 6, 8, 10, 11, 13 and 14 have all undergone one or more instances of convergence or reversal, of which those involving brachial end-organs (Character 8), larval eye position (13) and a funnel valve (5) have already been discussed as examples above. Most of these latter characters have undergone but one or two instances of homoplasy, and we would hesitate, based on this observation alone, to enjoin caution in their use in future phylogenetic investigations, but Character 11 is an exception. Over the course of cranchiid phylogeny, digestive duct appendages appear to have migrated on and off the digestive duct and gland with abandon. Primitively situated on the digestive duct (see analysis for Character 11, above), appendages are here interpreted to have moved onto the digestive gland in the common ancestor of *Cranchia* and *Liocranchia,* and in the taoniin ancestor, to have reverted to the ancestral state in *Megalocranchia,* and to have vanished from the ducts entirely in *Cranchia* and *Liocranchia,* in the ancestor of the *Sandalops* group and in *Mesonychoteuthis* and some species of *Galiteuthis*. Evidently, the digestive duct appendages are evolutionarily labile structures, and it would be interesting to know what adaptive significance accruing to their anatomical positions makes them so.

CONCLUDING REMARKS

The form of a phylogenetic hypothesis, the topology of a tree diagram, results both from the analysis of individual characters and from the procedures subsequently employed to re-

solve character conflicts. We have endeavored to be as explicit as possible about each step that led us to adopt the hypothesis presented here so that would-be critics can discover exactly where we may have gone wrong and set about directly to correct the error. Because errors in phylogeny reconstruction, when they exist, usually consist of mistakes in determining homologies or in estimating polarities, the greater part of this paper is devoted to individual character discussions, and the future, critical tests of our phylogeny that we hope to have provoked will perforce consist either of discovering new characters or of more detailed analyses of the characters treated here. In neither case will materials be found wanting. As sources of new characters, for example, the myology of teuthoids remains little explored; the morphology of the cranial cartilages and the spermotophores likewise invites attention as does the comparative anatomy of the nervous, reproductive and circulatory systems. Of characters treated here, the histology of the ocular photophores and of the brachial end-organs is in need of study, and careful observations of courtship and mating behavior may confidently be expected to permit more informed treatment of the hectocotylus and of other male sexual modifications of the arms. Additionally, we know little or nothing of the functional significance of variations in the form of the funnel organ, of the larval eye stalks, or of the relative size of the caecum and stomach to name but three of many enigmatic aspects of cranchiid morphological variation. References provided in the individual character discussions will provide introductions to these and other promising areas of teuthoid morphological research; we know of few animal groups in which the potential for innovative and phylogenetically rewarding comparative studies appears so great.

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LETTER TO THE EDITORS

ON SOME APLACOPHORAN HOMOLOGIES AND DIETS

(1) The assertion by Salvini-Plawen (1981) that the subradular membrane of mollusks is a direct continuation of pharyngeal cuticle is not supported by Märkel (1958, fig. 35, p. 281), Runham (1963), or Peters (1978, fig. 3, p. 287), who demonstrated that the subradular membrane is distinct from, and at its anterior end overrides, the pharyngeal cuticle. The subradular membrane is secreted by the distal inferior epithelium of the radular sac and is firmly attached to both the inferior epithelium by apical processes and the radula membrane (Kerth, 1976, fig. 3, p. 275). The subradular membrane develops ontogenetically later than the radula membrane (Kerth et al., 1981). The radula membrane, which bears the radula teeth, is secreted by the most anterior odontoblasts at the posterior end of the sac (Kerth & Krause, 1969, fig. 11, p. 66; Wiesel & Peters, 1978, fig. 8, p. 84).

According to Salvini-Plawen, the "basal cuticle" upon which the teeth of neomeniomorphs (= Solenogastres) usually are born "is . . . not independently formed at the . . . blind end of the radula sheath," and on p. 378 he states that the "radula itself" is formed "as usual in a separate sheath by odontoblasts." Thus we are left to conclude that radula teeth, but not a radula membrane, are formed by odontoblasts; these teeth somehow become attached to a membrane presumably continuous with pharyngeal cuticle.

In my own experience with isolated entire radulae those of the Neomeniomorpha as well as the Chaetodermomorpha consist of teeth on a discrete membrane that is not continuous with buccal cuticle and looks just like any other radula ribbon. The concept of a "basal cuticle" seems to rest on no evidence.

(2) *Gymnomenia* is one of the primitive genera of neomeniomorphs but the lack of ventral salivary glands is a derived state. As the absence or presence of foregut glands does not define the primitive order Pholidoskepia, I apologize for attributing to Salvini-Plawen the idea that either state is primitive.

(3) My "non-homology" of the oral shield and the molluscan foot seems altogether correct to me. The oral shield is clearly derived from gut epithelium (Scheltema, 1981, fig. 2, p. 364); the continuous cuticle of the gut and oral shield is of course secondary to a noncuticularized state. Since the oral shield is not a vestigial foot, then there is no cladistic basis for splitting the Aplacophora into two classes (Salvini-Plawen, 1972). This split rests (a) on the assumption that the Chaetodermomorpha (Caudofoveata) and Neomeniomorpha (Solenogastres *sensu* Salvini-Plawen) evolved into a worm shape in two separate evolutionary events from an ancestor whose mouth opened through a gliding foot-sole and (b) on the homology between the molluscan creeping foot and the oral shield of the Chaetodermomorpha. The latter homology originated with Hoffman (1949), who compared the evolutionarily advanced *Chaetoderma nitidulum* with *Dorymenia hoffmani* (= *Proneomenia antarctica*). He wrote (p. 382, herein translated): "The oral shield integument consists . . . of elements which we are acquainted with in the integument of the ventral furrow: [a] cells which show signs that they were ciliary cells and which have gone through the same change as the most common cells in the inner surface of the lateral fold (cuticularization of cilia) [i.e., outside wall of the furrow]; [b] true sensory cells, and finally [c] gland cells which have the same form (deeply sunk, pyriform), the same arrangement to one another (joined in lobes), and the same secretion (strongly stained with Ehrlich's haemotoxylin) as the gland cells of the ventral furrow." (cf. Hoffman, 1949, figs. 16, 17, 18, 21, 30, 31, 34.)

Of this homology Salvini-Plawen wrote (1972: 295, herein translated): "The extremely detailed likeness of the foot-shield [oral shield] epithelium and ventral-furrow epithelium (Solenogastres) including the glands in themselves leaves no doubt as to the mutual homology." He then went on to illustrate and describe carefully for several species the direct connections between the cerebral ganglion and precerebral ganglia which give rise to the innervation of the oral shield. Hoffman had interpreted the precerebral ganglia as arising from fused cerebrolateral-cerebroventral connectives. According to Salvini-Plawen, in both Chaetodermomorpha and Neomeniomorpha the area around the mouth is innervated from the cerebral ganglion (1972, fig. 45). In the English summary (1972,

p. 376, paragraph 16) he wrote that the "foot-shield" as shown by histologic detail "represents a portion of a previously overall ventral gliding surface—merely distinguished from the recent molluscan foot by innervation. Consequently, the foot-shield represents the cerebrally innervated fragment of the overall ventral gliding-sole of the Archimollusca."

From my own observations (1978, 1981), I have concluded that: (a) The oral shield is a specialized (derived) structure formed from foregut epithelium; (b) The gland cells of the oral shield are diffuse in the primitive genus *Scutopus* and thus do not agree with Hoffman's homology. I do not follow the reasoning by which Salvini-Plawen on the one hand retains Hoffman's homology, which is based on the "deeply sunk" mucus glands "joined in lobes" in the more highly evolved genus *Chaetoderma,* while on the other hand he corrects the homology to include scattered, diffuse cells (i.e., of *Scutopus*). After all, diffuse mucous cells are ubiquitous among mollusks as an aid to all sorts of ciliary and muscular movement and are therefore not reliable markers of homologous structures.

The only sure evidence for a vestigial foot would be innervation from the ventral ganglia or from a branch from the lateroventral connectives, which Hoffman endeavored to show. The cerebral innervation of the oral shield detailed by Salvini-Plawen is precisely the evidence that determines that the oral shield is not a vestige of the archimolluscan foot.

(4) Finally, I do not believe that my paper "corroborates" Salvini-Plawen's wherein it is concerned with the diets of the Chaetodermomorpha. Organic debris, pieces of sponge spicules, bits of radiolaria, diatoms, and occasional inorganic sediment particles usually occur in the guts of chaetoderms. Salvini-Plawen himself states (p. 375) that because these animals are burrowers, "findings of other particles and/or stated organisms, therefore, may be an accidental by-product" of selective feeding. It is regrettable that Salvini-Plawen's Table 1 (pp. 376–77) does not distinguish between likely diets (recognizable crustacean eggs, crustacean parts, Foraminifera) and organic debris that may have been accidentally ingested.

In my experience there is no indication from gut contents that any species of Chaetodermomorpha is a deposit-feeder, i.e. "seize[s] sediment particles without specific selection" (p. 375).

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An asterisk (*) denotes a new taxon

AWARDS FOR STUDY AT

The Academy of Natural Sciences of Philadelphia

The Academy of Natural Sciences of Philadelphia, through its Jessup and McHenry funds, makes available each year a limited number of awards to support students pursuing natural history studies at the Academy. These awards are primarily intended to assist predoctoral and immediate postdoctoral students. Awards usually include a stipend to help defray living expenses, and support for travel to and from the Academy. Application deadlines are 1 April and 1 October each year. Further information may be obtained by writing to: Chairman, Jessup-McHenry Award Committee, Academy of Natural Sciences of Philadelphia, 19th and the Parkway, Philadelphia, Pennsylvania 19103, U.S.A.

UNITAS MALACOLOGICA

Eighth International Malacological Congress

Organizing Committee: *László PINTÉR (president); Endre KROLOPP (secretary); Károly BÁBA; Tamás DOMOKOS; Levente FÜKÖH; Gyula KOVÁCS; László MERÉNYI; Ede PETRÓ; Andor RICHNOVSZKY; András VARGA*

First Circular

DATE: August 29—September 3, 1983.

PLACE: Budapest, Hungary.

TOPICS: The Congress is open to any topic of malacology (recent and fossil). Furthermore, in case of sufficient number of people, we organize Colloquia in the following subjects:

1. Quaternary malacology and fauna genesis
2. Non-marine mollusc biogeography
3. Sphaeriidae

We organize meetings for the EIS and for museum curators, also the General Assembly of the UM.

LANGUAGES: English, French and German.

EXCURSIONS: Preceding, during and after the Congress we shall organize collecting tours and excursions.

Further details shall be issued in the Second Circular.

László Pintér
Hungarian Natural History Museum
Budapest
———
Baross u. 13.
H-1088
Hungary

INSTRUCTIONS FOR AUTHORS

1. MALACOLOGIA publishes original research on the Mollusca that is of high quality and of broad international interest. Papers combining synthesis with innovation are particularly desired. Papers of local geographical interest should be submitted elsewhere, as should papers whose primary thrust is physiology or biochemistry. Nearly all branches of malacology are represented on the pages of MALACOLOGIA.

2. Manuscripts submitted for publication are received with the tacit understanding that they have not been submitted or published elsewhere in whole or in part.

3. Manuscripts may be in English, French, German or Spanish. Papers in languages other than English must include a translation of the Abstract in English. Authors desiring to have their abstracts published in other languages must provide the translations (complete with main titles). Include all foreign accents. Both American and British spellings are allowed.

4. Unless indicated otherwise below, contributors should follow the recommendations in the *Council of Biology Editors (CBE) Style Manual* (ed. 4, 1978), available for U.S. $16.00 from CBE, 9650 Rockville Pike, Bethesda, MD 20014, U.S.A.

5. Be brief.

6. Manuscripts must be typed on one side of good quality white paper, double-spaced throughout (including the references, tables and figure captions), and with ample margins. Tables and figure captions should be typed on separate pages and put at the end of the manuscript. Make the hierarchy of headings within the text simple and consistent. Avoid internal page references (which have to be added in page proof).

7. Choose a running title (a shortened version of the main title) of fewer than 50 letters and spaces.

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