REPRODUCTIVE BEHAVIOR AND ANATOMY OF THREE CENTRAL CALIFORNIAN SCAPHOPODS

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By

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Thanks for letting me use your computer, Jeff R.

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INTRODUCTION

There is a remarkable lack of information on the entire class of mollusks known as the Scaphopoda. Certain papers do exist that deal with specific topics such as taxonomy (Emerson 1962), general behavior (Gainey 1972), and anatomy (Lacaze-Duthiers 1856, Yonge 1937, and Morton 1959). The literature that is available on reproduction focuses on certain aspects of fertilization and development of the egg (Kowalewsky 1883, Verdonk et al. 1971, Geilenkirchen et al. 1970, and Timmermans et al. 1970). These studies contain no information on gonad morphology and are all confined to individuals of the genus Dentalium. A single study on the timing of reproductive activity of Cadulus californicus in the San Diego Trench by Rokop (1977) is of limited value because of infrequent sampling and through-the-shell observation of gonad development. It is a start, however, and serves to compare the scaphopod's reproductive behavior to that of other deep water invertebrates.

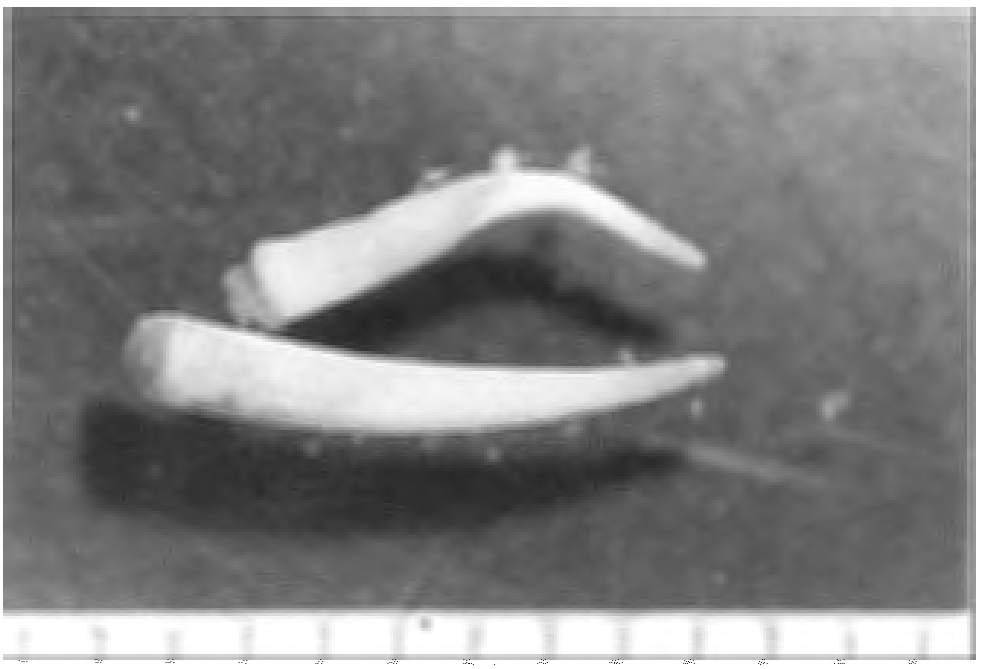
This study investigates two separate aspects of reproductive activity in three species of scaphopods. The species are Dentalium rectius (Pilsbry and Sharp 1898), Cadulus fusiformis (Pilsbry and Sharp 1898), and

Plate I. Dentalium rectius

These full-sized individuals show the long and thin structure of the species as well as the reddish-brown deposits often left near the anterior opening. Scale in centimeters/millimeters.

Plate II. Cadulus fusiformis

Note the simple structure of the shell at the apex. The upper individual has had its shell dissolved, revealing the white testis. Simple apex structure and lack of an orange band before the posterior appendix permits easy discrimination between this species and Siphonodentalium quadrafissatum. Scale in millimeters.

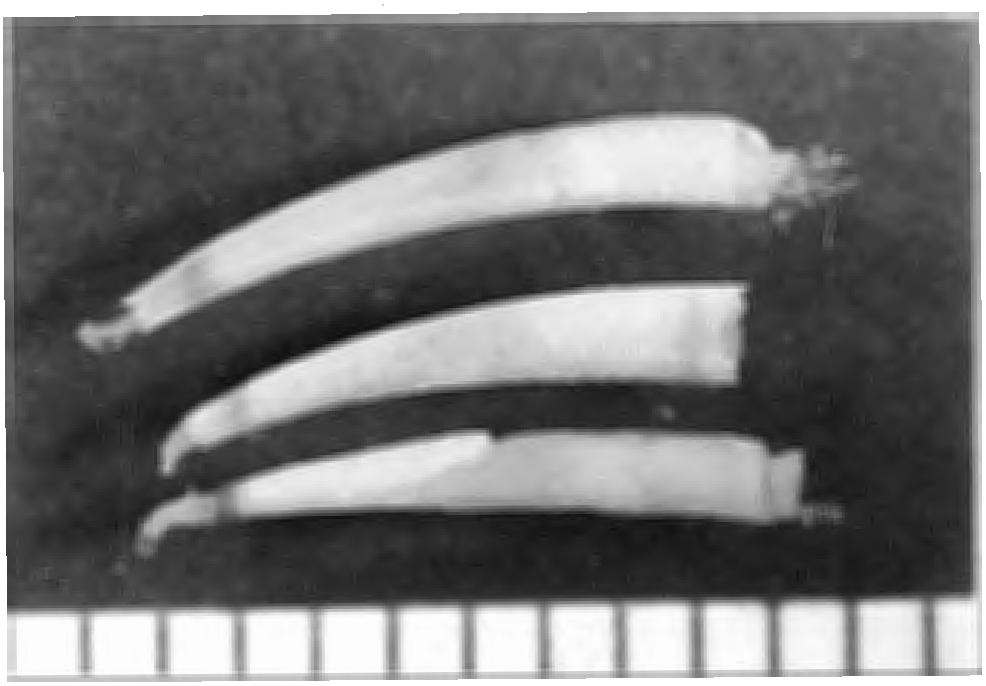


Siphonodentalium quadrifissatum (Pilsbry and Sharp 1898). The first aspect investigated was the seasonal timing of spawning; the second, the structure of the gonads and its effect upon the mechanism of gamete release.

Dentalium rectius is a slender and delicate animal ranging from Alaska to Central California, and specimens collected in this study were from 11 to 56 mm. in length (Plate I). Cadulus fusiformis ranges from Monterey Bay south to Baja California and individuals taken were from 3 to 12.5 mm. in length (Plate II). The size and geographical range of Siphonodentalium quadrifissatum (Plate III) are similar to that of Cadulus fusiformis. Siphonodentalium quadrifissatum differs from C. fusiformis in that instead of having a smooth and robust shell, it has an apex with four notches and a more delicate structure. This light shell is often semi-transparent and the sex of the individual can frequently be determined from color differences in the gonad that can be seen through the shell. It is not usually possible to tell the stage of gonad development by this method, however, and even attempts to relate ripeness to gonad size are invalid.

Plate III. Siphonodentalium quadrifissatum

Note the four notches at the apex and the orange band just in front of the posterior appendix. Captacula can be seen protruding from the anterior opening in the upper and lower animals. The lower animal has had the shell dissolved, revealing the gonad and mantle. The testis is the white structure in the posterior half of the body. Scale in millimeters.



MATERIALS AND METHODS

Benthic samples were taken at monthly intervals from March 1983 through July 1984. Several follow-up non-quantitative samples were also taken in October 1984, February 1985, April 1985, and May 1985. The sampling station was near the head of the Monterey Submarine Canyon in Central California, approximately 1.8 miles northwest of the Moss Landing Harbor entrance (36°49'N, 121 50'W). Depth during sampling runs ranged from 60 to 80 meters.

Samples were taken using a Menzies Interfacial Biological Trawl with an extra 50 pounds of weight attached to guarantee contact with the bottom. The net mesh on the trawl was 0.571 mm., ensuring retention of very small individuals. Towing time was approximately one minute at one knot, resulting in a sampling surface of approximately 30 square meters. This number is merely a rough guide since the actual towing time might increase up to as much as two minutes and the water speed of the boat increase or decrease as much as half a knot, depending upon the sea conditions. Samples initially weighed about 135 kilograms and were routinely towed behind the boat at the surface for five to ten minutes to do some preliminary labor-free sieving. The average sample brought on board weighed about 90 kilograms.

Replicate samples were taken during many of the sixteen trips. In other months single samples were taken due to time and weather constraints. Samples were obtained that supplied sufficient numbers of individuals for dissection and histology in all months but December 1983.

Samples were sieved through a 0.5 mm. mesh upon return to the laboratory. All live scaphopods were removed as well as a selection of empty shells. Live animals were immediately placed in Bouin's fixative to prepare them for histologic examination but were not relaxed beforehand. After 24 hours in the fixative the samples were cleared through two soakings in 70% ethanol and then stored in 70% ethanol until dehydration and embedding.

The shell length of each animal was measured to the nearest 0.5 mm. with a ruler and dissecting microscope. Shell length was defined as the straight line distance from the ventral lip of the aperture to the dorsal lip of the apex.

Since some shells were eroded by the action of the acid in the Bouin's fixative, a correction factor between body length and shell length was determined as follows. Twenty specimens were measured, had their shells dissolved, and were re-measured. By comparing these lengths the mean ratio of shell length to body

length was determined to be 1.22. This agreed well with values obtained by comparing the average length of 44 intact C. fusiformis from one replicate in January 1985 to 35 eroded C. fusiformis taken from another replicate in the same month. This correction factor was then applied to records of body length from the specimens exhibiting shell erosion. The corrected length was then used in the size distribution data.

Individuals were selected from each month and species for histological preparation. An attempt was made to select equal numbers of males and females from each month, but for various reasons this was not always possible. Between six and thirteen individuals were selected, measured and decalcified with RDO Rapid Bone Decalcifier (Dupage Kinetic Labs). These specimens were measured again, wrapped in cheesecloth, and put through a succession of dehydration washes with Dioxane and alcohol. The samples were then soaked in warm paraffin, removed from the cheesecloth and embedded in a poured paraffin block. After the block hardened, 8 micrometer serial sections were cut with an American Optical rotary microtome.

Most sections were stained with Delafield's Haematoxylin (Carleton and Leach 1947) and Eosin. Some sections were stained with Gomori's trichrome (Gomori 1950) for comparison. The condition of the gonads was

then observed through a compound microscope, and photomicrographs were taken. In all, over 150 specimens were sectioned: 14 *D. rectius*, 49 *S. quadrifissatum*, and 87 *C. fusiformis*.

The condition of the testis was recorded as a subjective judgement of maturity based on size, number of mature sperm present and the amount of empty space present in the gonad. Female reproductive maturity was ascertained more quantitatively by comparing the ratio of mature and maturing oocytes to the total number of oocytes and oogonia. These ratios in combination with relative egg sizes were used to determine sexual ripeness. By determining which months the specimens appeared most mature, or to have just spawned, the seasonality of the reproductive cycle of the populations could be determined. These data were also compared to the size distribution data for a more complete analysis. The extensive microscope investigations undertaken revealed aspects of gonad structure.

RESULTS

DESCRIPTION OF THE GONADS

The gonads of all three species are quite similar. Since this is the case, there is one description of the general gonad structure, using *C. fusiformis* as the specimen of reference, and variations between species are dealt with separately.

TESTIS

The testis can be distinguished from the ovary by its whitish, folded, and wrinkled appearance; the ovary is granular and yellowish or pink. The folds actually ramify throughout the thickness of the organ, separating its dorsal part into lobes (Plate IV). The ventral base of the testis lies just above the retractor muscles and is very closely associated with them (Plate V). The organ occupies the posterior two-thirds of the animal from the digestive glands back to the apex (Lacaze-Duthiers 1857, Deshayes 1825). From the ventral base of the testis, lobes of decreasing radius and size (anterior to posterior) curve up and around a central tubular cavity, almost encircling it. The lobes do not quite connect at the top, however, leaving the testis "tube" slightly open near the dorsal surface (Plate IV). Again, the folding seen from a top view of the entire organ is the delineation of these lobes by their

Plate IV.
Generalized gross form of a Cadulus fusiformis testis.
do... dorsal opening
gt... central gonad "tube"
lb... testis lobe

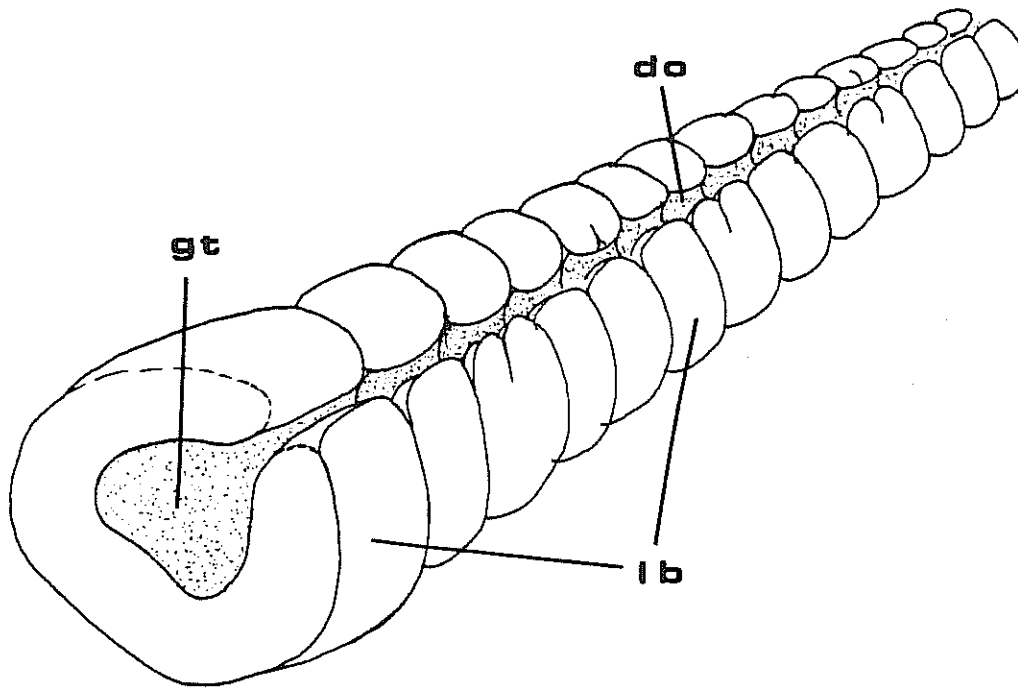
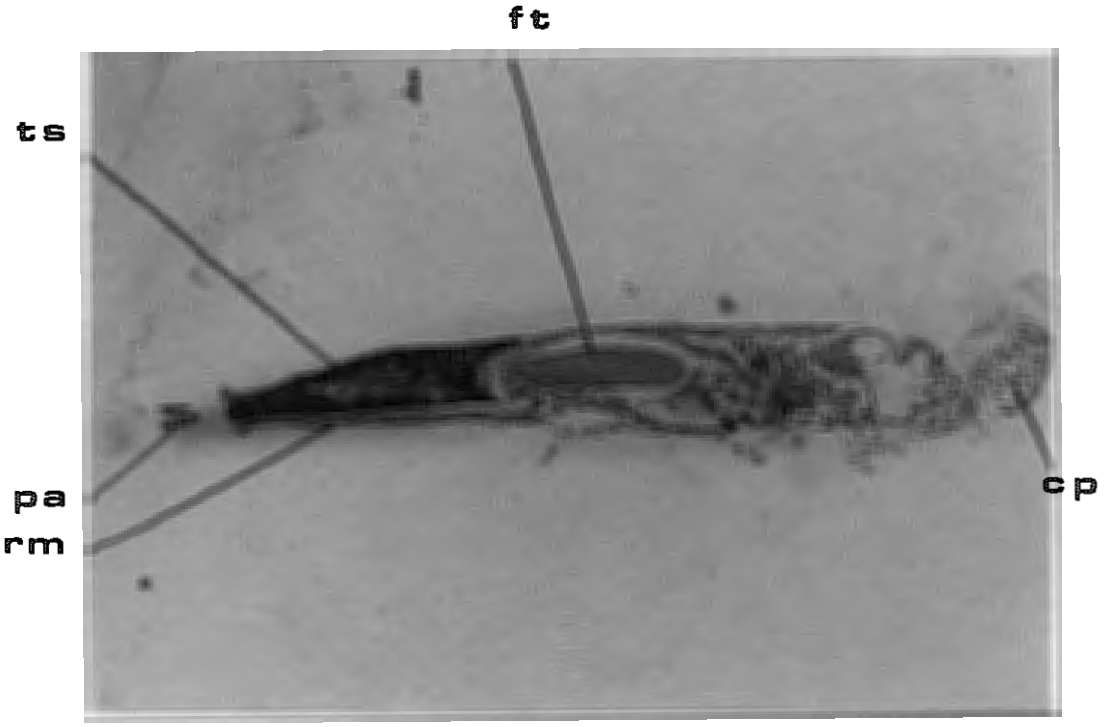

do
gt
lb

Plate V.

Longitudinal median section of a whole male *Cadulus fusiformis*. Total length is 11 mm.

cp... captacula
ft... foot
pa... posterior appendix
rm... retractor muscle
ts... testis


ft
ts
pa
rm
cp

outlining membranes. In addition to curving around dorsally, the lobes also lean forward, with the dorsal part of a lobe being more anterior than the ventral. This leaning is most pronounced in the anterior part of the organ, and as one moves posteriorly it becomes less noticeable. There is an extension of the digestive gland that occupies much of the space within the "tube" formed by the curving of the testis. Digestive gland material may be found nearly as far as the apex in some individuals but more commonly only proceeds about half the length of the testis.

Mature spermatozoa line up head to tail on small branches of the testis ducts that ramify throughout the cores of the testis lobes (Plate VI, VII, and VIII). These "feeder" ducts connect with larger ducts that presumably combine with a central gonoduct that leads to the nephridial gland. Some observations indicate that this gonoduct is probably closely associated with the the retractor muscle (Plate VIII), although the degree of association between the two has yet to be thoroughly invesigated. Material that appears to be gonoduct has been observed connecting to the right nephridial gland in specimens collected in May 1985. Whether the connection is present and functional during other months is unknown.

Some results indicate that this gonoduct stains green with Gomori's trichrome, suggesting tissue of a

Plate VI.
Idealized mid-testis cross section showing relative sizes and positions of important structures.
bm... outer body membrane
gt... central gonad "tube"
rm... retractor muscle
td... testis "feeder" ducts
tm... testis membrane

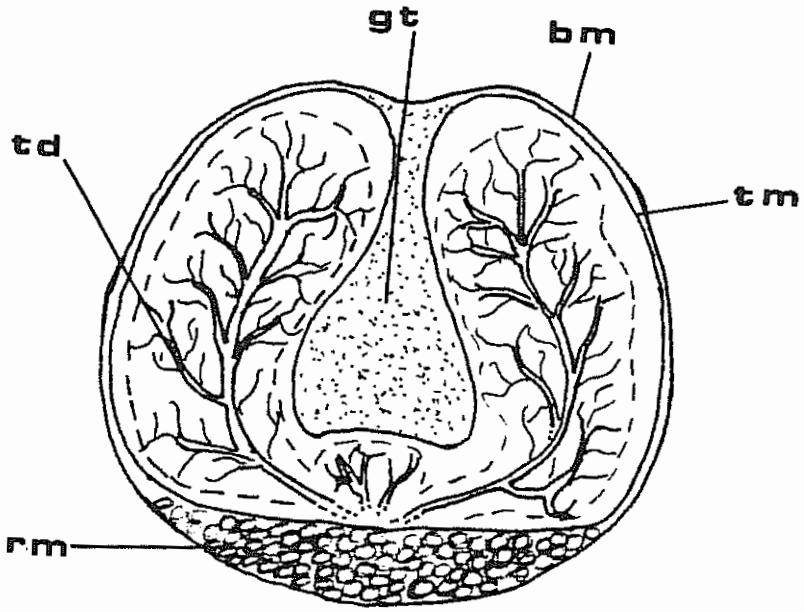

gt
bm
td
tm
rm

Plate VII. Cadulus fusiformis

Longitudinal section of a moderately mature testis. Maturing spermatids are found in the dark blue areas near the center of each lobe and the ventral testis base. Lighter grey areas near the periphery of the organ are where the primary spematocytes and spermatogonia are found. The pink-staining ductile tissue can be seen throughout the organ. Note the gonad "tube" formed by the testis lobes. The space between the testis and the retractor muscle is an artifact of cutting. Haematoxylin and Eosin, magnification is 200x.

dt... ductile tissue
gt... central gonad "tube"
im... inter-lobe membranes
rm... retractor muscle
sd... spermatids
sg... spermatogonia

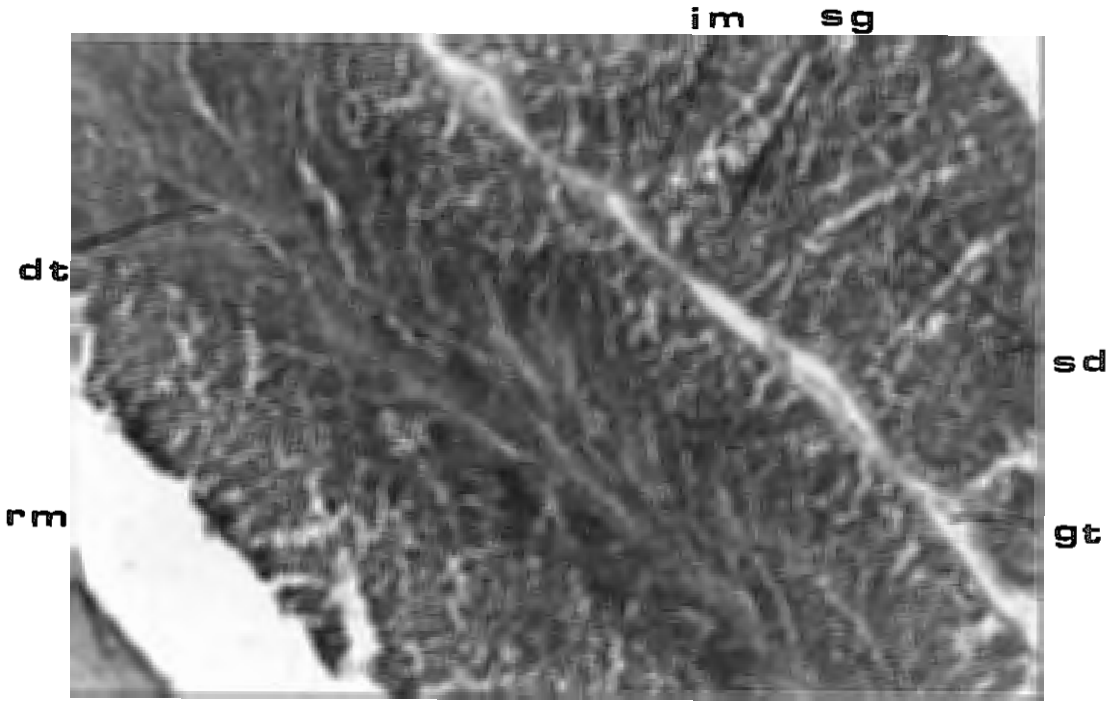
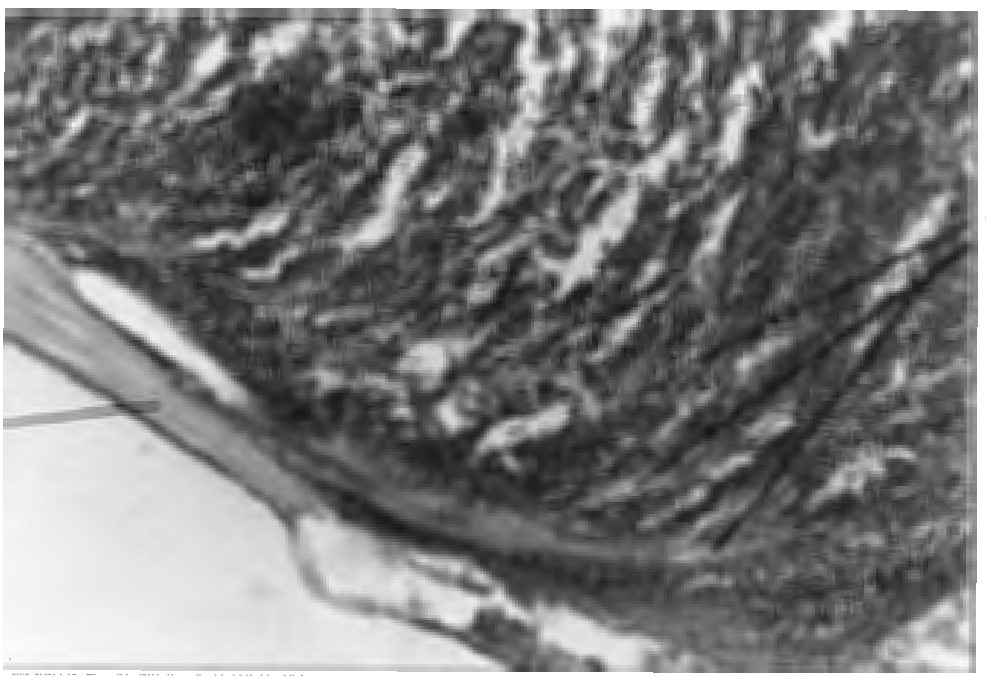

im
sg
dt
sd
rm
gt

Plate VIII. Cadulus fusiformis

Note the relationship between the pink-staining testis ductile tissue and the retractor muscle. Two clear junctures can be seen, one is quite massive. The sperm are very closely associated with the pink-staining tissue, and at higher magnification can be seen to line up head to tail descending down along it. Haematoxylin and Eosin, magnification is 200x.
dt... ductile tissue
rm... retractor muscle


rm
dt

collagenous nature (U.S. Armed Forces Institute of Pathology, 1960). Unfortunately, this hypothesis must remain somewhat in doubt as the staining procedures and results in this particular trial were not of the highest quality. Much of the connecting ductwork that leads sperm to this gonoduct also appears green in this preparation, especially in the anterior and ventral parts of the gonad. As one proceeds more posteriorly and dorsally (further away from the opening) this ductwork stains red, suggesting the presence of muscular tissue. The posterior ductwork connects directly to the retractor muscle and arises directly from it. The retractor muscle also stains an identical shade of red. In very mature specimens that have recently ejected sperm, the posterior parts of the testis are empty of sperm (which stain red/purple) and this ductwork can be seen very clearly.

These observations suggest that the accessory ducting in the male gonads is muscularized at some distance from the gonoduct opening and leads to a green staining, collagenous gonoduct that connects to the nephridial gland. Presumably sperm escape through the nephridal coelomoducts and are carried out the posterior apex with the efferent water current.

In all three species the spermatogonia and primary spermatocytes occupy positions near the

membranes at the surface of each testis lobe. This layer is usually four to seven cells thick. Moving inwards towards the core of one lobe, one finds a layer of secondary spermatocytes that stain a lighter blue-purple than the primary spermatocytes. Even more inwardly located are the spermatids, differentiating spermatozoa, and mature sperm. These cells all stain quite similarly and can only be separated on the basis of shape. The core of the lobe is infiltrated by numerous branches of "feeder" ductile tissue that move the sperm to the branches of the main gonoduct. The overall organization of the different layers of cell types could be summarized as two concentric, hollow cylinders, the outermost made of spermatogonia and primary spermatocytes, and the inner of secondary spermatocytes. The one fits just inside the other, and both surround a central core of spermatids, spermatozoa, and ductile tissue.

The sperm themselves are typical of simple molluscan sperm (Franzen 1955). The sperm of C. fusiformis and S. quadrifissatum are essentially identical and appear to lack acrosomes. The sperm of D. rectius are somewhat different in that they are larger than the cells of the other two species, have acrosomes, more prominent mitochondria, an they are produced in immensely greater quantities due to the larger organ that forms them.

OVARY

The ovary occupies approximately the same percentage volume and the same position in the body in both *C. fusiformis* and *S. quadrifissatum* as does the testis (Plates IX and X). The ovary's yellowish color and grainy appearance make it discernable from the testis. However, occasionally specimens of *C. fusiformis* and *S. quadrifissatum* may be dredged that have bright pink gonads. This color is quite easy to see through the thin *S. quadrifissatum* shell, but can also be seen even through the more robust shell of *C. fusiformis*. Five specimens of *C. fusiformis* from May 1985 that had pink gonads were sectioned and all of these were extremely mature individuals with enormous numbers of very mature yolk-packed oocytes. These numbers were so overwhelming that no other stages of eggs in development could be seen. Even though Shimek (personal communication) has suggested that the pink colored gonad can be used as a diagnostic trait in keying out various scaphopods, it appears that this coloration may only be indicative of the relative state of maturity in some females.

The overall shape of the ovary is the same as that of the testis, but the manner in which it is partitioned is somewhat different. Instead of membranes separating the organ into a few relatively large lobes,

Plate IX.

Longitudinal median section of a whole female *Cadulus fusiformis*. Total length is 10mm.

cp... captacula
ft... foot
mf... mantle fringe
ov... ovary
pa... posterior appendix

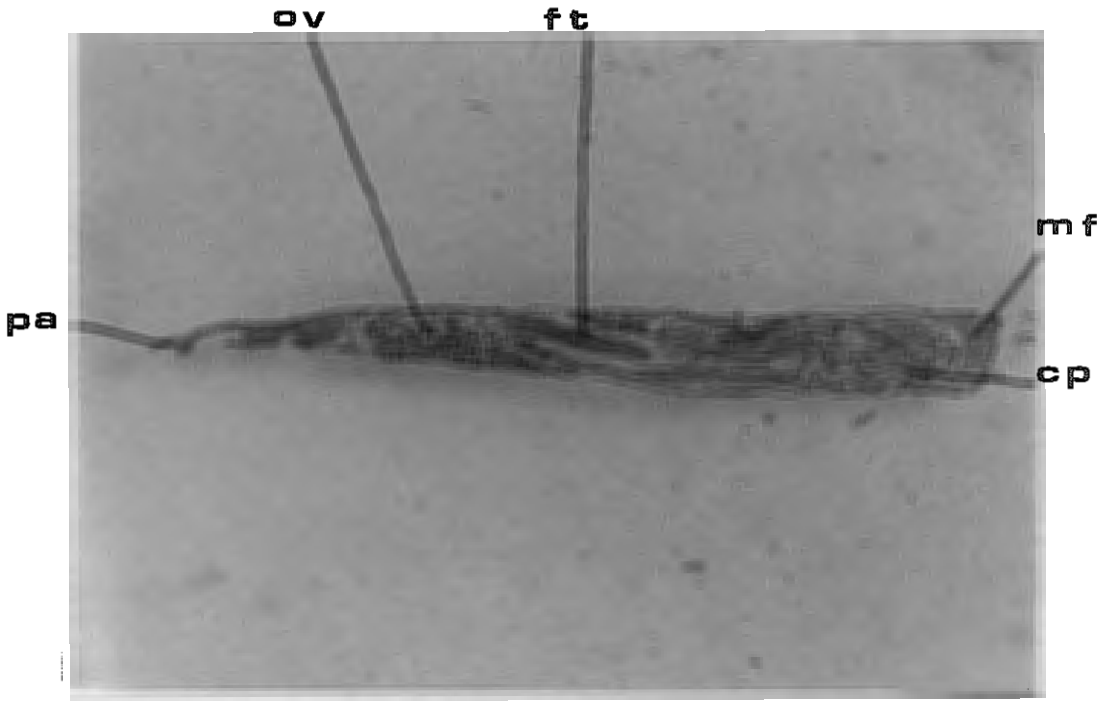

ov
ft
mf
pa
cp

Plate X.

General surface anatomy of a female scaphopod with the shell removed. Right: whole animal, dorsal view. Left: ventral view with digestive gland peeled away from body and mantle musculature sliced and moved. From Deshayes (1825).

dg... digestive gland
ft... foot
mn... mantle
mo... mouth
ov... ovary
pa... posterior appendix
rm... retractor muscle

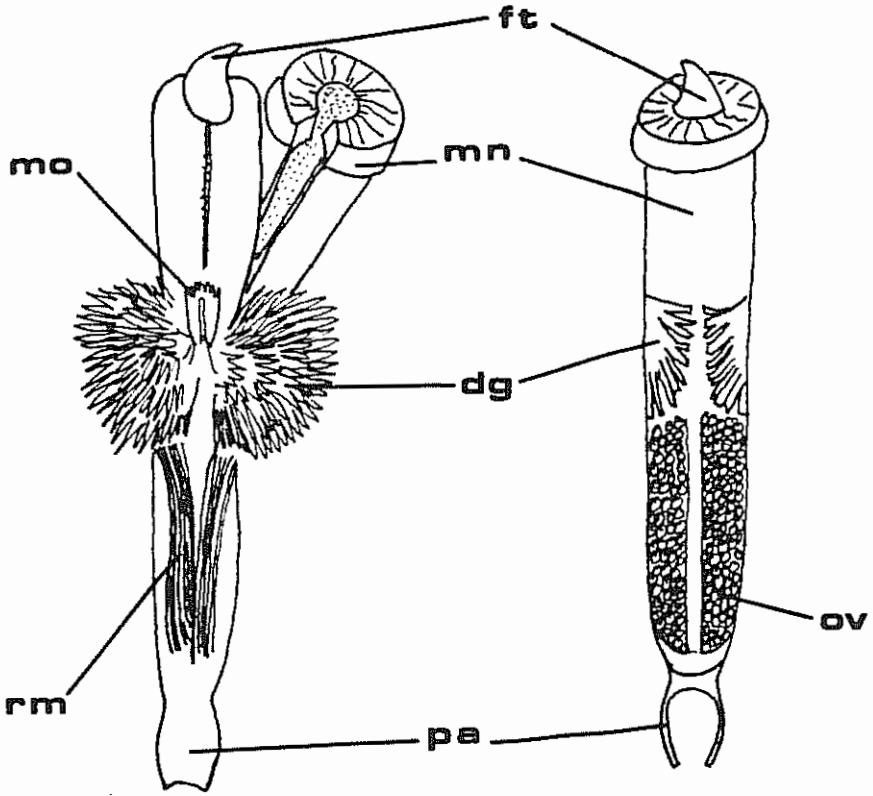

ft
mo
mn
dg
ov
rm
pe

Plate XI.

Generalized gross form of a Cadulus fusiformis ovary.

do... dorsal opening
gt... central gonad "tube" that forms the oviduct
vs... vesicles in which oocyte development takes place

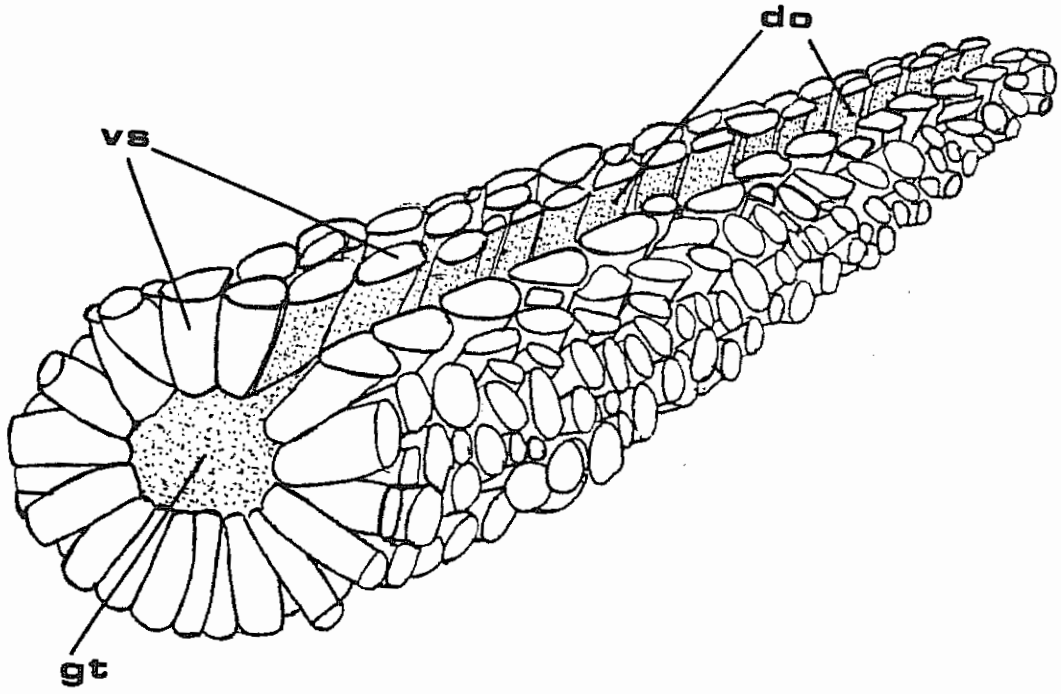
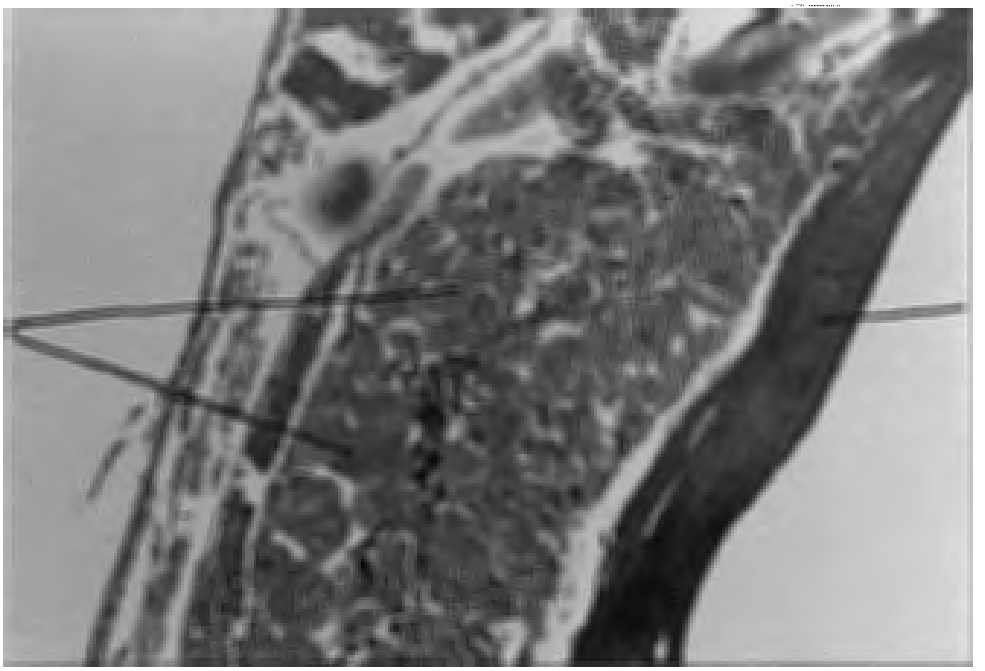

do
vs
gt

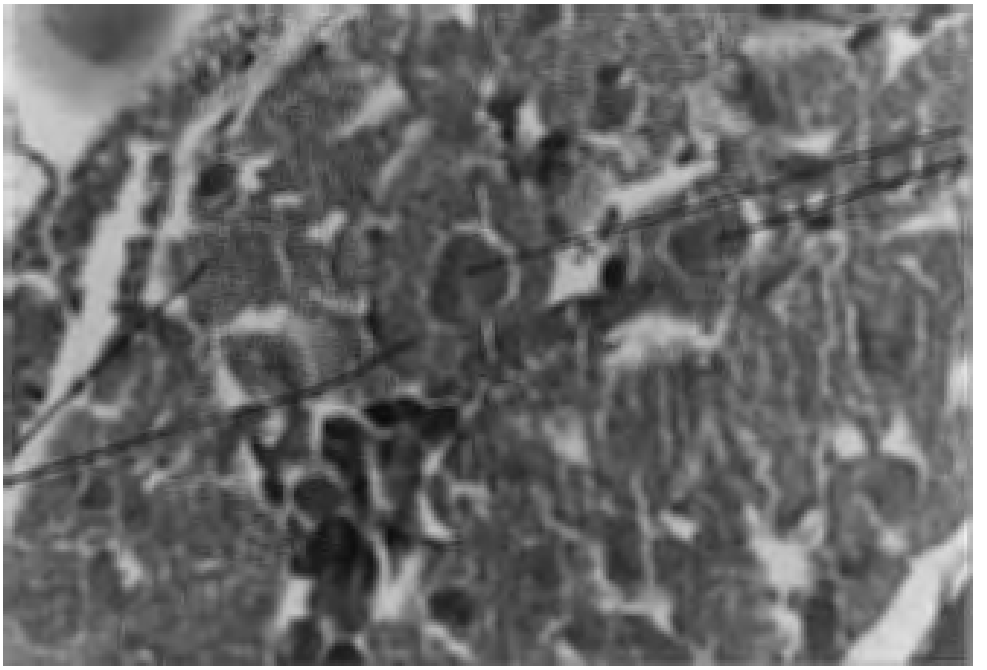
Plate XII. Cadulus fusiformis

Note the quantity of pink-staining eggs in this mature ovary. Some clusters of oocytes at the left are still attatched to the lining vesicle membrane. Space between retractor muscle and ovary is an artifact of cutting. Gomori's Trichrome, magnification is 100x.
ov... ovary
rm... retractor muscle

Plate XIII. Cadulus fusiformis

A closer view of the above section. The large oocyte in the center of the photo is approximately 300 micrometers in diameter. Nucleoli have all dispersed and these oocytes are all in stage IV. Gomori's Trichrome, magnification is 200x.
nu... nucleus
yk... yolk granules


ov
rm


nu
yk

delicate membranes enclose very numerous small vesicles (Plate XI). These vesicles are oriented perpendicular to the long axis of the body and are roughly cylindrical in shape, with the ends of the cylinder at the inner and outer surfaces of the organ. Oocyte development takes place within the vesicles.

Ovaries may contain anywhere from hundreds of maturing eggs to none, but an average would be 50-75 eggs. Oocytes do not develop synchronously throughout the gonad (Arvy 1950) and examples of eggs in all stages of development is the norm during most months. Mature oocytes of all three species are about 300um in diameter. Each contains a nucleus, the nucleolus having dispersed, surrounded by a membrane that encloses a cytoplasm that is packed with lipid-rich yolk grains. These yolk particles color very strongly pink with the two staining procedures used, and this makes the mature ovary quite easy to identify (Plates XII and XIII).

Developing eggs can be separated into four relatively distinct stages. The first is the oogonium (Stage I). This cell is quite small, about 5 um. in diameter, only a tiny fraction of the size of the mature oocyte. When subjected to haematoxylin and eosin staining its cytoplasm appears dark purple, its nucleus quite a bit lighter, and the nucleolus about the same hue as the cytoplasm. The oogonium is attached strongly

to the membranes that line the individual vesicles of the ovary. The second stage is the pre-vitellogenic primary oocyte (Stage II). At this stage the developing oocyte has greatly enlarged, up to as much as 200 um., but not yet to the size of a fully mature egg (Plate XIV). The nucleus is clearer and is slightly grainy in appearance. The nucleolus has expanded in size along with the rest of the cell and still stains strongly purple with hematoxylin and eosin. The third stage is the vitellogenic primary oocyte (Stage III). This cell is nearly full size and the nucleolus may have begun to disperse (Plates XIV and XV). After the nucleolus has dispersed the oocyte enters Stage IV and has completed prophase of meiosis I. From this stage through the second meiotic division no change in appearance can be noted, and the polar bodies themselves were actually never observed. It is possible that meiosis II occurs immediately after meiosis I so that it is only rarely that an oocyte may be observed between stages. It is also possible that meiosis II may actually occur after fertilization. Finally the mature oocyte has a fully packed cytoplasm, has reached the full size of about 300 um. in diameter, and has no visible nucleolus (Plates XII and XIII).

Throughout the process of maturation the cell becomes progressively less attached to the vesicular

Plate XIV. Cadulus fusiformis

Note the several stages of oocyte development, and the exit pore at the apex. This female is not very mature, few well developed oocytes are present. Note the nucleus and nucleolus in the previtellogenic oocyte, and the vesicle membranes at the lower left. Gomori's Trichrome, magnification is 200x.

ep... exit pore
od... oviduct
pa... posterior appendix
rm... retractor muscle
I ... stage I oogonia
II... stage II previtellogenic oocyte
III.. stage III vitellogenic oocyte with yolk
IV... stage IV mature oocyte

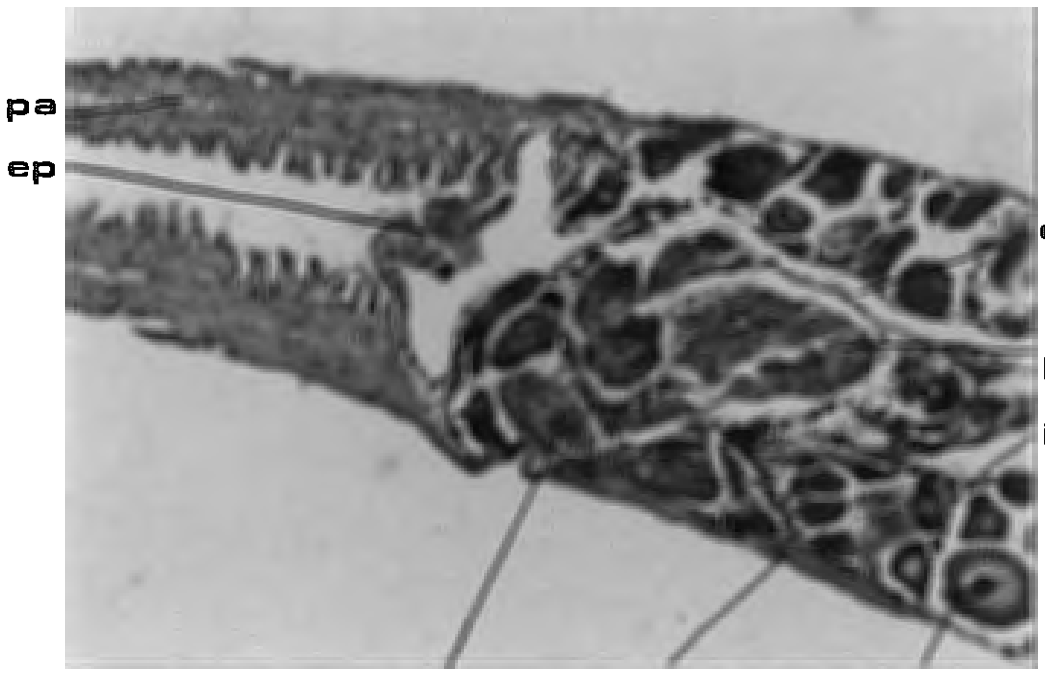
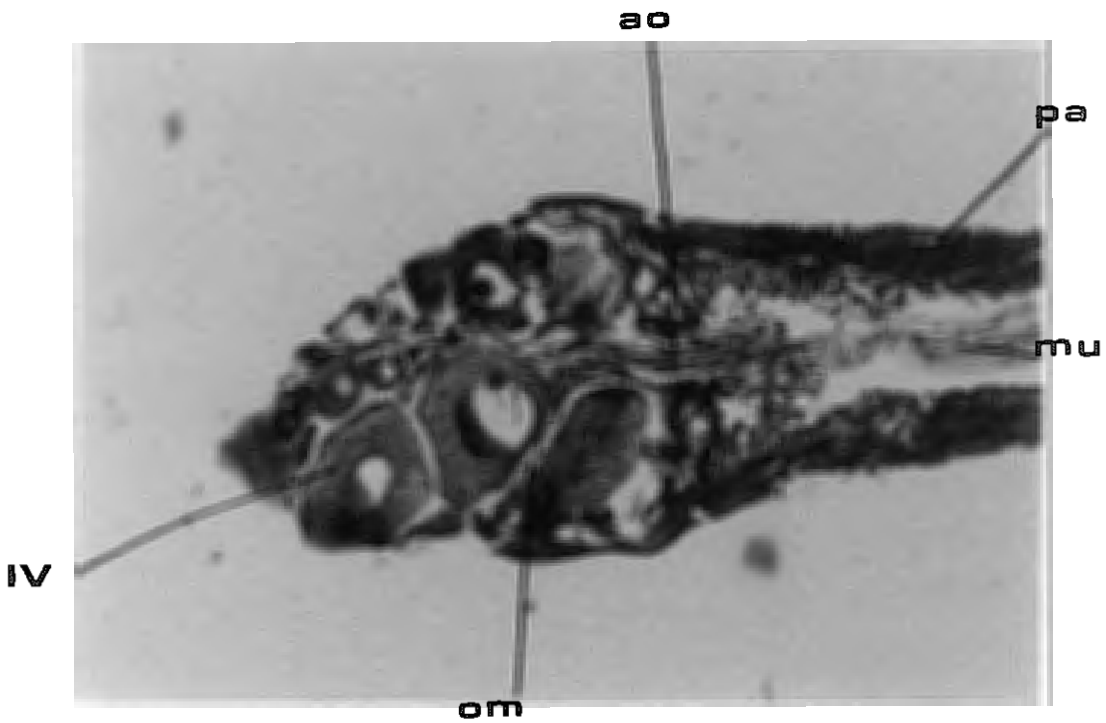

pa
ep
od
IV
I
III
rm
II

Plate XV. Cadulus fusiformis

This specimen is oriented such that only a small section of the apex is cut in this pass. Note the mature eggs approaching the apex exit pore where a stream of blue-staining mucus will envelop them in the fold of the posterior appendix. The apex opening has enlarged in relation to plate XIV. Gomori's Trichrome, magnification is 200x.

ao... apex exit pore
mu... mucus ribbon
om... ovary vesicle membrane
pa... posterior appendix
IV... mature oocytes


ao
pa
mu
IV
om

membrane (Arvy 1950), finally becoming free floating within the vesicle. Then the epithelium enclosing the vesicle becomes quite thin and finally dissolves, releasing the oocytes into the central gonoduct.

The gonoduct occupies the "tube" of the central cavity formed by the ovary's overall structure. It connects with the outside through an opening at the apex that is closed by a muscular sphincter (Plates XIV and XV). The gonoduct does not lead forward into a nephridial gland as has been reported for Dentalium entalis (Lacaze-Duthiers, 1857) or for Dentalium tarentinum (Fol 1889), although the anterior portion may be somewhat enlarged and serve as a holding area for mature, released eggs waiting to be ejected.

Near the inner edges of the sphincter in a very few individuals of C. fusiformis are structures that stain bright red with Gomori's trichrome. They are clearly not muscle tissue, and although they appear to be glandular, their function is actually unknown. They are only present in females that are most thoroughly filled with mature oocytes.

The gonoduct's diameter decreases as oocytes mature and take up more space in the ovary. It is still large and clear, however, in contrast to the male gonoduct. There is no muscular tissue associated with the duct and therefore oocytes are most probably moved posteriorly

along the duct by ciliary action, although this has not been observed.

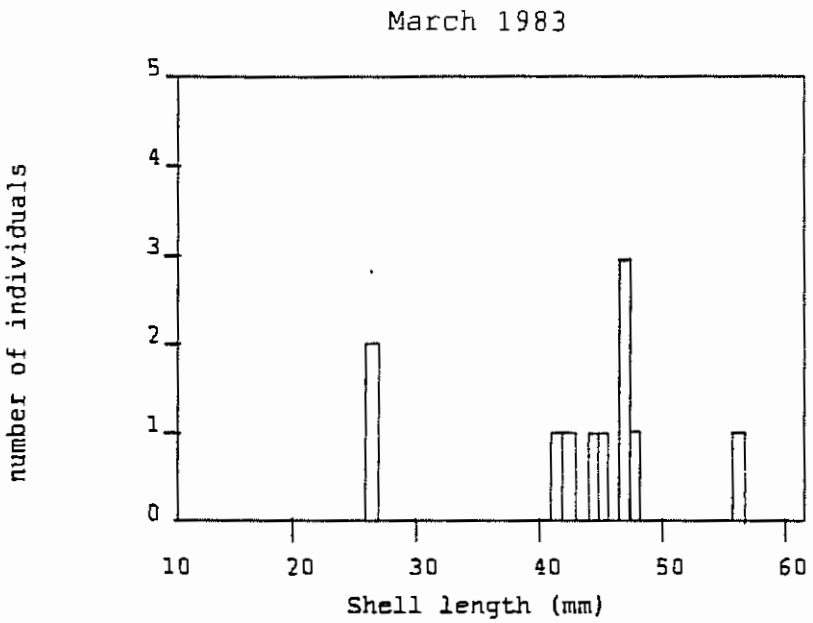
Finally, it appears clear that the relative size of the ovary is no indication of the condition of maturity of the organ. For proper determination of maturity in the these species the animals must be dissected or sectioned and stained.

POPULATION DYNAMICS AND THE SEASONALITY OF REPRODUCTIVE ACTIVITY

Although dredges were taken at the sampling station for a period of two years, circumstances did not allow all species to be followed for the entire period. In the size distribution graphs all replicates from each month are combined (Figs. 1-26). Data are not presented for months when less than five individuals of a species were found even though some of those animals may have been used for histological study.

The size distribution graphs for *D. rectius* (Figs. 1-5) show that *D. rectius* was found in substantial numbers only from March 1983 to July 1983. After that time essentially all traces of this species disappeared from the sample site, including empty shells. In light of this inadequate sampling period, *D. rectius* will not be discussed further and this section will focus on *S. quadrifissatum* and *C. fusiformis*.

Fig. 1



Size Distribution graphs for Dentalium rectius

Fig. 2

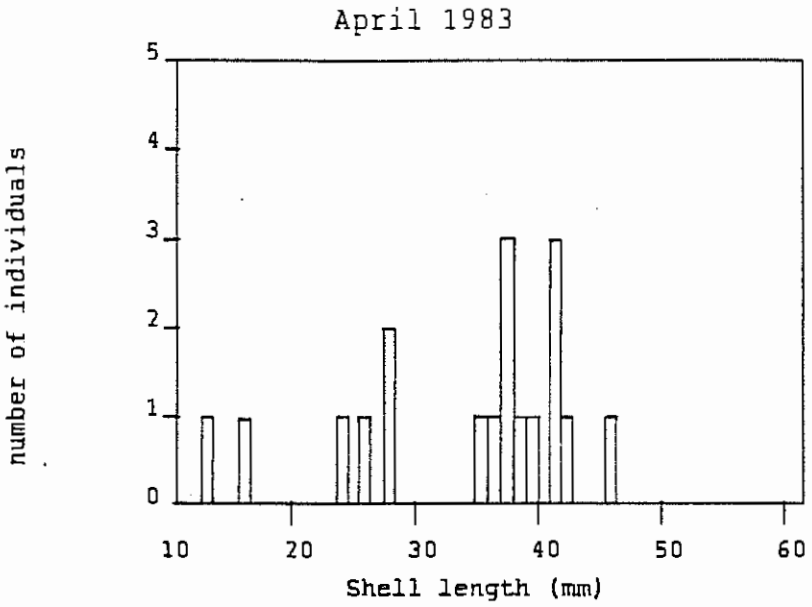
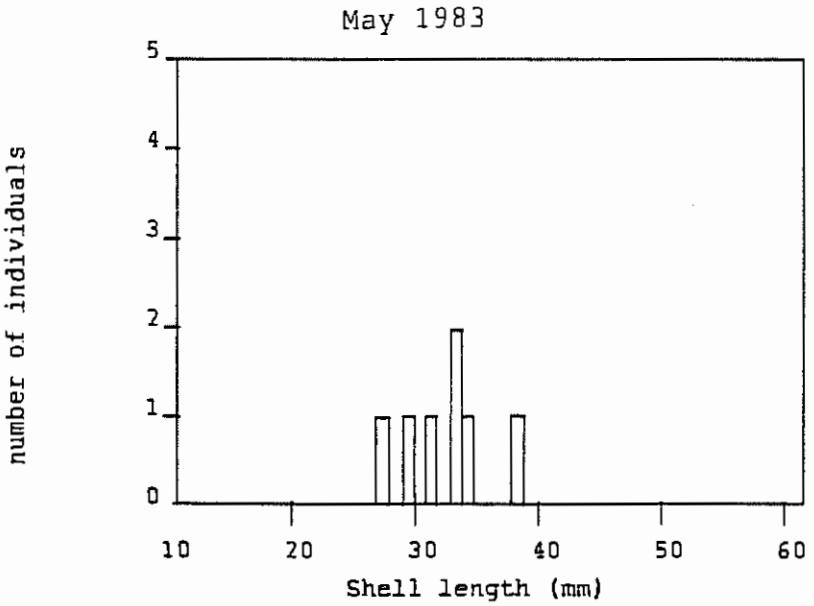


Fig. 3



Size Distribution graphs for Dentalium rectius

Fig. 4

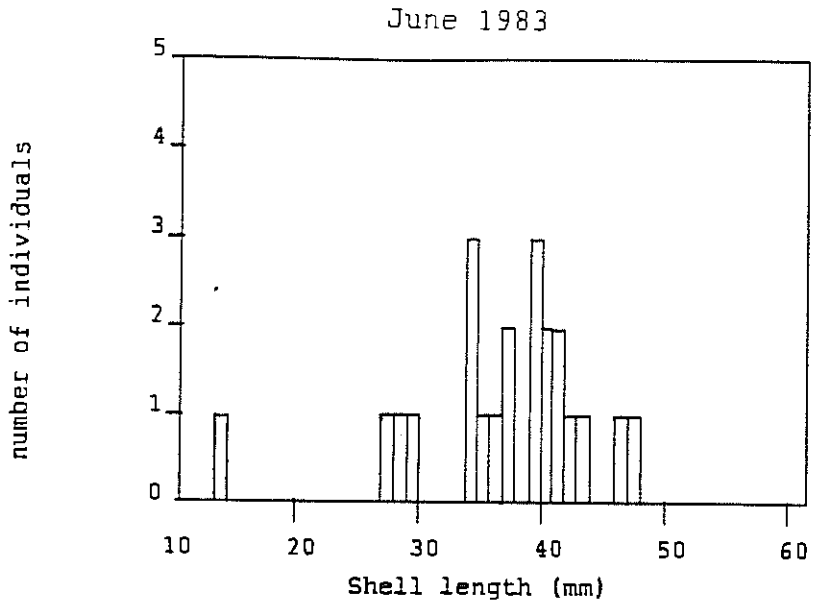
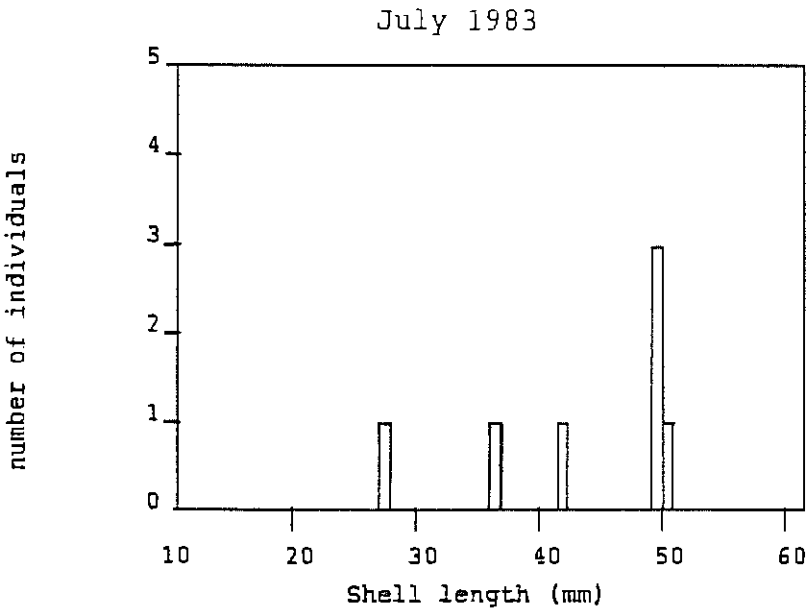
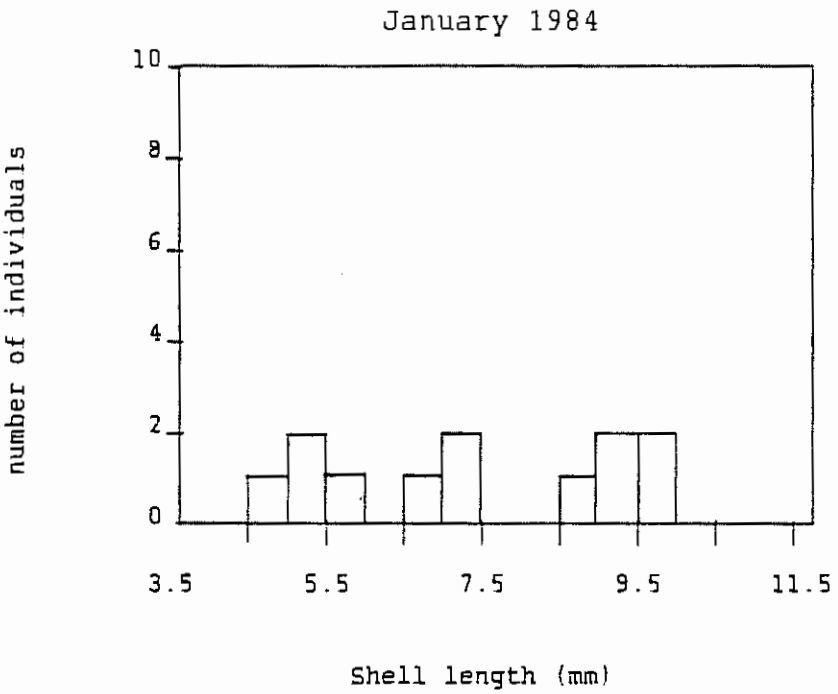


Fig. 5



Size Distribution graphs for Dentalium rectius

Fig. 6



Size Distribution Graphs for Siphonodentalium quadrifissatum

Fig. 7

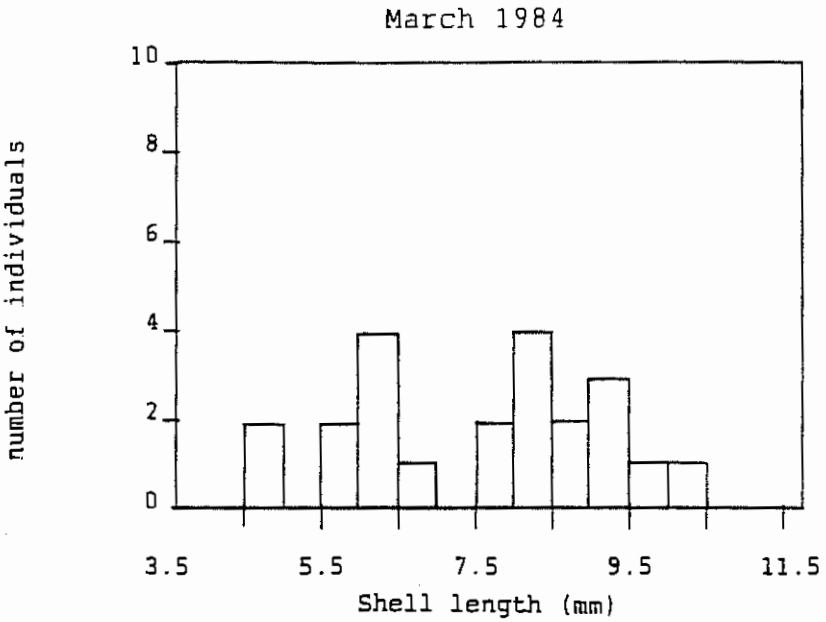
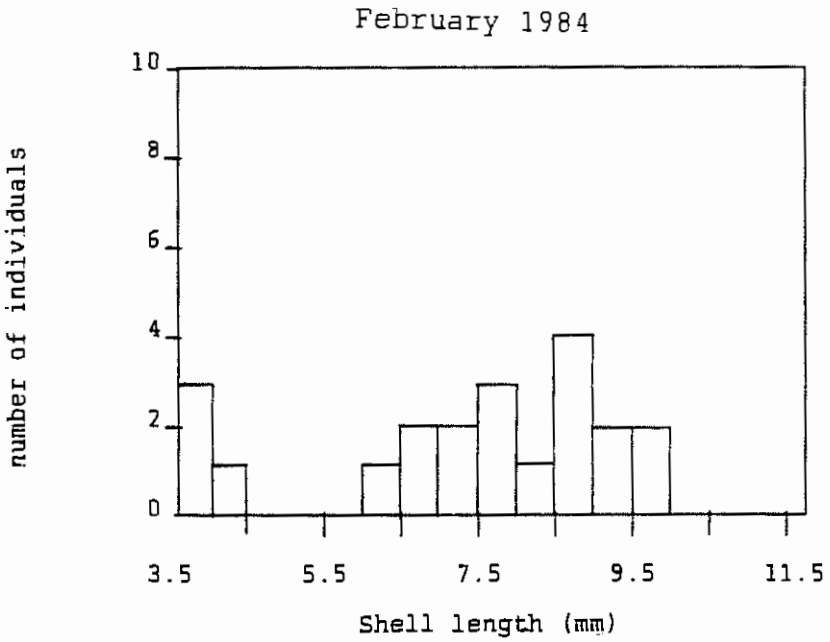


Fig. 8



Size Distribution Graphs for Siphonodentalium quadrifissatum

Fig. 9

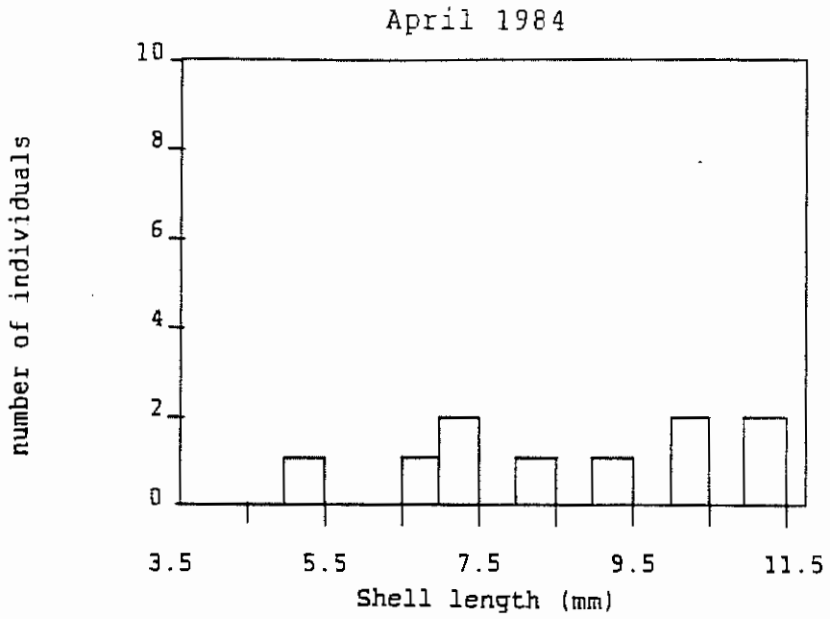
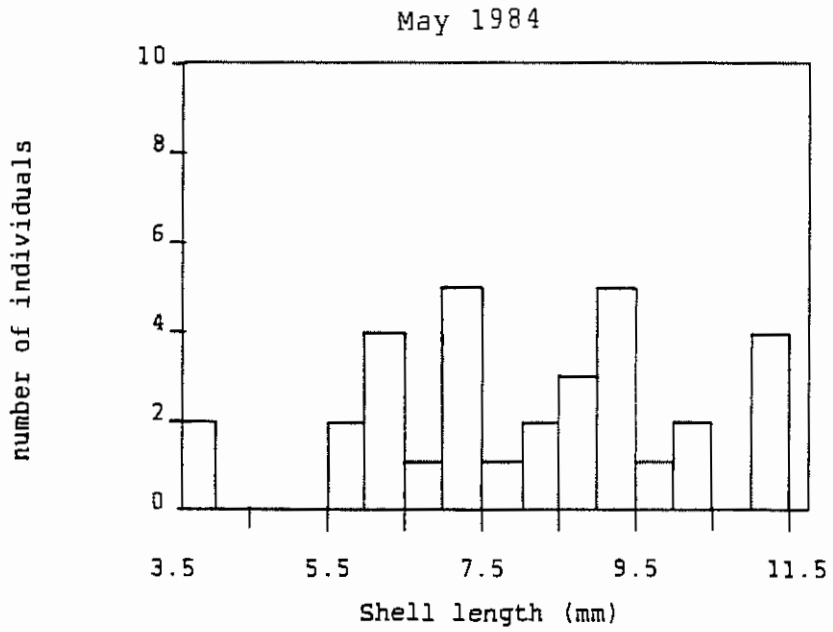


Fig. 10



Size Distribution Graphs for Siphonodentalium quadrifissatum

Fig. 11

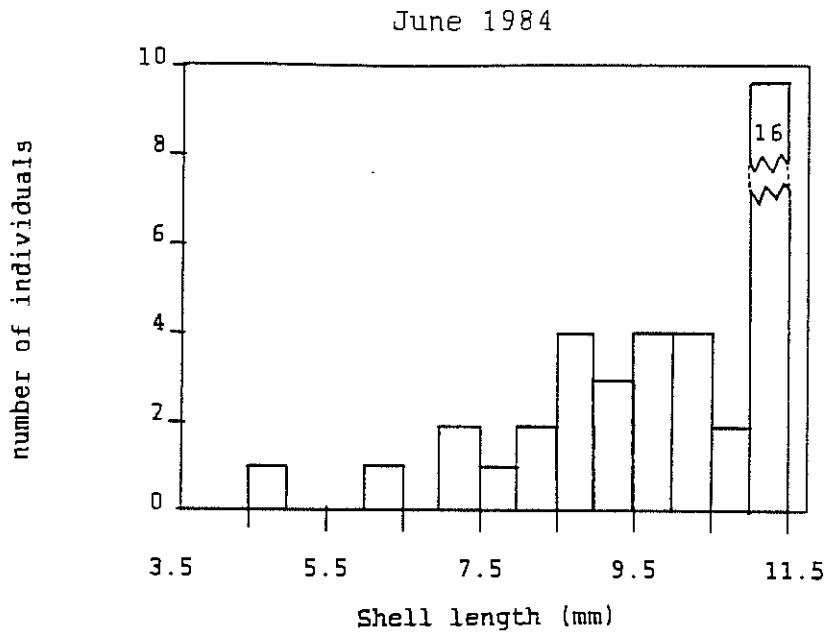
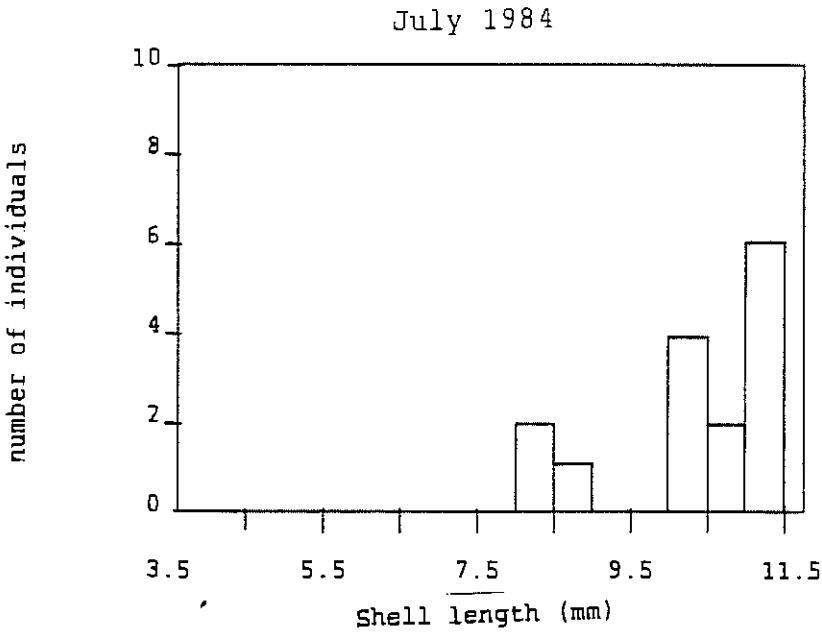
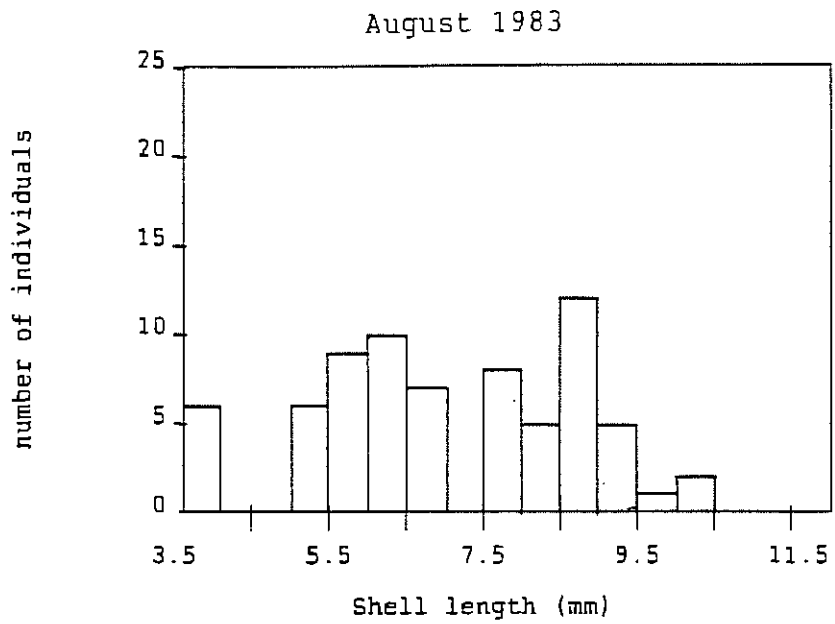


Fig. 12



Size Distribution Graphs for Siphonodentalium quadrifissatum

Fig. 13



Size Distribution graphs for Cadulus fusiformis

Fig. 14

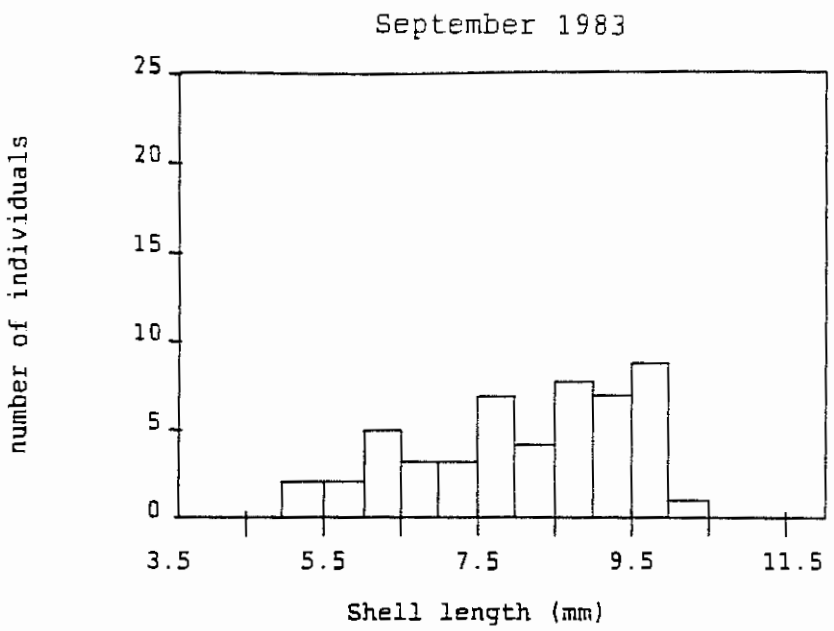
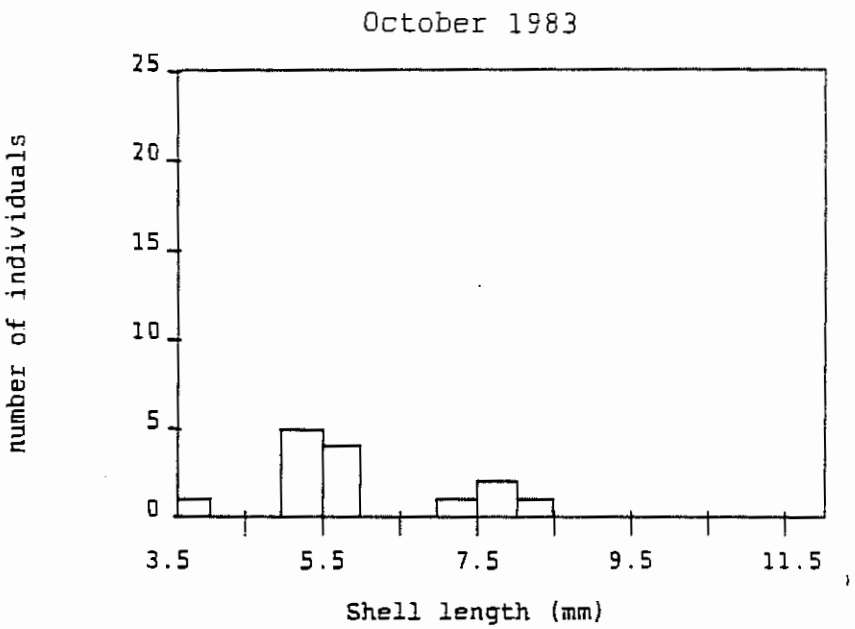


Fig. 15



Size Distribution graphs for Cadulus fusiformis

Fig. 16

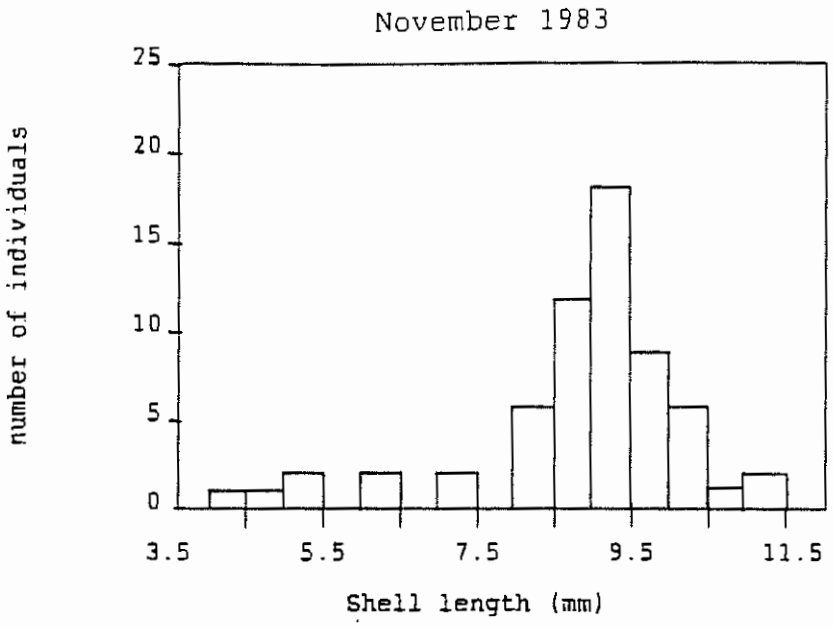
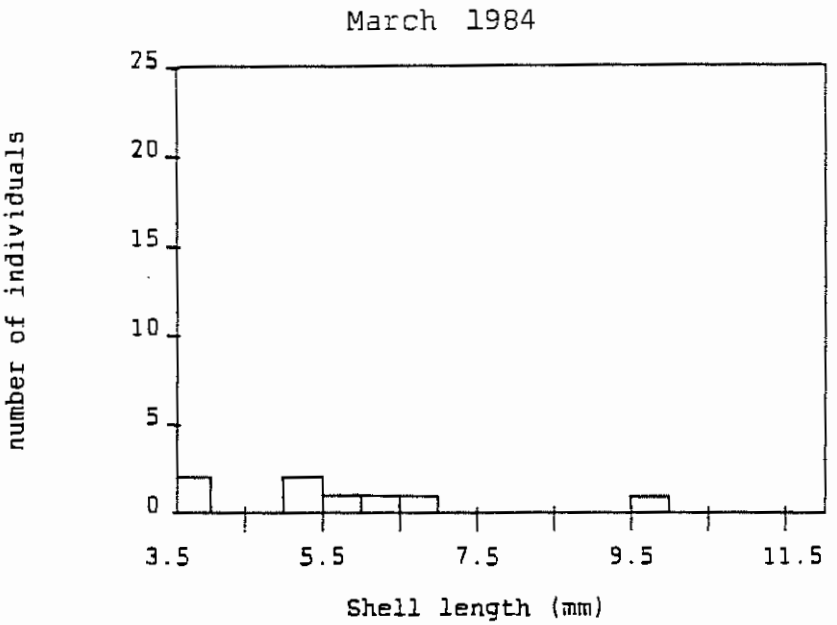


Fig. 17



Size Distribution graphs for Cadulus fusiformis

Fig. 18

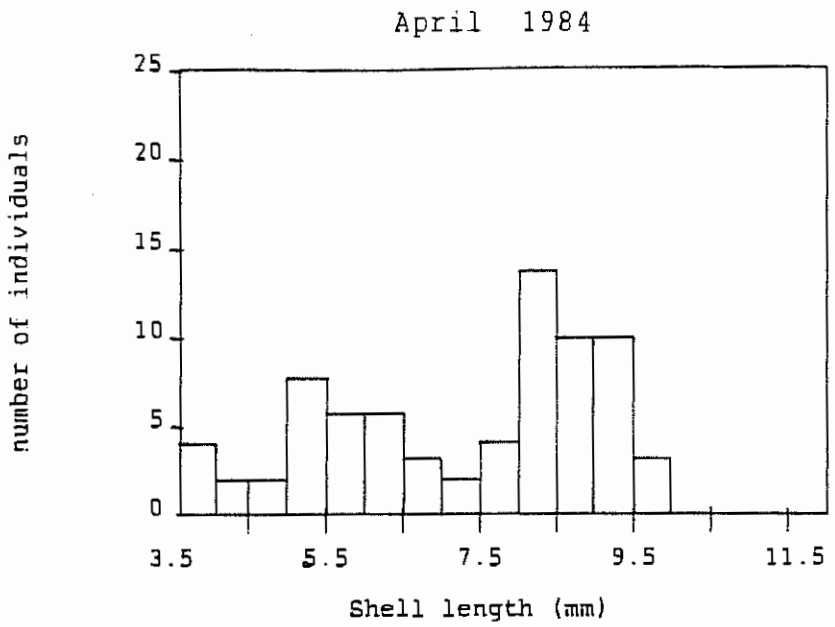
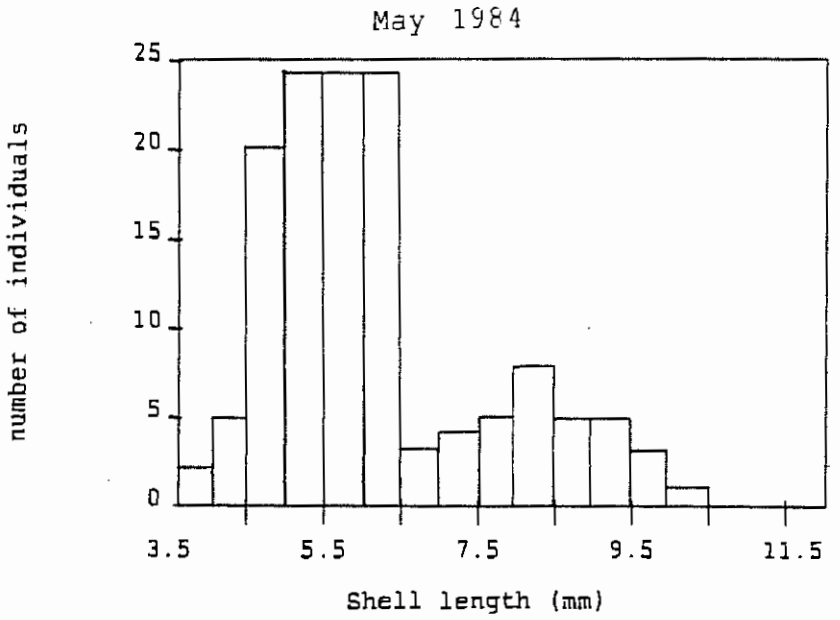


Fig. 19



Size Distribution graphs for Cadulus fusiformis

Fig. 20

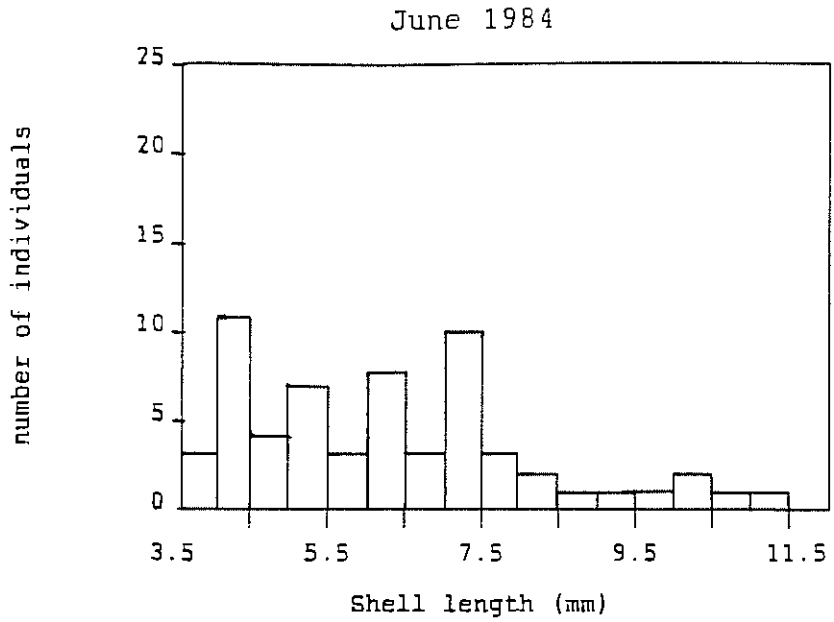
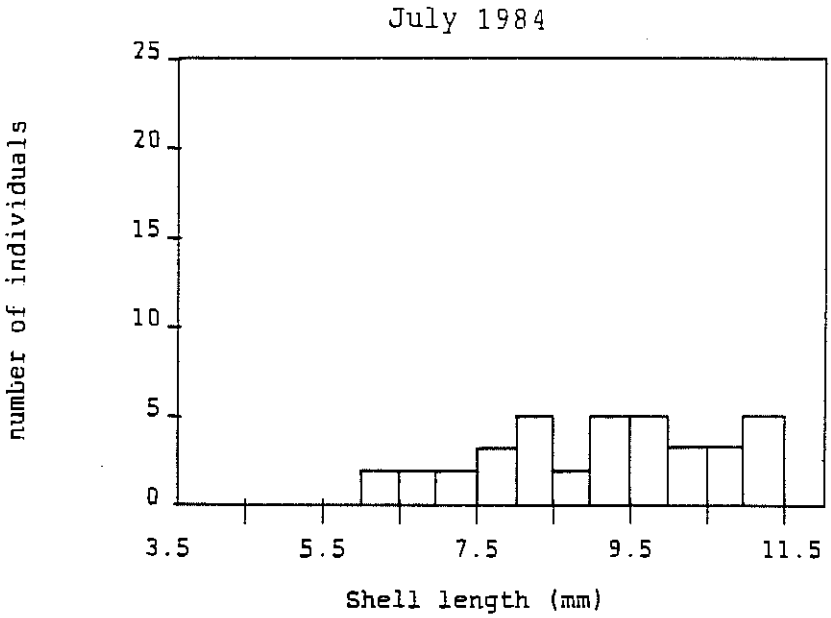


Fig. 21



Size Distribution graphs for Cadulus fusiformis

Fig. 22

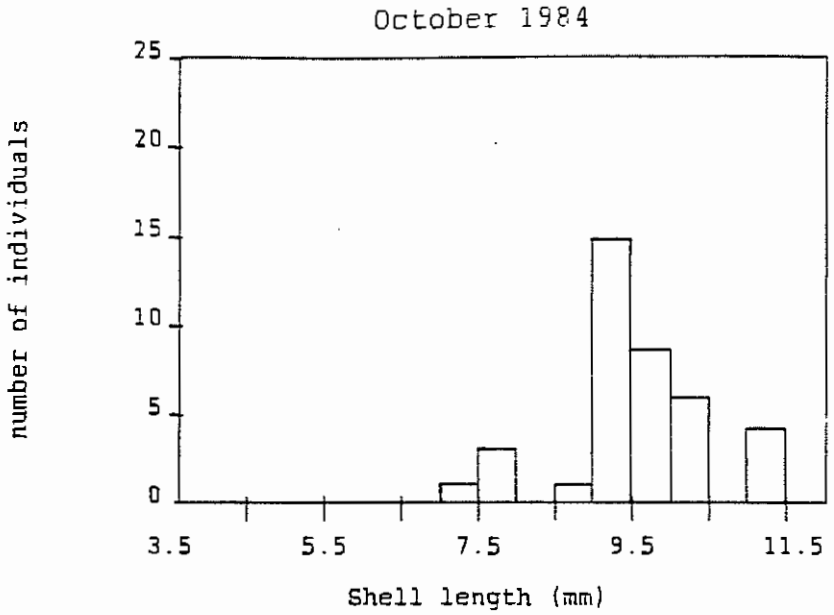
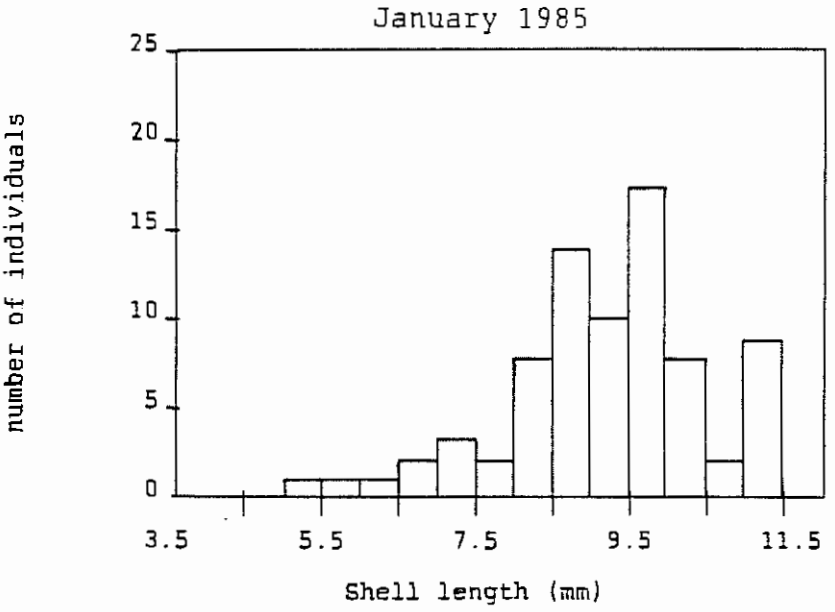


Fig. 23



Size Distribution graphs for Cadulus fusiformis

Fig. 24

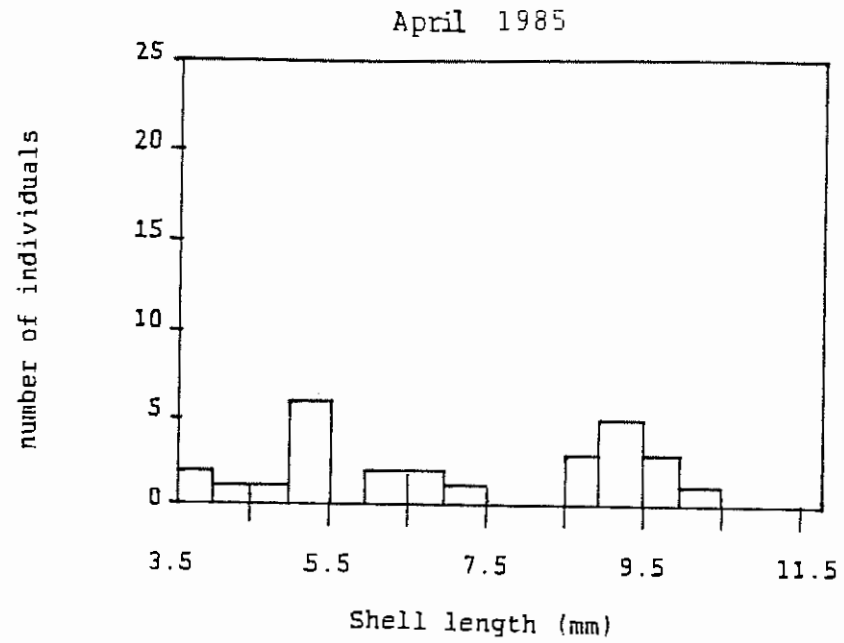
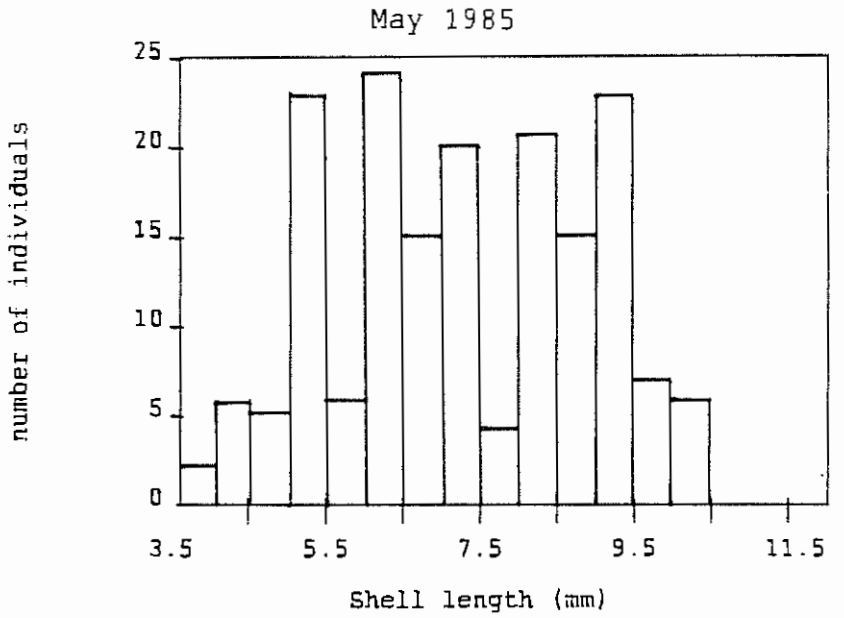


Fig. 25



Size Distribution graphs for Cadulus fusiformis

Month	Species	Females	N	Males	N
4-83	D.r.	Active	4	Mixed	3
5-83	D.r.	NA	-	NA	-
6-83	D.r.	Active	3	Active	4
7-83	C.f.	Inactive	1	Too Small	
8-83	C.f.	Mixed	4	Active	5
9-83	C.f.	Mixed	2	Mixed	4
10-83	C.f.	Active	1	Inactive	4
11-83	C.f.	Active	5	Mixed	2
12-83	-	-	-	-	-
1-84	S.-4	Mixed	2	Inactive	3
2-84	S.-4	Mixed	3	Mixed	4
3-84	S.-4	Active	5	Active	7
4-84	S.-4	Active	3	Active	3
5-84	S.-4	Mixed	4	Inactive	4
	C.f.	Active	4	Active	3
6-84	S.-4	Inactive	2	Mixed	2
	C.f.	Active	4	Active	1
7-84	S.-4	Active	4	Active	8
	C.f.	Active	5	Active	5
10-84	C.f.	Inactive	6	Mixed	4
1-85	C.f.	Mixed	3	Mixed	3
4-85	C.f.	Mixed	4	Active	4
5-85	C.f.	Active	4	Active	4

Fig. 26: Subjective judgment of gonad activity based on numbers of gametes, developmental stage, and empty space in the gonad. Mixed refers to varying activity levels in different gonads from one month.
C.f. ...Cadulus fusiformis
S.-4 ...Siphonodentalium quadrifissatum
D.r. ...Dentalium rectius

Month	Species	Egg Ratios	Month	Species	Egg Ratios
4-83	D.r.	too many to count	4-84	S.-4	.40 .28
				C.f.	.35 .74
5-83	D.r.	N.A.	5-84	S.-4	.30 .22
					.48 .55
6-83	D.r.	too many to count		C.f.	.53 .15
					.03 .92
7-83	C.f.	.37	6-84	S.-4	.38 .42
8-83	C.f.	.24 .29		C.f.	.58 .79
		.43 .26			
9-83	C.f.	0.0 .38	7-84	S.-4	.69 .52
					.59 .28
10-83	C.f.	.42		C.f.	.52 .51
					.30 .23
11-83	C.f.	.39 .70			.20 .59
		.58 .34			
		.52	10-84	C.f.	.47 .40
					.17 .22
12-83	-	-			
			1-85	C.f.	.55 .29
1-84	S.-4	.44 .38			
			4-85	C.f.	.19 .30
2-84	S.-4	.17 .51			.23 .17
		.32			
			5-85	C.f.	.53 .68
3-84	S.-4	.18 .27			.53 .92
		.43 .55			.97 .90
		.54			.90 .95

Fig. 27: Ratios of Stage III plus Stage IV oocytes to the total number of oocytes and oogonia in each ovary. This number provides a quantitative measurement of ovarian activity, with either very high or very low numbers associated with reproductive activity. Each number in the "Egg Ratios" column refers to the ratio obtained from one female sectioned that month.
C.f. ...Cadulus fusiformis
S.-4 ...Siphonodentalium quadrifissatum
D.r. ...Dentalium rectius

Cadulus fusiformis was initially found in small numbers in June 1983 and persisted at the sampling site until January 1984 (Figs. 13-25). In February 1984 very large numbers of dead C. fusiformis shells were gathered along with a new population of S. quadrifissatum replete with many full grown individuals (Figs. 6-12). In March 1984 a few C. fusiformis were found along with the S. quadrifissatum and by April 1984 a mature population of C. fusiformis was co-existing with the S. quadrifissatum. This condition lasted until the end of the monthly sampling regime in July 1984.

More samples were taken at the site in October 1984 and January and April 1985. The same sampling apparatus was used, but on a much larger ship without the author present. The author received these samples at a later date. In May 1985 the author returned to the site and sampled again with the standard apparatus and original boat. In all of these later samples C. fusiformis was essentially the only scaphopod present.

In the entire two years spent sampling the only other species found was Dentalium neohexagonum. This species is apparently somewhat rare at the sampling site since only three live animals were taken over the two year period. Dead shells were found in several months, however.

There are several things one may notice from the size distribution graphs. 1) There are small, possibly recently settled individuals about 3.5 to 4.5 mm in length present in the samples in almost all months. The microscopic examinations reveal that sexual maturity is reached at about 5mm. in S. quadrifissatum and C. fusiformis. These two facts strongly suggest that spawning is a year-round process in the two species. 2) There are large numbers of small C. fusiformis that recruit into the population during June and July 1983, April, May, and June 1984, and April and May 1985. 3) There are bimodal distributions in August 1983, April and May 1983, and April 1985. These months correspond to months of high recruitment of small individuals. This bimodal distribution is not a permanent attribute of the population.

It is most informative to compare these points to the results of microscopic examination of the gonads. Figures 26 and 27 present the results of the examination. Figure 27 presents the number of individuals of each species sectioned each month that were retained in a satisfactory enough condition to allow subjective judgements of the stage of development of their gonads. Figure 27 presents a quantitative measurement of ovarian activity by recording the ratios of Stage III and IV oocytes to the total number of

developing oocytes in any stage. Individuals with high numbers in Figure 27 are well packed with very mature oocytes. Individuals with very low numbers have almost no mature oocytes and may have recently released their eggs. In some cases the number of females noted in one figure does not match that noted in the other. This is because some animals partially disintegrated when being sectioned, making accurate counts of eggs impossible. In some cases, however, enough ovary was left to make a subjective judgement of activity. Thus the numerical results from one figure must be combined with the judgement from the other to form the complete picture.

Results show that S. quadrifissatum is active during the months of March and April, 1984. February, May and June are mixed or inactive months, and the population becomes active again in July.

The picture is more clear for C. fusiformis. Reproductive activity was centered in April, May, June, and July in 1984, and in April and May 1985. In the winter months the population is relatively inactive. The size distribution graphs show that a large influx of small, recently settled individuals recruited into the population during the gametogenically active months of April and May 1985.

Another trend that may be noticed is that all three species become reproductively active in the month

or two immediately prior to a drastic drop in the population count. This can be seen in D. rectius in June 1983, C. fusiformis in October and November 1984, and S. quadrifissatum in July 1984.

At no time of the year were adult scaphopods found with gonads that had absolutely no mature gametes in them. Egg numbers varied but there were always some mature or maturing eggs in the ovary. Males always had some regions of the testis that contained mature sperm. This feature of constant activity was more obvious in males than in the females. This phenomenon is, in part, what dictated combining numerical counts of egg ratios with subjective judgements of degrees of ripeness. Members of these species appear to be placed somewhere in a continuum of activity with some high points rather than in an on/off situation.

DISCUSSION

Careful review of the literature reveals only two studies that have investigated the structure of scaphopod gonads. Fol (1884 and 1889) concludes that some sort of cavity brings the mature sperm and eggs anteriorly to a point where the forward epithelium of the gonad contacts the right kidney. At this point he states that a tear or suture takes place that allows gametes to pass into the kidney and then out into the mantle cavity. Fol's work was done on Dentalium tarentinum, and he believes both sexes evacuate the sexual products through openings in the same location.

Earlier work by Lacaze-Duthiers (1857) on Dentalium entalis claims that both males and females possess an epithelium-lined gonoduct that passes gametes into the right nephridia and out the nephridiopores. This latter opinion is the currently held summary for all scaphopods. The structure of the testis in C. fusiformis and S. quadrifissatum is very like that outlined by Lacaze-Duthiers for Dentalium entalis. The major difference seems to be that in both species investigated in this study the female gonoduct evacuates eggs through the apex opening, contrary to Lacaze-Duthiers' assertion that it utilizes the same nephridiopore exit as that of the testis.

The structure of the ducts that leads mature sperm into the central gonoduct is quite interesting. As noted previously, this duct is closely associated with the retractor muscle. It stains exactly the same color as the muscle, both with Haematoxylin and Eosin as well as Gomori's trichrome. While this arrangement sounds unusual, it may serve to explain some spawning observations that have been attributed to Lacaze-Duthiers by McFadien-Carter (McFadien-Carter 1979). She reports that Lacaze-Duthiers observed sporadic clouds of sperm being ejected from the posterior apex. This might occur when contractions of the muscularized ductile tissue forced large quantities of sperm into a central gonoduct and out the nephridiopores. There they could be picked up by the efferent respiratory current and blown out the opening at the apex.

The fact that only the posterior and dorsal parts of the testis lobes are muscularized may explain why there are always mature sperm in the testis. Contractions far from the nephridiopore can only force sperm along the system so far, and since sperm are only activated after immersion in salt water (Geilenkirchen et al. 1970) some of the cells will be stranded in the anterior ducts. The analogy could be made to trying to empty a tube of toothpaste by only squeezing the end farthest from the opening.

It is interesting to note briefly how Fol (1889) described the structure of the gonads. He claimed that the gonads became swollen when mature, and where the anterior membrane of the gonad came in contact with the posterior membrane of the nephridia a tear or perforation would result, allowing gametes to pass into the kidney and out the nephridiopores. He also claimed that the structures Lacaze-Duthiers had called gonoducts were not true ducts in the sense of having a lining epithelium. The staining methods employed in this study, however, suggest that the ducts may in fact be lined with epithelium, and therefore may be true ducts in both the testis and ovary.

While there is significant agreement on the basic structure of the testis between this work and that of Lacaze-Duthiers, the same is not true of ovarian structure. The ovaries of C. fusiformis and S. quadrifissatum are radically different from what has previously been thought. In an effort to reconcile these differences several dissections of large female Dentalium vernedei from Taiwan were performed. These dissections did not reveal any ductile structure leading from the ovary to the right or left nephridial glands. Of course these animals may not have been sexually active when collected and therefore according to Lacaze-Duthiers' hypothesis would not have an oviduct.

However, the dissections, along with other observations, lead me to believe that the relative sizes of the different species of scaphopods have profound effects upon their gonad structure. In large scaphopods such as Dentalium entalis and Dentalium vernedei there are tremendous numbers of eggs that are very small in relation to the body size. While a 300um oocyte will require quite a large nephridiopore to exit from, there is room in these animals for a duct of this size to exist in addition to the central cavity . In the two small species investigated in this study the situation is quite different. The oocytes are extremely large in relation to the diameter of the posterior parts of the animal; as much as 1/3 or 1/2 of the diameter.

If an oviduct of appropriate size were to exist along with the central cavity that leads to the apex, there would be almost no room left for ovary! It would be most advantageous for females of small species to utilize this central cavity as an oviduct and have it connect directly to the outside environment. This is, then, precisely the situation one sees in the small scaphopods S. quadrifissatum and C. fusiformis.

The suggestion of glands at the opening of the oviduct in C. fusiformis raises interesting questions. Dinamani (1964) reported that he observed Dentalium conspicuum laying its eggs in a long ribbon 6-7 cm.

long. His account is somewhat sketchy, but nonetheless it suggests that the eggs are bound together in the ribbon by some matrix. It is possible that other scaphopods, including C. fusiformis, also lay their eggs in ribbons or strings. Therefore the function of these glands at the sphincter could be to secrete the material of the matrix of the ribbon. It is interesting to note that the only time these glands were seen was in months when the individuals were at their highest point of ripeness, and that several females from May 1985 showed ribbons of blue-staining material (mucus) streaming from the apex sphincter into the posterior appendix (Plate XI).

If, in fact, Cadulus fusiformis does lay its eggs within a mucous or protein matrix, then they are probably deposited already fertilized. No other mollusk on the Pacific coast that lays its eggs in a ribbon or string lays them un-fertilized. Actually, when considering the potential fecundity of a mature female (60 eggs at best) of C. fusiformis, internal fertilization would be advantageous. The norm with broadcast spawning invertebrates is to produce massive quantities of eggs with very little energy invested in them. This is not the case with C. fusiformis where total numbers are low and packing the cytoplasm with energy-rich yolk is the standard.

Males of C. fusiformis produce many thousands of sperm during the active months of April and May. An unusual feature of the scaphopod populations was noted as follows. Non-quantitative dredge samples taken one immediately after another, and immediately adjacent to each other, often produced vastly different numbers of scaphopods while raising equivalent amounts of bottom sediment. This occurred in several months and suggests that these species may be patchy in their distribution. By combining these observations one may produce a coherent hypothesis of spawning behavior as follows.

Males release copious quantities of sperm into the water under the proper stimulus. Since the distance between individuals is small, the concentration of sperm is great and females detect the sperm in their respiratory currents. They take sperm-laden water into the "central cavity" gonoduct by relaxing the sphincter at the apex. Fertilization occurs within the gonoduct, where mature eggs are being stored prior to release. After fertilization the eggs are released and laid in a ribbon or string of mucus secreted by the glands along the inner lip of the sphincter at the apex. Some development of the larvae may take place within the ribbon matrix.

The most probable reason that this series of events has not been reported by previous authors that

have worked on fertilization and development is that many of these workers have obtained eggs to fertilize by breaking the shell and squeezing eggs from the ruptured ovary. This, of course, precludes any natural process of egg laying. The only descriptions of scaphopod spawning to be found in the literature at this time are those of Dinamani (1964) and (Lacaze-Duthiers 1857).

While one may not necessarily claim that this system is the exact one employed by Cadulus fusiformis and Siphondentalium quadrifissatum, nonetheless it is consistent with the facts presented. We should begin to rethink the current hypothesis that all scaphopods are broadcast spawners.

Of course the most solid piece of evidence that might be easily gathered to support this hypothesis would be to observe the animals actually in the act of spawning. This was attempted several times without success. In trying to maintain C. fusiformis and S. quadrifissatum in aquaria it was discovered that the animals are very temperature sensitive. A change of just a few degrees would kill all the animals in the aquarium. Therefore due to the limits of time and equipment no actual observations of spawning were made, although they are now of the highest priority for future investigations.

Other questions about the reproductive biology of the scaphopoda have been raised by this work. Among the most interesting would be that of sperm morphology, especially concerning the lack of acrosomes in two species and the presence of a large one in the third. Could sperm structure be used to elucidate details of the very confused taxonomic hierarchy of the Scaphopoda? More work could be done on the possibility of scaphopods laying fertilized eggs in mucous ribbons, and whether this behavior is only seen in smaller species or also in larger animals that have the potential fecundity to distribute gametes freely in the water for external fertilization. These are only a few questions for the future. It is to be hoped that the Scaphopoda will not be as neglected by future researchers as they have been in the past.

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