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FUNCTIONAL MORPHOLOGY OF REPRODUCTION IN SELECTED NEOGASTROPODS FROM THE PUERTO PENASCO REGION OF SONORA, MEXICO

by

Roy Seamands Houston

A Dissertation Submitted to the Faculty of the

DEPARTMENT OF BIOLOGICAL SCIENCES

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY
WITH A MAJOR IN ZOOLOGY

In the Graduate College

THE UNIVERSITY OF ARIZONA

1974

THE UNIVERSITY OF ARIZONA

GRADUATE COLLEGE

I hereby recommend that this dissertation prepared under my

direction by Roy Seamands Houston

entitled Functional Morphology of Reproduction in Selected Neogastropods from the Puerto Penasco Region of Sonora, Mexico

be accepted as fulfilling the dissertation requirement of the

degree of Doctor of Philosophy

Mead 27 March 1974

Dissertation Director Date

After inspection of the final copy of the dissertation, the following members of the Final Examination Committee concur in its approval and recommend its acceptance:*

Walter S. Miller 27 March 1974
Peter E. Pickens March 27, 1974
Thomson March 27, 1974
March 28 1974
Mead 19 April 1974

*This approval and acceptance is contingent on the candidate's adequate performance and defense of this dissertation at the final oral examination. The inclusion of this sheet bound into the library copy of the dissertation is evidence of satisfactory performance at the final examination.

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ABSTRACT

The reproductive systems in six species of Neogastropods are described in depth. Species studied were *Acanthina angelica* I. Oldroyd, 1918; *Columbella fuscata* Sowerby, 1832; *Crassispira pluto* Pilsbry and Lowe, 1932; *Olivella dama* (Wood, 1828 ex. Mawe MS); *Nassarius tiarula* (Kiener, 1841); and *Solenosteira macrospira* Berry, 1957. Specimens were collected at Puerto Penasco, Sonora, Mexico, in the Gulf of California and transported alive to The University of Arizona. Studies on the genitalia were accomplished through dissections and histological sections. In addition, twenty-five species from other regions of the world were examined briefly for comparative purposes.

The reproductive systems of the Gulf of California species studied are similar to those of species from other regions. Hence, phylogenetic trends, based on reproductive systems, are difficult to establish. Parallel evolution appears to have occurred among the subgroups of Neogastropoda.

In *Columbella fuscata*, sperm ingestion occurs within the pericardial cavity of the female. Ingested sperm probably serves as a nutrient source because the digestive gland atrophies in reproductively active females. In the male, a region of the vas deferens is open to the mantle cavity and apparently acts as a "safety valve" for the release of spermatozoa.

In many species, homologies exist between the seminal receptacle, bursa copulatrix, ventral channel, and gonopericardial duct of the females. In males of many species, there is communication between the vas deferens and the mantle cavity. These openings appear to be derived primarily from the primitive open condition.

INTRODUCTION

Previous studies have indicated that among the Neogastropods there is a similarity in the organization of reproductive systems. This has been most clearly shown in studies of the families Muricidae, Buccinidae, and Nassariidae (Fretter 1941; Johansson 1942, 1957; Fretter and Graham 1962; Purchon 1968). In addition, there are descriptions of the reproductive system of a few species of other Neogastropods from the families Olividae, Terebradae, Fasciolaridae, Columbellidae, and the Turridae (Marcus and Marcus 1959a, 1960, 1962a, 1962b; Robinson 1960; Smith 1967). The Panamic region, including the Gulf of California, is enriched with a vast assemblage of gastropods including many endemic species. To date very little work has been done on these species. It would be valuable to study the function of and the phylogenetic relationships between reproductive systems of all Gulf species, and compare them with those of species in other regions of the world. However, with the limited amount of time available, it was decided that a few selected species would be examined and not all with the same intensity.

The species examined in depth are *Acanthina angelica* I. Oldroyd, 1918; *Solenosteira macrospira* Berry, 1957; *Nassarius tiarula* (Kiener, 1841); *Columbella fuscata* Sowerby, 1832; *Olivella dama* (Wood, 1828 ex. Mawe MS); and *Crassispira pluto* Pilsbry and Lowe, 1932 (Fig. 1). According to Keen (1971), *A. angelica*, *S. macrospira*,

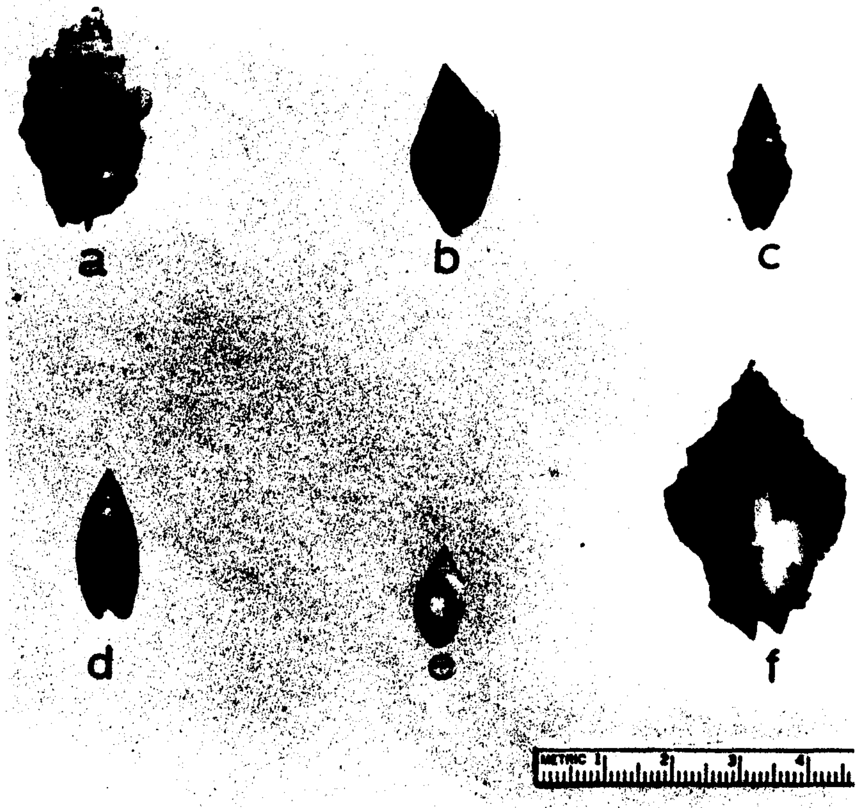

Fig. 1. Selected Species of Neogastropods Examined in this Study

a - Acanthina angelica, b - Columbella fuscata,
c - Crassispira pluto, d - Olivella dama,
e - Nassarius tiarula, f - Solenosteira macrospira

O. dama, and *Crassispira pluto* are endemic to the Gulf of California. In contrast, *N. tiarula* occurs throughout the Gulf and south to Panama, while *Columbella fuscata* can be found as far south as Peru. In addition, twenty-five other species were studied. These species are listed in Table 1.

Table 1. Additional Species and Their General Localities

Species	Locality
I. Muricidae	
A. *Thais haemostoma* (Linne, 1767)	Sao Paulo, Brazil
B. *Thais emarginata* (Deshayes, 1835)	California, U.S.A.
C. *Thais canaliculata* (Dulcos, 1832)	California, U.S.A.
D. *Ocenebra japonica* (Dunkie, 1860)	California, U.S.A.
E. *Urosalpinx cinerea* (Say, 1822)	California, U.S.A.
F. *Acanthina spirata* (Blainville, 1832)	California, U.S.A.
G. *Ceratostoma foliatum* (Gemlin, 1791)	California, U.S.A.
H. *Drupa nodulosa* (Adams, 1862)	Sao Paulo, Brazil
I. *Trophon truncatus* (Strom, 1768)	Millport, Scotland
J. *Trophon barvicenses* (Johnston, 1825)	Millport, Scotland
II. Buccinidae	
A. *Neptunea antigua* (Linneaus, 1758)	Millport, Scotland
B. *Colus gracilis* (da Costa, 1778)	Millport, Scotland
C. *Pisania janerensis* (Philippi, 1848)	Sao Paulo, Brazil
D. *Cantharus d'orbigny* (Link, 1807)	Sao Paulo, Brazil
E. Fusus rostratus (Olivi, 1880)	Naples, Italy

I.	*Trophon truncatus* (Strom, 1768)	Millport, Scotland
J.	*Trophon barvicenses* (Johnston, 1825)	Millport, Scotland
II.	Buccinidae	
A.	*Neptunea antigua* (Linneaus, 1758)	Millport, Scotland
B.	*Colus gracilis* (da Costa, 1778)	Millport, Scotland
C.	*Pisania janerensis* (Philippi, 1848)	Sao Paulo, Brazil
D.	*Cantharus d'orbigny* (Link, 1807)	Sao Paulo, Brazil
E.	*Fusus rostratus* (Olivi, 1880)	Naples, Italy
F.	*Tritonalia erinacea* (Linne, 1767)	Naples, Italy
III.	Columbellidae	
A.	*Columbella strombiformes* (Lamarck, 1822)	Sonora, Mexico
B.	*Anachis nigrofusca* (Carpenter, 1857)	Sonora, Mexico
C.	*Anachis coronata* (Sowerby, 1832)	Sonora, Mexico
D.	*Anachis sanfelipensis* (Lowe, 1935)	Sonora, Mexico
E.	*Anachis varia* (Sowerby, 1832)	Sonora, Mexico
F.	*Anachis hilli* (Pilsbry and Lowe, 1932)	Sonora, Mexico
G.	*Parametaria duponti* (Kiener, 1849-1850)	Sonora, Mexico
H.	*Mitrella guttata* (Sowerby, 1832)	Sonora, Mexico
IV.	Nassariidae	
A.	*Nassarius incrassatus* (Strom, 1768)	Millport, Scotland

METHODS AND MATERIALS

Monthly samples of specimens were collected over a period of two years from Puerto Penasco, Sonora, Mexico. There were ten individuals of each species in each sample. Three species, *Acanthina angelica*, *Columbella fuscata*, and *Crassispira pluto* occur intertidally at Station Beach, while *Olivella dama*, *Nassarius tiarula*, and *Solenosteira macrospira* can be found on the mudflats of Cholla Bay (Fig. 2). In addition to sampling, field observations were made on copulatory and spawning behavior. Water temperature was also taken for each sample.

The snails were then transported alive in styrofoam containers to The University of Arizona where they were maintained in plastic aquaria for laboratory investigations.

General descriptions of the genitalia were made after careful dissections of both preserved and living material, while stained sections of the reproductive tract were examined in order to elucidate the components and cellular details. The soft parts were removed from their shell with the aid of a vice. They were then relaxed in propylene phenoxytol (Owen 1955) and fixed in Bouin's fluid. Through the use of standard histological techniques, the animals were infiltrated with 52.5°C and 56.5°C paraffin and sectioned at 10 micra. The sections were stained with Kornhouser's Hemalum and Eosin B. Alcian Blue and Mallory's Trichrome were used for identifying

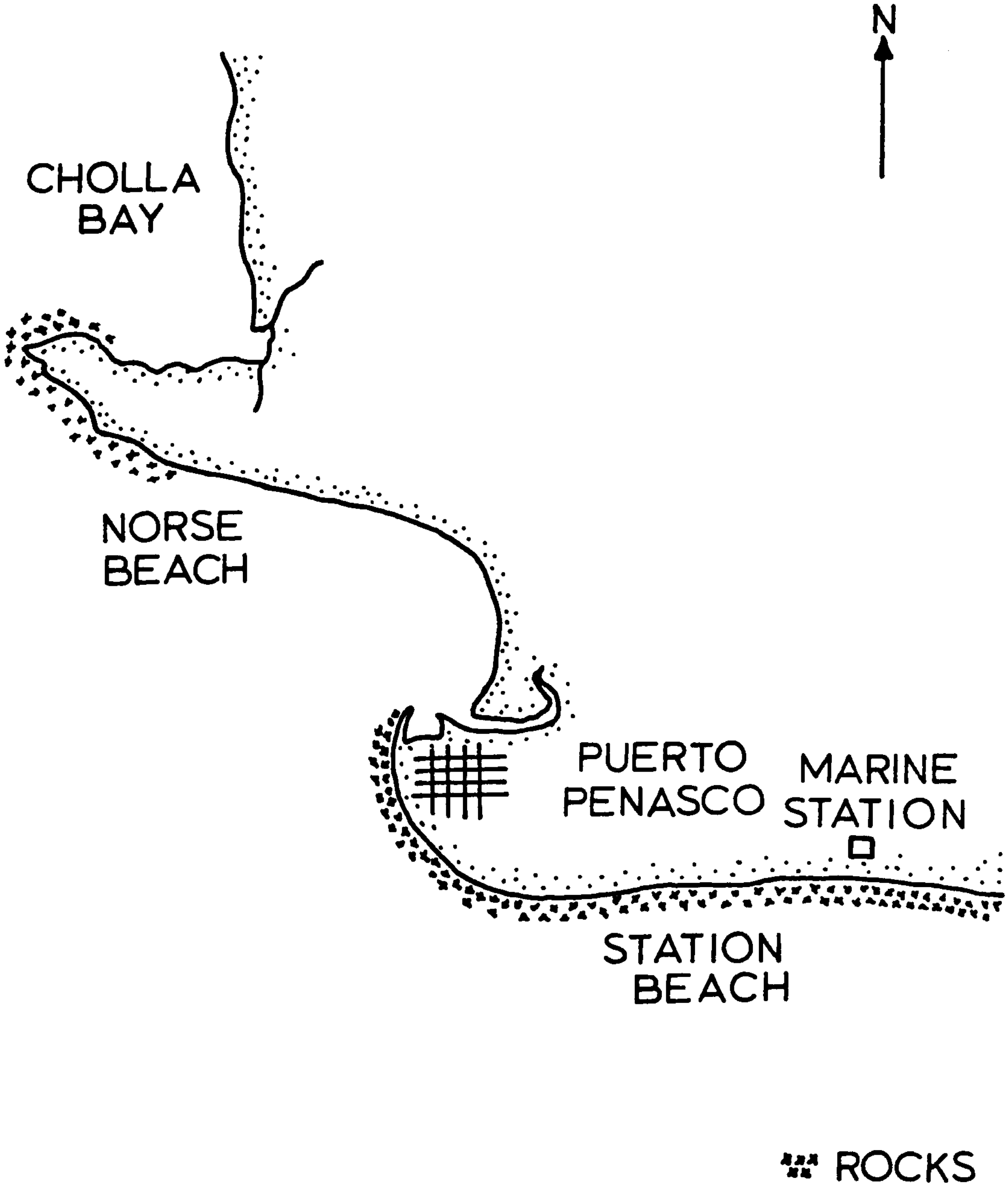

Fig. 2. Collecting Areas at Station Beach and Cholla Bay

glandular parts of the reproductive system. Ciliary currents were observed by using suspended carmine particles in sea water.

A detailed analysis of the functional aspects of reproduction were studied for *Columbella fuscata* only. The snails were periodically observed, and during times of reproductive activity, both males and females were preserved before, during, and after copulation. They were later dissected and sectioned to determine sperm and egg movements, site of fertilization, egg capsule formation, and the mechanism of sperm ingestion.

Juveniles of *Acanthina angelica* and *Columbella fuscata* were also collected to study the development of the genital systems. Their shell length was determined by measuring with vernier calipers the distance from the top of the spire to the tip of the siphonal canal.

RESULTS

Ecology

Acanthina angelica

Acanthina angelica inhabits the high-mid rocky intertidal zone at Station Beach. These muricids can be observed on and under the basaltic boulders which occur in this zone. *A. angelica* is quite gregarious and clusters of twenty or more individuals are found feeding on barnacles which are attached to these boulders. At low tide during the day the snails hide underneath in crevices between the rocks. However, at dusk and at high tide they are exposed, and can be seen crawling on top of the boulders.

Columbella fuscata

This columbellid also occurs at Station Beach; however, it inhabits the tide pools which pocket the limestone substrate of the middle and low intertidal zones. Unlike *A. angelica* it is herbivorous and feeds on clusters of *Padina* and *Sargassum* which occur in these pools. Occasionally individuals of *C. fuscata* may be observed crawling in the sand among these clumps of algae.

Crassispira pluto

On the limestone reef adjacent to the tidepools where *Columbella fuscata* occurs is *Crassispira pluto*. This small black turrid crawls about, half buried in sand pockets, apparently feeding on interstitial metazoans.

Nassarius tiarula

This colorful nassariid occurs at Cholla Bay where it can be seen crawling on the mudflats among broken bivalve shells. At times these snails are completely buried except for their siphons, which protrude above the surface of the mud. Because *N. tiarula* is a carrion feeder, it is common to observe these snails clustered on dead bivalves and fish.

Solenosteira macrospira

On the mudflats of Cholla Bay are several outcroppings of limestone substrate. In mud pockets on these reefs is found *S. macrospira*, a buccinid which appears to be feeding on interstitial metazoans. These snails are usually half buried and quite often they also occur on the mudflats immediately surrounding the reef.

Olivella dama

This small attractive olivid lives on the sandflats farther out in Cholla Bay. These snails spend almost all of their time crawling beneath the sand with only their thin white siphons visible above the surface. If it were not for the characteristic trail, these animals would be extremely difficult to collect. *O. dama* secretes copious amounts of mucus which are utilized in trapping small interstitial organisms upon which they feed.

Reproductive Systems

Because this study concentrates on six species of Neogastropods, detailed descriptions of their genital systems are treated first. The reproductive morphology of the remaining twenty-five species is included for comparative purposes. Hence, they are discussed secondarily with a brief account of the major structural differences and similarities.

The Male Duct

The male ducts of all six species are illustrated in Figs. 3 through 8.

Acanthina angelica. Posteriorly, both the testis (te) and the digestive diverticula share the visceral mass. From the testis the straight vas deferens (vd) leads anteriorly, ventral to the intestine and passes dorsal to the pericardial cavity. Here it joins the prostate gland (pg) in the right posterior region of the mantle cavity. Anteriorly, the large curved prostate gland runs beneath the hypobranchial gland until it connects with the anterior vas deferens. At this point the vas deferens lies embedded in the body wall and continues anteriorly until it joins the penis (pn), which is situated just posterior to the right cephalic tentacle. This system is similar to that of *Nucella lapillus* and *Ocenebra erinacea* described by Fretter (1941), except for the posterior region of the vas deferens which is coiled.

Histologically the entire posterior vas deferens is thin-walled and surrounded by a thin layer of connective tissue. The lumen is lined with cuboidal ciliated epithelium with round, centrally located nuclei. There is no seminal vesicle in this species, and at no time even during the breeding season was sperm observed in this

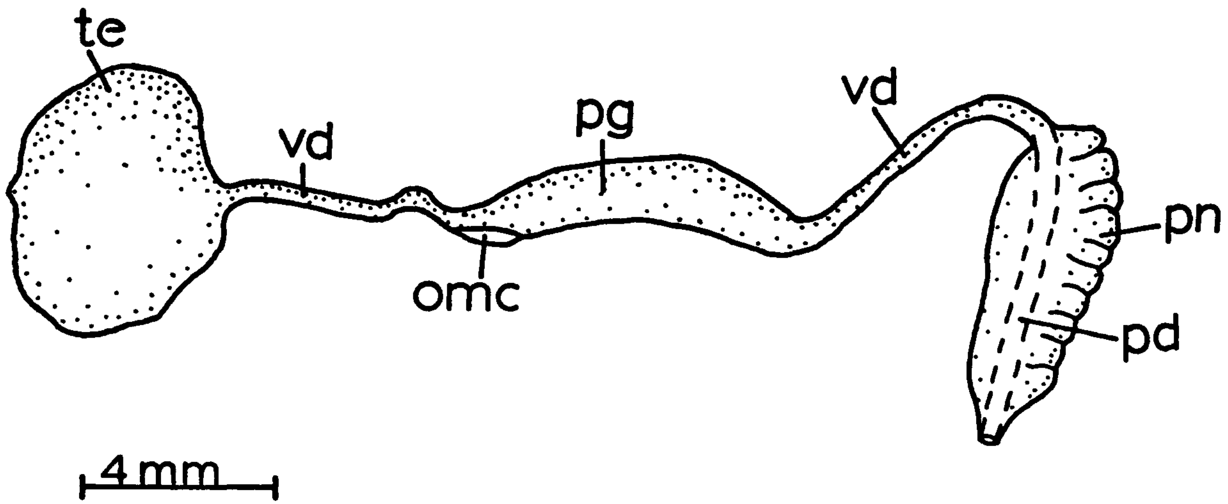

Fig. 3. The Male Duct of Acanthina angelica

omc - opening to the mantle cavity, pn - penis, pd - penial duct,
pg - prostate gland, te - testis, vd - vas deferens

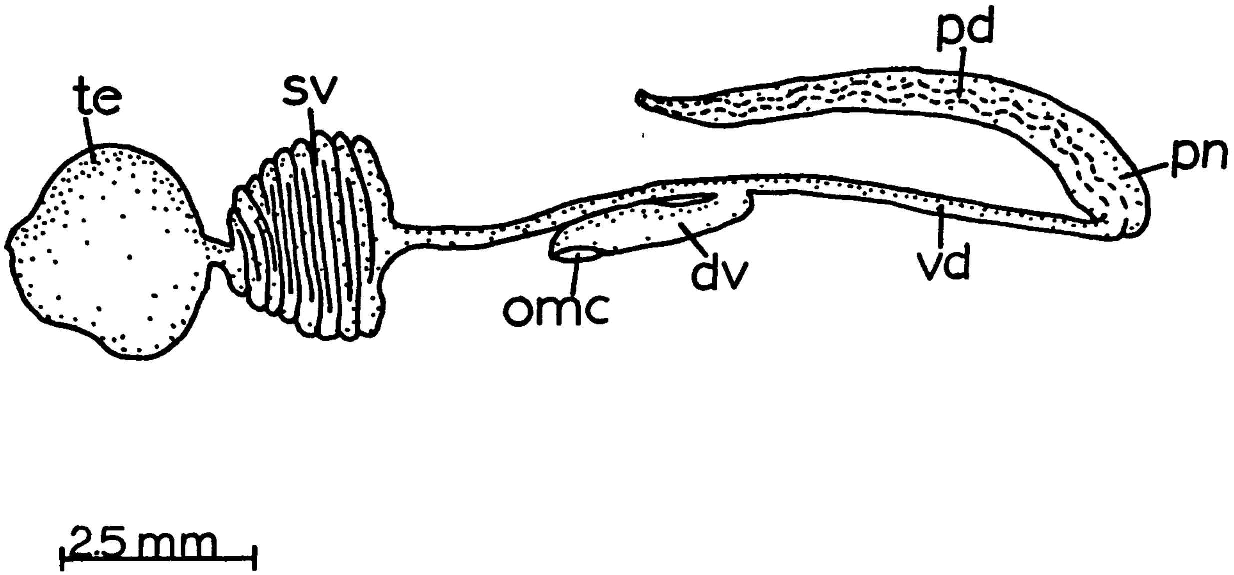

Fig. 4. The Male Duct of <u>Columbella</u> <u>fuscata</u>

dv - diverticulum, omc - opening to the mantle cavity, pn - penis,
pd - penial duct, sv - seminal vesicle, te - testis, vd - vas deferens

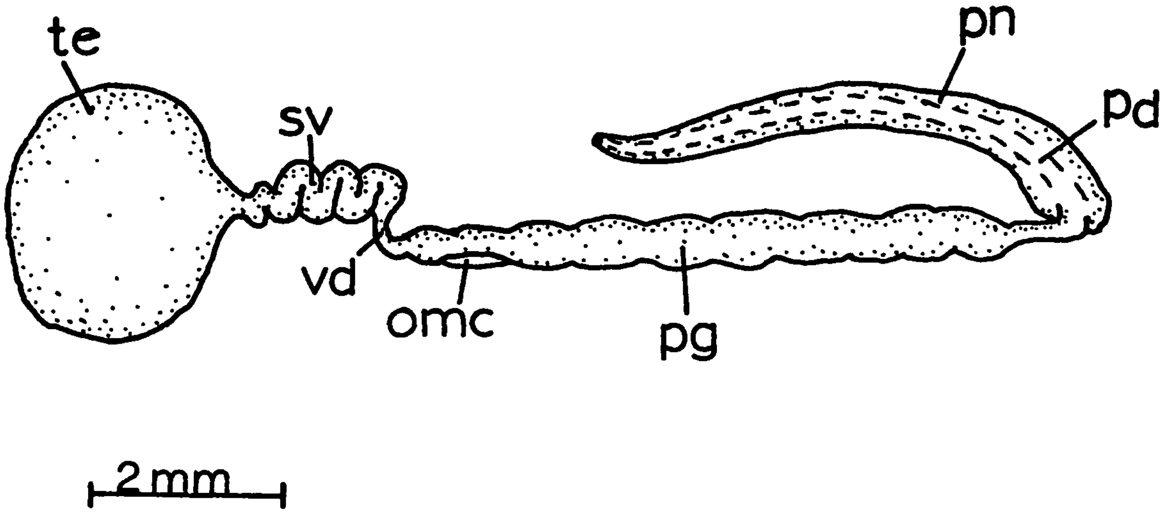

Fig. 5. The Male Duct of <u>Crassispira</u> <u>pluto</u>

omc - opening to the mantle cavity, pn - penis, pd - penial duct,
pg - prostate gland, sv - seminal vesicle, te - testis, vd - vas deferens

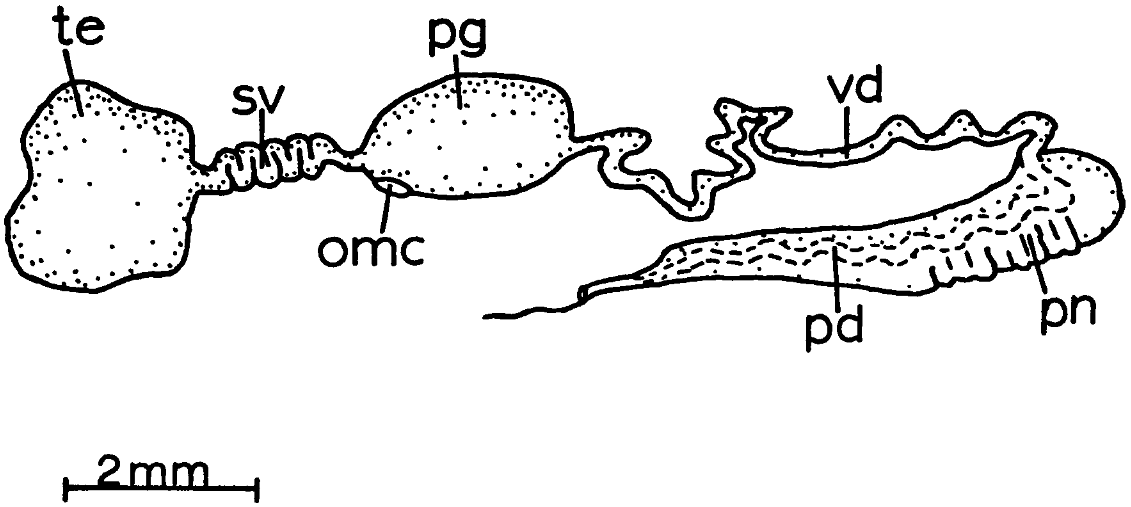

Fig. 6. The Male Duct of <u>Olivella dama</u>

omc - opening to the mantle cavity, pn - penis, pd - penial duct,
pg - prostate gland, sv - seminal vesicle, te - testis, vd - vas deferens

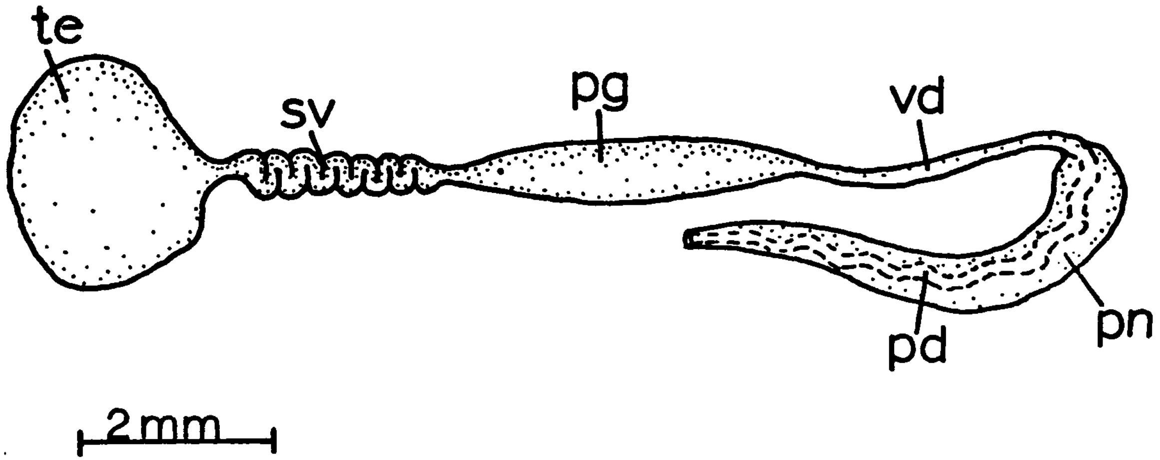

Fig. 7. The Male Duct of <u>Nassarius</u> <u>tiarula</u>

pn - penis, pd - penial duct, pg - prostate gland, sv - seminal vesicle,
te - testis, vd - vas deferens

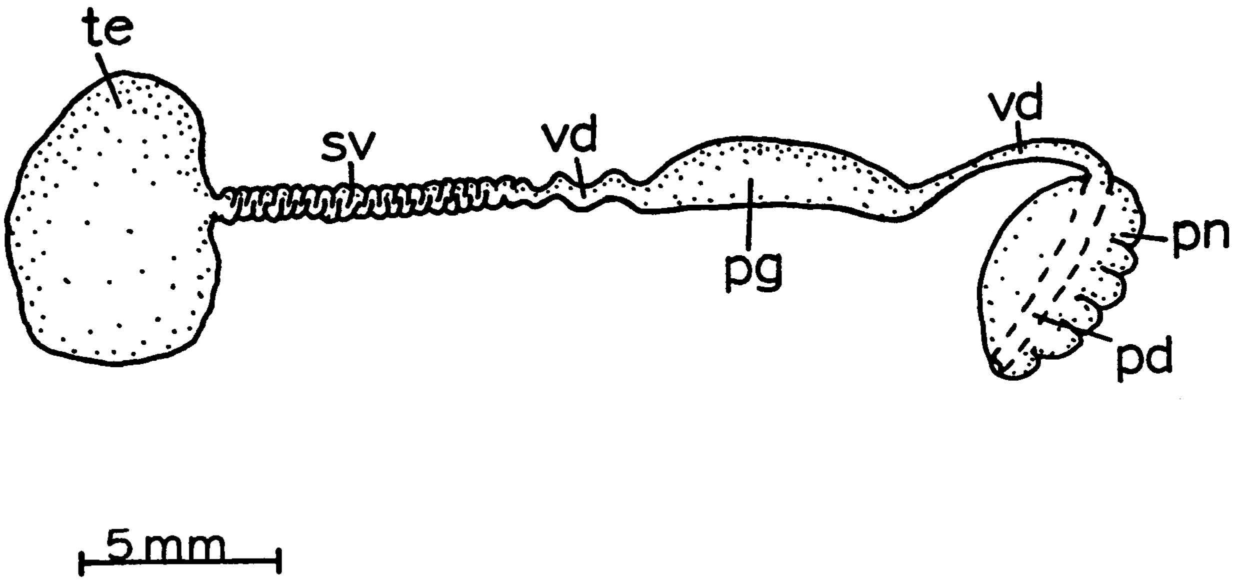

Fig. 8. The Male Duct of _Solenosteira macrospira_

pn - penis, pd - penial duct, pg - prostate gland, sv - seminal vesicle,
te - testis, vd - vas deferens

duct. In Nucella lapillus, Fretter (1941) observed that the coiled vas deferens acts as a seminal vesicle. The entrance to the prostate gland is guarded by a sphincter composed of circular muscles. The lateral lobes of the prostate (Fig. 9) bulge due to the development of glandular tissue. The subepithelial gland cells (sgc) (Fig. 10) are in the shape of teardrops containing large round nuclei (n). The cytoplasm contains eosinophilic granules and is highly vacuolated. These gland cells are arranged in clusters where secretions empty into common ducts that lead to the lumen of the prostate. The lumen appears as a dorsoventral slit which is lined with ciliated columnar epithelium. The epithelial cells contain basal oval nuclei and lie on a basement membrane. Interspersed regularly between these epithelial cells are the ducts (d) leading from clusters of subepithelial gland cells. The entire prostate is held together by a thin layer of connective tissue. It is interesting to note that the epithelium lining the lumen merges ventrally as a double row of cells, which is the result of fusion (lf) of the duct from an originally open groove. The prostate is fused throughout its length except for a posterior region of about 450 microns. The unfused region appears as a slit-like aperture (omc) which is in communication with the posterior region of the mantle cavity (Fig. 11). This differs from Littorina littorina, L. obtusata, and L. rudis in which the prostate is composed of two glandular lobes and is open throughout its entire length (Linke 1933).

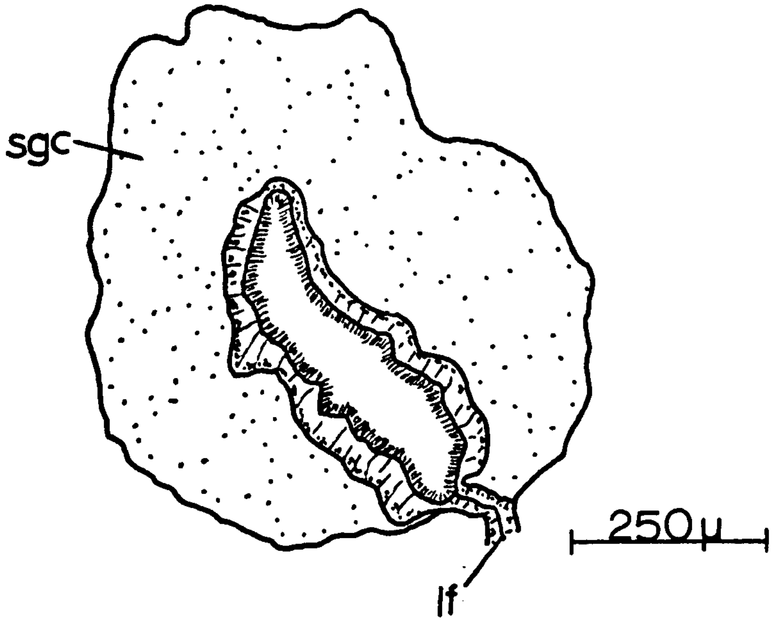

Fig. 9. Cross Section through the Prostate Gland of *Acanthina angelica*

lf - line of fusion, sgc - subepithelial gland cells

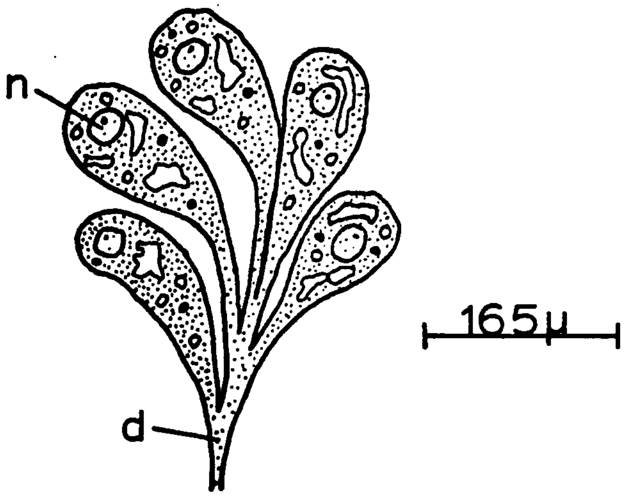

Fig. 10. Subepithelial Gland Cells from the Prostate Gland of *Acanthina angelica*

d - duct, n - nucleus

Anterior to the prostate the vas deferens is highly muscular and the lumen is lined with ciliated cuboidal epithelium. The line of fusion is evident throughout the anterior vas deferens and the penial duct (pd). The penis is flattened dorsoventrally with the penial duct appearing as a straight canal just dorsal of center. The histology of the penial duct is similar to that of the vas deferens. In cross section the penis appears as a complex network of longitudinal and circular muscle fibers loosely arranged around haemal sinuses. The surface is lined with ciliated columnar epithelium interspersed by ducts leading from subepithelial mucus cells.

Columbella fuscata. The genital system of *Columbella fuscata* differs markedly from that of *Acanthina angelica* in several respects. The upper vas deferens is highly convoluted and acts as a seminal vesicle (sv). This seminal vesicle is divided into two regions. When examined, the posterior half appears white due to the presence of stored sperm. The anterior region is yellowish brown, and it is in this area where sperm are apparently resorbed. The walls of the seminal vesicle are quite thin and appear rather rigid when filled with sperm. It is lined with ciliated cuboidal epithelium resting on a basement membrane below which is a thin layer of connective tissue. The epithelial cells contain large round basal nuclei and distal yellowish brown inclusions which may be the products of sperm resorption (s) (Fig. 12). Anteriorly, the seminal vesicle straightens out for a short distance until it reaches the posterior region of the mantle cavity, where it becomes the true vas deferens. It passes anteriorly and parallel to the rectum, where in this region it bends

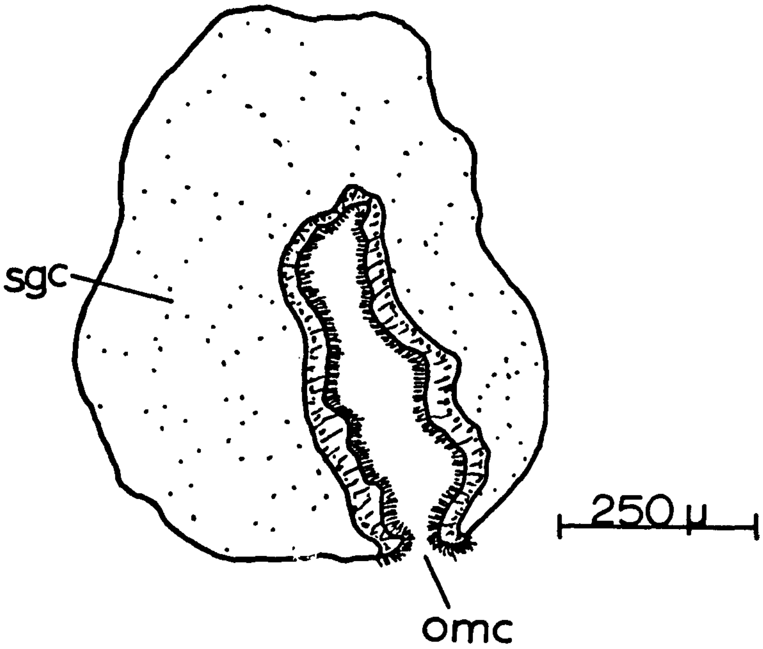

Fig. 11. Cross Section through the Unfused Region of the Prostate Gland of Acanthina angelica

omc - opening to the mantle cavity, sgc - subepithelial gland cells

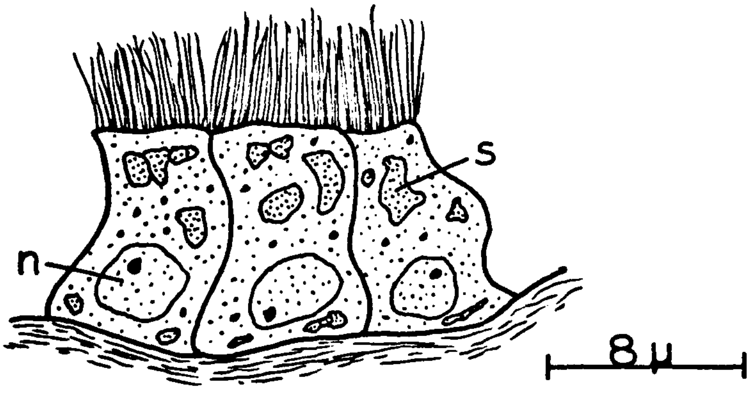

Fig. 12. Epithelial Cells Lining the Seminal Vesicle of Columbella fuscata

n - nucleus, s - sperm breakdown products upward and runs along the body wall until it joins the base of the penis. There is no prostate gland in *C. fuscata*. A study by Marcus and Marcus (1962b) on Brazilian columbellids revealed the absence of a prostate in *Anachis veleda*, *A. brasiliana*, *A. sparsa*, and *Nitidella dichroa*. A sphincter is present between the seminal vesicle and vas deferens, which controls entry of sperm into the latter. The epithelial cells lining the vas deferens are not as strongly ciliated as those of the seminal vesicle. However, the vas deferens is extremely muscular and assumes an ejaculatory function. As shown in Fig. 4, the vas deferens ventrally gives rise to a long, thin-walled diverticulum (dv), which passes ventrally and parallel to the duct. A sphincter guards the entrance between the vas deferens and the diverticulum. The posterior region of the diverticulum is open to the mantle cavity by a ventral slit 450 microns in length. On several occasions sperm masses (sp) were observed escaping through this opening (Fig. 13). The function of this opening will be discussed later. No line of fusion is evident as in the case of *Acanthina angelica*.

The penis, while dorsoventrally flattened, is much longer than that of *A. angelica*. Moreover, it is wide at the base and then tapers to a point. The penial duct is centrally located and appears as a convoluted tube which opens at the tip of the penis. In resting males, the penis is curved back along the right side of the body wall. When the mantle edge is pulled back, one can see that the tip is tucked in a pouch in the posterior mantle wall. This has been observed in other columbellids (Marcus and Marcus 1962b).

Fig. 13. Section through the Opening to the Mantle Cavity in a Male <u>Columbella fuscata</u>

sp - sperm escaping through the opening, dv - diverticulum, omc - opening into the mantle cavity

Crassispira pluto. The gross anatomy of this species resembles closely that of *Philbertia leufroyi boothi* described by Smith (1967). The testis interdigitates with the digestive diverticula in the upper whorls of the visceral mass. The upper region of the vas deferens is coiled and acts as a seminal vesicle. However, it is much shorter than that of *Columbella fuscata*. It is thin-walled and lined with ciliated cuboidal epithelium. The cytoplasm is highly vacuolated and contains yellowish staining granules. Hence, this area may serve as a site for sperm resorption in which the granules are breakdown products. Smith (1967) reports that yellowish staining granules are situated along the apical borders of gland cells just anterior to the seminal vesicle of *Haedropleura septangularis*. Anterior to the seminal vesicle the duct straightens out and passes ventral to the kidney. As it approaches the prostate gland, it passes in close proximity to the pericardial cavity. In fact, histological sections reveal a thin strand of connective tissue joining the vas deferens with the pericardial cavity. This may be the remnant of a gonopericardial duct, as is the case in *Cenodagreutes aethus* (Smith 1967). A sphincter controls the entrance to the long convoluted prostate gland. Upon dissection, it can be seen that this gland extends from the area of the kidney and continues beneath the mantle until it reaches the anterior region near the anal gland. An opening into the mantle cavity occurs where there is incomplete fusion of the lobes. This posterior opening appears as a ventral slit about thirty micra in length. In cross section the prostate is circular with subepithelial gland cells arranged in clusters around the lumen.

These cells give rise to ducts which intersperse the ciliated cells lining the lumen. The cytoplasm of the gland cells is highly eosinophilic like those in the prostate of Acanthina angelica. The short duct from the prostate to the penis is ciliated and surrounded by a thick coat of circular muscle cells.

The penis is long and similar to that of Columbella fuscata. The surface is highly ciliated with the ciliary currents running at right angles to the long axis. There are numerous subepithelial mucous cells which open onto the surface. The muscular penial duct is central and straight, with the opening directly at the tip. When not in use, the penis is tucked posteriorly in the mantle cavity.

Olivella dama. The male duct of this species differs widely in several respects. During the reproductive season, the large testis occupies most of the visceral mass. It begins at the apex, passes behind the digestive gland and extends almost to the body whorl. Its large bright yellow acini are easily visible in dissections. Anteriorly the short testicular duct leads to a convoluted but relatively short seminal vesicle. This duct is lined with ciliated cuboidal epithelium containing central round nuclei. The cytoplasm is filled with brownish staining granules which appear to be breakdown products from spermatozoa. Anterior to the seminal vesicle the vas deferens straightens out for a very short distance before entering the prostate gland. Again, a sphincter muscle guards the entrance to the gland. The gland appears as a large oval orange mass lying ventral to the hypobranchial gland. The histology of the prostate is interesting because, instead of being a straight duct bordered by glandular tissue, it is highly coiled and surrounded by a single layer of gland cells alternating with ciliated epithelial cells. The posterior region of the prostate is in communication with the mantle cavity by means of a ventral slit about thirty micra in length. This differs from the pallial opening in Olivella verreauxii, which is a short ciliated duct (Marcus and Marcus 1959a). The epithelial gland cells are cuboidal with central round nuclei. The nuclei are strongly basophilic while the cytoplasm is filled with red eosinophilic granules. Anterior to the prostate gland the vas deferens straightens out and passes along the right body wall for a short distance. Before it enters the penis, it becomes convoluted, straightens out and again is coiled upon entering the base of the penis (Fig. 6). In cross sections the vas deferens is not as muscular as in the other species, however, it is strongly ciliated. In the coiled regions of the duct the cilia are long and again alternate with gland cells which stain similar to those in the prostate gland. Hence, it appears that these convoluted regions are accessory prostate glands.

The penial duct is also convoluted and centrally located. The penis is highly muscular with the penial nerve located to the left of the duct. The penis is a massive organ which can be extended almost the length of the snail. It is flattened dorsoventrally and the outer edge is slightly scalloped when contracted. It has a marked taper that terminates in a fine filamentous structure, the function of which is unknown. The penial duct opens just ventral to the base of this structure.

Nassarius tiarula. The testis leads directly to the ciliated seminal vesicle, which is tightly coiled and rather long. In dissections it can be seen bulging with spermatozoa. This organ is quite elastic and when filled with sperm its diameter is increased two-fold. Ventral to the kidney and anterior to the seminal vesicle, the vas deferens straightens out before entering the prostate gland. It is covered with a thin layer of circular muscles and lined with ciliated cuboidal epithelium. There is no sphincter at the posterior entrance. Moreover, this gland is closed throughout its entire length. In contrast, Fretter (1941) observed a posterior mantle opening in Nassarius reticulatus. In dissections the prostate appears as a dull orange tube of slightly larger diameter than the vas deferens. The histology of this gland is similar to that of Olivella dama, in that the lumen is lined with alternating gland and epithelial cells. There are no subepithelial gland cells present in the prostate gland of this species. The staining properties of these cells are similar to those of the other species previously discussed. Anterior to the prostate the ciliated vas deferens can be traced along the right body wall to the base of the penis. In this region the duct is actually embedded in the musculature of the body wall. This probably aids in the movement of sperm, for there is only a thin layer of circular muscle cells around the duct itself.

The penis is flattened dorsoventrally but not as much as in the other species. Moreover, it is not as long and the penial duct is central and convoluted throughout. This duct is covered with a thick layer of circular muscles, and peripherally are haemal sinuses that run laterally throughout the length of the penis. Surrounding the blood spaces is a network of oblique and longitudinal muscles covered by a coat of circular muscle cells that traverse the length of the organ.

Solenosteira macrospira. The anatomy of this species is similar in many respects to *Acanthina angelica* and *Nassarius tiarula*. The testis leads directly into the extremely long seminal vesicle. Posteriorly the duct is tightly coiled, and extends from the upper whorls of the visceral mass to the posterior region of the mantle cavity. Here it directly opens into the prostate gland, for there is no uncoiled region of the vas deferens between the two structures. The seminal vesicle resorbs sperm and the breakdown products again appear as brown staining granules within the cytoplasm of the epithelial cells. The prostate gland is large and similar to that of *Acanthina angelica*. However, there is no sphincter muscle guarding the posterior entrance. The lumen appears as a dorsoventral slit lined with columnar ciliated epithelium lying on a basement membrane. The gland is divided into right and left lobes, each containing many clusters of subepithelial gland cells. These cells empty into ducts which are interspersed between the epithelial cells. The gland cells have round nuclei and a highly eosinophilic cytoplasm. There is no opening from the prostate into the mantle cavity in this species. Anteriorly the vas deferens passes along the right body wall to the base of the penis, which is situated just posterior to the right cephalic tentacle. In this region, it is ciliated and covered with a thick layer of circular muscle cells.

The penis is not flattened as much as that in Acanthina angelica, but assumes the same general shape. It is short with a central straight penial duct. When males are not reproductively active, the penis is reduced to a small tubercle. The musculature is a latticework of longitudinal and oblique muscle cells surrounded by a thick layer of circular fibers. Interspersed between these muscle cells are numerous lateral haemal sinuses.

The Female Duct

Since material of Acanthina angelica and Columbella fuscata is obtained more easily than for the other species, their female systems were studied in greater detail. The female ducts of all six species are presented in Figs. 14 through 19.

Acanthina angelica. The genital duct of this species is similar to that of Nucella lapillus described by Fretter (1941). The The ovary (oa) and the digestive diverticula share the upper whorls of the visceral mass. From the ovary the thin-walled oviduct (ov) leads anteriorly and ventrally along the right side of the viscera. Upon dissection, it appears as a large blood vessel just below the integument. In the region of the kidney it approaches the albumin gland (ag), which doubles back on itself like a hairpin, thereby making a sharp angle with the oviduct. The albumin gland then opens into the capsule gland (cg), which is divided into right and left glandular lobes. These lobes are joined together by dorsal and ventral sutures and covered with a thin layer of connective tissue. Lying between the albumin and capsule glands is a deep brown, highly

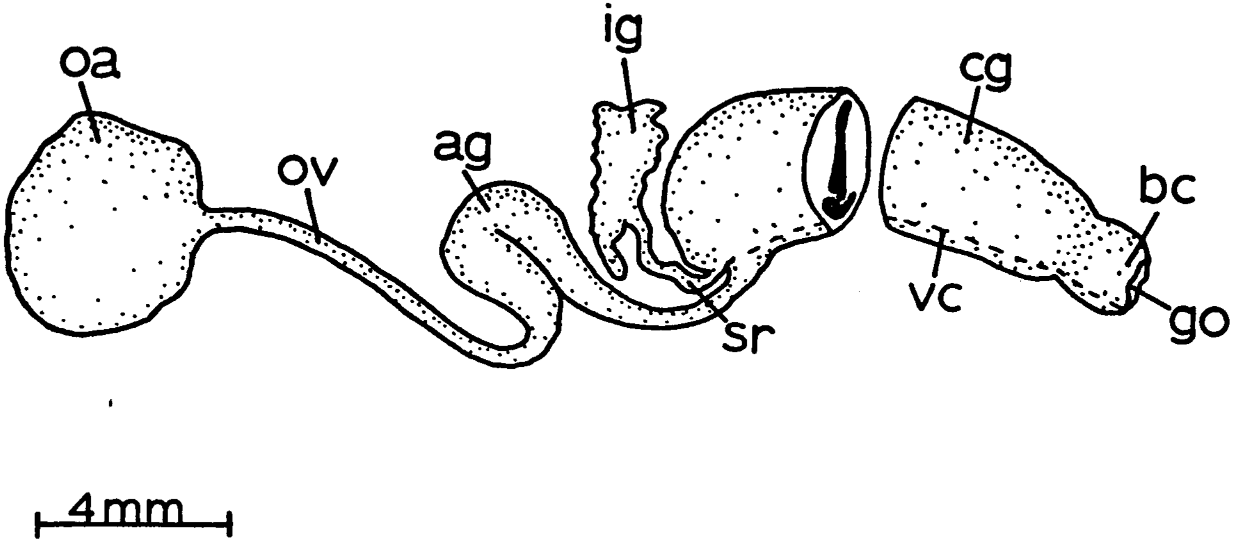

Fig. 14. The Female Duct of Acanthina angelica

ag - albumin gland, bc - bursa copulatrix, cg - capsule gland,
go - genital opening, ig - ingesting gland, oa - ovary, ov - oviduct,
sr - seminal receptacle, vc - ventral channel

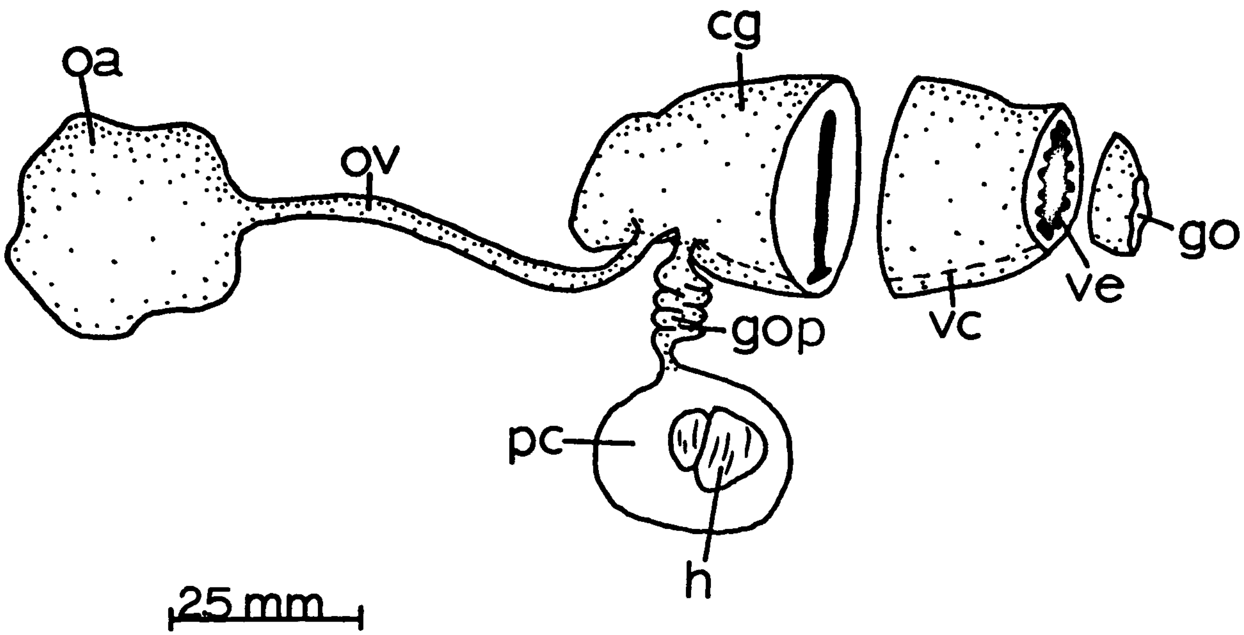

Fig. 15. The Female Duct of <u>Columbella</u> <u>fuscata</u>

cg - capsule gland, go - genital opening, gop - gonopericardial duct,
h - heart, oa - ovary, ov - oviduct, pc - pericardial cavity,
vc - ventral channel, ve - vestibule

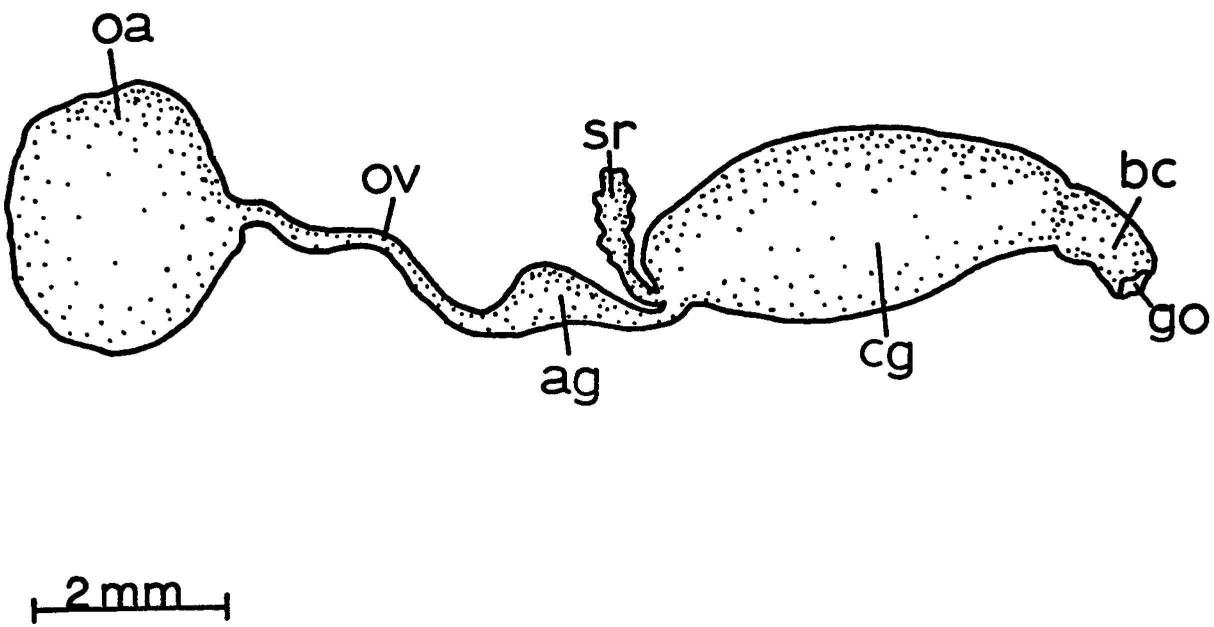

Fig. 16. The Female Duct of <u>Crassispira</u> <u>pluto</u>

ag - albumin gland, bc - bursa copulatrix, cg - capsule gland,
go - genital opening, oa - ovary, ov - oviduct, sr - seminal receptacle

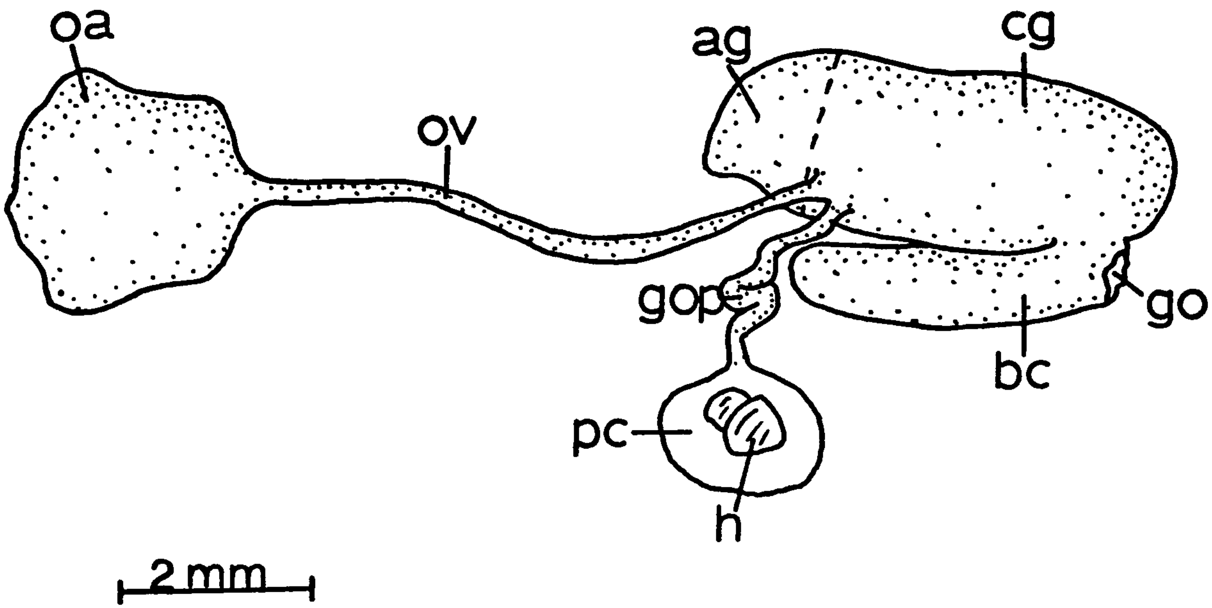

Fig. 17. The Female Duct of Olivella dama

ag - albumin gland, bc - bursa copulatrix, cg - capsule gland, go - genital opening, gop - gonopericardial duct, h - heart, oa - ovary, ov - oviduct, pc - pericardial cavity

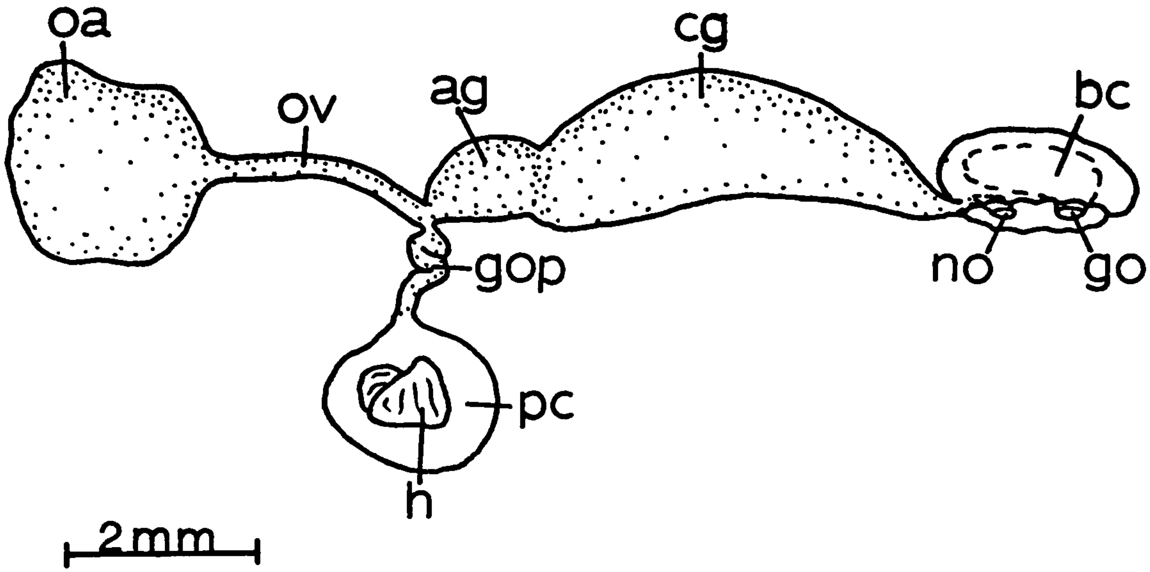

Fig. 18. The Female Duct of Nassarius tiarula

ag - albumin gland, bc - bursa copulatrix, cg - capsule gland, go - genital opening, gop - gonopericardial duct, h - heart, no - nidimental opening, oa - ovary, ov - oviduct, pc - pericardial cavity

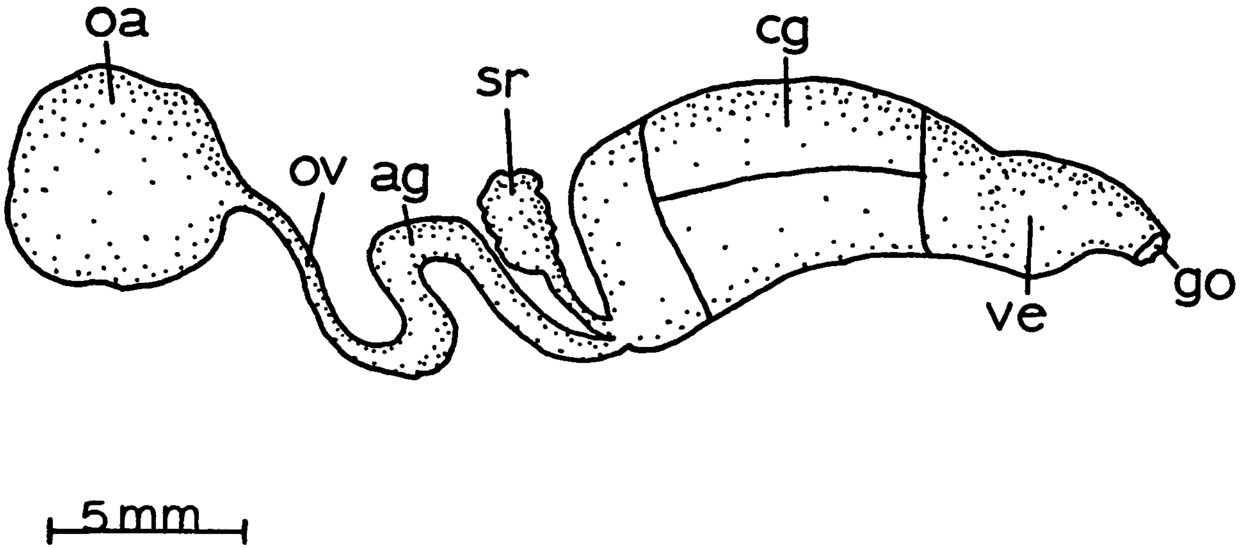

Fig. 19. The Female Duct of Solenosteira macrospira

ag - albumin gland, cg - capsule gland, go - genital opening, oa - ovary, ov - oviduct, sr - seminal receptacle, ve - vestibule diverticulated glandular mass, the ingesting gland (ig). A duct from this gland opens into the posterior region of the capsule gland. The capsule gland continues forward and ventral to the anal gland, where it leads to a vestibule. Anterior and ventral to the vestibule is a blind muscular sac, the bursa copulatrix (bc), which receives sperm from the male. The bursa and vestibule join to form the short ciliated vagina that leads to the genital opening (go) proximal to the anus.

The oviduct is thin-walled and lined with a single layer of ciliated cuboidal epithelium resting on a basement membrane. As it approaches the albumin gland, the oviduct becomes heavily laden with circular muscle cells, which act as a sphincter controlling the entrance to the albumin gland.

When the shell is removed from a living specimen, the albumin gland can be seen as a white opaque mass lying dorsal and just posterior to the pericardial cavity. Internally it is ciliated throughout, with the lumen containing transparent secretory droplets. Sections reveal a circular gland with a ciliated dorsoventral lumen. There are two major staining regions in this gland (Fig. 20). The right side of the gland is composed chiefly of mucous cells (muc) which stain bright blue, while the left half is made up of groups of subepithelial gland cells that stain pink with Eosin B (prc). The latter cells are oval with large round nuclei and have ducts which open into the lumen between the epithelial cells. A short duct connects the albumin gland to the posterior region of the capsule gland. Transverse sections reveal two lobes of equal size with the lumen appearing as a dorsoventral slit. The lumen is ciliated throughout. Running the length of the gland are two longitudinal flaps of tissue, which form the roof of the ventral channel. The left flap is more developed and lies over the right one, thereby possibly closing off the ventral channel and making it a functionally separate duct.

The subepithelial gland cells (sgc) are teardrop shaped and have a long thin duct (d) which opens into the lumen between the epithelial cells (ec) (Fig. 21). Figure 22 is a series of sections through the capsule gland showing the distribution of different staining properties and how they change throughout the gland. Anteriorly the gland is composed of mucous cells, which are scattered around the ventral channel and extend throughout both lobes. This configuration continues throughout the anterior one-fourth of the gland, until it is replaced by two other cell types. Cells around the ventral channel stain dark purple with Kornhouser's Hemalum, while the cells in the medial region of the gland stain pink with Eosin B. Mucous cells are limited to the dorsal region. About one-half way through the gland, the purple cells extend into the medial regions, leaving only a thin strip of pink staining cells in this area. Finally, in the posterior quarter, the purple staining cells occupy the ventral and medial regions, while mucous cells are scattered throughout the dorsal region.

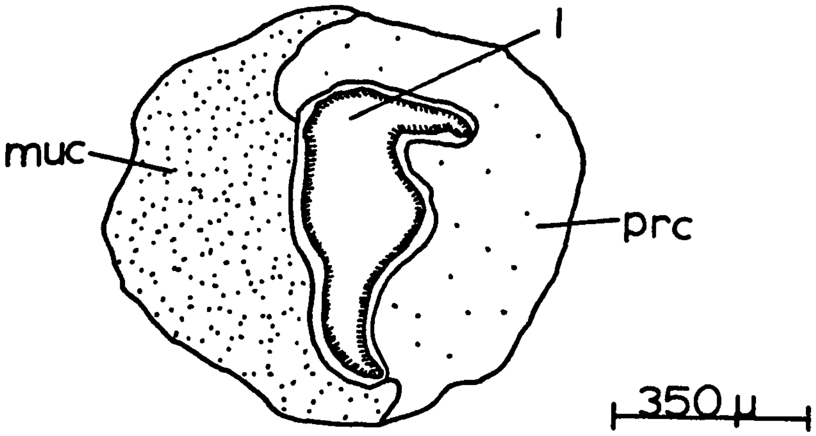

Fig. 20. Section through the Albumin Gland of <u>Acanthina angelica</u>

l - lumen, muc - mucous cells, prc - protein cells

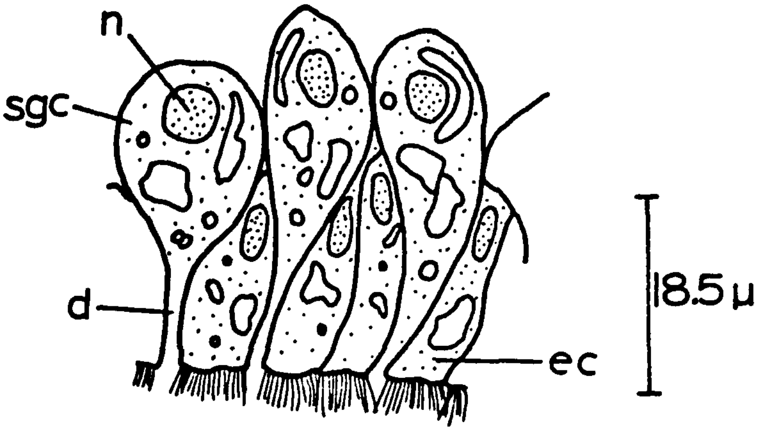

Fig. 21. Subepithelial Gland Cells from the Capsule Gland of <u>Acanthina angelica</u>

d - duct, ec - epithelial cells, n - nucleus,
sgc - subepithelial gland cell

The cilated ventral channel (vc) passes ventrally from the bursa copulatrix, extends posteriorly through the capsule gland and joins the duct to the ingesting gland. This duct is without cilia and functions as a seminal receptacle (sr). Here spermatozoa are neatly aligned, with their heads embedded in the epithelium, and their tails projecting into the lumen. The ingesting gland is a sac composed of many blind tubules, lined with glandular epithelium, which break down and metabolize the sperm (Fretter 1941). Figure 23 is a drawing of the bursa copulatrix, which comprises the anterior end of the capsule gland. It is covered by a layer of circular muscle cells (m) and lined with cilated columnar epithelium lying on a basement membrane. This anterior bursa receives sperm deposited by the male.

The eggs are released from the oviduct into the albumin gland upon relaxation of the sphincter muscle. In the gland, ciliary tracts beat at right angles, swirling the secretions from the epithelium and mixing them with the eggs while driving the entire mass forward. As the mass enters the capsule gland, it comes under the influence of additional ciliary currents (Fig. 24). In this region the currents are weak and the egg mass is slowed down considerably for egg capsule formation. Some of these currents beat in the direction of the long axis of the gland, while others beat at right angles to the ventral channel. As the secretions are poured into the lumen, they are distributed around the eggs, forming the capsule. The cilia in the anterior region of the gland are directed forwards,

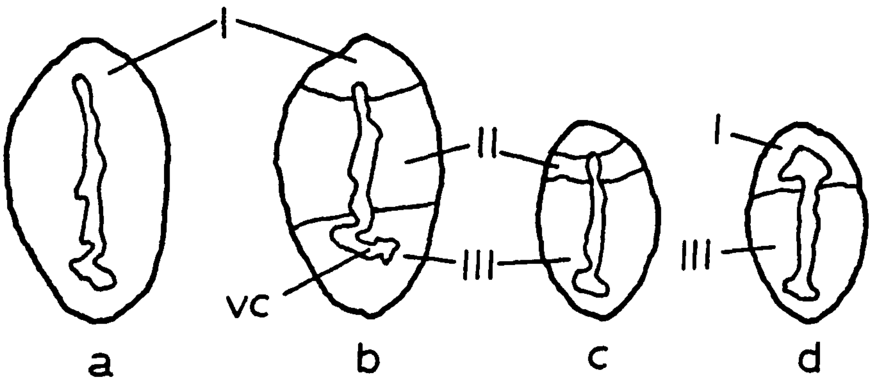

Fig. 22. Sections through Various Regions of the Capsule Gland of *Acanthina angelica*

I - mucous cells, II - pink cells, III - purple cells, vc - ventral channel, a through d - sections from posterior to anterior end of gland

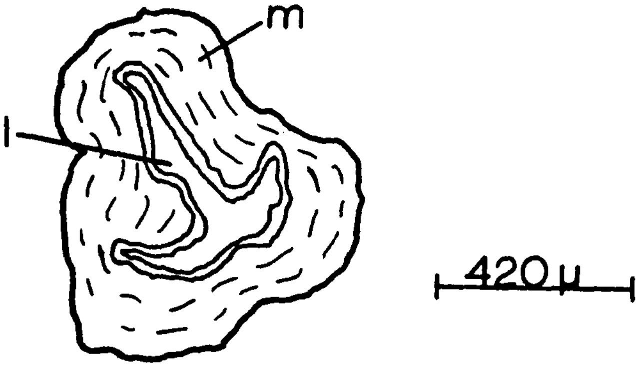

Fig. 23. Section through the Bursa Copulatrix of *Acanthina angelica*

l - lumen, m - muscle layers

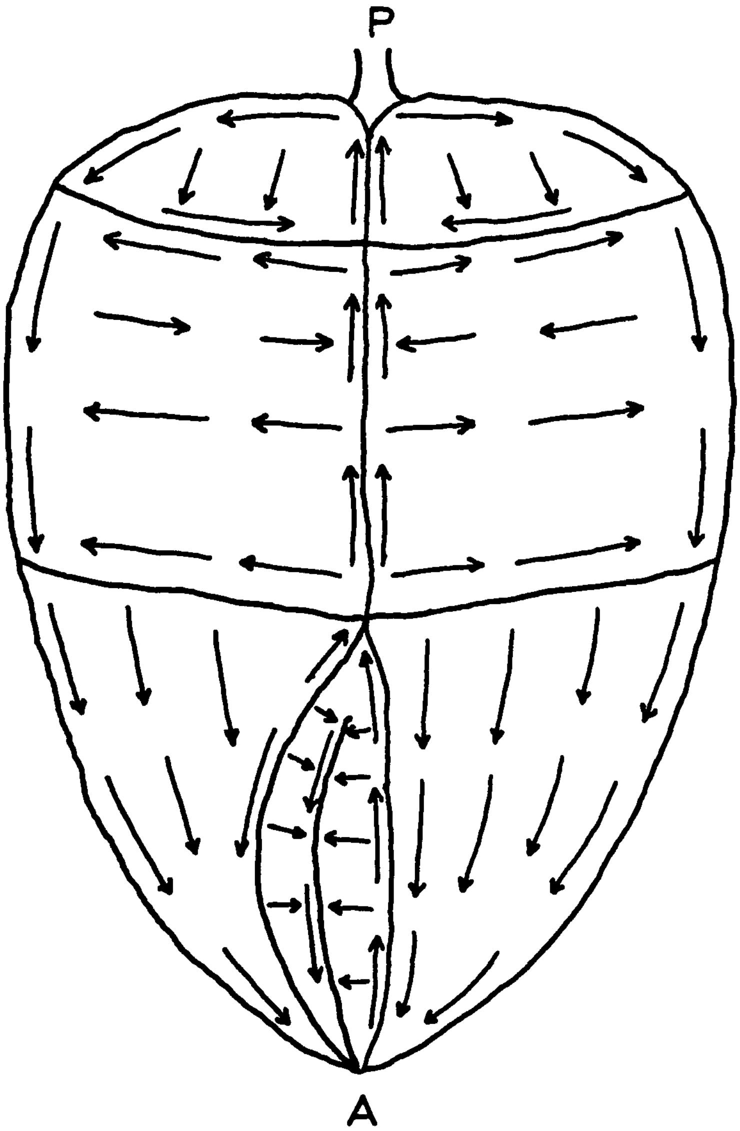

Fig. 24. Ciliary Currents in the Capsule Gland of *Acanthina angelica*

A - anterior, P - posterior

which enables the capsule to pass through the vestibule and out of the genital opening. In addition, the release of the egg capsule is assisted by muscular action.

Columbella fuscata. The female system of this species is quite different from that of *Acanthina angelica*. The bright yellow ovary lies on the outer surface of the visceral mass and is separated from the digestive gland by a thin layer of connective tissue. The ovary consists of many acini which collectively funnel into the upper end of the oviduct. The individual acinus is lined with various cell types, including epithelial cells, secretory cells, and primordial sex cells. Oogenesis occurs in a developmental gradient from the periphery to the center of the acinus. The mature ovum (e) is attached by a peduncle (pe) to a peripheral nurse cell, and pink staining yolk granules and other secretory droplets fill the lumen of the acinus (Fig. 25). The long, transparent oviduct runs along the ventral edge of the ovary and passes down the right side of the viscera. Ventral to the kidney it opens into the capsule gland. There is no albumin gland in this species. The entrance to the posterior region of the capsule gland is guarded by a sphincter muscle. The oviduct is thin-walled and lined with cuboidal ciliated epithelium.

In the living animal, the capsule gland is an opaque, creamy white, glandular mass. In reproductively active individuals this gland will attain a thickness of about two millimeters and a length of five millimeters. It is ciliated throughout, and in cross section

Fig. 25. Section through the Ovary of <u>Columbella</u> <u>fuscata</u>

e - ovum, pe - peduncle

the lumen appears as a dorsoventral slit with short diverticula extending into the lobes. The ventral channel, extending the length of the gland, has fewer cilia which, however, are longer than those lining the lumen. This channel is similar to that of Nassarius reticulatus described by Fretter (1941). Since the staining regions of the capsule are complex, they are listed in Table 2 and their distribution is illustrated in Fig. 26. The posterior region of the gland is largely composed of mucous cells and protein secreting cells, while the remainder consists of a complicated interrelationship of protein, mucous, and muco-protein secreting cells. Figure 27 is a view of the capsule gland dissected along the mid-dorsal line exposing the ciliary tracts. It can be seen that the gland is subdivided into three functional regions based upon the predominant direction of the currents. Posteriorly in region A, the cilia beat in a cyclic manner, while in regions B and C the currents are directed obliquely towards and away from the ventral channel. The distribution of the staining areas in relation to the ciliary tracts are shown in Table 3.

Posteriorly the ventral channel gives rise to two openings. The posterior opening leads to the oviduct, while the anterior one opens into the convoluted gonopericardial duct (gop) (Fig. 28). This latter is quite large and can be easily removed from the living animal. When extended it reaches a length of three millimeters and measures about 70 micra across. It is surrounded by a thin layer of circular muscle cells and lined with cuboidal ciliated epithelium, the cilia of which beat towards the pericardial cavity (pc). The

Table 2. Cell Types in the Capsule Gland of Columbella fuscata

Type	Description
I	Mixed mucous and pink staining eosinophilic cells
II	Mucous cells that stain with Alcian Blue
III	Basophilic cells that stain purple with Hemalum
IV	Neutrophilic cells that stain maroon in color
V	Metachromic cells that stain dark blue
VI	Eosinophilic cells that stain pink with Eosin B

Table 3. Relationship of Ciliary Tracts to Staining Regions in the Capsule Gland of Columbella fuscata

Region	Thickness (micra)	Staining Pattern*	Cell Type	Secretion
A	400	a, b, c	II, VI	mucous and protein
B	1950	d, e	II, III, IV	mucous and muco-proteins
C	2100	f, g, h, i, j, k	I, II, III, IV, V	muco-proteins

* From Fig. 26.

Fig. 26. Sections through Various Regions of the Capsule Gland in *Columbella fuscata* Showing the Differential Staining Patterns

a through k - sections from posterior to anterior end of gland, I - mixed mucous and eosinophilic cells, II - mucous cells, III - basophilic cells, IV - neutrophilic cells, V - metachromic cells, VI- eosinophilic cells

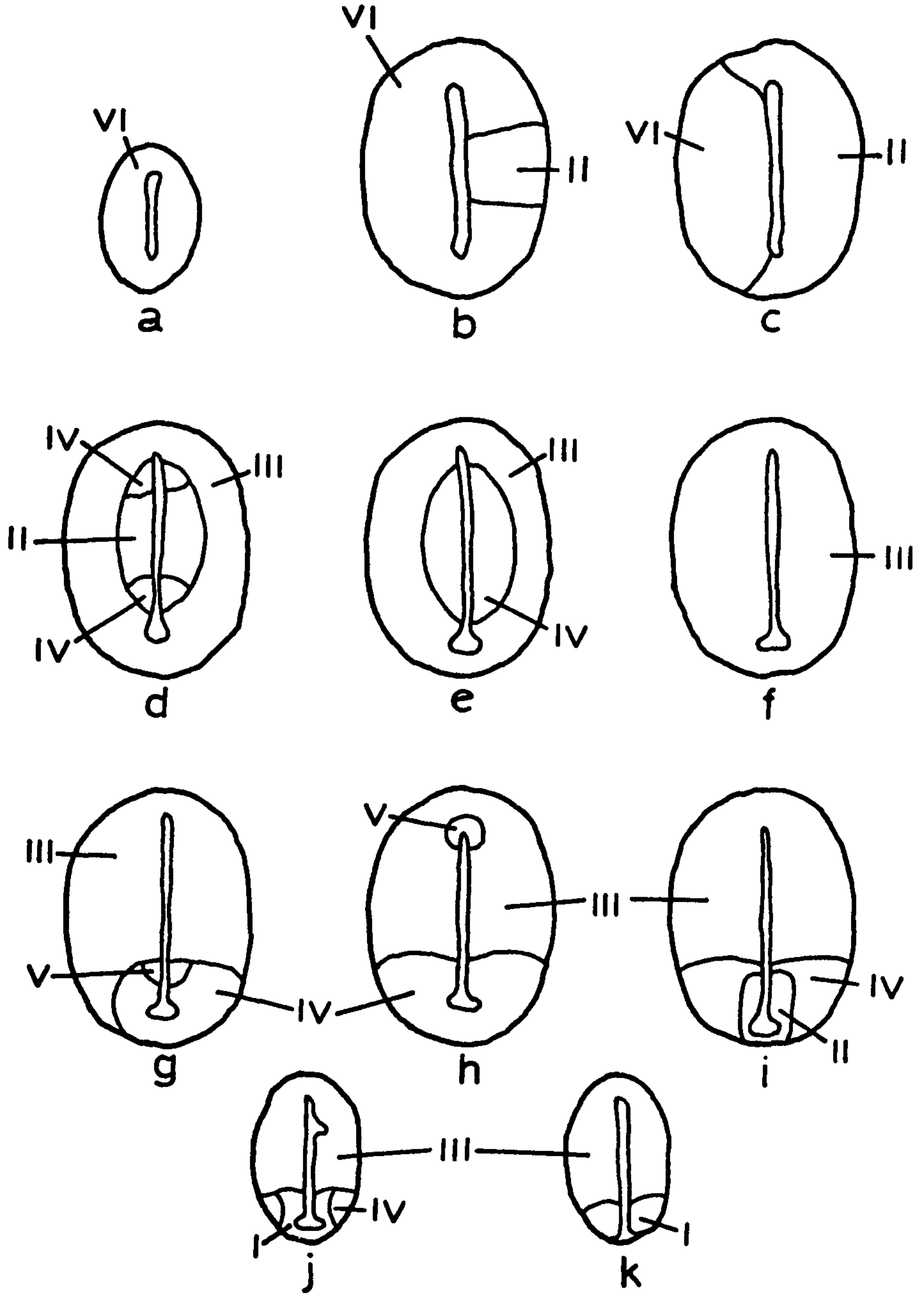

Fig. 26. Sections through Various Regions of the Capsule Gland in *Columbella fuscata* Showing the Differential Staining Patterns

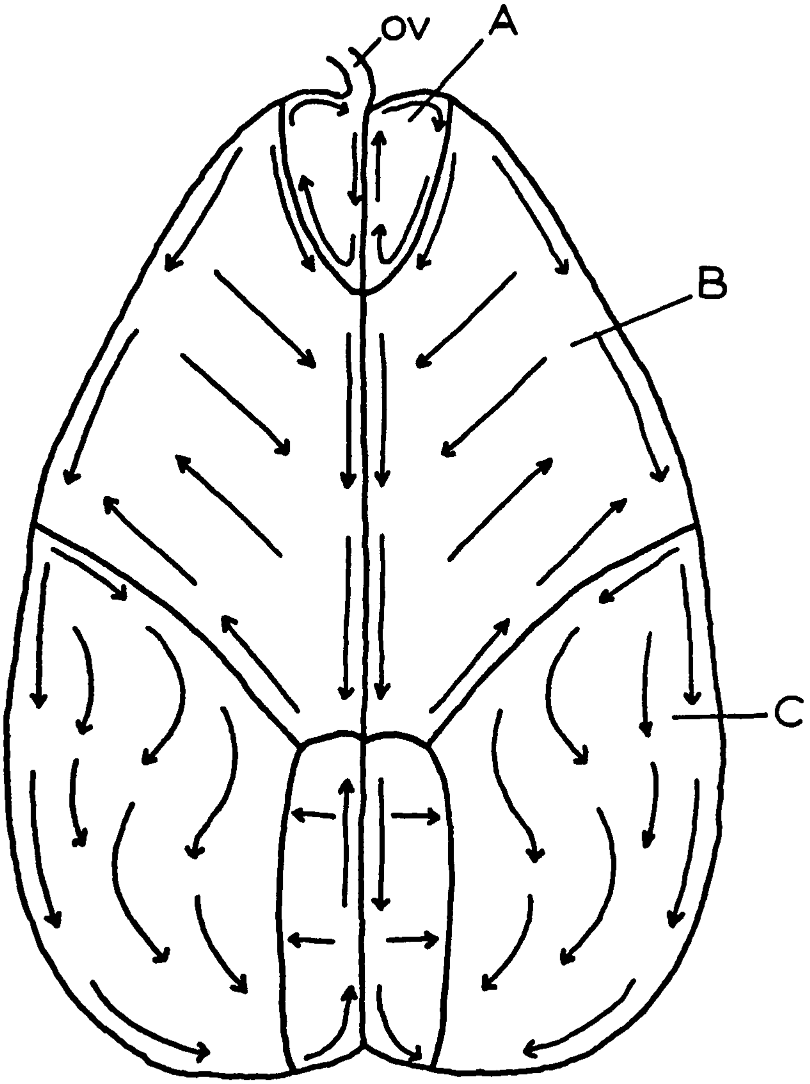

Fig. 27. Ciliary Currents in the Capsule Gland of *Columbella fuscata*

A, B, and C - ciliary regions, ov - oviduct

Fig. 28. Section through the Gonopericardial Duct of <u>Columbella fuscata</u>

cg - capsule gland, gop - gonopericardial duct,
pc - pericardial cavity

opening into the cavity is guarded by a thick sphincter. The pericardial wall is ciliated on the left side and glandular on the right. The pericardial cavity is the site for sperm ingestion, which is discussed below.

The lumen of the capsule gland can be traced anteriorly where it empties into the deeply folded vestibule (ve). It is large and bulges into the mantle cavity when occupied by newly formed egg capsules. The vestibule is ciliated and contains scattered subepithelial mucous cells. There is no musculature and the entire structure is surrounded by a thin layer of connective tissue. A short ciliated vagina connects the vestibule with the external genital opening. Histologically the vagina and vestibule are similar. In spawning females, a ciliated groove can be traced from the genital opening down to the region of the ventral pedal gland in the foot.

Crassispira pluto. The female system of this species is similar to that of Acanthina angelica; however, there are some noteworthy differences. From the ovary the thin-walled oviduct passes anteriorly along the inner side of the visceral mass. Here, in the region of the kidney it joins the albumin gland. There is no sphincter muscle at the posterior entrance. In the living animal the opaque albumin gland is not doubled back on itself, but appears as an enlargement of the oviduct with right and left lobes. It is ciliated and contains subepithelial cells of two types. Those on the left color deeply with Alcian Blue, while those in the right wall stain with Hemalum. A short region of the pallial oviduct connects the albumin and capsule glands. This duct is ciliated and covered with a thin sheet of circular muscle cells.

Posteriorly the ciliated capsule gland is composed of two types of subepithelial cells, which are mixed throughout this region, One type stains with Hemalum while the other stains pink with Eosin B. About halfway through the gland, the dorsal region contains only eosinophilic cells, while basophilic cells make up the rest of this area. The anterior part of the capsule gland is composed chiefly of mucous cells and a thin medial strip which colors deep purple with Hemalum.

The ventral channel is ciliated throughout and separated partially from the main lumen by two equal size flaps of tissue. The lumen of the capsule gland opens into the large, deeply folded, ciliated vestibule, while the ventral channel gives rise to the bursa copulatrix. Therefore, it can be deduced that the vestibule receives the egg capsules prior to deposition. There are subepithelial mucous cells present, and on several occasions the lumen of the vestibule contained copious amounts of mucus. Hence, it appears that mucus, with the aid of cilia, moves the egg capsules to the exterior.

Just dorsal to where the pallial oviduct opens into the capsule gland is a short ciliated duct which leads to an elongated sac. This sac occupies a similar position to that of the ingesting gland of _Acanthina_ _angelica_. It contains sperm stored in an orderly fashion and, therefore, functions as a seminal receptacle. The epithelium which lines the lumen stains with Alcian Blue and has basal, oval nuclei that stain red with Hemalum.

Olivella dama. The bright yellow ovary is located in the two uppermost whorls behind the digestive gland. The oviduct that connects the ovary to the albumin gland is extremely long. The upper end is thin-walled, non-muscular, and lined with squamous non-ciliated epithelial cells. Anteriorly, however, the oviduct is covered by a thin layer of circular muscle cells and lined with ciliated cuboidal epithelium. As the oviduct opens onto the right side of the junction of the albumin and capsule glands, the musculature increases to form of a sphincter. There is no duct separating the two glands, as in Acanthina angelica and Crassispira pluto.

The histology of the albumin gland is similar to Acanthina angelica, in that two gland cells are present. In this species, however, those that stain with Alcian Blue are on the right and those with Hemalum occur on the left. The lumen is ciliated and is continuous with that of the capsule gland.

The capsule gland consists of three types of subepithelial cells. The medial and dorsal regions color deeply with Hemalum, while the ventral area is mainly eosinophilic with scattered groups of mucous cells. Each group consists of five flask-shaped cells which empty into common ducts that intersperse the epithelium lining the lumen. The lumen is ciliated with no distinctive ventral channel. However, in this region the cilia are long and may function as a ventral channel.

Anterior and adjacent to the oviduct is a gonopericardial duct which connects the capsule gland with the pericardial cavity.

It is ciliated and covered with a thin layer of circular muscle cells. A sphincter is located at the opening into the capsule gland. The opening from the duct into the pericardial cavity is in the shape of a ciliated funnel with a diameter of thirty micra. No sperm cells were observed in either the duct or the cavity.

The long cilia in the ventral region of the capsule gland pass anteriorly to the bursa copulatrix. The bursa is extremely long and muscular and composed of longitudinal muscle fibers surrounded by a thick layer of circular muscle cells. The bursa opens into a short muscular vagina, which in turn communicates with the exterior. There is no vestibule in *O. dama*.

Nassarius tiarula. The female genital tract of this species differs from that of *O. dama* in several respects. The ovary occupies the upper whorls of the visceral mass and interdigitates with the digestive diverticula. The oviduct is not as long as that of *Olivella dama*, but is divided into two regions. The upper end is thin-walled and non-ciliated, while the lower region is ciliated and muscular. This duct runs along the inner side of the visceral mass and passes very close to the pericardial cavity. As it enters the albumin gland, it bifurcates and the ventral arm becomes the gonopericardial duct. It is ciliated throughout and empties into the dorsal region of the pericardial cavity as a funnel. There is a large sphincter muscle at the point of bifurcation which controls the entrance to both the gonopericardial duct and the albumin gland.

The bright orange albumin gland is attached directly to the posterior end of the capsule gland. It is ciliated throughout and contains three types of subepithelial cells. Those in the right lobe are of one type that stains purple with Hemalum. In contrast, the other two types are in the left wall and stain with Alcian Blue.

The capsule gland is quite long and tapers suddenly at its anterior end. Posteriorly the gland is composed chiefly of eosinophilic cells, with a few mucous cells scattered throughout the dorsal region. Sections through the rest of the gland show that eosinophilic cells are limited to the medial regions, while mucous cells comprise the roof of the gland. In addition, basophilic cells line the ventral area. The lumen is ciliated throughout and the ventral channel is similar to that of Crassispira pluto. However, the flaps of tissue which make its dorsal wall are less pronounced. Anteriorly this channel opens into a muscular vestibule that is ciliated and deeply folded. When occupied by an egg capsule this region becomes highly distended.

Upon dissection, the distal end of the female duct appears as a brown kidney bean-shaped mass joined to the capsule gland by a short duct. Ventrally are two flaps of ciliated tissue which enclose two openings that lie in tandem. The posterior opening is in direct communication with the duct that opens into the vestibule. This opening is ciliated and assumes a nidimental function (no). Dissections of several gravid individuals induced the passage of egg capsules through this opening. The anterior opening leads to the bursa copulatrix. Sections reveal a non-ciliated blind sac partially divided into right and left chambers by a dorsal flap of tissue. Since there is no direct communication between the bursa and the ventral channel, spermatozoa deposited in this chamber migrate back through the aperture and are carried by cilia to the posterior opening. Here they migrate up the duct, through the vestibule, and subsequently to the ventral channel. A similar condition exists for Nassarius reticulatus; however, there is direct communication between the bursa and the vestibule (Fretter 1941).

Solenosteira macrospira. From the ovary, the thin-walled oviduct opens into the albumin gland. It is doubled back on itself, but in a more loose fashion, giving a U-shaped appearance. The histology of the albumin gland is similar to that of the previous species. A short duct separates the albumin and capsule glands. Lying between these glands is a brown elongated sac which may function as a seminal receptacle, although no sperm was observed in this region.

Anteriorly the large capsule gland can be seen underneath the right mantle wall. In cross sections the ciliated lumen divides the gland into equal right and left lobes. The well defined ventral channel is much like that of *Acanthina angelica*. The glandular components of the gland are not as complex as those in *Columbella fuscata*. The posterior tips largely consist of mucous cells, while the rest of the gland, except the anterior region, is chiefly a mixture of mucous cells and protein secreting cells. The anterior region is mostly mucous cells.

Anteriorly the ventral channel enters the vestibule. Here it runs along the floor of the chamber and to the outside through a short ciliated vagina. The vestibule is not as deeply folded as that of *Columbella fuscata*, however, it is muscular. The musculature is complex, consisting of a lattice network of longitudinal and oblique fibers interspersed by subepithelial mucous cells.

Reproductive Systems of Additional Species

The gross anatomies of the following species investigated in this study are illustrated in Figs. 29 and 30.

Family: Muricidae

The Male Duct. The vas deferens can either be convoluted as in *Thais emarginata*, *T. canaliculata*, *T. haemostoma*, *Trophon barvicenses*, *Urosalpinx cinerea*, and *Ocenebra japonica*; or more or less straight as in *Drupa nodulosa*, *Trophon truncatus*, *Acanthina spirata*, and *Ceratostoma foliatum*. The upper end of the convoluted vas deferens acts as a seminal vesicle. Openings from the vas deferens into the mantle cavity in *Trophon truncatus*, *Acanthina spirata*, and *Ceratostoma foliatum* occur by means of a small ciliated duct located just posterior to the prostate gland. In these species there is also a sphincter located anterior to the seminal vesicle.

The prostate glands are typical of those found in *Ocenebra erinacea* (Fretter 1941). In *Thais canaliculata* and *Trophon barvicenses* the posterior region is not fused, and there is communication to the mantle cavity by a ventral slit.

Fig. 29. The Male Ducts

a - *Thais emarginata*, b - *Thais canaliculata*, c - *Thais haemostoma*, d - *Drupa nodulosa*, e - *Trophon truncatus*, f - *Trophon barvicenses*, g - *Acanthina spirata*, h - *Ocenebra japonica*, i - *Urosalpinx cinerea*, j - *Ceratostoma foliatum*, k - *Colus gracilis*, l - *Neptunea antigua*, m - *Cantharus d'orbigny*, n - *Fusus rostratus*, o - *Pisania janerensis*, p - *Nassarius incrassatus*, q - *Mitrella guttata*, r - *Columbella strombiformes*, s - *Anachis coronata*, t - *Anachis nigrofusca*, u - *Anachis sanfelipensis*, v - *Anachis varia*, ct- *connective tissue*, omc - opening to the mantle cavity, pc - pericardial cavity, pd - penial duct, pg - prostate gland, pn - penis, sv - seminal vesicle, te - testis, vd - vas deferens

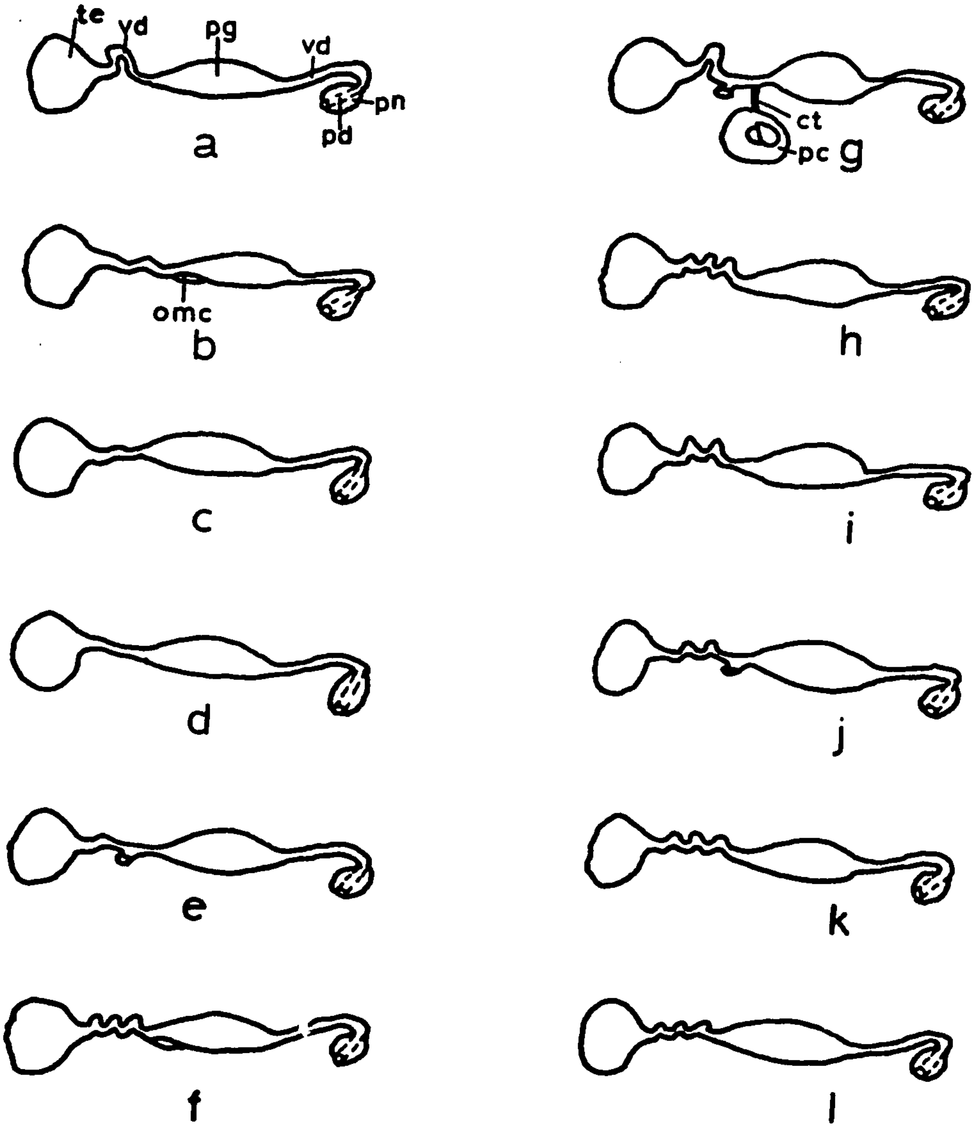

Fig. 29. The Male Ducts

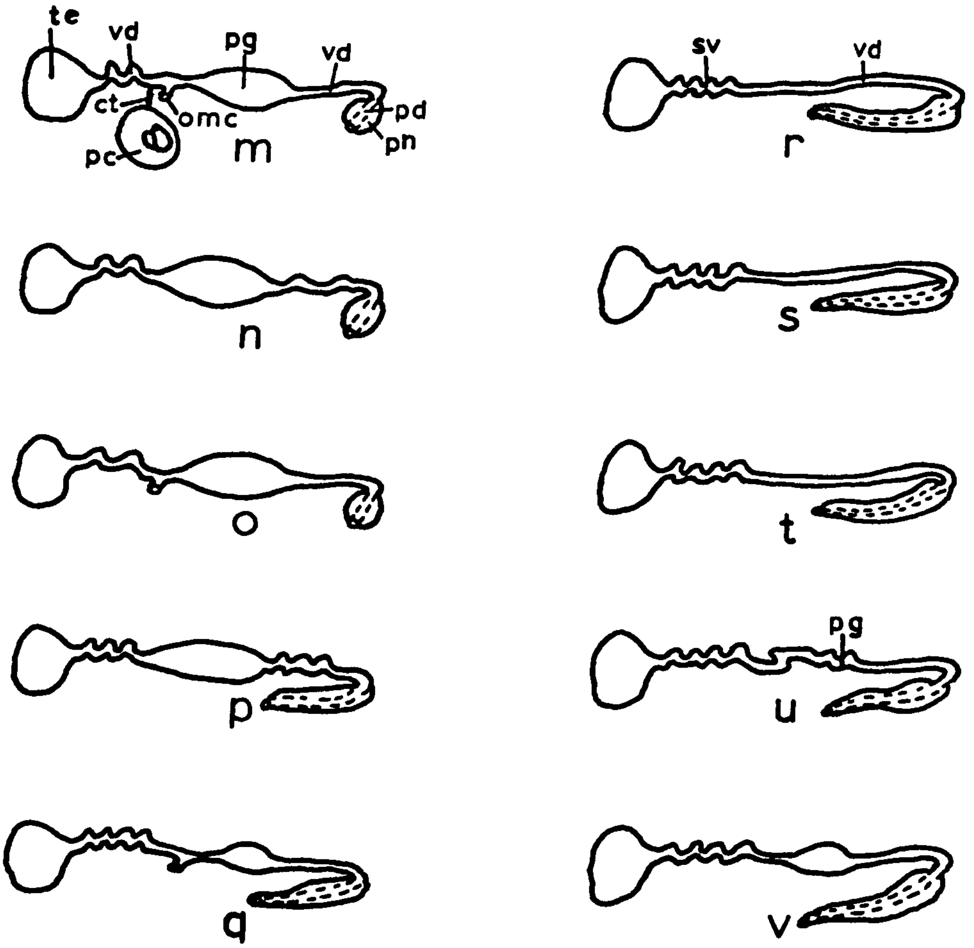

Fig. 29. The Male Ducts (continued)

Fig. 30. The Female Ducts

a - *Thais emarginata*, b - *Thais canaliculata*, c - *Thais haemostoma*, d - *Drupa nodulosa*, e - *Trophon truncatus*, f - *Trophon barvicenses*, g - *Acanthina spirata*, h - *Ocenebra japonica*, i - *Urosalpinx cinerea*, j - *Ceratostoma foliatum*, k - *Colus gracilis*, l - *Neptunea antigua*, m - *Cantharus d'orbigny*, n - *Fusus rostratus*, o - *Tritonalia erinacea*, p - *Pisania janerensis*, q - *Nassarius incrassatus*, r - *Mitrella guttata*, s - *Columbella strombiformes*, t - *Parametaria duponti*, u - *Anachis coronata*, v - *Anachis hilli*, w - *Anachis nigrofusca*, x - *Anachis sanfelipensis*, y - *Anachis varia*, ag - albumin gland, bc - bursa copulatrix, cg - capsule gland, ct - connective tissue, gop - gonopericardial duct, go - genital opening, ig - ingesting gland, oa - ovary, omc - opening to the mantle cavity, ov - oviduct, pc - pericardial cavity, sr - seminal receptacle

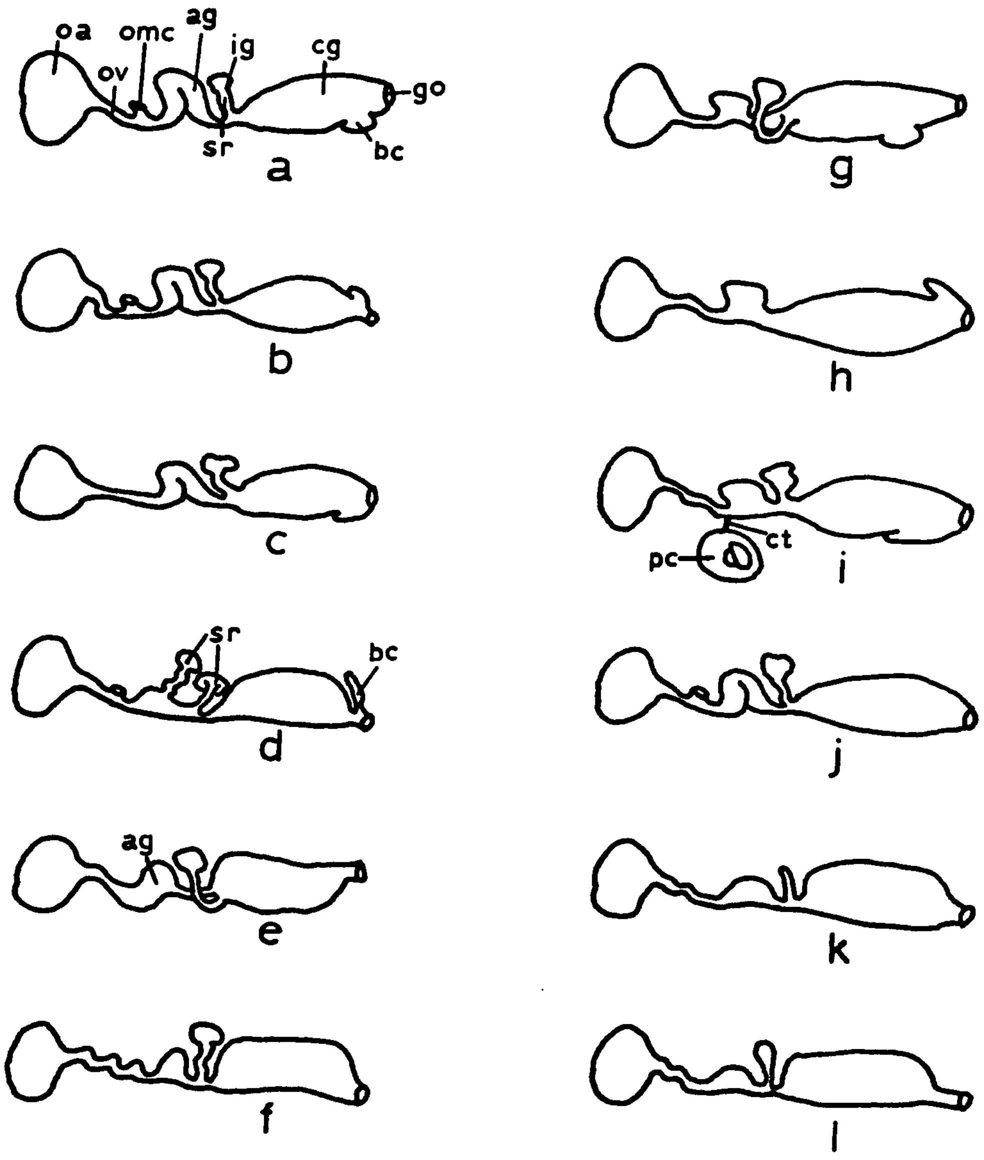

Fig. 30. The Female Ducts

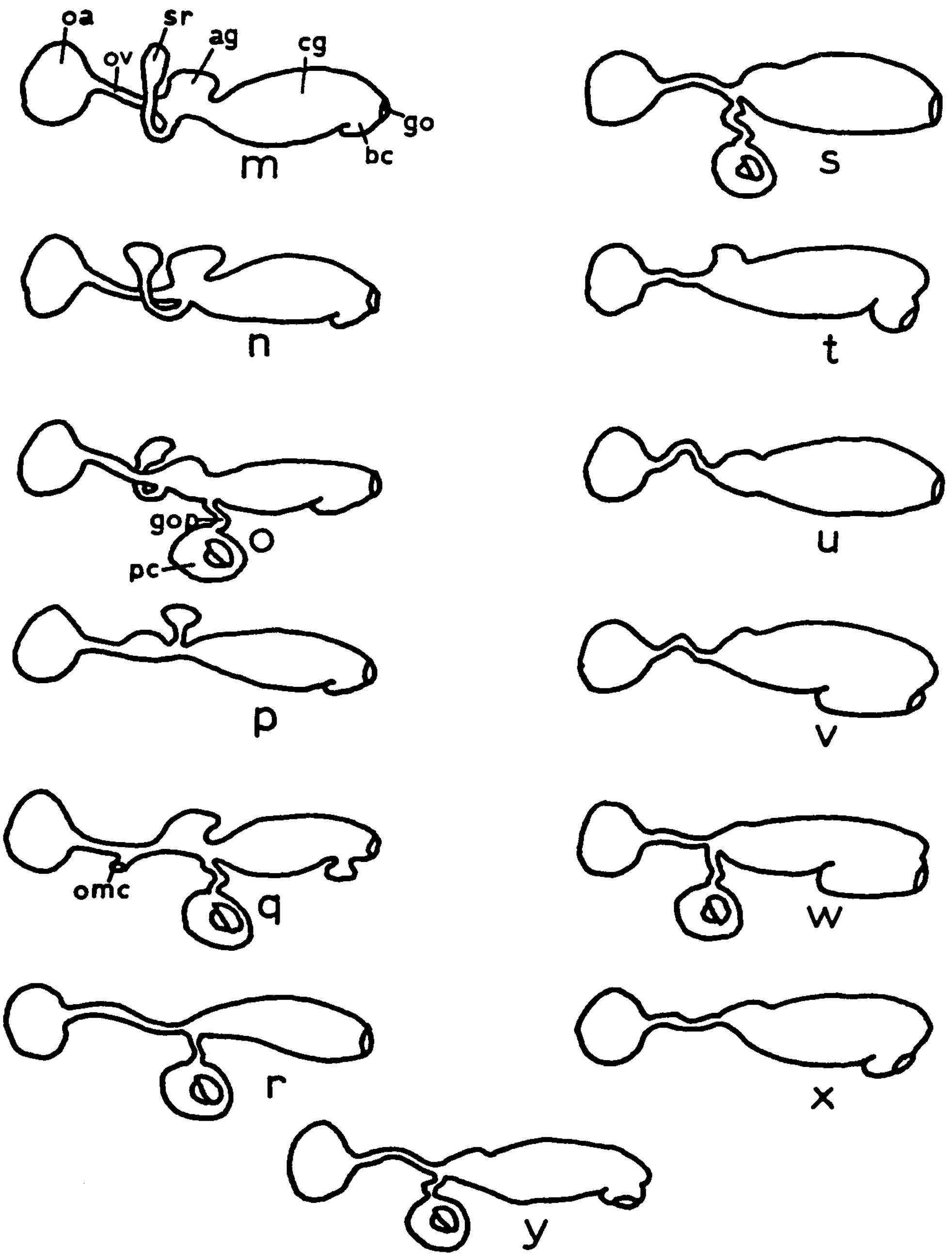

Fig. 30. The Female Ducts (continued)

No functional gonopericardial duct occurs in the males examined. There is, however, a remnant of a duct in *Acanthina spirata*. Histologically it appears as a thin strand of connective tissue (ct) joining the pericardial wall to the vas deferens.

All of the species have a penis which is flattened dorsoventrally. The penial ducts are either centrally located or off to one side as in *Thais emarginata*, *T. canaliculata*, and *T. haemostoma*. This agrees with the findings of Fretter (1941) for *Nucella lapillus* and *Ocenebra erinacea*. However, in *O. japonica* the penial duct is centrally located. In *Trophon truncatus* and *T. barvicenses* the penial duct is highly convoluted, and the anterior vas deferens runs for only a short distance before joining the prostate gland. This duct is much longer in the other species.

The Female Duct. Generally the vagina is a ciliated, muscular, and sometimes glandular tube running parallel to the rectum. It is, however, reduced to a small cavity in *Thais emarginata*, *T. canaliculata*, *T. haemostoma*, and *Drupa nodulosa*. This condition is also found in *Nucella lapillus* (Fretter 1941). The morphology of the bursa copulatrix is highly variable, and its location will vary depending upon the species. It has the appearance of a bulbous sac just anterior and ventral to the capsule gland in *Thais emarginata*, *Acanthina spirata* and *Urosalpinx cinerea*, as opposed to being dorsally located in *Thais canaliculata* and *Drupa nodulosa*. In *T. emarginata* sperm can be seen attached to the bursal wall. The bursa is quite large in *T. haemostoma*, while that of *Trophon barvicenses* is reduced to a small vesicle just posterior to the vagina.

The capsule glands are remarkably similar among the several species with the lumen appearing as a ciliated dorsoventral slit. The ventral channel in Acanthina spirata is almost a closed duct. There is no connecting duct, but only a slight constriction separating the albumin and capsule glands in Ocenebra japonica and Urosalpinx cinerea. In contrast, a duct joins the two glands in the other species. In Thais emarginata, T. canaliculata, T. haemostoma, and Ceratostoma foliatum, the albumin gland is doubled back on itself like a hairpin.

Thais emarginata, T. canaliculata, T. haemostoma, Urosalpinx cinerea, Drupa nodulosa, Acanthina spirata, and Ceratostoma foliatum have an ingesting gland. The ducts to these glands originate from the posterior region of the capsule gland. The lumina of the glands are composed of numerous blind diverticula containing masses of sperm. Sperm ingestion was observed in Thais emarginata, T. canaliculata and U. cinerea. Ocenebra japonica, Trophon truncatus and T. barvicenses do not have an ingesting gland.

The duct to the ingesting gland in Thais canaliculata is modified as a seminal receptacle. Although no sperm ingestion was observed in Trophon truncatus and T. barvicenses, similar ducts arise from the posterior region of the capsule gland, which end in glandular vesicles. In T. barvicenses sperm heads can be seen attached to the epithelial walls of the vesicle. Hence, these regions serve as areas for sperm storage. The seminal receptacle in Drupa nodulosa and Thais haemostoma is separate and lies posterior to the ingesting gland. It consists of a convoluted duct ending in a small bulb that is embedded within the nephridium. The ventral channels in *T. emarginata* and *Urosalpinx cinerea* give rise to elongated sacs for sperm storage. In *T. emarginata* the sac lies to the left of the ventral channel, and in *U. cinerea* it lies to the right. It can be seen as a bulging ridge along the right side of the capsule gland in the living animal. In both species this sac is open to the ventral channel throughout its entire length, until it reaches the posterior region of the gland. In this area it fuses into a duct that crosses over to the opposite side and opens into the ingesting gland. Spermatozoa within these sacs are neatly arranged with their heads attached to the epithelium, and their tails extending into the lumen. No seminal receptacles have been observed in *Ocenebra japonica*, *Ceratostoma foliatum*, and *Acanthina spirata*.

Both *Thais haemostoma* and *Drupa nodulosa* have a gonopericardial duct, which connects the pericardial cavity with the pallial oviduct, just anterior to the albumin gland. The ducts are ciliated and are guarded by a sphincter upon entering the cavity. In *Ocenebra erinacea* the duct opens into the cavity through a prominent ciliated funnel instead of a sphincter (Fretter 1941). The duct has disappeared in *Urosalpinx cinerea* and is represented by only a thin strand of connective tissue.

The posterior region of the oviduct in *Thais emarginata*, *T. canaliculata*, *Drupa nodulosa*, and *Ceratostoma foliatum* is open to the mantle cavity via a short ciliated duct.

Family: Buccinidae

The Male Duct. The gross morphology of the genital tracts of the buccinids in this study are similar to that of *Buccinium undatum* described by Fretter (1941). The upper end of the vas deferens is coiled and serves as a seminal vesicle. In *Cantharus d'orbigny* a sphincter muscle is present just anterior to the seminal vesicle. The presence of connective tissue joining the pericardial wall to the vas deferens indicates a remnant of a gonopericardial duct in this species. Also, the vas deferens is open to the mantle cavity via a short ciliated duct. The prostate gland of *Fusus rostratus* is surrounded by a thin circular muscle layer. No males of *Tritonalia erinacea* were observed.

The Female Duct. The major difference in the gross morphology of the genital tracts of buccinids, as compared to those of muricids, is the location of the duct to the ingesting gland. In *Cantharus d'orbigny*, *Fusus rostratus*, and *Tritonalia erinacea* this duct originates from the albumin gland, as opposed to the capsule gland in muricids. *Neptunea antigua*, *Colus gracilis*, and *Pisania janerensis* have no ingesting gland. The seminal receptacle in *N. antigua* and *C. gracilis* is divided with one sac on each side of the ventral channel. There is a convoluted, ciliated gonopericardial duct connecting the pericardial cavity to the posterior region of the capsule gland in *T. erinacea*. Another interesting note is that the ventral channel of *T. erinacea* is sickle shaped.

Family: Nassariidae

The reproductive system of Nassarius incrassatus is quite different from that of N. reticulatus described by Fretter (1941).

The Male Duct. The vas deferens is greatly coiled at the upper end like that of the buccinids. The prostate gland is interesting in that it is surrounded by a thick muscle layer and becomes convoluted at its anterior end. Leaving the prostate is the coiled, muscular, anterior vas deferens that terminates in a tubular penis.

The Female Duct. The muscular vagina leads into the bursa copulatrix, which appears as a pouch located ventral to the capsule gland. According to Johansson (1957), a seminal receptacle and ingesting gland exist in this species. However, both organs are absent in the specimens examined for this study. There is a ciliated gonopericardial duct that joins the ventral channel near the posterior region of the capsule gland. Within this gland was a partially formed egg capsule containing numerous eggs. A sphincter muscle controls the posterior entrance to the large albumin gland. A short duct from the oviduct opens into the mantle cavity about halfway between the albumin gland and the ovary.

Family: Columbellidae

The Male Duct. Unfortunately no male specimens of Anachis hilli and Parametaria duponti were collected. Therefore, their genitalia are not included.

The upper vas deferens in all the columbellids in this study is modified into a seminal vesicle. It is interesting to note that one specimen of *Anachis varia* had a vas deferens which bifurcated and rejoined, resulting in a double duct. In *Mitrella guttata* a sphincter muscle is present just anterior to the seminal vesicle.

A prostate gland is present in *M. guttata*, *A. varia*, and *A. sanfelipensis*. It appears as an elongated glandular mass in *M. guttata* and *A. varia*, as opposed to a coiled duct in *A. sanfelipensis*. The anterior vas deferens is highly muscular and weakly ciliated in all of the species examined. The penes are similar to that of *Columbella fuscata* in that they are wide at the base and taper to a pointed tip. In addition, all of these species have a pouch in the posterior mantle roof into which the penis is tucked when not in use.

An opening from the vas deferens into the posterior mantle cavity is present only in *M. guttata*. This opening differs from that of *C. fuscata*, in that it appears as a short ciliated duct arising from the posterior vas deferens.

The Female Duct. The female systems of these species are basically similar to that of *Columbella fuscata*, except for the following features. *Anachis sanfelipensis* has a large muscular bursa copulatrix which is non-ciliated. This structure acts as a seminal vesicle because females that had previously copulated were found to contain sperm neatly arranged in rows. Sperm storage also occurs in the bursae of *Anachis brasiliana*, *A. sparsa*, and *A. veleda* (Marcus and Marcus 1962b). *Anachis coronata*, *A. varia*, and *Parametaria duponti* have an albumin gland which occupies the posterior tip of the capsule gland. Columbella fuscata has no albumin gland. A gonopericardial duct is present in A. varia, A. nigrofusca, Columbella strombiformes, and M. guttata.

As in Columbella fuscata, sperm ingestion occurs in the pericardial cavity of Mitrella guttata. Here the sperm are shunted to a diverticulum off the main cavity, where they are broken down by the epithelial cells.

Egg Capsules

The egg capsules of Columbella fuscata, Acanthina angelica, Nassarius tiarula, and Solenosteira macrospira are illustrated in Fig. 31.

Columbella fuscata

In this species, spawning occurs throughout the year with peak periods from June through August, and November through the middle of February. Females deposit their capsules on Padina and Sargassum. Individual females can deposit up to 40 capsules; however, the average mass contains about 20. The light brown capsules are deposited in alternating rows and average 2.2 mm in height and 1.5 mm in width. They are flask shaped and are attached to the algae by an adhesion disk. The top is surrounded by a flanged collar, and the mucous plug is centrally located.

There are about 50 eggs (e) in each capsule which are distributed in a loosely packed mass. There is no apparent albumin present around the eggs.

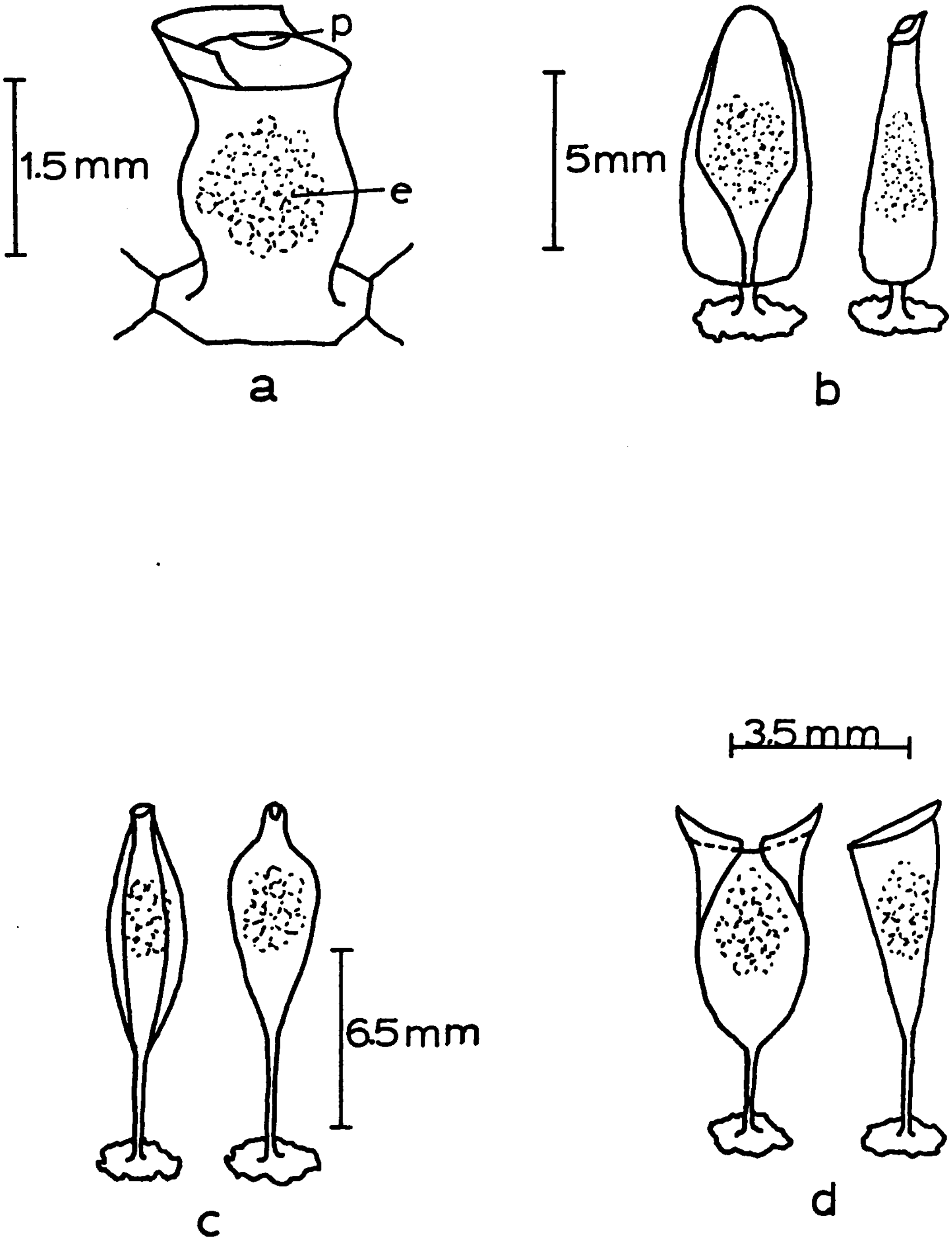

Fig. 31. Egg Capsules

a - Columbella fuscata, b - Acanthina angelica,
c - Nassarius tiarula, d - Solenosteira macrospira,
e - eggs, p - mucous plug

The histology of the capsule wall is shown in Fig. 32a. The wall of the capsule is made up of four layers. The thin innermost mucous layer is homogeneous and transparent. The two middle layers consist of oblique protein fibers directed in a crisscross network. The outer layer which is secreted by the ventral pedal gland, is composed of mucus. The formation of the egg capsule is discussed below.

Acanthina angelica

The egg capsules of this species were described earlier by Wolfson (1970). A. angelica spawns from the middle of December through the last week in March. Females aggregate in large numbers underneath the basaltic boulders to deposit their capsules. These egg masses can be quite large, sometimes numbering up to 500 capsules.

The bright yellow capsule averages 5 mm in height and 2 mm in width. It is vase shaped and attached to the substratum by a short stalk. The surface of one side is flat and bordered by two longitudinal ridges running the length of the capsule. The top is sealed by a round mucous plug which dissolves away when the juveniles hatch. The walls are translucent and the egg mass can be seen within.

The yellow eggs are rather large and yolky, reaching a diameter of about 250 micra. In freshly laid capsules, the eggs are compacted into a mass of 400-500 in number and surrounded by a coat of albumin.

Histological sections reveal the three layers that compose the capsule wall (Fig. 32b). The innermost layer is thin and

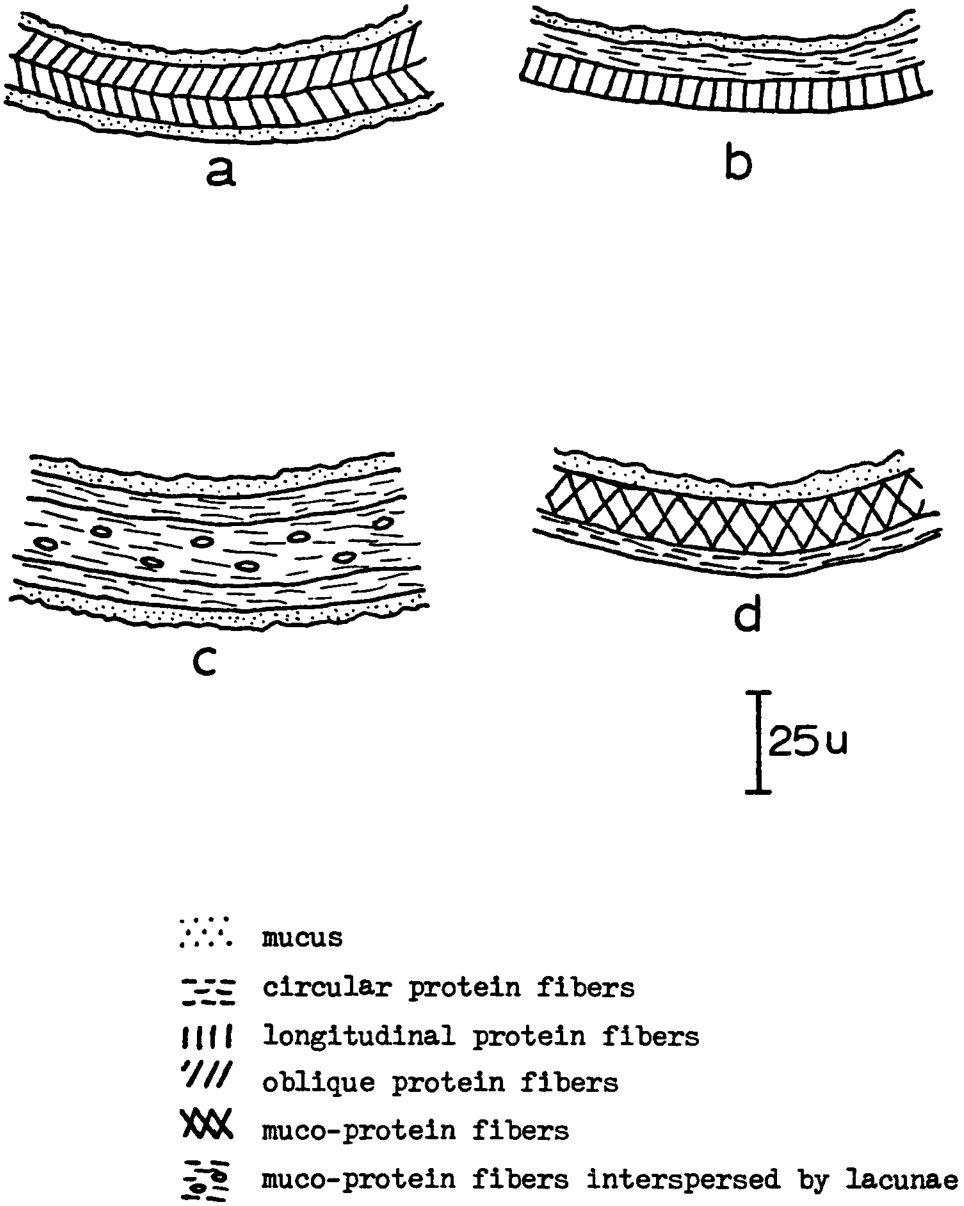

Fig. 32. Egg Capsule Histology

a - *Columbella fuscata*, b - *Acanthina angelica*,
c - *Nassarius tiarula*, d - *Solenosteira macrospira*

homogeneous and stains with Alcian Blue. The middle and outer layers are fibrillar in appearance and stain pink with Hemalum and Eosin B. In addition, a homogeneous substance is dispersed among the fibers. The direction of the fibers in the middle layer are circular, while those in the outer layer are longitudinal. In capsules from Nucella lapillus, Fretter (1941) showed that the circular fibers make up the outer coat, while the middle layer is longitudinal. Ankel (1937) proposed that the egg capsule of the same species is composed of two substances, the protein and conchiolin intermixed with mucus or a mucoid substance. This has been substantiated in histochemical studies by Bayne (1968), in which acid mucopolysaccarides and conchiolin were found to be the major components of the capsule wall.

Based upon the histological evidence and ciliary currents within the capsule gland, formation of the egg capsule may occur in the following manner. As the egg mass enters the capsule gland, mucoid substances are secreted around the eggs by the posterior tips of the gland. Cilia in this region beat forward to drive the mass, with its mucoid covering, into the protein secreting areas of the gland. Here the cilia beat towards and away at right angles to the main axis of the gland, thereby mixing some of the mucoid material with the protein in forming the middle layer. Ciliary tracts in the anterior region of the gland beat parallel to the main axis, and it is here that the outer or longitudinal layer is secreted. In the anterioventral region of the right lobe is a strip of mucous cells. Here the cilia beat in opposing directions perpendicular to the ventral channel. This region possibly functions in the synthesis of the mucous plug. The newly formed capsule passes through the vestibule and out the genital opening. It is then transported along a ciliated groove to the ventral pedal gland in the foot, where the layers are compressed. Finally, it is attached to the substratum and hardened into its final shape.

Crassispira pluto

No egg capsules were observed for this species.

Olivella dama

No egg capsules were observed for this species.

Nassarius tiarula

Spawning can be observed from April through the last of June for this species. Females deposit their pale yellow egg capsules in clusters of 15 to 30 on empty shells lying just beneath the surface of the mud. They are vase shaped and have four sides. The front and back are flat, while the sides are slightly convex. The opening at the top is fitted with a mucous plug. The bottom of the capsule tapers to a long narrow stalk, which is attached to the adhesive disk. The entire capsule measures 13 mm high and about 3 mm wide. Each capsule contains about 50 eggs which float in an albuminous fluid.

During dissection of gravid females, egg capsules were released from the nidimental opening. First a small transparent bubble appeared which proved to be the basal region of the capsule.

As the bubble was squeezed out, the opening was dilated to about three times its normal diameter. Shortly after, the eggs were released and appeared first as a single string surrounded by albuminous fluid. Prior to release of the capsule, the eggs clumped into a central mass. The top of the capsule with the mucous plug intact was the last to leave the nidimental opening. This newly formed capsule was soft and transparent. It was then transported along a ciliated groove to the ventral pedal gland.

In cross section the capsule wall appears to have five layers (Fig. 32c). An innermost mucous layer is surrounded by a coat of circular fibers. Peripheral to this is a loose network of fibers interspersed by lacunae, which are filled with a mucoid substance. The fourth layer is another coat of circular fibers. The final layer is a tenuous mucous coat surrounding the entire capsule.

Solenosteira macrospira

For this species, spawning begins in March and continues through the first week of June. This is an interesting species because the female deposits the egg capsules on the shell of the male. On many occasions the shell of the male was completely obscured by the capsules.

The capsules are transparent and the reddish-brown eggs can be observed inside floating in the albuminous fluid. The flask shaped capsules stand about 7 mm high and are attached to the shell by a long slender stalk like that of Nassarius tiarula. The top is concave and bordered by a flanged collar extending around almost the entire circumference. One side of the capsule is flat and bordered by two longitudinal ridges that are continuous with the edge of the collar. In each capsule there are up to 500 eggs averaging 200 micra in diameter.

The wall of the egg capsule consists of three layers (Fig. 32d); however, there is no mucous inner layer as in the other species discussed. The inner region is a muco-protein layer bordered by a thick fibrous muco-protein coat. This is covered by an outer layer consisting of circular fibers.

Reproduction in Columbella fuscata

Copulatory Behavior

Copulation in Columbella fuscata occurs in the sand around the bases of Padina and Sargassum. When the mates pair, the male crawls onto the shell of the female and grasps the anterior siphonal canal with his epipodial palps. At this point, both male and female are lying side by side, with their anterior canals facing in the same direction. The penis is removed from the posterior pouch and inserted into the genital aperture of the female. When this occurs there is a slight twisting movement of both partners. Ejaculatory movements occur at 5 second intervals for about one minute. At this time, the male jerks violently while the female remains more or less motionless. Soon after ejaculation is completed, the male withdraws the penis while the female raises up on her foot and twists rapidly from side to side until the male falls off. The entire process takes up to twenty-five minutes. During this time, ten to fifteen minutes are spent in precopulatory attachment.

Egg String Movement

Eggs are released, one at a time, from the ovary into the oviduct in females dissected prior to copulation. At this time, the oviduct is distended to one and one-half its original diameter. The eggs are surrounded by large amounts of yolk and situated toward the edge of the yolk mass (Fig. 33). This orientation exposes a region of the egg membrane for sperm entry during fertilization. The yolk and eggs are transported in single file by cilia along the oviduct. As they enter the capsule gland, the ova can be seen disappearing one by one. The journey from the ovary to the capsule gland takes from ten to fifteen minutes.

Sperm Movement in the Male

Spermatozoa are released from the seminal vesicle upon relaxation of the sphincter muscle. From this point, they are transported mainly by ciliary action along the posterior vas deferens. The ciliary tracts are arranged in spiral fashion, thereby drawing the sperm mass out into a helicoid configuration (Fig. 34). This probably aids in mixing the sperm evenly with prostatic secretions. When the sperm enters the anterior vas deferens, it is moved mostly by peristaltic contractions towards the penis. The sphincter near the entrance to the diverticulum closes, thus preventing any loss of seminal fluid. However, in snails that are dissected during

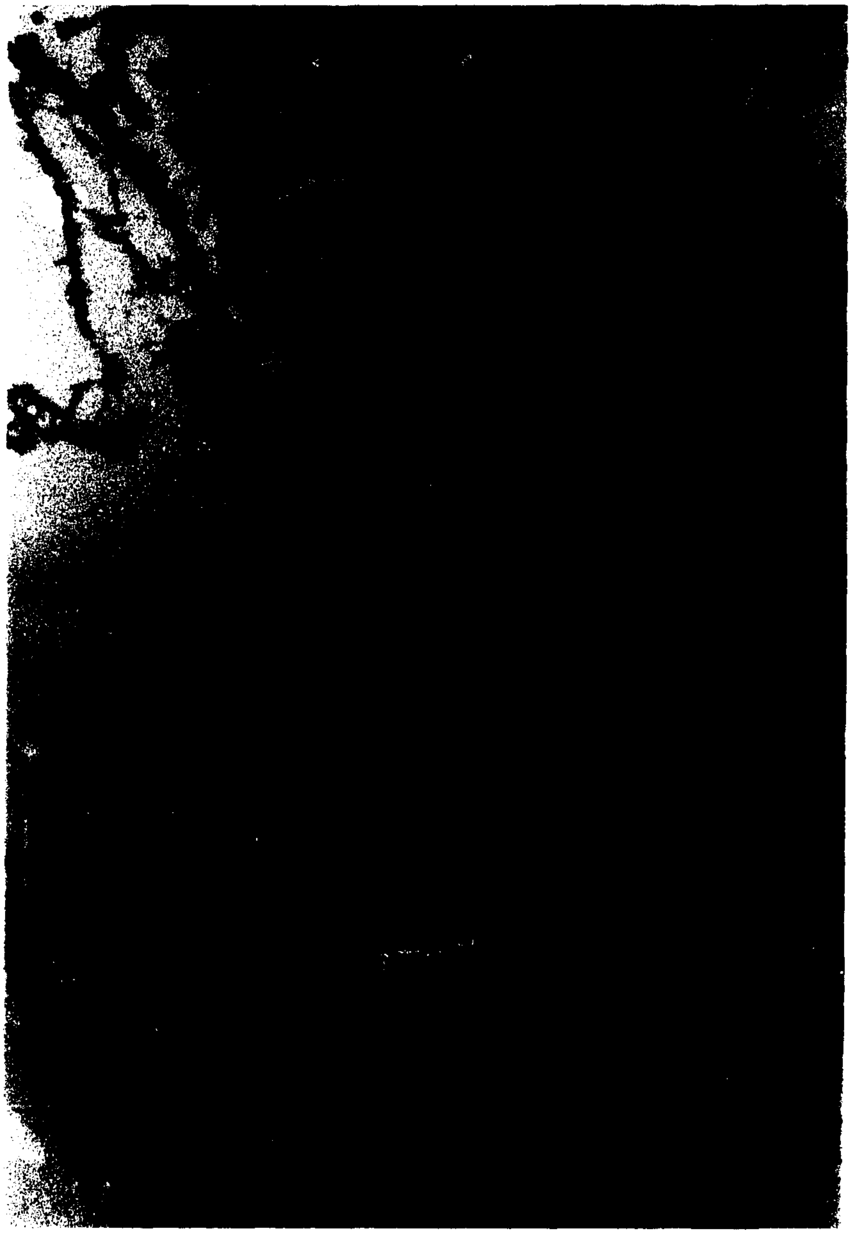

Fig. 33. Section through the Oviduct of Columbella fuscata Showing the Position of an Egg within the Yolk Mass

e - egg, ov - oviduct, yo - yolk

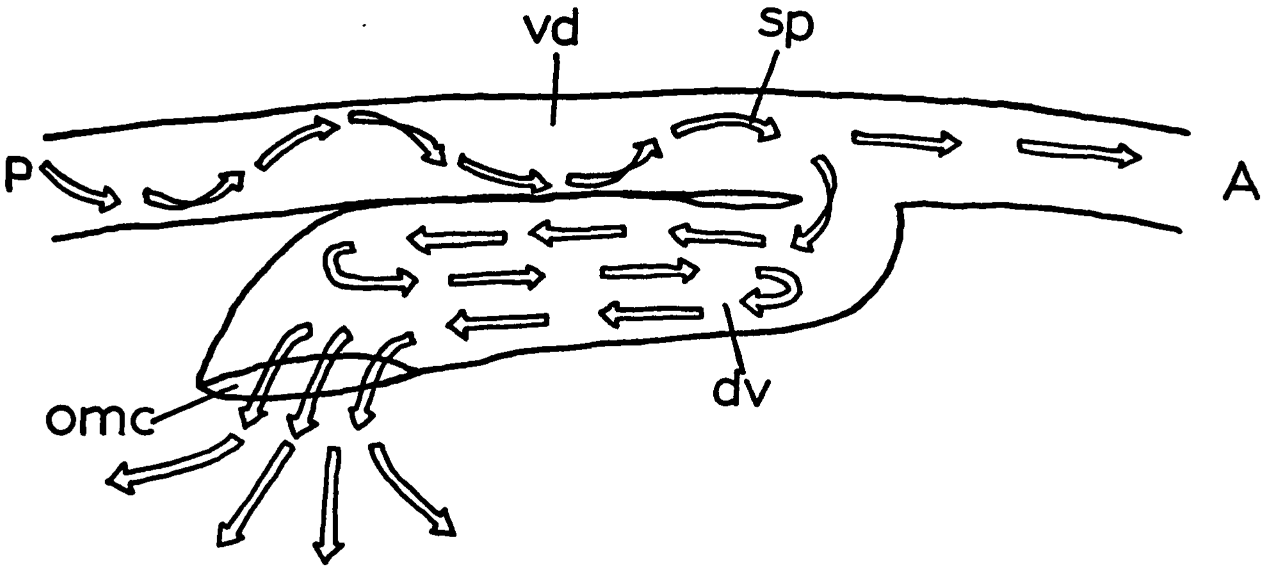

Fig. 34. Sperm Movement in a Male <u>Columbella</u> <u>fuscata</u>

dv - diverticulum, omc - opening into the mantle cavity, sp - sperm,
vd - vas deferens, A - anterior, P - posterior

copulation, sperm can be observed escaping into the mantle cavity through the opening in the diverticulum. The sphincter at the entrance to the diverticulum relaxes and the sperm is shunted into this region. Concurrently the sphincter at the anterior end of the seminal vesicle closes, thereby preventing any backward movement. As the sperm enters the diverticulum, it congregates into a large mass completely filling the region before it is extruded (Fig. 34). Cilia in the diverticulum then direct the entire sperm mass through the opening and into the posterior mantle cavity. Small quantities of sperm are also extruded through the penial opening.

Sperm Movement in the Female

During copulation the penis is inserted into the female aperture. The apical portion of the penis passes through the vestibule, where the tip is directed downwards and inserted directly into the ventral channel. Two muscular strips posterior to the genital opening hold the penis in place. Males observed after copulation show a constriction near the anterior end of the penis, which is produced by these muscular strips. The region anterior to the constriction is about 1.5 mm in length, which is the same length as that of the vestibule. Sperm released in the ventral channel move, with the aid of cilia, to the posterior region of the capsule gland where fertilization takes place.

In females dissected soon after copulation, sperm not utilized in fertilization can be observed moving through the gonopericardial duct and filling the pericardial cavity (Fig. 35). The cavity is

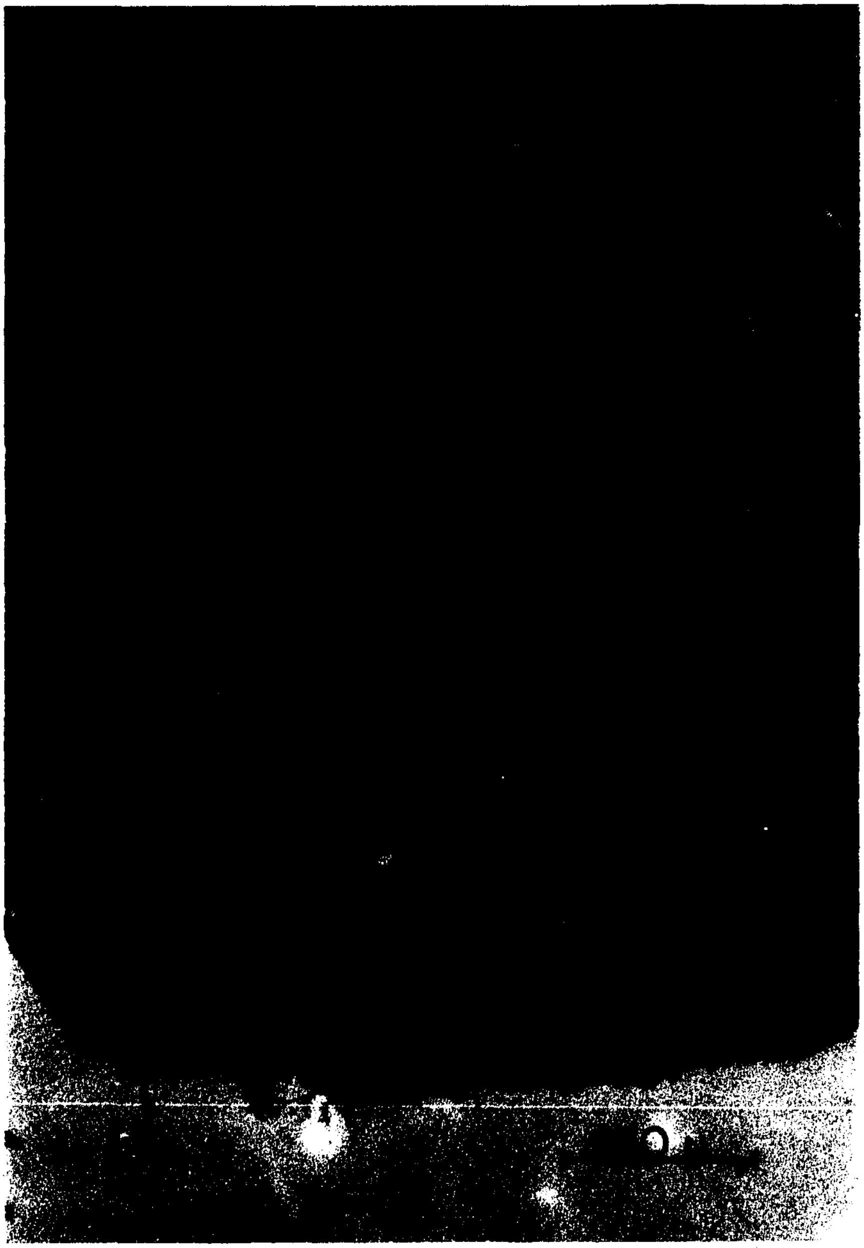

Fig. 35. Section through the Pericardial Cavity of <u>Columbella fuscata</u>

ce - cercaria larva, sp - sperm

about one-half full in females dissected five minutes after copulation, and completely full after ten minutes. The normally transparent cavity now attains a white opaque appearance.

Fertilization

Fertilization occurs in the lumen of the posterior region of the capsule gland. As the unfertilized eggs (ue) enter the capsule gland, they come into contact with spermatozoa from the ventral channel (Fig. 36). Dissections of females during the first ten minutes after copulation show the eggs being mixed with the sperm mass.

Sperm Ingestion

As the pericardial cavity becomes filled with sperm, cilia lining the left side of the pericardial wall drive the sperm towards the right side, which is lined with ingesting cells. These cells are columnar with basal oval nuclei and highly vacuolated cytoplasm. As shown in Fig. 37, these cells send out long pseudopodia which reach out and phagocytize the sperm. The breakdown products appear as dark staining granules in the cytoplasm, and in some cases pieces of heads and tails are visible. In addition to sperm, yolk platelets and occasionally ova pass into the cavity and are catabolized.

Egg Capsule Formation

As the fertilized eggs (fe) pass through the posterior region of the capsule gland, they are covered with the inner mucous coat secreted by cells in this region. The two muco-protein layers are

Fig. 36. Sperm and Egg Movement in a Female *Columbella fuscata*

cg - capsule gland, fe - fertilized egg, gop - gonopericardial duct,
h - heart, ov - oviduct, pc - pericardial cavity, sp - sperm,
ue - unfertilized egg

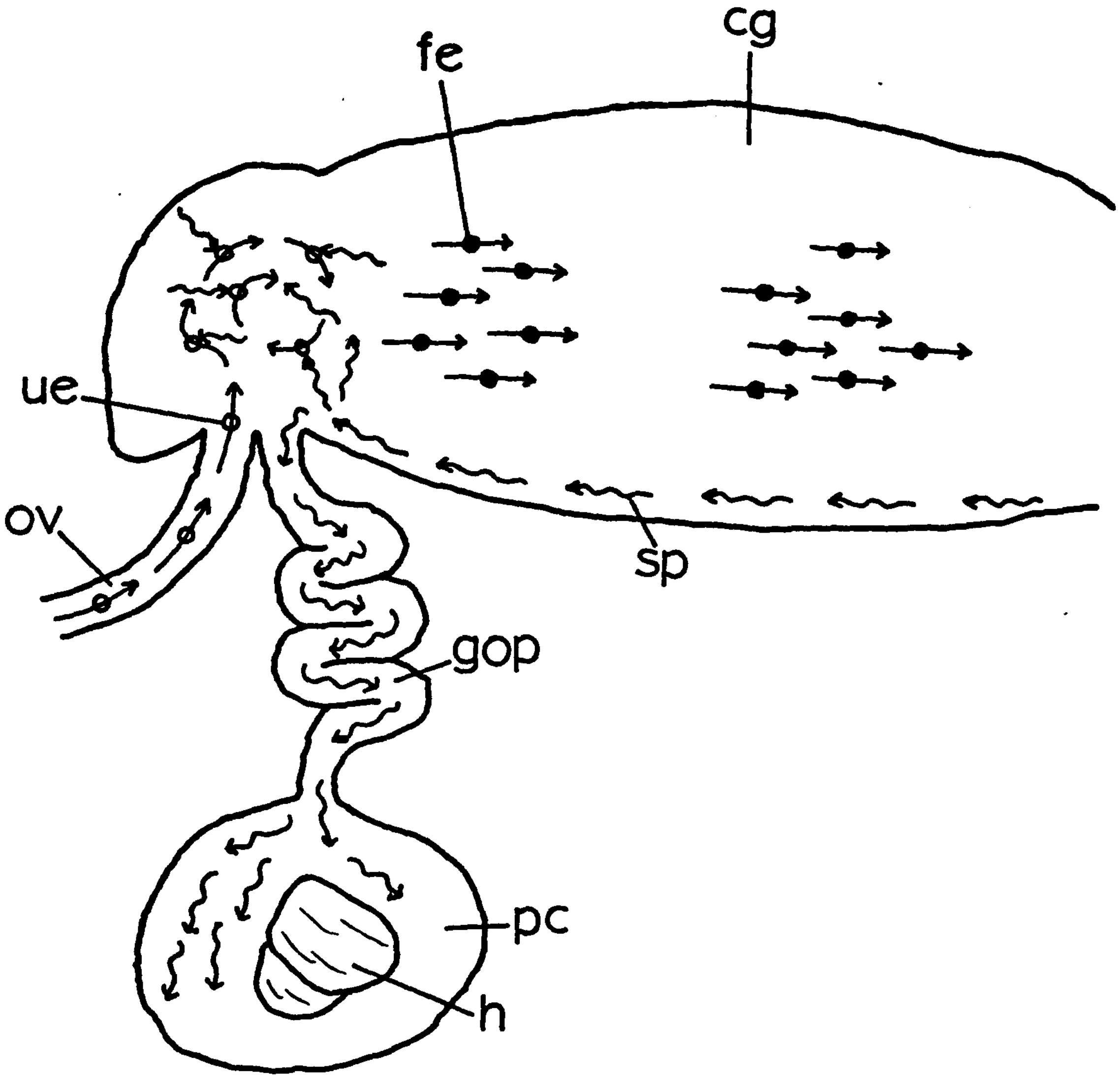

Fig. 36. Sperm and Egg Movement in a Female *Columbella fuscata*

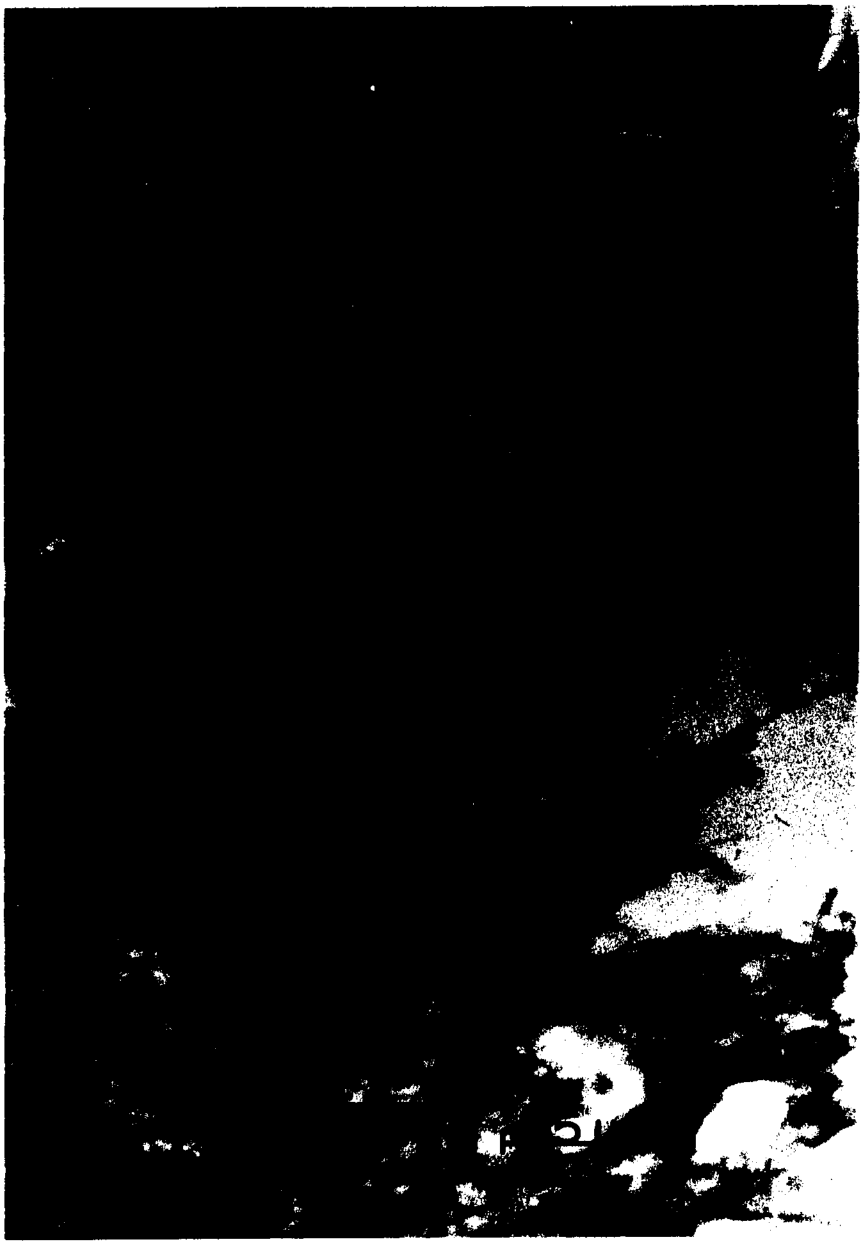

Fig. 37. Section through the Pericardial Cavity Showing Sperm Ingestion in Columbella fuscata

ic - ingesting cells, ps - pseudopodium, sp - sperm spread obliquely around the egg mass at right angles to each other. By the time this first capsule enters the vestibule, a second is being secreted. Connecting these capsules is a muco-protein string. At this stage the egg capsules do not assume their final shape, but appear as soft irregular masses lined with many ridges, probably produced by folds in the vestibule. After the first capsule is released from the vestibule, a second one enters the chamber, while a third is being formed posteriorly. This process continues and produces what appears to be a string of beads joined together by the muco-protein strands. The string is then transported along the ciliated groove down to the ventral pedal gland, where an outer mucous coat is secreted.

The pedal gland apparently hardens the capsule and gives it the final shape. The capsules are then cemented to the algae in alternating rows to insure maximum support. Spawning takes up to several hours depending on how many capsules are deposited.

Larval Development

Larval development of *Acanthina angelica* and *Columbella fuscata* was observed in the laboratory.

Acanthina angelica

Five females spawned in the laboratory, and the average number of capsules laid by an individual was five. After eleven days veligers were observed (Fig. 38). These larvae fed on the undeveloped eggs which had fused into the yolk mass. After 22 days juveniles crawled out through the opening in the top of the capsule.

As shown in Fig. 39, juveniles of this species are about two mm long when they hatch. Looking like miniature adults, they are pale yellow and the spine (spi) on the outer lip is already evident.

Columbella fuscata

The development time for this species is shorter than that of *Acanthina angelica*. Approximately sixteen days after spawning, all of the eggs developed into veligers (Fig. 40). Two days later they metamorphosed into juveniles, which emerged through the opening on top of the capsule.

As shown in Fig. 41, these young snails are about one millimeter long and appear quite different than the adult.

Ontogeny of the Genital Ducts

The development of the reproductive systems of *Acanthina angelica* and *Columbella fuscata* are illustrated in Figs. 42 and 43.

Acanthina angelica

The Male Duct. When the males are 12.1 mm long, the testis is undifferentiated with only a thin layer of connective tissue lining the upper end of the testicular duct. This thin-walled duct is fused throughout its entire length and filled with loosely packed strands of connective tissue. Anteriorly, the duct straightens out and passes along the right side of the visceral mass. As it approaches the region of the kidney, it opens up into a seminal groove (sg), which continues underneath the right mantle wall until it reaches

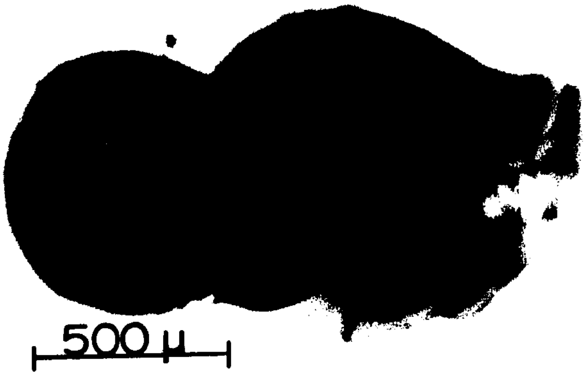

Fig. 38. Veliger Larva from <u>Acanthina</u> <u>angelica</u>

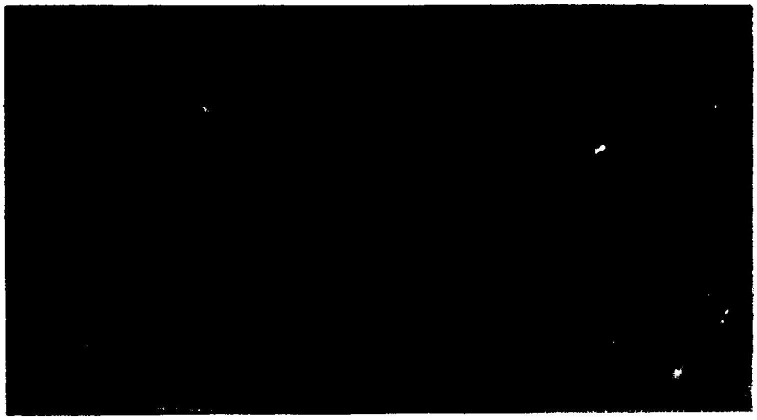

Fig. 39. Juveniles of <u>Acanthina</u> <u>angelica</u>

spi - spine

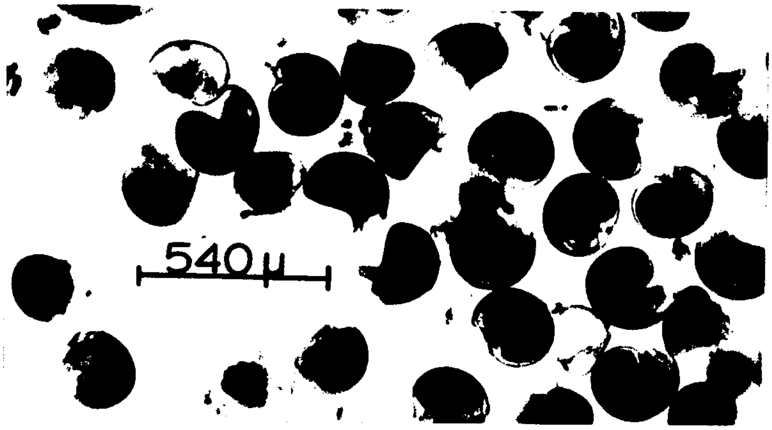

Fig. 40. Veliger Larvae from Columbella fuscata

Fig. 41. Juveniles of Columbella fuscata

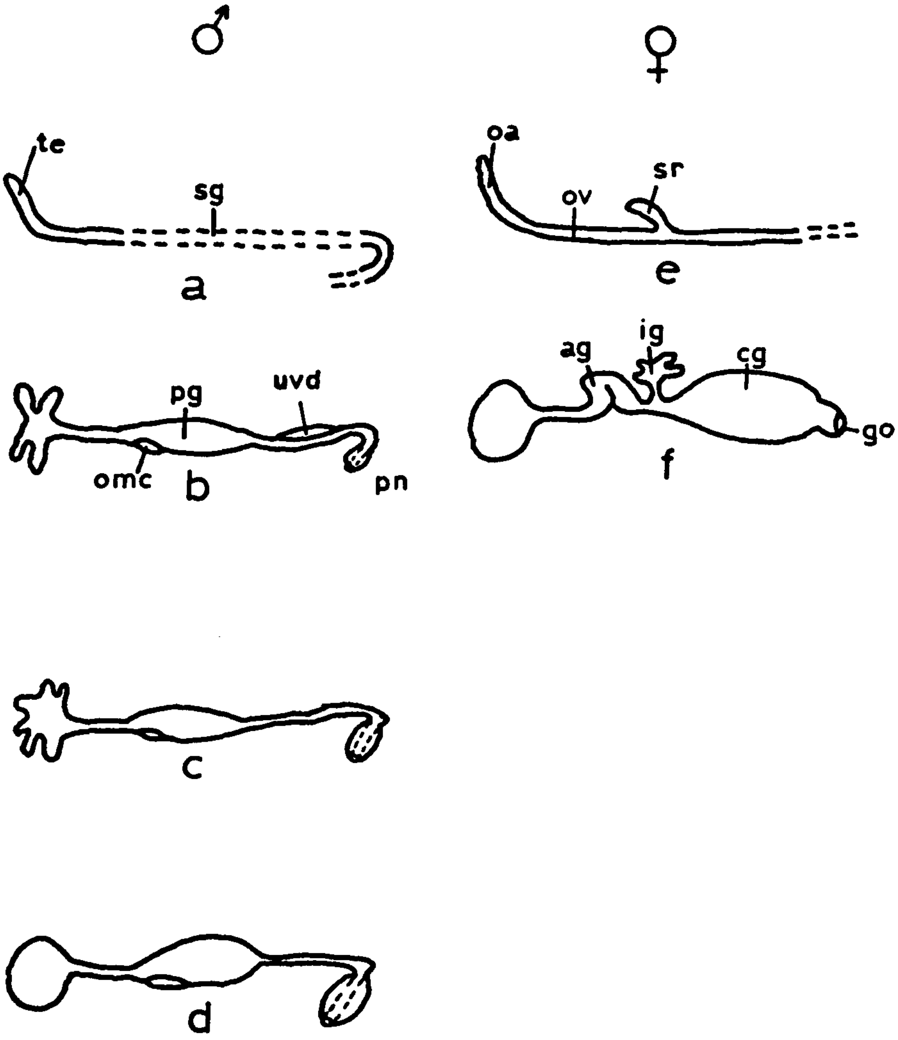

Fig. 42. Ontogeny of the Reproductive System in <u>Acanthina</u> <u>angelica</u>

Male: a - 12.1 mm., b - 13.5 mm., c - 15.0 mm., d - mature, omc - opening into the mantle cavity, pg - prostate gland, pn - penis, sg - seminal groove, te - testis

Female: e - 15.8 mm., f - mature, ag - albumin gland, cg - capsule gland, go - genital opening, ig - ingesting gland, oa - ovary, ov - oviduct, sr - seminal receptacle

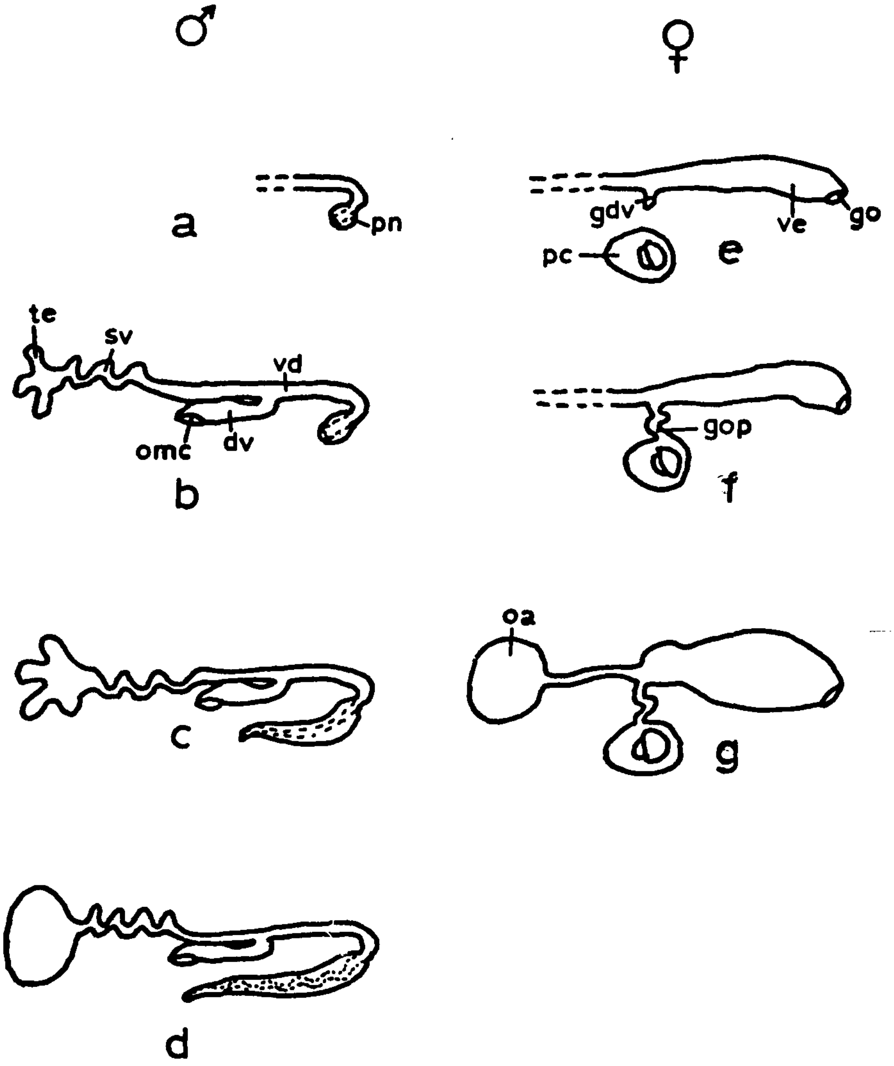

Fig. 43. Ontogeny of the Reproductive System in Columbella fuscata

Male: a - 10.0 mm., b - 11.0 mm., c - 13.0 mm., d - mature, dv - diverticulum, omc - opening to the mantle cavity, pn - penis, sv - seminal vesicle, te - testis, vd - vas deferens

Female: e - 16.5 mm., f - 18.0 mm., g - mature, gdv - diverticulum towards pericardial cavity, go - genital opening, gop - gonopericardial duct, pc - pericardial cavity, ve - vestibule the mantle fold. Here it curves left and passes along the right body wall to the base of the rudimentary penis. There is no evidence of a prostate gland or prostatic tissue at this stage of development. The penis appears as a bulge of pedal tissue, 200 micra long, posterior to the right cephalic tentacle. The penial duct is fused for about 110 micra, then reopens near the anterior end of the penis. A double row of columnar cells define the line of fusion in the duct.

When the juveniles reach a length of 13.5 mm, the testis begins to differentiate. It consists of several layers of proliferating round cells that have large nuclei. A thin sheet of connective tissue separates the young testis from the digestive diverticula. The testicular duct still contains connective tissue. Anteriorly the vas deferens is now fused into a closed duct which opens into the rudimentary prostate region. This region is also fused throughout its length, except for the posterior area. No glandular tissue has developed yet, and the epithelium lining the lumen is composed of tall ciliated columnar cells. Anterior to the prostatic region, the pallial vas deferens is fused along its entire length and joins the base of the penis. The well differentiated penis is 1.5 mm long and contains a centralized penial duct, of which only the anterior region is a ciliated groove.

By the time juveniles attain a length of 15.0 mm, the testis is well developed with many acini and primordial germ cells. The testicular vas deferens is clear of connective tissue and the prostatic region is becoming glandular. The pallial vas deferens and penial duct are completely fused throughout their entire length.

The Female Duct. Only one juvenile collected was a female; it measured 15.8 mm in length. The ovary was undeveloped and only a thin layer of connective tissue surrounded the upper end of the gonadal oviduct. This duct was lined with cuboidal epithelium and contained strands of loosely packed connective tissue. Anteriorly the oviduct was fused along its entire length. The pallial oviduct, however, was only a straight tube without accessory glands and structures, except for a small diverticulum about 160 micra in length. The diverticulum originated dorsally just anterior to the kidney. It was non-ciliated and probably gives rise to the seminal receptacle and ingesting gland.

Columbella fuscata

The Male Duct. The development of the male genital duct in this species is remarkably different than that of *A. angelica*. In juveniles of 10.0 mm and 11.0 mm in length, the penis is fairly well developed and about one millimeter long. The penial duct is fused but there is no line of fusion present. Posteriorly the closed pallial vas deferens passes along the right body wall, then disappears in the region of the mantle fold. It is lined with ciliated cuboidal epithelial cells and covered with a thin layer of connective tissue. The remainder of the genital duct, along with the testis, is not discernible. When the juveniles attain a length of 13.0 mm, the reproductive system is complete. The testis is not completely developed. However, it consists of many acini, and spermatogenesis is occurring with many spermatogonia and primary spermatocytes present. The seminal vesicle is highly convoluted but it is devoid of sperm. The penis and anterior vas deferens are extremely muscular and well developed. In addition, the diverticulum leading to the opening into the posterior mantle cavity is present.

The Female Duct. In females the development is again from anterior to posterior. Juveniles which are 16.5 mm long have a genital opening that leads to a well developed ciliated vestibule. This opens into a slightly smaller duct, which is lined with ciliated columnar epithelium and surrounded by a thin layer of connective tissue. This region will apparently become the capsule gland; however, at this time there is no glandular tissue present. Posteriorly the duct bifurcates with the dorsal branch giving way to the oviduct, while the ventral branch is a diverticulum projecting towards the pericardial cavity (gdv). The oviduct continues posteriorly for a short distance then disappears as a diffuse mass of connective tissue. Moreover, the ovary is not apparent at this time.

When the females reach a length of 18.0 mm, the diverticulum has joined the pericardial cavity, forming the gonopericardial duct. In addition, the pallial oviduct is enlarged but there is still no indication of any glandular tissue.

Parasites

During this study it was noted that Columbella fuscata carries the larva of an unidentified trematode parasite. The larvae, which appear to be cercariae, are found in the seminal vesicle of males, distributed within the sperm mass. As shown in Fig. 44, they

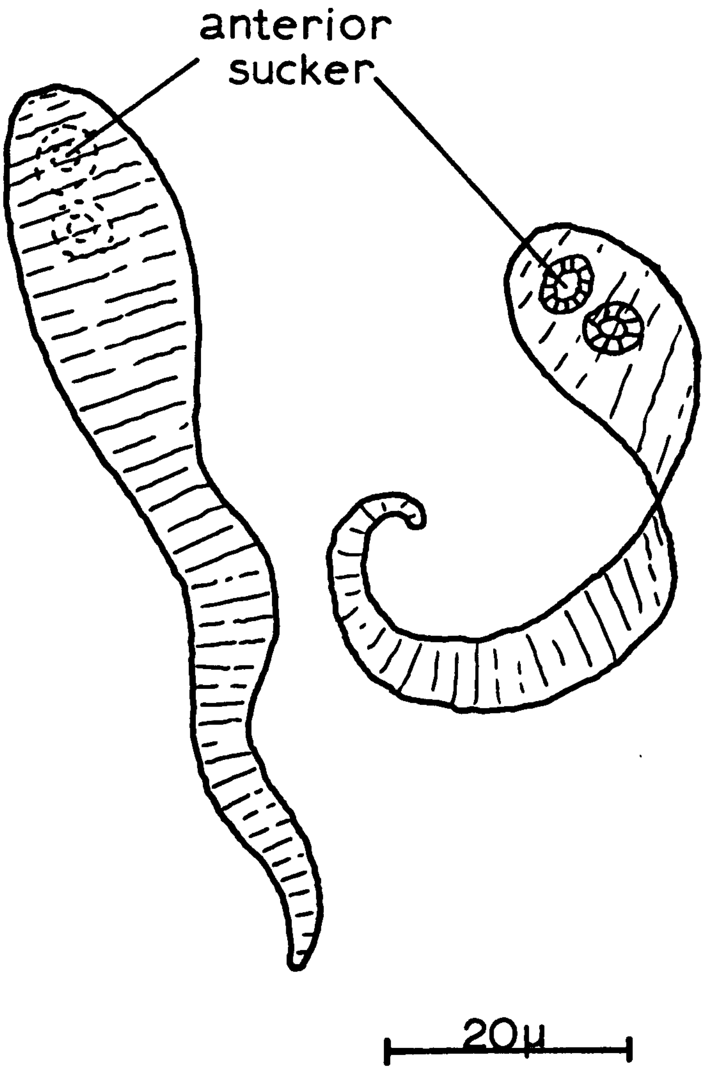

Fig. 44. Cercariae Larvae from the Seminal Vesicle of Columbella fuscata

are long and slender with pointed tails. Anteriorly they have two suckers which lie in tandem. They are transmitted venereally to females and can be observed among the spermatozoa in the pericardial cavity (Fig. 36). Hence, this insures distribution throughout the snail population, which is advantageous in continuing their life cycle. In *C. rustica*, Pagenstecher (1863) discovered sporocysts and cercariae of the trematode *Cercaria columbellae*.

DISCUSSION

The general plan of the genital systems in the species studied is relatively uniform; however, both anatomical and functional differences occur within the accessory structures. Of particular interest are the occurrence of mantle openings in the male, and sperm receiving and storage organs, and gonopericardial connectives in the female.

Evolution of the Open Reproductive System

A hypothesis for the evolution of mantle (pallial) gonoducts was proposed by Johansson (1942), and Fretter (1946). They suggested that the gonoducts in both sexes were originally open, ciliated grooves, bordered by glandular tissue. This condition is quite evident among the Mesogastropods. Studies by Johansson (1946) of *Turritella communis*, *Bittium reticulatum* (Johansson 1947) and *Cerithiopsis tubercularis* (Fretter 1951) revealed the fact that open ducts occur in both sexes. In addition, Johansson (1953) examined *Cerithium vulgatum* but unfortunately was able to collect only male specimens, all of which, nevertheless, had open seminal grooves.

At this place it is appropriate to mention that several species of mitriform Neogastropods retain the primitive open condition. Woodward (1901) wrote that the entire pallial duct of *Adelomelon ancilla* is a long groove. In a study of *Alcithoe arabica*, Ponder (1970) notes that the prostate is completely open along the ventral surface. Also, in a later report, Ponder (1972) observed a seminal groove in *Peculator hedleyi*.

As the Mesogastropods and Neogastropods evolved, the pallial groove became fused into a duct along its entire length (Fretter 1946). Krull (1935) found this to be true for the hydrobiid, *Assemania grayana*.

The Male Duct

Incomplete fusion of the male duct occurs sporadically throughout the higher Prosobranchia. Previous studies suggest that incomplete fusion arose independently in different genera. In *Trivia monacha* and *T. arctica* (Fretter 1946) and *Circulus striatus* (Fretter 1956), the posterior region of the prostate gland is open to the mantle cavity by a ventral slit. As mentioned earlier, similar openings occur in many of the British Stenoglossa (Fretter 1941), columbellids (Marcus and Marcus 1962b), olivids (Marcus and Marcus 1959a), and turrids (Smith 1967). Moreover, this condition occurs in most of the species in this study (Figs. 3, 4, 5, 6, 29). It is interesting to note that the opening occurs in several different ways. In *Thais canaliculata*, *Acanthina angelica*, and *Crassispira pluto* it appears as a ventral slit in the posterior region of the prostate. In contrast, it is a short ciliated duct in *Cantharus d'orbigny* and *Acanthina spirata*. In *Columbella fuscata* the opening is unique in that it occurs on the surface of a diverticulum ventral to the vas deferens.

In accordance with the open groove theory, it would appear that these openings are a retention of the primitive condition and therefore are of primary derivation. The ontogenetic studies of Columbella fuscata and Acanthina angelica give support to this hypothesis. Posterior openings are present even in the youngest of juveniles, which would indicate they are primary. This condition probably exists in other species, but has yet to be determined.

The pallial opening is doubtfully pleiotropic because of its widespread occurrence in different genera. Fretter (1941) suggests that it acts as a "safety valve" in providing an escape for sperm when males are disturbed during copulation. The author agrees with her hypothesis because this was observed repeatedly in C. fuscata. Also, there is a close relationship between the pallial opening and the presence of a sphincter muscle anterior to the seminal vesicle. Species that have a mantle opening also have the sphincter. Further, the seminal vesicle is thin and fragile. Conversely, the opening and sphincter muscle are absent in those species that have an elastic seminal vesicle. Therefore it is apparent that if the opening was not present, pressure resulting from seminal fluid would rupture the vas deferens, because the sphincter contracts and prevents any backward movement of seminal fluid. In those species without the opening, the seminal vesicle can expand and absorb the excess pressure. It is important to have some protective mechanism. Because the copulatory period in gastropods can be rather long, a protective mechanism of this sort has high survival values. Marcus and Marcus (1959b) observed that copulation lasts up to three hours for Olivella verreauxii. During this time, vulnerability to predation and physical parameters, such as waveshock, is increased.

The length and shape of the prostate gland are quite variable. The gland is especially long in Crassispira pluto and other turrids (Smith 1967), probably owing to the narrowness of the mantle cavity. It is well known that the prostate gland provides an aqueous medium for the spermatozoa, for transference from male to female. This gland, however, is absent in several species of columbellids. Instead, the seminal vesicle is glandular and appears to assume a prostatic function in addition to sperm storage and resorption.

The penes can be separated into two morphological types. Those that are wide, blunt, and considerably flattened dorsoventrally, and those that are wide, tapering to a point, and flattened or tubular. The first type occurs in the muricids and buccinids, whereas the second type occurs in nassariids, columbellids, olivids, and turrids. Studies by Ponder (1972) on mitriform gastropods indicate they have penes similar to those of the olivids. The penis, in addition to functioning as an intromittent organ, also aids in holding the copulating partners together. In Littorina irrorata, Bingham (1972) observed that the penis is directed into the mantle cavity of the female, where it quickly inflates. It is possible that penes which are wide and blunt never actually enter the female aperture, but only the mantle cavity, where they release their gametes. Respiratory currents would then carry the gametes to the female opening. This has been suggested for *Buccinium undatum*, because the gigantic penis could in no way be inserted into the minute distal bursa copulatrix of the female (Johansson 1953). Also, ciliary currents are directed towards the bursa. This appears to be the case for *Acanthina angelica*, *Solenosteira macrospira*, and other muricids and buccinids. On the other hand, in *Columbella fuscata* the penis does enter the female aperture and is held in place by muscular strips bordering the vestibule. This most likely occurs in other columbellids and other groups with this type of penis. An exception is *Olivella verreauxii*, which has a long narrow penis that is inserted only to the mantle cavity of the female. Here the sperm are transported by cilia to a groove leading to the oviducal opening (Marcus and Marcus 1959b). The mucous glands in the penis may assist in the insertion and securement in the female. Fretter and Graham (1962) believe this to be true for *Littorina irrorata*.

The Female Duct

Johansson (1953) suggests that when the oviducal groove was transformed into a closed duct, a posterior or proximal opening would have been necessary for those forms lacking a penis because sperm transport and reception were dependent on ciliary currents. In the Neritidae and Helicinidae, Bourne (1911) remarks that the vagina resulted from incomplete fusion of the oviducal groove, and is homologous with the posterior mantle opening in males. This condition is also present in *Nerita birmanica* (Berry, Lim, and Kumar 1973), where the vagina is separate from the nidimental opening.

A proximal opening, however, occurs in some species that have a penis. Both the females of *Skeneopsis planorbis* and *Rissoella diaphana* have a proximal opening, and the males have a penis (Fretter 1948). Johansson (1947) tried to associate the open oviduct with the absence of a penis. He found, however, that *Melania crenulata* and *M. tuberculata* have no penis and the females have a closed oviduct. Conversely, in a later study Johansson (1953) showed that *Melanella intermedia* has a penis and the female has a closed oviduct. As shown in Fig. 30, females of *Thais emarginata*, *T. canaliculata*, *Ceratostoma foliatum*, and *Drupa nodulosa* also have posterior openings in their oviducts. Fretter (1951) believed that the open condition is secondary and tried to correlate it with the shape of the shell and the narrow mantle cavity. This is applicable for such genera as *Bittium*, *Cerithiopsis*, and *Triphora*. However, in *Trivia* and *Littorina*, which also have the open condition, the shell is quite different and the mantle cavity is spacious. Fretter realized this and proposed that the alteration in shell shape may be recent and that the anatomy of the reproductive system has not had enough time to change from its ancestral condition. The author suggests that an external shell is more subject to environmental changes than internal systems. However, Johansson (1953) remarks that a retention of a primarily open condition may also be due to the initial shape of the shell. Either hypothesis could be clarified by studies of the fossil record and ontogeny of these species.

The higher Prosobranchs have evolved a distal bursa copulatrix to receive the sperm during copulation. This is a definite advantage, especially in those species with a narrow mantle cavity. If the opening was posteriorly located, it would be difficult to accommodate a large penis (Fretter 1953). This would interfere with respiratory currents and other vital functions of the mantle cavity. As mentioned earlier, the bursa in the muricids and buccinids is much too small to receive the penis. Therefore, the sperm are released in the mantle cavity and carried to the bursa by ciliary currents. In the higher Neogastropods this structure is compatible and functions as a penial receiving organ. This is exemplified in <u>Olivella</u> <u>dama</u> where the bursa is extremely muscular, indicating that it functions in holding the penis securely during copulation.

The bursa copulatrix, as a penial receiving organ, has evolved throughout the hermaphroditic gastropods. In the Opisthobranchiata the mantle cavity is reduced or absent, and in copulating partners the penes are inserted directly into the vaginal openings (Eales 1921, Pelseneer 1935). Moreover, in the Pulmonata, the genital duct has separated from the mantle and now lies within the haemocoele. Here again, the penis is inserted directly into the vagina. In <u>Ariolimax</u> <u>californicus</u>, the vagina is surrounded by a huge intrinsic muscle which firmly grasps the penis (Mead 1943). It is interesting to note that sometimes partners will apophallate to effect separation. To date, this phenomenon has not been observed in the Prosobranchs.

Seminal receptacles occur throughout the Mesogastropods and have probably evolved in connection with the posterior copulatory opening (Johansson 1942). This condition is retained in many Neogastropods, in which it is located posteriorly between the albumin and capsule glands. In these, the sperm received by the female is transported posteriorly along the ventral channel to the seminal receptacle, where it is stored. In *Thais emarginata* and *Urosalpinx cinerea* the ventral channel is modified into an anterior sperm sac. This structure reveals a clear homology between the ventral channel and the seminal receptacle. Observations by Marcus and Marcus (1962b) indicate that in some species of columbellids the bursa copulatrix is divided into two sacs. One sac serves as a site for sperm ingestion, which is discussed below, while the other is used for sperm storage. A similar case is known from the Rissoidae (Johansson 1957). It appears, at least in some species, that a homology exists between the seminal receptacle and the bursa copulatrix.

Fretter (1941) has observed in *Nucella lapillus* that the seminal receptacle opens into a diverticulated sac, which functions as an ingesting gland. This also occurs in *Acanthina angelica*, *Thais emarginata*, *Urosalpinx cinerea*, and several other Neogastropods. Fretter (1941) suggests that the ingesting gland is homologous with the seminal receptacle of other species. The diverticulum which arises from the oviduct in juvenile *Acanthina angelica* was found in the present study to be the common root for both the seminal receptacle and ingesting gland. This evidence clearly supports Fretter's view that a homology exists.

The gonopericardial duct, which occurs in several of the species studied, warrants some discussion. Marcus and Marcus (1962b) indicate that it is homologous to the right ureter of Archaeogastropods. The author disagrees with this hypothesis because the ontogeny of the gonopericardial duct in Columbella fuscata reveals a double origin. Both the pallial oviduct and the pericardial wall produce diverticula that eventually join in forming the canal. A similar condition occurs in Littorina saxatilis (Cousin 1972). Marcus and Marcus (1962b) have observed sperm storage in this duct and conclude that it functions as a seminal receptacle. It therefore appears that the gonopericardial duct is indeed homologous with the seminal receptacle of other species. Although this duct is usually confined to females, Smith (1967) found that one exists in the male of Lora trevelliana. Moreover, in Acanthina spirata a dense strand of connective tissue connects the vas deferens with the pericardial cavity, which suggests that it is the remnant of a duct.

As far as the capsule and albumin glands are concerned, it is apparent that homologies exist among those species with closed oviducts and those with open oviducts. Johansson (1957) remarks that there is a definite homology between the albumin gland of Neogastropods and the uppermost gland of Littorina saxatilis and Alvania reticulata. He also indicates that the ventral channel of Neogastropods is equivalent to the vaginal lumen of the Rissoacea.

The ventral channel is interesting because, as previously mentioned, in *Thais emarginata* and *Urosalpinx cinerea* it is modified into a seminal receptacle. Also, in *Acanthina angelica* and other muricids, its roof is composed of two longitudinal flaps of tissue which make it a functionally separate duct. This may indicate a homology to the functionally separate spermoviducts in hermaphroditic gastropods. If this were true, it would support the hypotheses of Duncan (1960) and Ghiselin (1965) in that the separation of the spermiduct and oviduct in the pallial region represents an advanced condition. Furthermore, this would disagree with Solem (1972) in that the monaulic pallial spermoviduct is the advanced situation. This, however, is speculation and much work is needed in this area. In addition, the hermaphroditic turrid, *Lora turricula*, described by Smith (1967) has morphologically separate male and female systems.

Several hypotheses are presented to account for sperm ingestion in gastropods. In *Eupleura caudata* and *Urosalpinx cinerea*, Hargis and MacKenzie (1961) argue that if sperm left over from one mating is not completely ingested, then it is viable for the next spawning period. They also suggest that sperm ingestion would be a way of disposing of "sick sperm", which would otherwise penetrate ripe ova and block the passage of viable sperm. In a recent study on *Helix pomatia*, Lind (1973) suggests that sperm ingestion is a way of possibly preventing self-fertilization. This, however, would not apply to Neogastropods because they are generally not hermaphroditic. The author agrees with the suggestion of Fretter (1941) that ingested sperm serve as nourishment for the adult. In reproductively active females the digestive gland is highly reduced; therefore, it would be advantageous to utilize excess sperm for nourishment. Also, in *Columbella fuscata*, occasionally ova and yolk granules are ingested by the pericardial epithelium.

Egg Capsules and Larval Development

The egg capsules of *Acanthina angelica*, *Solenosteira macrospira* and *Nassarius tiarula* are typically stenoglossan. This is also the case for *Thais emarginata*, *Thais canaliculata* (Houston 1971) and *Torvamurex territus* (Murray 1964). On the other hand, the oyster drill *Bedeva hanleyi* deposits lens-shaped capsules (Hedley 1916). The egg capsules of *Columbella fuscata* are similar to those of the columbellid *Anachis brasiliana* described by Marcus and Marcus (1962b). Fretter and Graham (1962) state that in Neogastropods the shape of the cavity of the ventral pedal gland resembles that of the egg capsule. In these species it was extremely difficult to detect any relationship.

Egg capsules of *Acanthina angelica* and *Solenosteira macrospira* contained upwards of 500 eggs, of which only 2-3 percent developed into adults. On opening the capsules it was noted that most of the eggs had fused into a yolk mass on which the developing larvae fed. Nurse egg feeding occurs in *Nucella lapillus* (Costello et al. 1957) and other Neogastropods (Thorsen 1946, 1950). On the other hand, the capsules of *Columbella fuscata* and *Nassarius tiarula* contained only 50 eggs of which all developed. In *Nassarius tiarula* all of the eggs developed into veligers, which were then released into the plankton. Conversely, in *C. fuscata* development was exclusively within the capsule. In addition, development times were shorter for *N. tiarula* and *C. fuscata*. Shorter development time was probably due to the smaller amount of food reserves.

According to Thorsen (1950), Prosobranchs with wide zoogeographical ranges tend to have brood protection or direct development, or both, in colder waters, and free-swimming larvae in warmer waters. Species in the northern Gulf of California are subjected to wide temperature fluctuations (Fig. 45). Because the fauna of the Gulf is an assemblage of endemics and species with affinities to the Californian, Panamic, and West Indies provinces (Brusca 1973), it is expected that different modes of development exist. This would especially be true for *Columbella fuscata* and *Nassarius tiarula*, since they have such wide ranges.

Systematic Discussion

It is difficult to establish any definite phylogenetic relationships among major groups of Neogastropods, especially at the family and generic levels. At the superfamily level, however, evolutionary trends based on the genital system become evident. According to Thiele (1929-35), the Neogastropoda (Stenoglossa) is composed of four superfamilies: Muricacea, Buccinacea, Volutacea, and the Toxoglossa. The origin of the Neogastropods was probably some common

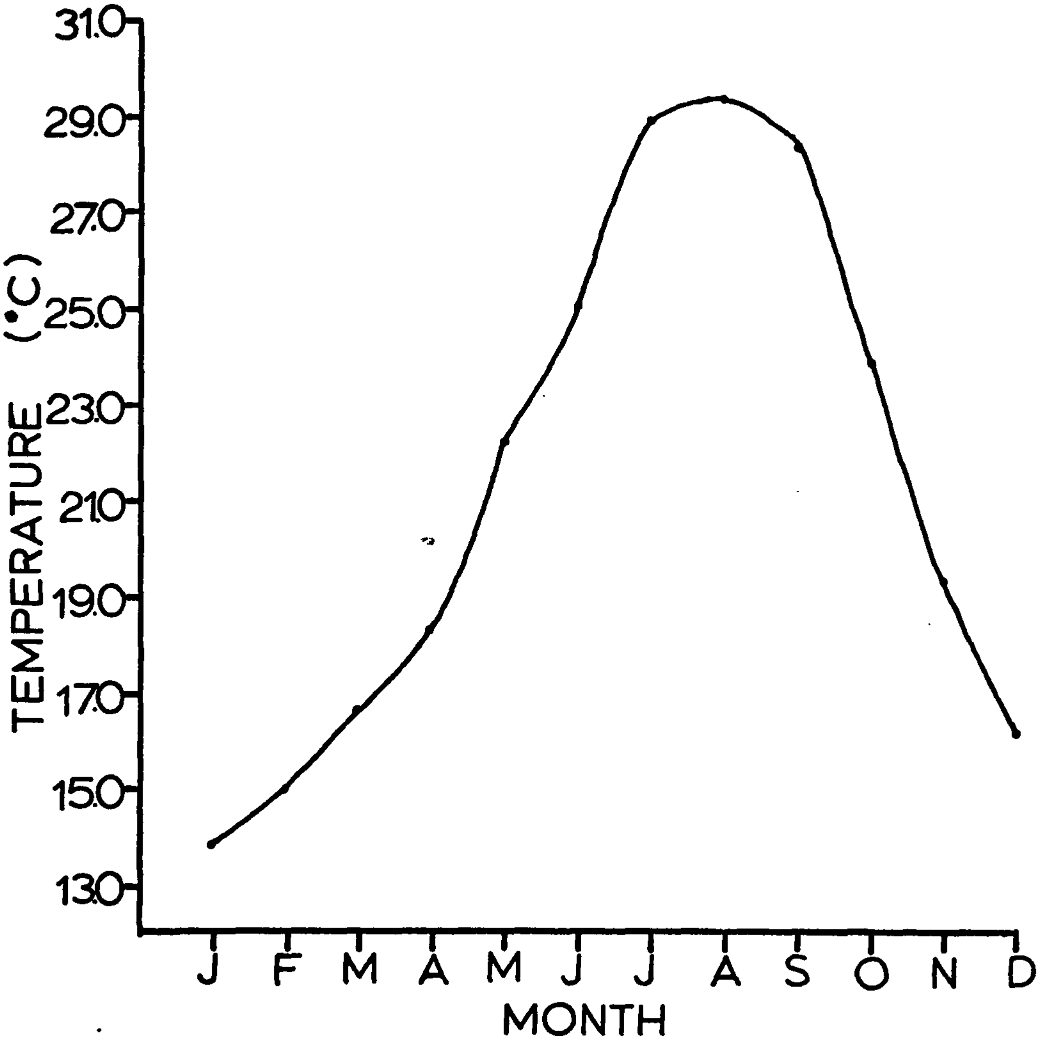

Fig. 45. Surface Water Temperatures at Station Beach

ancestor in the early Cretaceous. From this root arose these groups splitting off in four lineages. According to Schilder (1947), these groups evolved sometime during the late Cretaceous or early Paleocene. The presence of free-swimming larvae, which occur throughout these groups (Lebour 1937, Thorsen 1946), indicates a primitive condition. In the Muricacea, although it is a primitive group, there is a tendency to suppress the free-swimming larvae. In addition, the repeated occurrence of a gonopericardial duct and pallial openings suggest an early origin. Moreover, this early origin would allow time for specializations to occur, and at the same time retain primitive characteristics. The earliest North American and South American fossil records of the genera of the Gulf of California species studied are from the Tertiary (Wenz 1941-43). This is during the same period when the formation of the Gulf of California is estimated to have occurred (Larson, Menard, and Smith 1968). During this time, these genera probably invaded the newly formed gulf and speciated.

The reproductive systems of these species conform to those of species from other regions of the world in having both primitive and advanced structures. Hence, this suggests parallel evolution throughout the Neogastropods.

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