THE REPRODUCTIVE BIOLOGY OF THE RECENT ARTICULATE BRACHIOPOD *TEREBRATULINA RETUSA* (LINNAEUS).

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Declaration.

I hereby declare that this thesis represents, except where a note is made to the contrary, work carried out by myself. It has not previously been submitted for any degree.

Mark A. James

20th March 1989.

Dedicated to my parents.

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ABSTRACT.

The reproductive biology of the Recent articulate brachiopod Terebratulina retusa (Linnaeus) has been examined in detail.

Two populations of T. retusa were sampled. The first from a deep water brachiopod-Modiolus assemblage in the Firth of Lorn and the second from a shallower site at Kenmore Point, Loch Fyne. Limited assessements were made of local environmental conditions, including temperature and current profiles.

Aspects of the gross reproductive anatomy and important ultrastructural features of the gonads are documented in addition to detailed ultrastructural descriptions of gametogenesis. Using the information gained from the morphological studies, the reproductive cycles of two geographically separate populations of T. retusa have been analysed using a quantitative stereological technique.

T. retusa are dioecious. Maturity in both sexes is achieved when the shell length exceeds approximately 5.5 mm. There is no obvious external sexual dimorphism except for slight differences in the coloration of the gonads; testes are white/cream, ovaries are yellow/orange. The gonads occur as four palmate lobes, a pair in each valve. Gonads are formed within a mantle sinus, the vascula genitalia, which is an anterior extension of the coelom, that opens posteriorly into the visceral cavity and to the exterior via a pair of metanephridia. The latter serve as gonoducts during spawning. Gametes are borne on genital lamellae formed from a reticulate lattice of connective tissue. The lamellae are an extension of the ileoparietal band and are fused along one margin to the inner mantle epithelium.

Developing oocytes are closely affixed to the genital lamellae and originate from a pool of proliferating germ cells at its base. Vitellogenic oocytes that are at an advanced stage are released from the genital lamellae, but are retained within the vascula genitalia. Liberated oocytes continue to accumulate yolk and eventually occlude the vascula genitalia, before being spawned.

Histochemistry revealed the outer mantle epithelium to contain seasonally variable quantities of protein, glycogen and glycoprotein.

Functionally, the outer epithelium is presumed to act as a storage tissue and the cycling of reserves it contains is thought to be intimately associated with reproduction.

Two forms of phagocytic cell were identified within the gonads of spent animals. These appear to be actively involved in the resorption of atretic material.

Oogenesis has been divided into six stages, defined according to ultrastructural changes. Each stage is thought to be a key factor in the development of the gamete, particularly the process of vitellogenesis. Special attention has been given to the possible mechanisms involved in the aquisition and assimilation of nutrients within the oocyte and the possible physiological consequences.

Each vitellogenic oocyte is contained within a follicular envelope. As maturation proceeds, accessory cells proliferate within the follicular envelope. A variety of intra-accessory cell and oocyte/accessory cell communications were identified. The process of elaboration of the oolemma was observed. Lipid was identified as the major nutrient reserve of the oocyte. Late mature oocytes measure approximately 130 μm in diameter when spawned.

Spermatogenesis was also examined ultrastructurally. As with oogenesis, gametes are derived from a pool of proliferating germ cells which are distinct from other gonial tissues. Primary spermatocytes form clusters at the base of the genital lamellae, giving rise to a short-lived secondary spermatocyte stage which divides again to produce spermatids. The spermatids undergo structural modifications, eventually forming mature sperm. Successive stages of development are displaced from the genital lamella by division of cells beneath. Sperm are of the 'primitive type'. The head consists of an axially displaced acrosome, spherical nucleus and large doughnut-shaped mitochondrion.

Stereological analysis revealed a single synchronised spawning event between late November and the end of January in the Firth of Lorn. The Loch Fyne population spawned repeatedly throughout the spring and summer, with the greatest spawning activity occurring in the late autumn. T. retusa from Loch Fyne were more fecund than those from the Firth of Lorn.

The initiation of gametogenesis appears to be mediated by the mobilisation of reserves stored in the outer mantle epithelium during the winter. Primary productivity increases dramatically in the spring and both vitellogenesis and spermatogenesis show a corresponding increase. T. retusa from Loch Fyne successfully overwinter gametes and as a consequence are capable of spawning during the spring.

Productivity in the Clyde Sea area is approximately five times higher than in the Firth of Lorn. Higher fecundities and spawning frequencies of the Loch Fyne population are attributed, in part, to greater food availability.

Details of the reproductive cycles of both the Firth of Lorn and the Loch Fyne populations are discussed. The reproductive strategy and the mechanisms of physiological and environmental constraint which may have dictated the adoption of such a strategy are examined. Finally, the limitations and possible evolutionary consequences of brachiopod reproduction are addressed with reference to their palaeoecology.

TABLE OF CONTENTS.

CHAPTER 4. GAMETOGENESIS.

CHAPTER 5. THE REPRODUCTIVE CYCLE.

LIST OF FIGURES.

LIST OF PLATES.

FUNCTIONAL MORPHOLOGY OF THE GENITALIA.

OOGENESIS.

Plate 41-43. Light micrographs of semi-thin sections indicating the number of accessory cells which may be present.
Plate 44. The ooplasm of a Stage IV vitellogenic oocyte.
Plate 45. The ooplasm gradually increases in complexity with the initial proliferation of organelles.
Plate 46. Proteinacious membrane bound granule.
Plate 47. Vesicle.
Plate 48-49. Lipid granules.
Plate 50. Golgi complex.
Plate 51. Agranular endoplasmic reticula.
Plate 52. Two follicular cells.
Plate 53. Throughout vitellogenesis the nuclear envelope becomes increasingly plicate.
Plate 54. Mitochondria and agranular endoplasmic reticula.
Plate 55. Intra-accessory cell communications.
Plate 56. Direct communication between accessory cells.
Plate 57. The porifery of a Stage V vitellogenic oocyte.
Plate 58. Higher magnification micrograph of endocytotic vesicle formation.
Plate 59. Semi-thin sections reveal the concentration of yolk granules.
Plate 60. The Stage IV oocyte.
Plate 61. Two late mature vitellogenic oocytes in contact.
Plate 62. Section through the edge of a mature oocyte.
Plate 63. The lipid granules of the mature oocyte.
Plate 64. SEM of a vertical section through the ovary of a mature female in spawning condition.
Plate 65. Higher magnification micrograph of a prespawn ovary.
Plate 66. Surface view of a spent ovary.
Plate 67. Light micrograph of a vertical section through the ovary of a female approaching maturity.
Plates 68-73. A series of SEM's showing the manner in which the follicular envelope retreats.
Plate 74. The highly convoluted surface of the mature oocyte.
Plate 75. When highly magnified the density of the microvilli which form the convoluted surface can be observed.
Plate 76. TEM of a section through the microvillar fringe.
Plate 77. The margin of the mature oocyte.
Plate 78. SEM showing the surface of the genital lamella from a recently spawned female.
Plates 79-81. A series of light micrographs displaying various stages in the atretia of oocytes.
Plate 82. Pseudopodial extensions of an amoeboid phagocyte engulfing coarse granular material.
Plate 83. The cytoplasm of the phagocytes.
Plate 84-85. Two morphologically distinct types of phagocyte.

SPERMATOGENESIS.

Plate 86. TEM of a section through part of the genital lamella of a male.
Plate 87. A spermatogonium.
Plate 88. Light micrograph showing the structure of the

testis.

Plate 89. Primary spermatocytes.

Plate 90. Primary spermatocytes divide meiotically.

Plate 91. Secondary spermatocytes undergo a second meiotic division.

Plates 92-93. Spermatids.

Plate 94. Tangential section through the head of a mature spermatozoon.

Plate 95. Transverse section through the asymetric ring of the mitochondrion.

Plate 96. Transverse section of the tail of a spermatozoon.

Plate 97. The acrosome.

Plate 98. The acrosomal vesicle of the mature spermatozoon.

Plate 99. SEM of a vertical section through a mature male gonad.

Plate 100. Light micrograph illustring the density of developing cells within maturing testes.

Plate 101. A cluster of mature spermatozoa.

Plate 102. Clusters of lipid granules.

Plate 103. Lipid granules.

Plate 104. Light micrograph of a vertical section through a testis showing lipid material.

CHAPTER 1.

INTRODUCTION: SAMPLING LOCATIONS AND LOCAL ENVIRONMENTAL CONDITIONS.

CHAPTER 1.

1.0 INTRODUCTION.

Brachiopods are exclusively marine and widely distributed, but their occurrence is patchy. They range from the intertidal to depths in excess of 6000 meters (Cooper, 1973). The majority of species are known from the Continental Shelf down to 500 meters (Zezina, 1970). There are approximately 280 species of living brachiopod, which represents but a fraction of the 30,000 described fossil species that flourished in the seas of the Paleozoic and Mesozoic eras.

The phylum made its appearance in the Cambrian period and reached its peak in diversity and abundance during the Palaeozoic. The fossil record reveals that early in the Permo-Triassic period, there was in geological terms a rapid impoverishment in both abundance and diversity of many marine invertebrate phyla (Williams and Rowell, 1965), an event from which the Brachiopoda never recovered. The robust calcareous valves of this phylum have ensured that the Brachiopoda exhibit one of the oldest and most complete fossil records of any organism yet studied, (Rudwick, 1970). As such, the majority of our present knowledge of this phylum derives from the annals of palaeontology.

The investigation of extant faunas closely related to those of past assemblages, adds a new dimension to the great palaeoecological and biostratigraphic importance of the Brachiopoda. Few extant species have ever been studied. The longevity of many of the living genera and there affinity with analogues in the rich and diverse fossil record, provide a unique opportunity for the biologist and palaeontologist to gain valuable information of the functioning of this little known group of organisms.

Many authors have suggested that further studies should be firmly grounded in the biological processes underlying the palaeontological data. The literature is now beginning to reflect this trend. A growing number of publications have now examined extant faunas with a view to the interpretation of past assemblages (Percival, 1944; Paine,

1969; Logan and Noble, 1971; McCommon, 1973; Logan, 1975; Thayer, 1975; Doherty, 1979; Stewart, 1981; Curry, 1982a; Witman and Cooper, 1983; Tunnicliffe and Wilson, 1988).

To date 21 species of 17 genera, assigned to 12 families and representing six superfamilies, have been recorded in British waters (Brunton and Curry, 1979). Some are locally abundant, off the west coast of Scotland and in the Western Approaches (Curry, 1982a; Atkins 1959a, b, 1960a, b; Davidson, 1886-1888; Chumley, 1918; Brunton and Curry 1979).

A number of populations have recently been located, (or as the literature often reveals, relocated) in the Firths and Lochs of the west coast of Scotland. The reasonable accessability of some species, has prompted an integrated biological and palaeontological study.

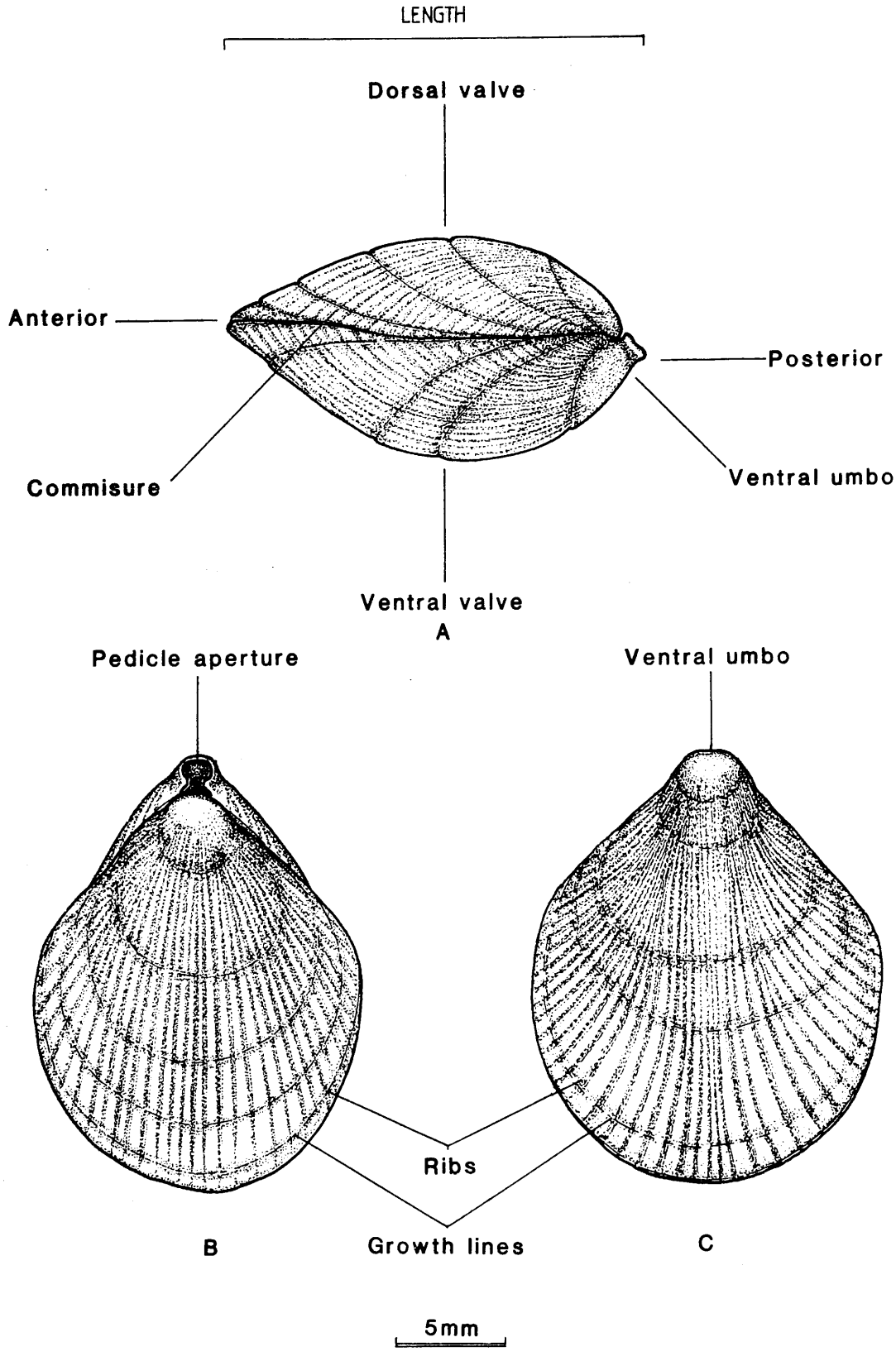
The articulate Terebratulina retusa (Linnaeus) and the inarticulate Neocrania anomala (Muller) are the most common British brachiopods, often occurring together.

The present investigation is centred on the reproductive biology of T. retusa. This species is generally considered to typify many of the aspects of articulate brachiopods and has therefore been chosen to serve as a reference around which future studies may develop.

T. retusa is a morphospecies which is widely distributed in the North Atlantic from the Mediterranean to Scandinavia. Terebratulina has a world wide distribution in present seas in addition to an extensive and well documented fossil record, which displays a high degree of continuity dating back to the Jurassic; a time span of approximately 150 million years (Curry, pers. comm.).

The success of the reproductive strategy of any organism is paramount in determining its ability to transmit genetic material to subsequent generations. It is also fundamental to any informed appreciation of the mechanisms by which an organism maximises its ecological fitness. These factors are of primary importance in moulding the evolutionary patterns of Phyla.

Fig. 1.0 External features of the valves of the Recent Articulate Brachiopod Terebratulina retusa.



Many of the most basic aspects of the reproductive ecology of the Brachiopoda are unknown. Much of the available data are disjointed, lack detail and are the result of fortuitous observations, rather than dedicated investigations. As a result, sparse information has been extrapolated throughout the phylum, inhibiting ecological and palaeontological interpretation. The present study has attempted a comprehensive and detailed review of the reproductive anatomy of T. retusa. The main objectives were to describe both the gross functional morphology of the genitalia and to follow the processes of gametogenesis at the ultrastructural level.

Once a clear understanding of gonad function had been achieved, a stereological technique was employed to trace the reproductive cycles of two discrete populations of T. retusa. This information was used to assess the differences in the reproductive stategies and provide data of direct relevance to their present physiological ecology and that of their fossil ancestors.

1.1 LOCATIONS.

1.1.1 Firth of Lorn.

Collections of Terebratulina retusa (Linnaeus) were made from two sites on the west coast of Scotland (Fig. 1.0). The main sampling area was a deep depression between the island of Kerrera and the south west of the island of Mull (Grid ref. NM745 265) (Figs. 1.1, 1.2). This is considered to be a part of the Firth of Lorn Fault; a subsidiary splay of the Great Glen Fault (Barber et al., 1979). The bathymetry of this area is characterised by steep sided valleys up to 275 m deep; a result of intense Pleistocene glaciation (Binns et al., 1974). In this location T. retusa typically occurs as an epizoite of the horse mussel Modiolus modiolus, and on fragments of hard substrate and rock walls.

An underwater television survey of the area by Collins (1986), has defined five facies based on faunal and sediment associations. T. retusa commonly occurs in categories 2 and 3, namely the "Rock wall facies" and the "Modiolus facies", within the depth ranges of 100 -

Fig. 1.1 Map showing the position of the Firth of Lorn and the location of the sampling area.

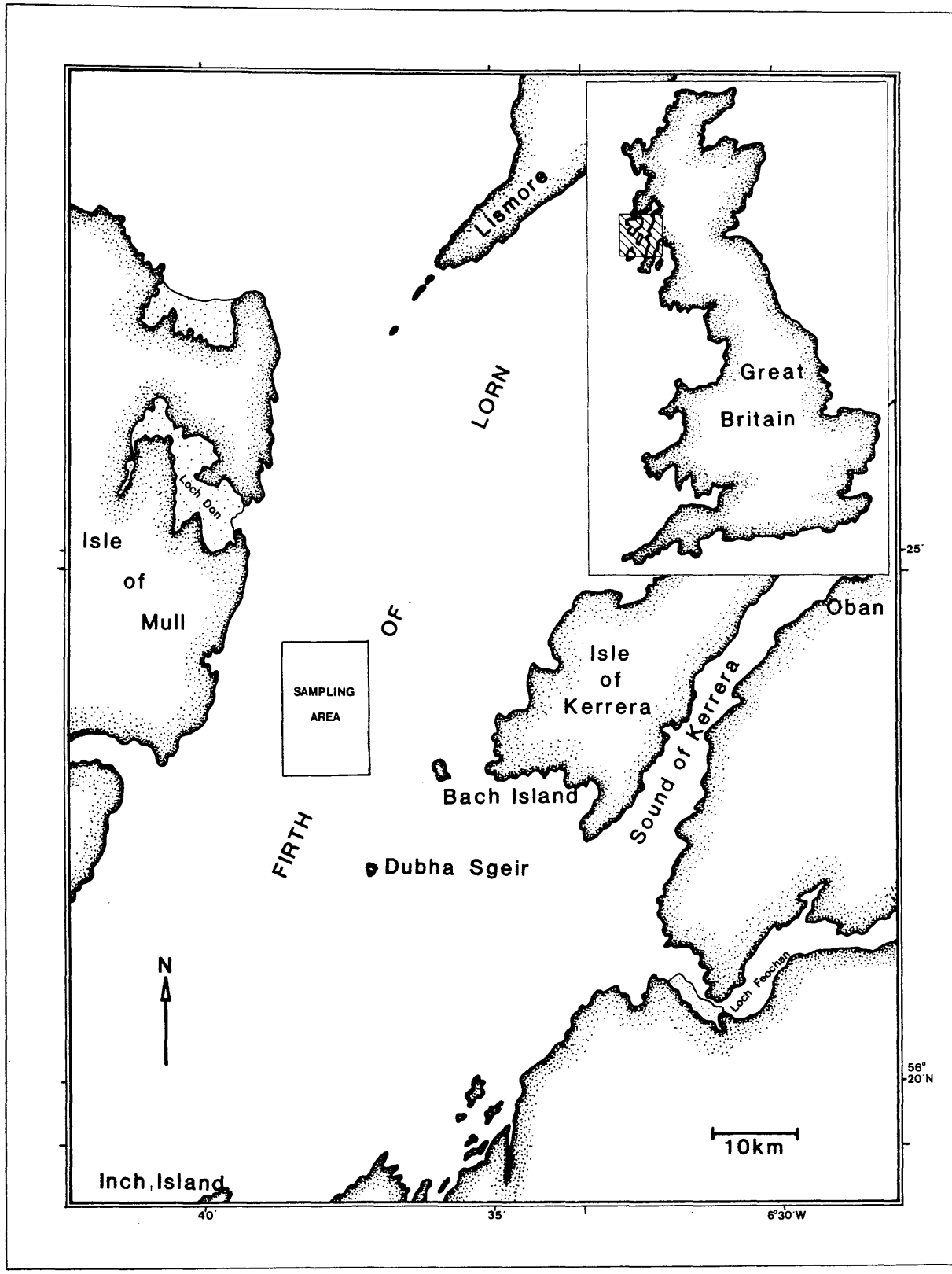
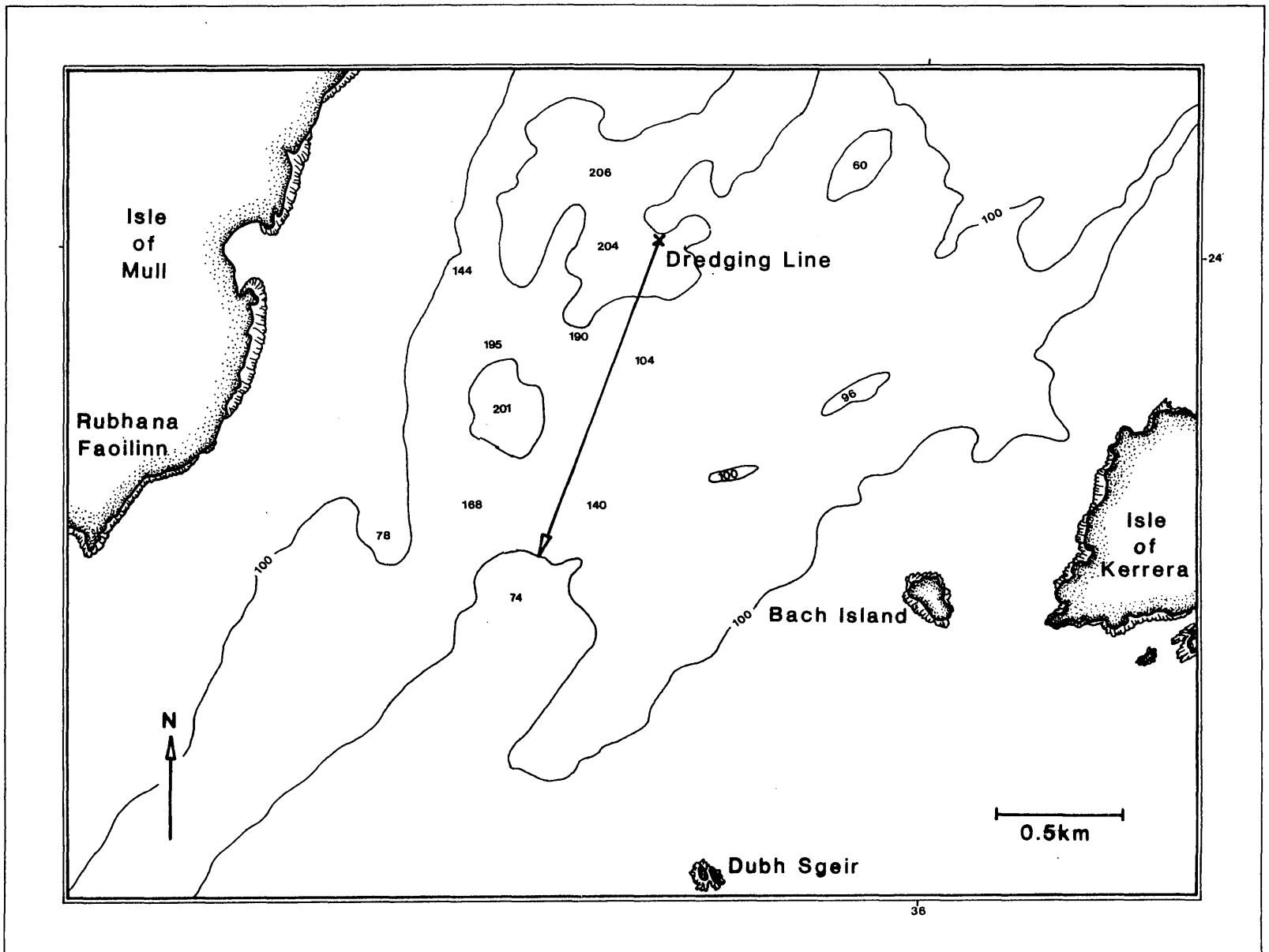


Fig. 1.2 Detailed map of the sampling area in the Firth of Lorn.



160 m and 160 - 195 m respectively. Exposed rock on the slopes of the depression provide attachment sites for large numbers of T. retusa. The base of the slope is populated by extensive clumps of M. modiolus which forms the basis for a diverse epifaunal community including T. retusa (Collins, 1986). This community is found in an elongated channel (Fig. 1.2), which attains a maximum depth of 206 m.

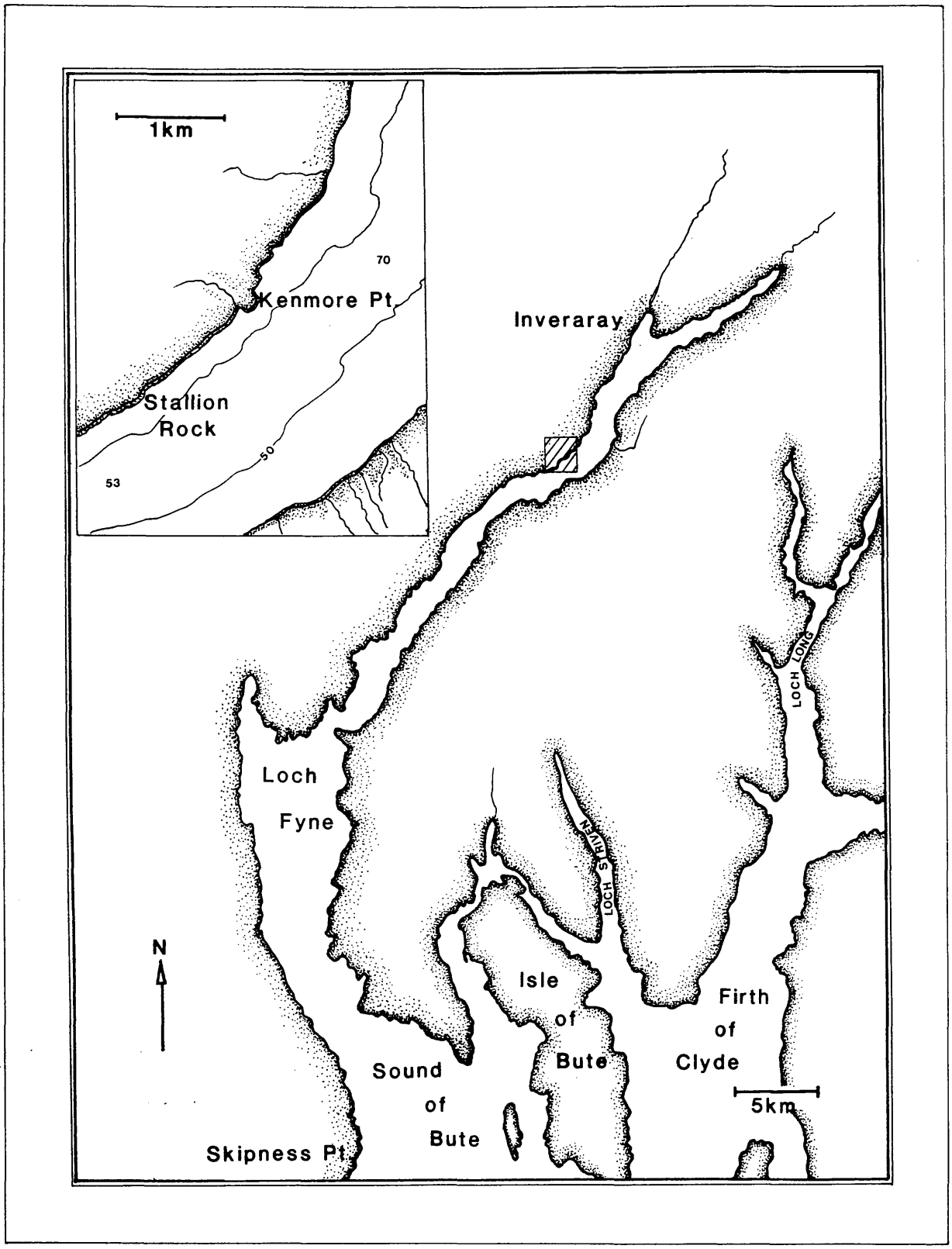
1.1.2 Kenmore Point.

A second sampling site was selected at Kenmore Point; Loch Fyne (Grid ref. NN 075 050)(Fig. 1.3). Loch Fyne is situated to the west of the Firth of Clyde. The loch extends in a north-easterly direction from the Sound of Bute for about 60 km attaining a maximum depth of 185 m with a mean depth at low water of 55.5 m (Edwards and Sharples, 1985). Loch Fyne is also the product of Pleistocene glaciation and its bathymetry is typically fjordic.

Kenmore Point lies in the upper reaches of the loch approximately 6 km south west of Inverary. This site consists of a small peninsula of Dalradian mica-schists which dominate the geology of the loch (Steers, 1973). The bed of the loch slopes steeply away from the shore at an angle of about 45 degrees to a depth of 12 m. Below this depth a cliff descends to 30 m at which point a fine muddy slope appears and continues down at an inclination of about 30 degrees. The cliff continues in a south-easterly direction parallel to the shoreline and is broken in places by flumes of fine sediment and terestrial detritus which form large, steeply-sloping, sediment fans. The cliff face is sheer and overhanging in places, and the rock surface is generally smooth with few cracks or crevices.

A clear faunal zonation is apparent on descending the cliff. Both T. retusa and N. anomala occur at depths between 25 m and 30 m attached to the rock surface, particularly in the more cryptic habitats. A layer of detritus covers the upper surfaces of promontories and ledges, effectively excluding brachiopods, presumably by preventing settlement or by a smothering effect. Although the distribution of T. retusa is patchy, densities of 5 m^{-2} are not uncommon. N. anomala is more evenly distributed over the surface of

Fig. 1.3 Map showing the position of the sampling area at Kenmore Point.



the cliff, and can achieve densities of up to 50 m^{-2}.

1.2 HYDROGRAPHY

In order to provide more detailed information on the hydrography of the area than was available in the literature, an Aanderaa Instruments recording current meter (RCM5) was deployed at each site for about a year. (For a technical decription of these instuments refer to TECHNICAL DESCRIPTION No. 119, June 1981).

The current meters were secured approximately 2 m above the sea bed with single point moorings. The Firth of Lorn mooring was set at a depth of 175 m. Kenmore Point offered few suitable mooring sites, the meter was eventually placed 2 m from the base of the cliff at a depth of 30 m. Records of current speed, velocity and water temperature were taken every hour. Meters were left in situ for up to six months before retrieval to allow overhaul and reclamation of stored data. The current meters were redeployed as soon as practically possible to minimise disruption to the data accumulated.

The information was processed on a PDP 11/23 computer, running the RT11 program using 'HILOW' filters, (Griffiths, pers. comm. 1988). Current meter data from the Firth of Lorn were curtailed prematurely due the loss of the recording instrument during recovery and is therefore of limited application.

1.2.1 Hydrography of the Firth of Lorn.

The hydrography of the Firth of Lorn displays a fjordic pattern of circulation which is superimposed on the North Atlantic current, the main oceanic current system (Collins, 1986). It is presumed that meteorological conditions dictate the balance of inflows. Deep water is drawn in from coastal shelf and the North Channel by a compensation current, increasing the distribution of the nutrients regenerated in the deep basins.

Fig. 1.4.1 Graph of annual temperature records from the Firth of Lorn.

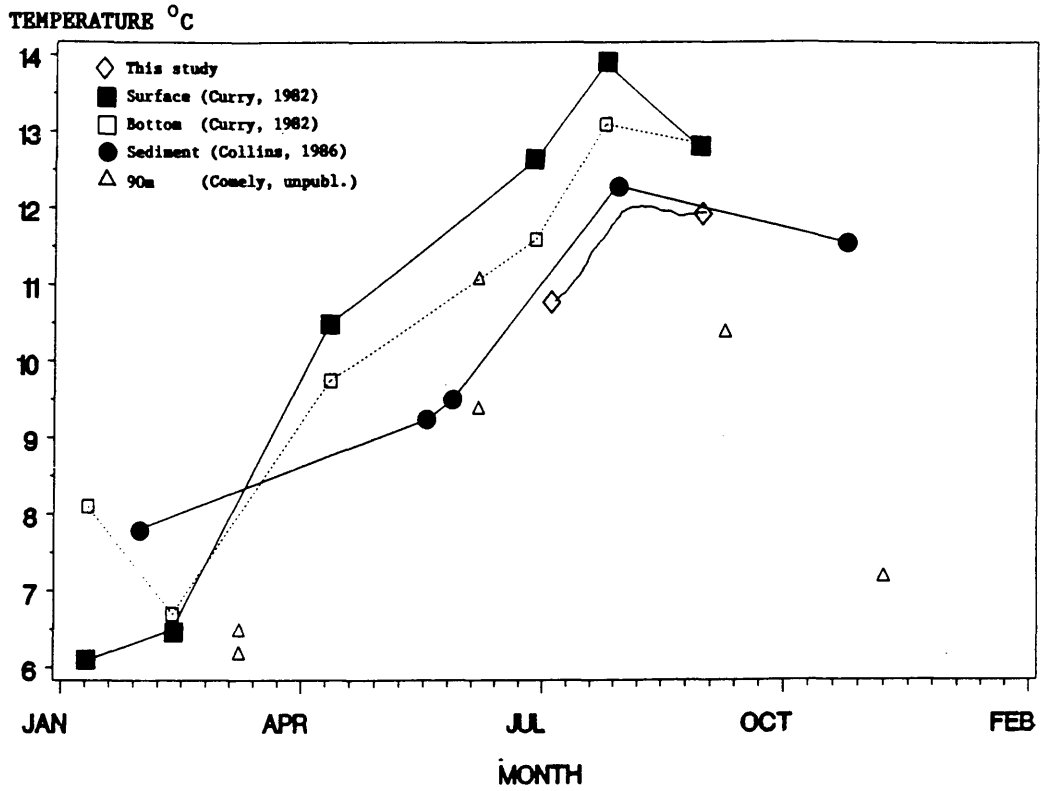
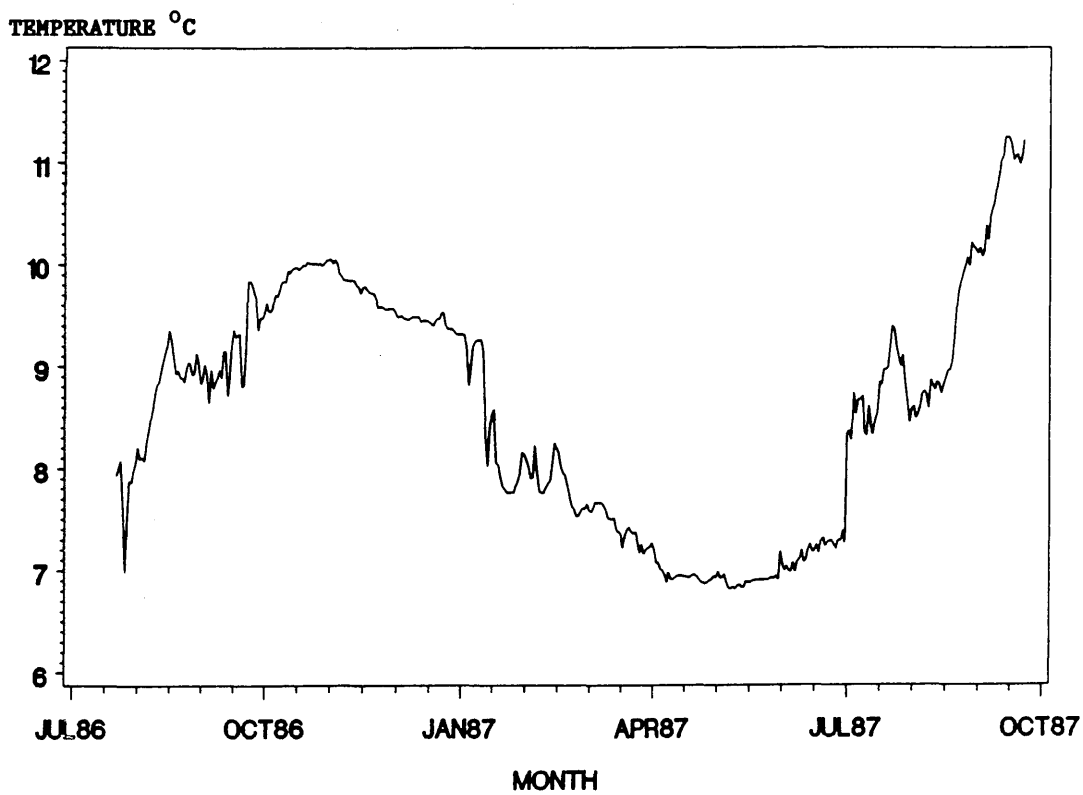


Fig. 1.4.2 Graph of the annual temperature record from Loch Fyne.



High phosphate concentrations recorded by Grantham et al. (1983) are thought to reflect the fact that the inflow contains a proportion of nutrient-rich Clyde water. In winter the water column is well mixed with little physio-chemical variation with depth.

Some thermal stratification has been reported during the summer in the lower reaches. The bottom waters achieve a minimum temperature of 6.5°C in February and a maximum of 13°C in August (Curry, 1982a, and Fig. 1.4.1). Temperatures within the sediment fluctuate to a lesser extent, from 7.25°C in February to 12.25°C in August (Collins, 1986). Records provided by the in situ current meter (RCM5), show reasonable agreement with sediment temperatures between July and October (Fig. 1.4.1). Comely (pers.comm) has also recorded temperature and salinity profiles off the southern tip of Lismore to a depth of 90m, from 1962 to 1963. Both the range and maximum temperatures are lower than in the Firth of Lorn depression (Fig. 1.4.1). Admiralty charts (Nos. 2387 and 2378) indicate current flows of 28 to 112 cms sec^{-1} (0.5 to 2.0 knots) at the surface, although tidal streams in excess of 224 cms sec^{-1} (4.0 knots) have been recorded.

There are few data available on the velocity and duration of water currents at depth in the Firth of Lorn. General opinion favours quite rapid current flow. Grantham (pers. comm.) reports extremely rapid rates of turnover and suspects that the waters of the Firth of Lorn are exchanged in a period of less than two months. The presence of large numbers of Modiolus modiolus may serve as a biological indication of high current flow as they are known to require considerable water exchange (Shelford, 1935).

The limited current meter records (Fig. 1.5.1) indicate a strong southwesterly residual flow with currents in the order of 30 cms^{-1}. This is, however, a complex region hydrographically, where Loch and Shelf waters meet and mix. Both the bathymetry and the hydrography conspire to make conditions unpredictable, particularly with the present lack of data.

Measurements from a single point may be misleading. Displaced to one side of the Firth of Lorn, on the margin of a deep depression, the currents recorded may be part of a greater pattern of circulation.

Fig. 1.5 CURRENT SPEED AND DIRECTION.

Fig. 1.5.1 Record of current speed and direction from the Firth of Lorn.
24th July 1986 to 3rd October 1986.
Interval 60 minutes.

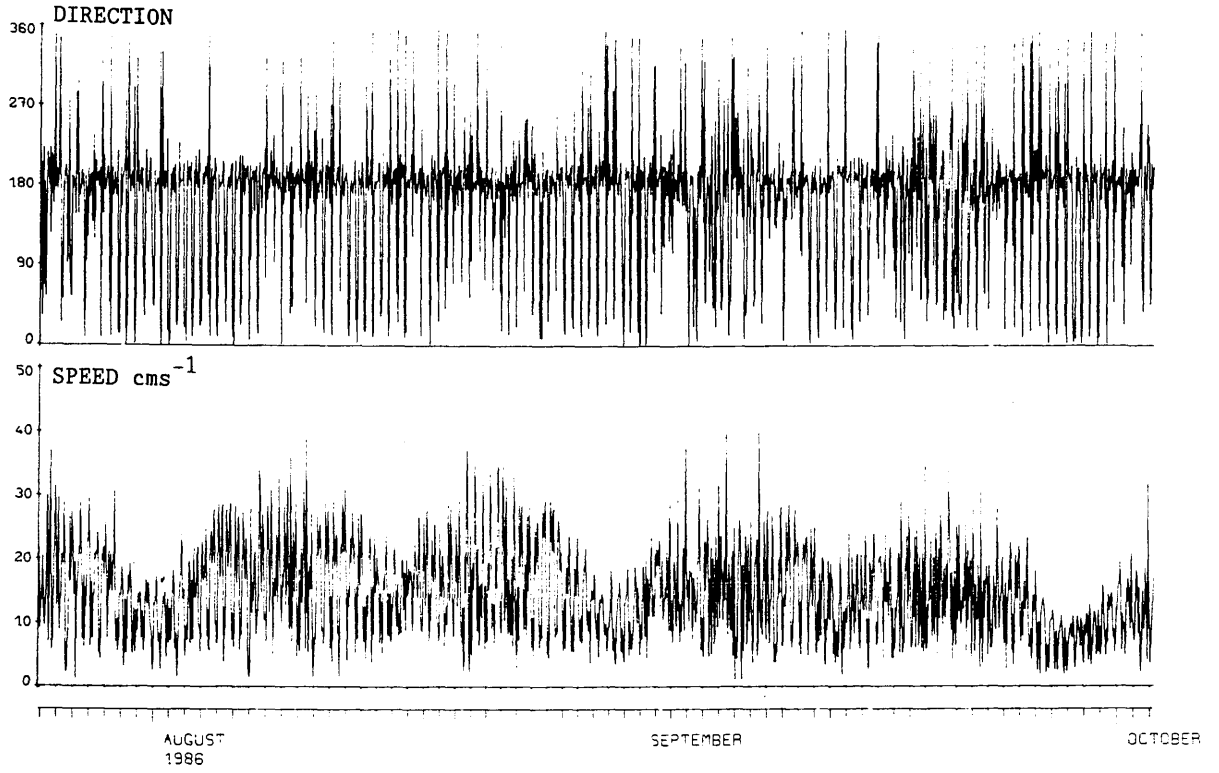
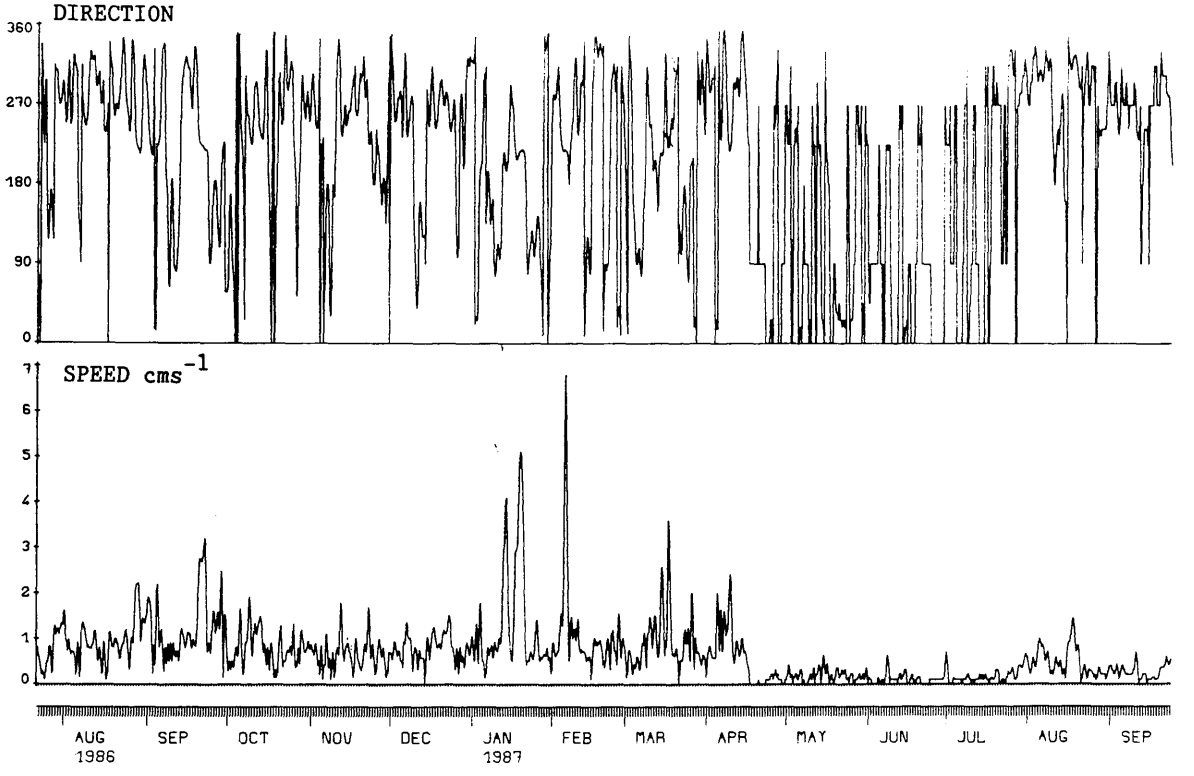


Fig. 1.5.2 Record of current speed and direction from Loch Fyne.
22nd July 1986 to 23rd September 1987.
Interval 360 minutes.



Allochthonous detritus, including shallow water fauna and terrestrial plant remains, dredged from this deep depression suggest that it is a sink, possibly the result of a deep gyral circulation.

1.2.2 Hydrography of Loch Fyne.

Loch Fyne is a deep-silled fjord opening into the Firth of Clyde, (Tett et al. 1986). Upper Loch Fyne is essentially a single basin, of maximum depth 150 m, separated from the Firth of Clyde by a long deep and complex sill between Lachlan and Oitir (Edwards and Sharples, 1986). The upper loch is one of the largest fjordic basins in Scotland but it receives relatively little fresh water runoff and has a relatively low rate of tidal exchange.

Work by Mill (1891) indicated that meteorological conditions, (as in the Firth of Lorn) are largely responsible for mediating the distribution of water types. Salinity and temperature data show that deep water may stagnate for a substantial part of the year. This isolated bottom water is renewed in the winter by density currents flowing in from the sills (Tett, et al., 1986). Freshwater input from local rivers, into the upper basin results in the formation of a stable brackish layer which favours phytoplankton growth in the surface waters. Nutrient input from the rivers and any supply of regenerated nutrient from stagnated deeper water also enhance production (Jones, et al., 1981). Terriginous particulate or dissolved organic matter may affect production by reducing light penetration and altering water chemistry, (Tett, et al., 1986).

The lowest annual temperatures at 30 m of approximately 6.8^{o}C, occur between May and June (Fig. 1.4.2). This is followed by a rise in temperature throughout the summer months to a maximum in late autumn of about 11^{o}C. Short term variations in temperature cause fluctuations of a degree Celcius or 25% of the annual range. The cause of these relatively rapid changes is unknown, but it has been suggested that density induced currents transporting colder surface water may be responsible (Griffiths, pers. comm.).

Current meter records from Kenmore Point show evidence of very low

speeds with a mean of less than 2 cms^{-1} (Fig. 1.5.2). The maximum recorded speed was 7 cms^{-1}. The velocity is generally confused, but there does appear to be a slight northerly residual flow. The record was disrupted from mid April, 1987. The reason for this disturbance is unknown.

1.3 PRODUCTIVITY.

TABLE: 1. Seasonal Productivity Measurements from Firth of Lorn and the Firth of Clyde.

Date	Station			
	Firth of Lorn(E4) (56°14'N 6°02'W)		Firth of Clyde(ASB30) (55°34'N 4°59'W)	
	Prod.	**Chl.**	**Prod.**	**Chl.**
May '86	71	33.1	584	73.6
September '86	61	56.7	134	50.9
January '87	2	3.5	3	12.4
March '87	25	18.6	48	70.3

Prod. is the integrated water column production in units of mg C $m^{-3}day^{-1}$.

Chl. is the integrated water column standing crop of chlorophyll in units of mg chlorophyll m^{-2} (Calculations were based on the mean monthly irradiance after Jones, 1988).

Comparative figures (TABLE 1) clearly show Clyde waters to be more productive than the Firth of Lorn. Indeed, in general, the Clyde waters are five times more productive (Jones, pers.comm.).

Fig. 1.6.1 Graph of integrated water column production (PROD. mg C m^{-2} day^{-1}).

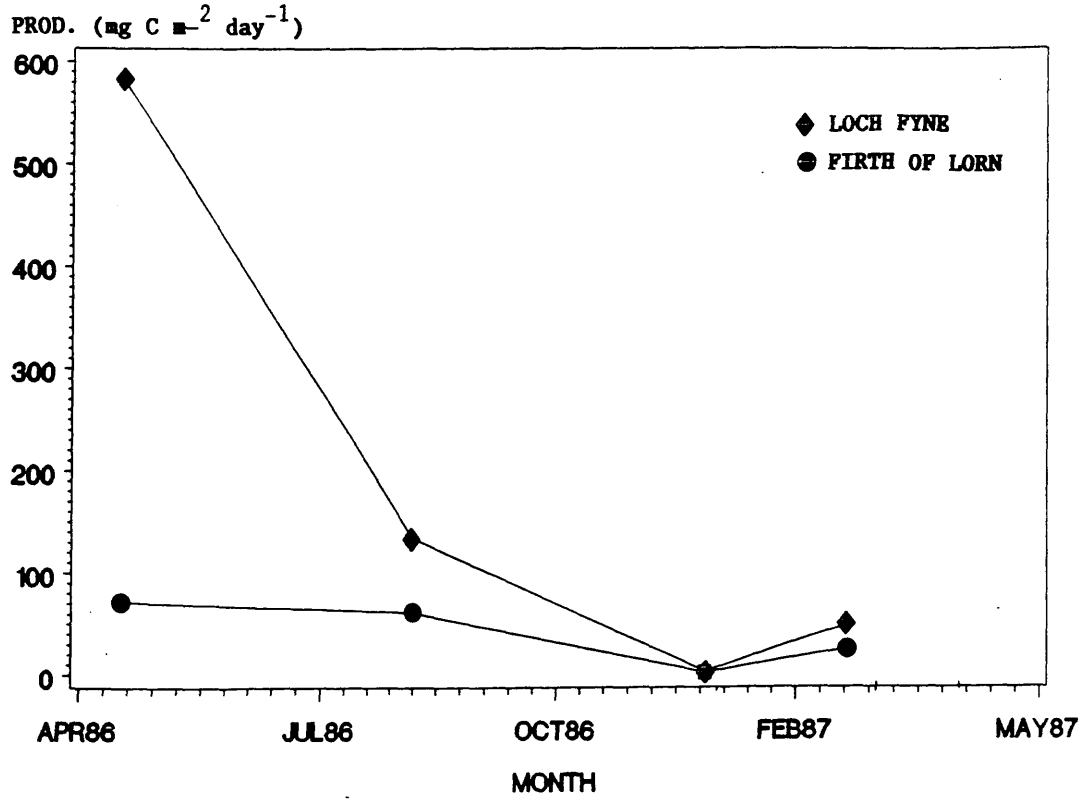
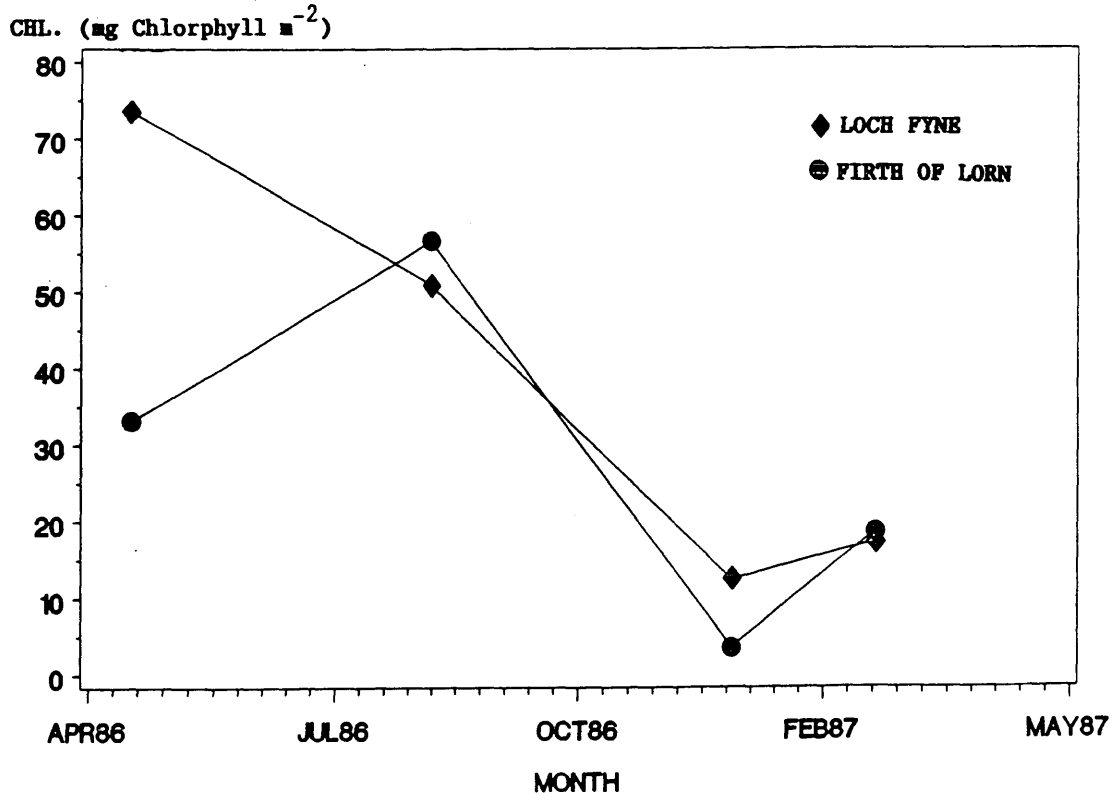


Fig. 1.6.2 Graph of integrated water column standing crop of Chlorophyll (CHL. mg Chlorophyll m^{-2}).



Jones (1988) reports a phytoplankton bloom in Loch Fyne between late March and early April with a strong possibility of a second in the autumn. In addition isolated blooms may occur throughout the summer (Figs. 1.6.1-2). Tett et al. (1986) report a succession of blooms along the length of Loch Striven, and Tett (unpublished data) has determined a similar pattern of events in Loch Creran. Although the data for Loch Fyne are limited, it would seem reasonable to suggest that it also experiences a succession of plankton blooms throughout the year.

In contrast the available data, while sparse, would indicate that the Firth of Lorn has a spring phytoplankton bloom in April, but a significant autumn event is unlikely, (Figs. 1.6.1-2).

The Clyde maintains a high phytoplankton biomass in its well illuminated stratified surface waters through much of the spring and summer, whereas strong vertical mixing in the outer Firth of Lorn appears to limit production by mixing the phytoplankton to poorly illuminated depths (Jones, 1988).

CHAPTER 2.

GENERAL PROCEDURES.

CHAPTER 2.

2.0 SAMPLING PROCEDURE.

The samples were collected by the R.V. CALANUS using a 1.2 m wide by 2 m long scallop dredge, with an external mesh of steel rings 75 mm in diameter and an inner nylon mesh of 15 mm diameter. When hauled for periods of approximately 15 minutes up the slope in a north-easterly direction (Fig. 1.2), the dredge proved to be particularly successful at sampling the semi-infaunal M. modiolus and associated fauna. Once on deck the samples were washed in fresh sea water and placed in plastic baths. Samples from Kenmore point were collected by diving.

2.1 SAMPLE TREATMENT.

Specimens of T. retusa were removed from the substrate by severing the pedicle with a scalpel. Thereafter, whole or partially dissected animals were immersed in fixative as soon as possible.

2.1.1 Fixation.

Two methods of fixation were adopted. Those specimens to be used for light microscopy were immersed in phosphate buffered 10% formal saline (pH 7.0). The valves of each individual were wedged open before immersion to allow rapid and thorough fixation.

For electron microscopy analysis, the two valves of the shell were separated and the gonads dissected out and cut into pieces approximately 1 mm^3. These were then fixed for 6 hours and 1 hour respectively. Fixation was carried out in a freshly prepared solution of glutaraldehyde (2.5%) buffered with 0.2M cacodylate buffer (pH 7.25) containing NaCl (1.9%). After the allotted time specimens were transferred into two changes of 0.2M cacodylate buffer (pH 7.25) containing NaCl (2.5%), for 15 minutes each. All the preceeding steps were carried out at approximately $4^{o}C$. At this stage individual valves were decalcified in a solution of 6% Ethylene Diamine Tetracetic Acid

(EDTA), adjusted to pH 7.25. Specimens were postfixed in osmium tetroxide (1%) buffered with 0.2M cacodylate containing NaCl (2.5%) for 1 hour, and finally washed with several changes of distilled water before further processing.

2.2 HISTOLOGICAL PREPARATIONS.

2.2.1 Light Microscopy.

Preserved specimens were decalcified in one of two ways: using a 10% saline solution of 'Rapid Decalcifier', a commercially available decalcifying agent, or a solution of 6% EDTA adjusted to pH 7.6. When necessary specimens were dissected before dehydration and wax embedding, (Appendix I). 5 μm sections were taken using a JUNG AG. microtome, and then floated out on to glass slides, dried and stained with Harris 'Haematoxylin' and counter stained with 'Papanicoloaus' OG6 and EA50. (Appendix II).

Some samples were subjected to further treatment:- 'Sudan black B', 'Alcian blue', 'mercuric bromophenol blue' (BPB) and periodic Schiff reaction (PAS) with and without a malt diastase digest. Sections were examined with a Watson microsystem 70 and/or a Lietz Wetzlar microscope and photographed with Kodak technical pan film ESTAR-AH Base.

2.2.2 Transmission Electron Microscope.

Glutaraldehyde-osmium tetroxide fixed material (Appendix III), was first embedded in Araldite resin (Appendix IV). Ultrathin sections (70 to 90 nm) were then cut using glass knives on a Reichert Ultramicrotome Om3.

Once mounted on copper grids, (300 Formvar coated or 400 uncoated) the sections were stained using uranyl acetate and lead citrate, (Appendix V). Specimens were viewed with a Corinth TEM and photographic images recorded on AGFA-GEVAERT 70 mm roll film.

2.2.3 Scanning Electron Microscopy.

Fixed material was dehydrated using acetone and critical point drying. Particularly small or delicate specimens were secured in a chamber between two Millipore filters (1 μm) and the solutions used for dehydration were forced though the chamber with a syringe. Dried specimens were then mounted on aluminium stubs with a silver based colloidal paint or with double sided sticky tape in the case of the filters. After coating with gold the specimens were viewed with a Philips SEM 500. Photographic images were recorded on Ilford FP4 120 roll film, (Appendix VI).

CHAPTER 3.

FUNCTIONAL MORPHOLOGY OF THE GENITALIA.

CHAPTER 3.

3.0 INTRODUCTION.

Reviews by Hyman (1959), Williams and Rowell (1965), and latterly Rudwick (1970) bear testimony to the lack of basic information regarding the reproductive anatomy, histology and functional morphology of the Brachiopoda. Mueller in 1776 (Joubin, 1886) was the first to recognise the genitalia in Neocrania anomala and in 1797 Cuvier gave some preliminary notes on the anatomy of Lingula. Not until the mid 19th centuary, however, did more comprehensive anatomical researches become available. Owen, Huxley, Hancock, Blochmann and Davidson all made notable contributions. The illustrations in Hancock (1859), and Blockmann (1892 - 1900) were for many years the best available figures of brachiopod anatomy. The articles of van Bemmelen (1883), Beyer (1886), Schaeffer (1926) and Senn (1934) are mainly histological. In 1861, Lacaze - Duthiers published his observations of the development of Lacazella mediteranea. He observed the egg with its surrounding follicular cells attached by a cellular pedicle to the ovary. Light microscope studies of the gonads in some inarticulates, and in Gryphus vitreus, Hemithiris psittacea, Macandrevia cranium and Terebratulina retusa were carried out by van Bemmelen (1883). Both Joubin (1886) and Blochmann (1892) studied the inarticulate Neocrania anomala. Yatsu (1902), Senn (1934) and Schaeffer (1926) have documented the lingulid genitalia.

An article by Prenant (1928) appears to be the first reference to deal specifically with the histology of Terebratulina. The more recent work of Tortell (1981) gives a brief description of the genitalia of Notosaria nigricans, Terebratella inconspicua, Terebratella sanguinea, Liothyrella neozelanica and Neothyris lenticularis, all from southern New Zealand. In offering a general introduction to the reproductive anatomy of the Brachiopoda it is impossible to overlook the invaluable contribution of Williams and Rowell (1965). To date, this work represents the most comprehensive review of the biology of extant brachiopods.

Typically brachiopods are dioecious, and a few are

hermaphroditic. The latter include three species of Argyrotheca from Naples (Senn, 1934), Pumilus and Platida (Atkins, 1958). It is also possible that an alternation in the production of sex cells occurs in Fallax, (Rowell and Williams, 1965). Sexual dimorphism, though unusual, has been reported in Lacazella mediterranea (Lacaze - Duthiers, 1861) in which the ventral valve of the female is distended to incorporate a brood pouch. Minor differences in shell morphology revealed by statistical analysis of some fossil assemblages has been taken to indicate possible dimorphism (Vandercarmen, 1959).

The sexes of many living brachiopods can often be assessed by the colour of the ripe gonad. Testes may be distinguished as being white or cream while ovaries exhibit yellow to orange-brown pigmentation. Usually the brachiopods have two pairs of gonads. In the inarticulate brachiopods the gonads are usually confined to the visceral cavity which is much larger than the mantle cavity. The mantle cavity is effectively a system of mantle canals (Fig. 1.8). The mantle canal pattern of inarticulates is less complex than that of the articulates, and only in the Crinidae are the gonads known to be partially inserted into the mantle canals. Articulates differ in that the relatively smaller visceral cavity houses a small part of the gonad, the greater part of which occupies spacious mantle canals.

Each gonad consists of a fold of germinal epithelium, which is part of the coelomic epithelium and is suspended in coelomic fluid. The germinal epithelium is supported on connective tissue membranes of the stomach; gastroparietal bands of the anterior chamber and ileoparietal bands of the posterior chamber. The gametes develop along the free margins of this lamellar structure. Mature gametes are released from the genital lamellae and are liberated via metanephridia into the brachial cavity during spawning. The metanephridia serve a dual purpose as excretory organs and gonoducts ocurring as one or exceptionally, as in the rhynconellids, two pairs (Rowell and Williams, 1965).

3.1 THE REPRODUCTIVE ANATOMY.

T. retusa is dioecious, and mature specimens with ripe gonads may be sexed because testes are white or cream while ovaries are yellow or orange in colour. The genitalia occur in four portions, (Fig.1.7), two in each valve. One partially occupies the dorsal mantle and the other larger portion is contained ventral mantle. The visceral cavity houses only a small part of the gonad, while the greater part is inserted within an extensive mantle canal system (Fig. 1.8).

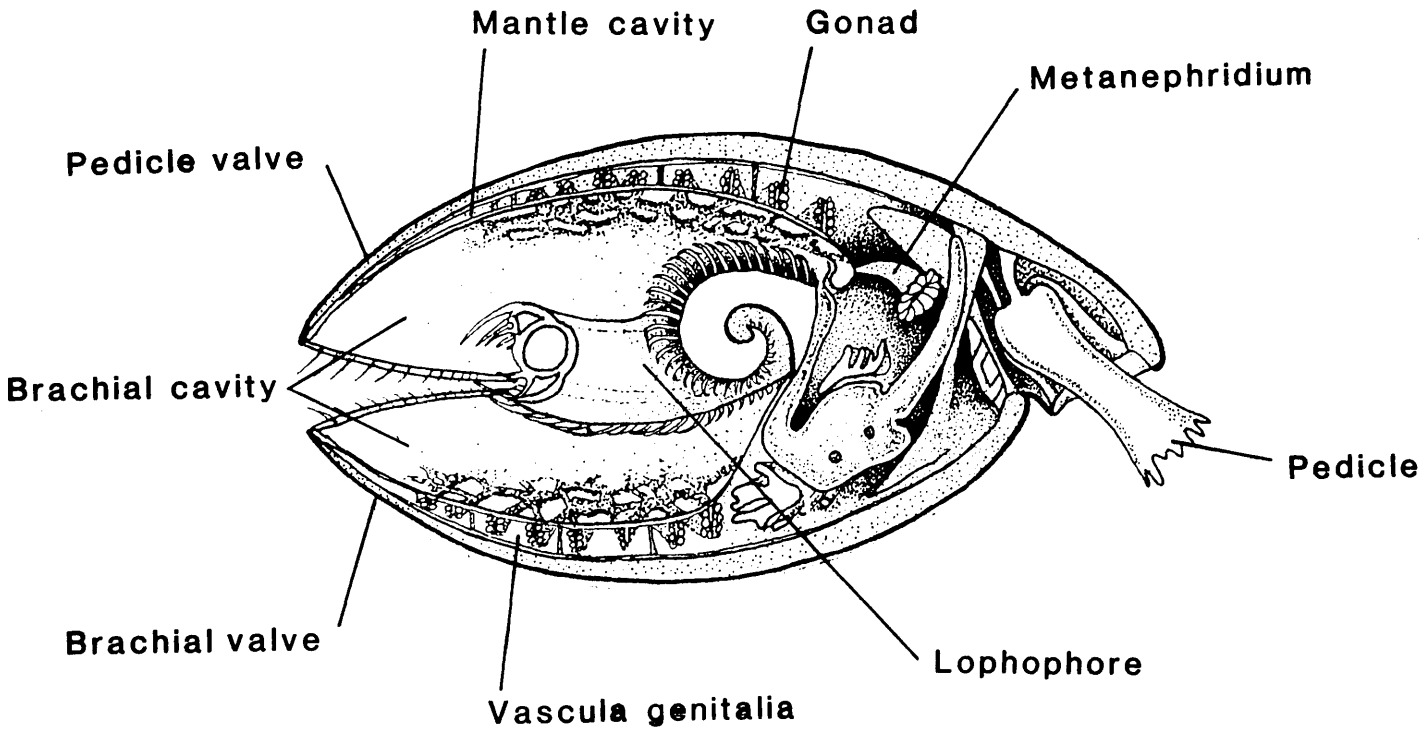
In surface view the gonads may be observed through a thin transparent epithelial membrane containing stellate calcareous spicules. The gonads appear as a flat reticulate lattice, which in its entirety is palmate. Each portion of the gonad emerges from the visceral cavity as a single strand branching in one plane to occupy the mantle sinus or vascula genitalia. The latter is the main repository of the gonad. Confined to the vascula genitalia, the gonad does not extend into the subordinate vascula media or the minor peripheral branches of the mantle canal system. As such the vascula genitalia constitutes a gonocoel within which the gametes develop (Plate 1). Recently post spawned individuals present a well defined lattice structure which is eventually obscured by developing gametes (Plates 2, 3. Fig. 1.8).

Coelomic epithelium on connective tissue membranes lines the gonocoel effectively forming an envelope, the surfaces of which are periodically united with connective tissue pillars. Pillars are evenly distributed and occur at the centre of the polygonal shapes described by the reticulations of the genital lattice (Plates 1-4).

Prolongations of the mesentaries supporting the stomach; the gastro- and ileoparietal bands, form folds of tissue. During development the lamellal folds extend into the mantle sinus; fused along one margin to the inner epithilium (Plates 18, 20. Fig. 1.9). Initially a single strand, the ileoparietal band bifurcates. Continuous bifurcation results in minor branches which anastomose to form a rudimentary genital lattice.

The gametes are borne on the folds of the parietal bands or

Fig. 1.7 Diagrammatic representation of a sagittal section through *Terebratulina retusa*.



Adapted from Williams and Rowell (1965).

genital lamellae (Plate 4), developing from a germinal epithelium. Mature gametes are released from the lamellae and liberated during spawning through two metanephridia (Plates 6, 7).

3.2 ULTRASTRUCTURE OF THE GONADS.

Fig. 1.9 shows a diagrammatic representation of a section through part of the vascula genitalia of a female. The structure is conveniently divisible into three major regions; (1)the inner mantle epithelium, (2) the mantle sinus containing the genital lamellae, and (3) the outer mantle epithelium.

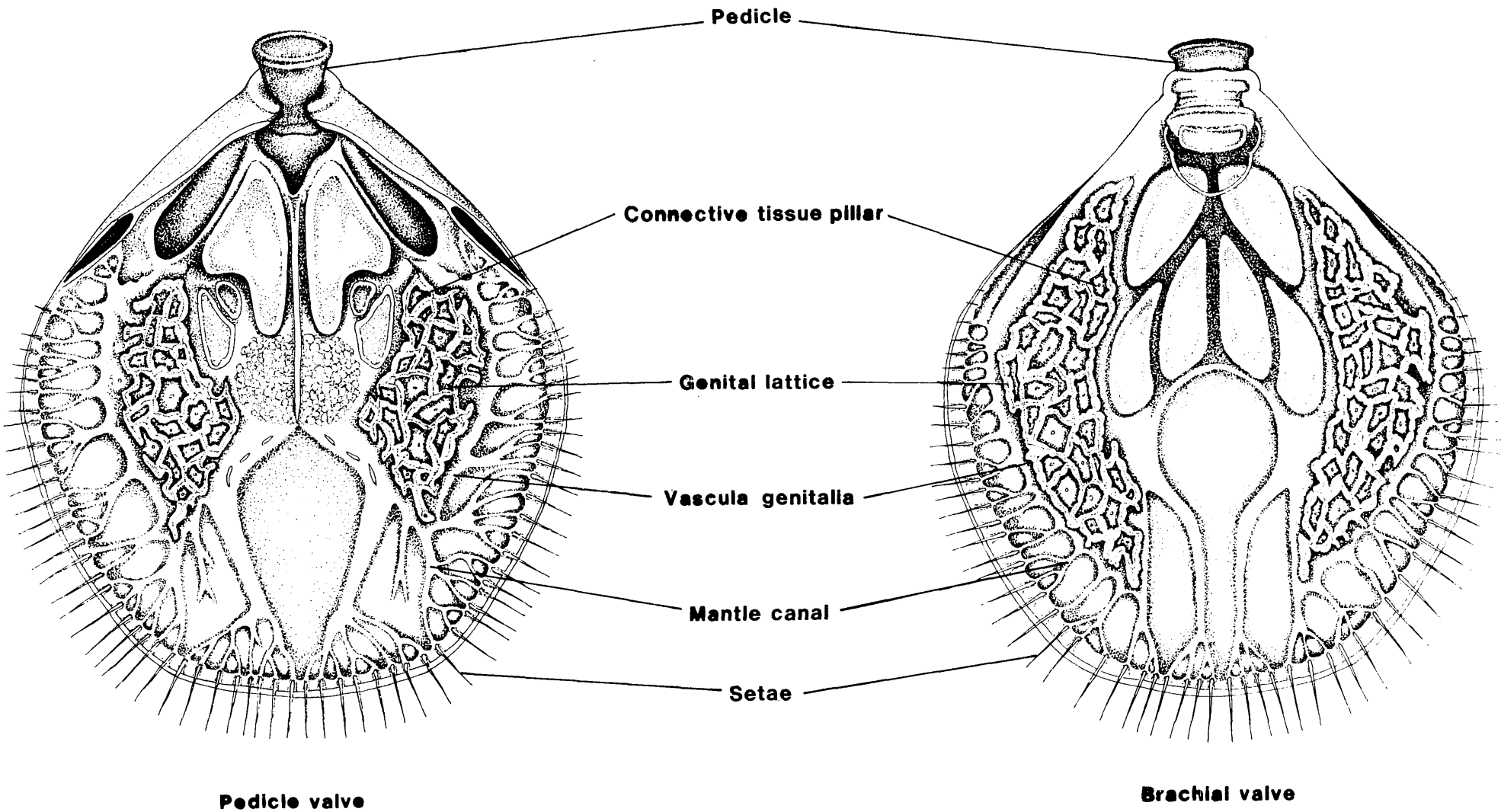
The mantle epithelia perform two distinct functions. They secrete the organo-mineral matrix of the valves thus providing the soft body parts with protection and support. They also enclose the brachial cavity; integrating with the lophophore to provide a milieu suitable for efficient feeding and ventilation (Williams, 1977; Westbroek et al., 1980). Both these functions are effected by epithelial specialisation.

3.2.1 The Inner Mantle Epithelium.

(i) The proximal epithelium of the inner mantle.

The inner mantle epithelium is made up of three distinct layers and is of variable thickness (Fig. 1.10). The innermost epithelium forms the lining of the brachial cavity consisting largely of a cuboidal, epithelium which becomes columnar near the margins of the mantle (Plates 8, 9). Some investigations reveal that the free margins of the cells carry a densely convoluted microvillar fringe, and that many are ciliated, while the entire epithelium is liberally punctuated by secretory cells. Typically, the intracellular organisation is polarised with various organelles disposed to the end of the cell nearest the basement membrane. Membrane bound droplets and secretory vacuoles accumulate beneath the microvilli. Golgi complexes, mitochondria and smooth endoplasmic reticulum are common inclusions, as are numerous vacuoles containing diffuse granular material, and

Fig. 1.8 Diagrammatic surface view of the mantle of Pedicle and Brachial valves illustrating the position and extent of the Genital Lattice.



membrane bound droplets of varying electron density. Basic histochemical tests indicate that these inclusions may be glycoproteins, mucoprotein and mucopolysaccharides interspersed with minor lipid droplets. Mucous cells are also common (Fig. 1.10).

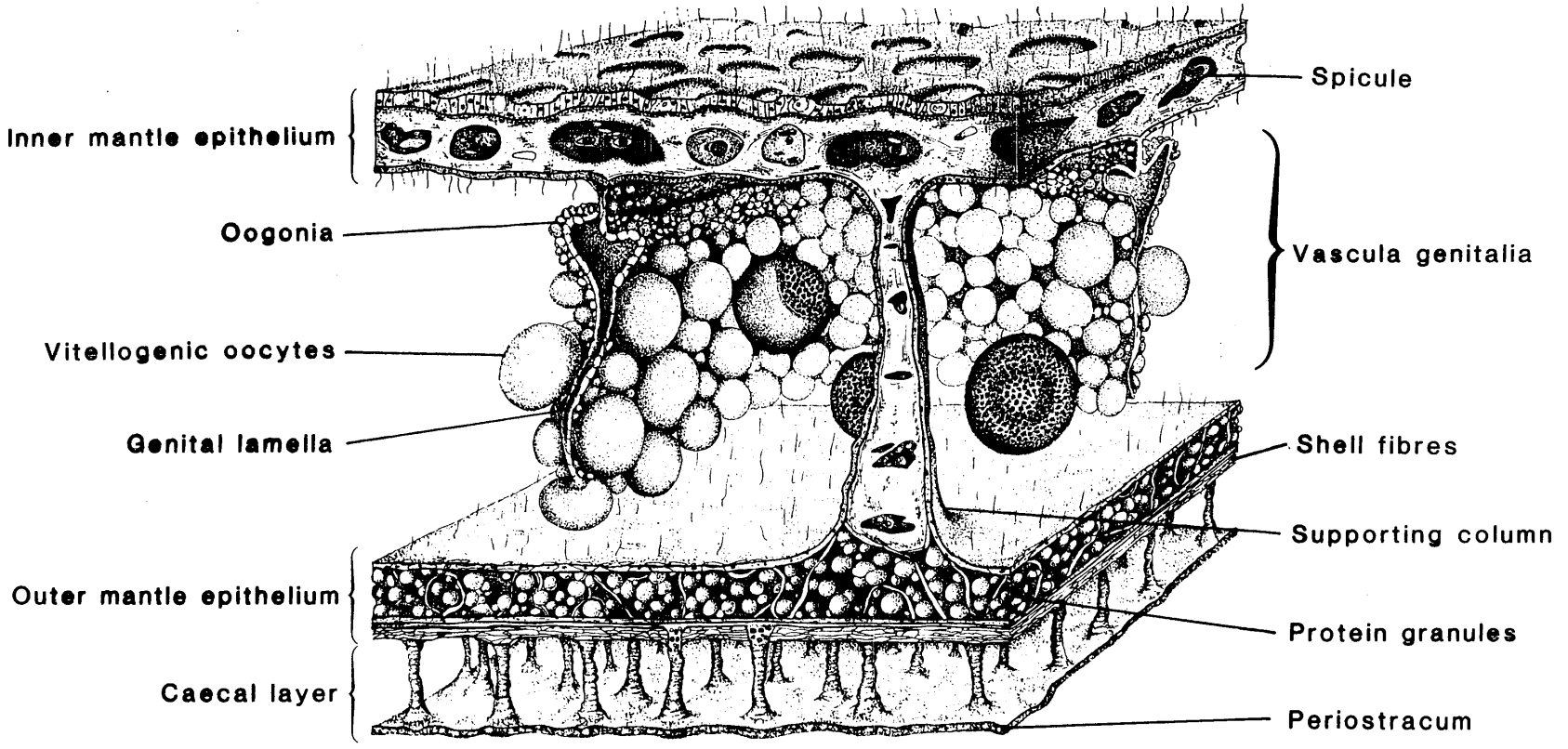
According to Williams (1977) the products of these cells are continuously exuded so that the fibrillar coats of the microvilli are always coated with a mucin-like substance. Williams (1977) suggests that the flow of mucin over the inner epithelium and the movement of the cilia would discourage microbenthic settlement and assist in the circulation of water within the brachial cavity. Westbroek et al. (1980) consider the lophophore and mantle as integrated organs which are active in the transport and capture of food, accumulation and removal of (pseudo)-faeces, the sorting of particles, the pumping of water and respiration.

(ii) Connective tissue.

The basal lamina to which the epithelial layer is affixed overlies a band of connective tissue (Plates 8, 9. Fig 1.10), of varying thickness. The layer is usually homogeneous but can contain apparently open cavities and mesenchyme cells. Close examination of the connective tissue reveals the presence of cartilaginous strands. At the centres of the polygonal shapes described by the genital lattice, the connective tissue forms pillar-like extensions which unite the inner and outer epithelia. These structures are continuous with the connective tissue layers in both epithelia, are widest at the points of insertion into them, and narrow medially (Plates 1, 4, 18, 19). Cartilaginous fibrils extend along the length of the connective tissue pillars, particularly towards the outer margin.

Both Blochmann (1892a), and Joubin (1887) mention structures which may be analogous to connective tissue pillars. The "genital pits" of some fossil brachiopods may also correspond to the positions of connective tissue pillars (Curry, 1982b). The morphology of connective tissue pillars would imply two functions: (1) to separate the inner and outer mantle epithelium thereby maintaining the lumen of the vascula genitalia, and (2) to prevent the inner epithelium from becoming overly

Fig. 1.9 Diagrammatic representation of a vertical section through part of an ovary.



distended by pressure from the increasing volume of gametes as the animal matures.

(iii) Spicules.

Mesenchymal cells are responsible for the secretion of the stellate calcite spicules which form a loose skeletal structure. Pre nant (1928) provides simple illustrations of spicular structure and notes the syncytial nature of the mesenchymetous scleroblasts. Mesenchymal cells occupy channels within the connective tissue. Many cells are attached to the walls of each channel and project into the lumen thereby creating a "vessel", within which it is assumed that an inorganic matrix is laid down. Although spicules or other irregular calcareous bodies occur within the connective tissue of the mantle and lophophores of other articulate brachiopods like Fallax (Atkins, 1960a) and have rarely been found loose in fossil Thecideans, they never contribute to the solid skeleton (Williams and Rowell, 1965). However, dense concentrations of spicules interdigitate, affording the inner epithelium some rigidity and offering a degree of protection to the mantle and that which it covers. Curry (1982a) remarks upon more dense spiculation in those areas of tissue immediately above the gonad, as does Atkins (1960a), in describing Fallax dalliniformis.

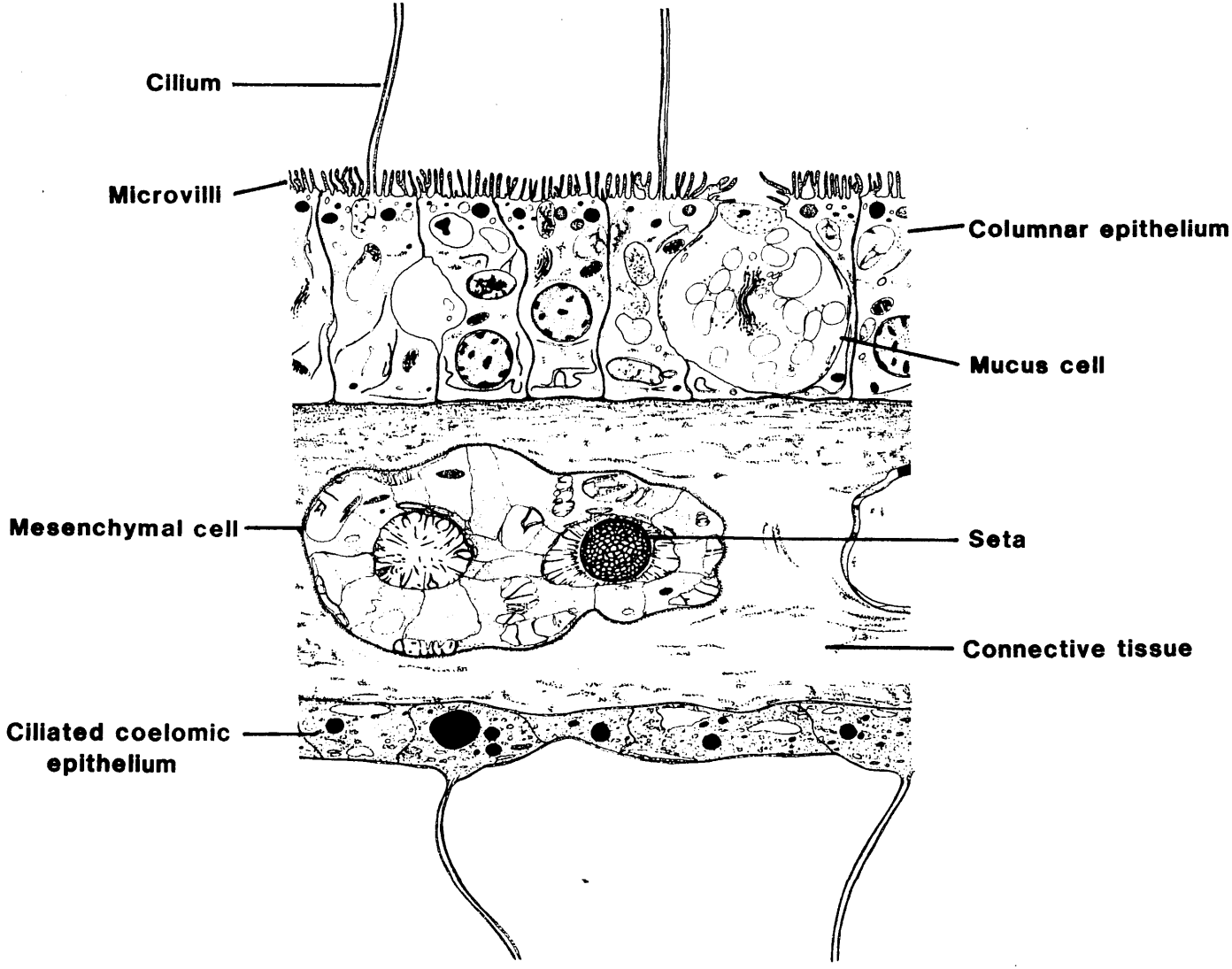
(iv) Peritoneum.

The inner epithelial membrane is lined by a thin flat peritonium which is ciliated, though sparsely so in regions occupied by the genital lamellae and attached gametes. The peritoneum is continuous over the inner surface of the coelom and by definition also covers the inner surface of the outer epithelial membrane. Ciliary action is therefore probably responsible for maintaining a circulation of the coelomic fluid contained within the mantle sinus and vascula genitalia.

3.2.2 The Outer Mantle Epithelium.

As befits its function, the outer epithelium is markedly

Fig. 1.10 Diagram of a vertical section through the Inner Mantle Epithelium.



dissimilar in structure to the inner membrane (Fig. 1.11). The outer epithelium may be considered as two functional units: (1) that part which lies within the shell matrix (the caeca), and (2) that which lines the mantle sinus proximal to the shell.

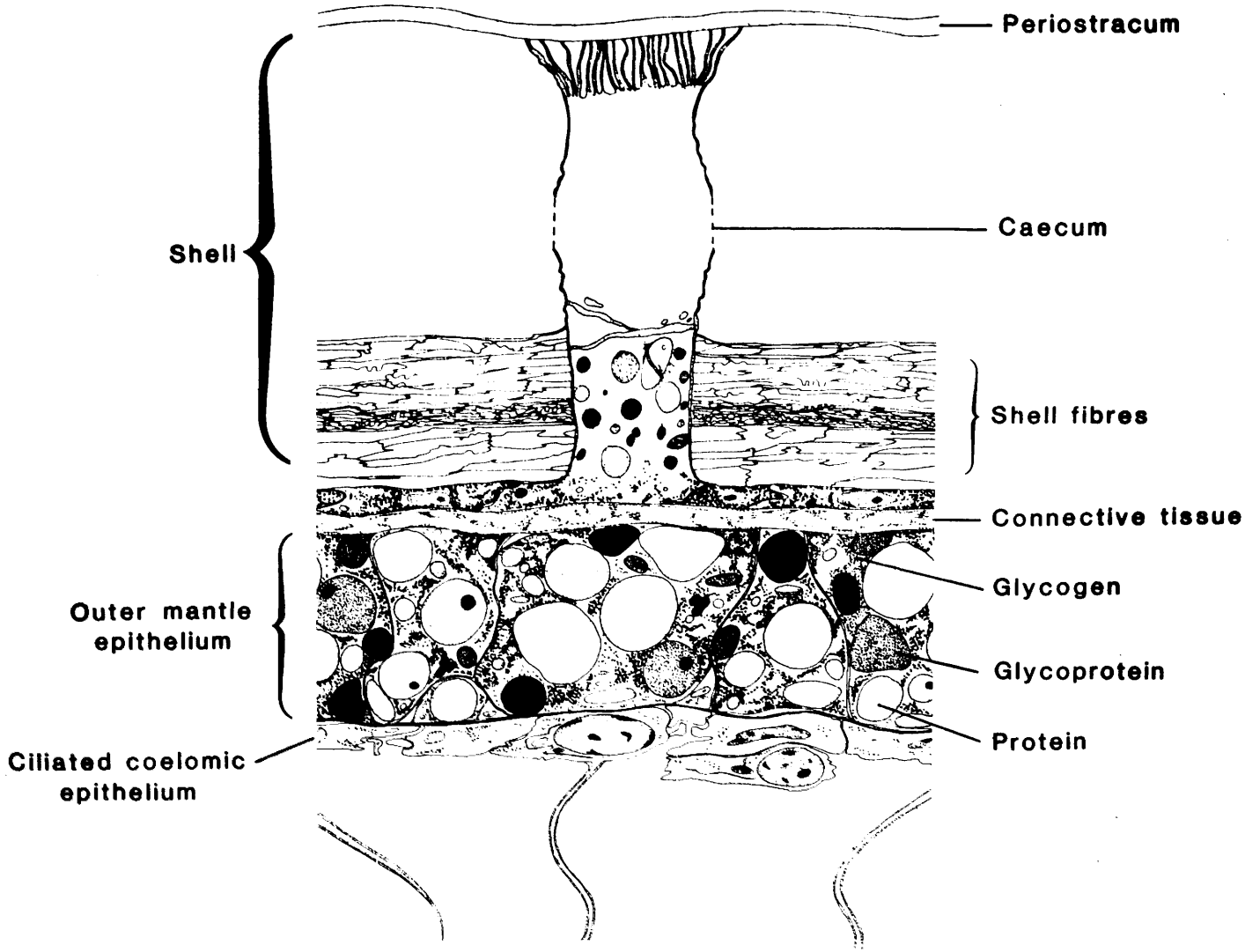
(i) The Proximal Epithelium of the Outer Mantle.

The proximal epithelium of the outer mantle is sandwiched between the peritoneum and collagenous connective tissue (Plates 10-13). This layer of cells is vesicular with dominant inclusions of variably electron dense droplets, lacking membranes and interspersed with small amounts of mucin and intimately associated with glycogen granules. BPB reveals these droplets to be largely proteineous, though a positive reaction with PAS is indicative of glycoproteins. Tonofibrils extend across the epithelial cells normal to the shell. Organelles are, where obvious, confined to the extreme margins of the cells and their nature is not easily determined.

Examination of monthly samples shows that and quality of these cellular inclusions changes seasonally. Subjective analysis of changes in the histochemical consistency of this layer point towards its functional and physiological significance, particularly with respect to the gametogenic cycle. Histochemically the tissue has two major components protein and glycogen, although there is evidence to suggest the presence of glycoprotein. Lipids droplets are relatively uncommon. The proportions in which these constituents are present, alters throughout the year (Plates 14-17). Specimens from the Firth of Lorn exhibit a well defined pattern. Protein droplets predominate, increasing in size and number throughout the spring and early summer, developing to a dense but even distribution within the cells.

Droplets begin to aggregate as the summer progresses and may coalesce. In the process their former structure becomes obscured by the occurrence of glycogen granules which first become abundant between May and June. Accumulation of both protein and glycogen continues at an apparently reduced rate until October/November, while the cells become distended and their contents amorphous. At this stage

Fig. 1.11 Diagram of a vertical section through the Outer Mantle Epithelium.



glycoprotein also forms a notable proportion of the intracellular material.

Glycogen and to some extent the glycoprotein component are strongly depleted early in the winter months. Specimens examined in January and February contain small numbers of ill-defined protein droplets contained within vacuous cells and by the end of the winter it is not uncommon for the cells of the outer mantle to be virtually devoid of inclusions. The start of protein accumulation in spring signals the beginning of another cycle (Plates 18-21).

(ii) Functional Morphology of the Outer Mantle Epithelium.

When viewed in isolation, these observations present a somewhat bizarre scenario, with protein, glycogen and glycoprotein forming a tripartite system, the constituents of which vary in quantity and relative proportion throughout the year.

In the light of recent work by Peck et al. (in press) it is perhaps possible to speculate as to the function of the outer mantle epithelium. In association with Peck et al. (in press) the metabolic rate of T. retusa from the Firth of Lorn was determined, in simulated summer and winter conditions. Metabolic rate was expressed as a function of the amount of oxygen consumed to nitrogen excreted. Results clearly indicate that the metabolism of T. retusa is strongly protein dependent in winter and somewhat less so in summer conditions, although protein probably constitutes more than 50% of the metabolite utilised during the summer. Feeding behaviour also had an effect on the substrate being utilised, with carbohydrates and lipids being used when available, while protein appears to have an important role in overcoming periods of starvation.

It is likely that the Firth of Lorn experiences a spring phytoplankton bloom in April (Jones pers. comm.). The increase in productivity at this time may be consistent with the accumulation of protein droplets in the outer mantle epithelia. This would be followed by the uptake of carbohydrate in the form of glycogen between May and June.

link with reproductive activity. L. uva brood their larvae and females are known to display declines in lipids and proteins which appear to coincide with gamete or larval release.

3.2.3 The Caecum.

Superficial analysis of the caeca was conducted in order to establish any relationship between the caeca within the shell and the adjacent outer mantle epithelium (Plate 5).

Owen and Williams (1969) in their paper, "The caecum of the articulate Brachiopoda" made some observations of the histochemistry and ultrastructure of the caeca of T. retusa. It would seem appropriate to include a brief summary of some of the conclusions derived from this study, with particular reference to the assimilation and storage of certain metabolites which may be closely associated with the gametogenic cycle.

The caeca are tubular evaginations of the outer mantle epithelium which penetrate the shell to connect the periostracum via a brush (Fig 1.9, 1.11). Functionally the caeca are; "indisputably concerned with the synthesis, storage and secretion of certain chemical components". However, it is debatable whether the secretory products move through the periostracum and or internally into the mantle. The morphology of the caecum and the fact that the distribution of the two main secretory products differs consistently within the structure suggests that two biochemical processes are operative concurrently. Protein occurs in the medial core cells but is more concentrated in the outer epithelial cells flanking the proximal part of the caecal lumen and especially in the distal peripheral flattened cells.

Owen and Williams (1969) drew attention to the presence of sloughed off secretory vesicle membranes which tend to be distributed towards the distal end of the caeca but noted that the differences in distribution may indicate that membranes are discharged into the lumen of the caeca and many disintegrate quickly. The lumen is chiefly involved in the circulation of materials taking part in the synthesis

and breakdown of glycoprotein and glycogen. Glycogen is also a common component which is stored without further synthesis.

Owen and Williams suggested that the main components of the caecum, the core cells, are concerned with the storage of glyco- and mucoprotein together with some protein, glycogen and a small amount of lipid. The distensible core cells act as storage centres by dilation of their freely hanging parts into the caecal lumen. The assumption was that most of the material released moves proximally into the lumen and ultimately enters the mantle.

Results of the present investigation appear to throw further light on the function of the caeca. The proximal end of the ceacum is closely married to the outer mantle epithelium, as described in the preceeding section. Although light micrographs show no clear evidence of a direct connection between the two, upon examining the evidence it seems likely, that they share an integrated purpose.

Ultrastructural investigation of the outer epithelium revealed these cells to be virtually devoid of the usual organelles which would normally be present. Such organelles are to be expected in cells whose contents are so obviously the products of secretion. The nature of these secretions is concomitant with those that are produced within the caeca which contains the infrastructure neccessary for both the synthesis and the breakdown of these compounds. Curry and Ansell (1986) estimate that the caeca could constitute upto 50% of the total living tissue mass of the animal. It might therefore be possible that the outer epithelium is inter alia a repository for storage compounds manufactured in the caeca and that the caeca also have the ability to remobilise these stored materials.

Thayer, (1986) presents physiological data supporting a respiratory role for the caeca. Thayer acknowledges however, that the caeca are not optimally "engineered", for this purpose and that it is probably not their sole function.

Although only a limited number of specimens were examined to detect any degree of seasonality within the contents of the caeca, it

coelomocytes in lingulids, which were later documented in detail by Yatsu (1902) and Ohuye (1937). Ohuye (1936,1937) described their occurrence in the articulates Terebratalia coresnica and Coptothyris grayi.

Morse (1902) and particularly Prenant (1928), gave diagrams of the coelomocytes of Terebratulina consisting of amoeboid cells and granulocytes, some of which are pigmented. A phagocytic form was also noted. The pigmented granulocytes contained red, orange or brown spherules.

Recently post-spawned females, and to a lesser extent males, have a distinctive band of red and brown droplets covering the distal end of the genital lamellae. These are assumed to correspond to those described by Hancock (1859) and Pren ant (1928) and may also be analogous to droplets noted in post spawned Neocrania by Joubin (1886). Many of these droplets give a strong positive response to lipid stains and some are clearly cellular. An ultrastructural view of these pigmented droplets is given in section 4.1.5, along with a more detailed description.

3.2.5 Metanephridia.

The metanephridia perform two roles. Primarily serving as excretory organs they also act as gonoducts during spawning. The nephridial opening within the coelomic cavity consists of a broad funnel-shaped nephrostome with a convoluted inner surface (Plate 7). Each nephrostome opens into the brachial cavity through a small nephridiopore (Plate 6) slightly ventral to the mouth. They extend continuing anteriorly as narrowing tubes, and are attached mediolaterally on the ventral side of the body cavity. The inner surface of the nephrostome is densely ciliated and interspersed with secretory cells. The ciliated cells cover a thin coelomic epithelium which in turn overlays a loosely fibrous and convoluted connective tissue.

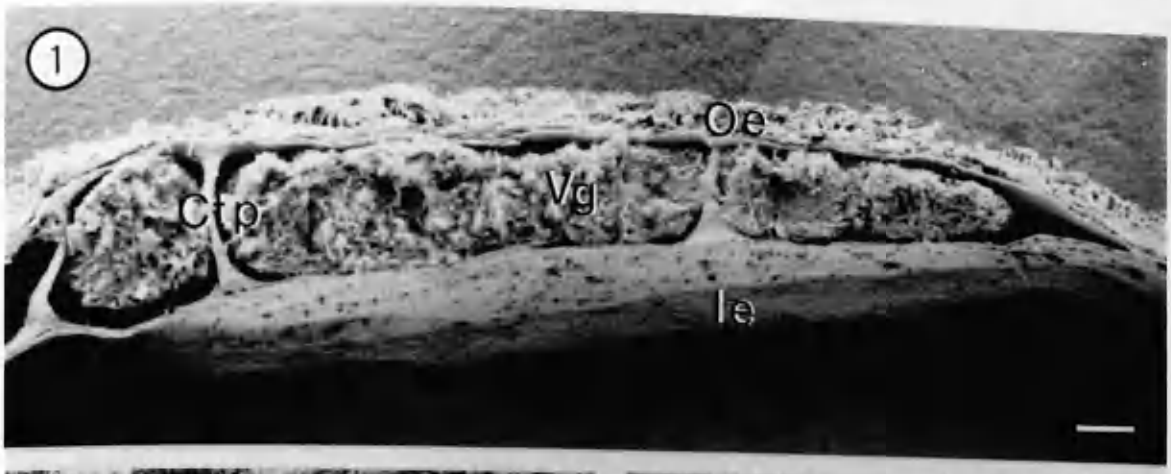
PLATES: 1–21

Plate 1. Scanning electron micrograph (SEM) of a vertical section through the the testes of a Terebratulina retusa. Scale bar = 200 μm

Plate 2. The reticulate genital lattice of the testis. The outer mantle membrane has been removed. Scale bar = 200 μm

Plate 3. The reticulate genital lattice of the ovary. The outer mantle membrane has been removed. Scale bar = 200 μm

Plate 4. SEM of vitellogenic oocytes attached to the margins of the genital lamella. Scale bar = 50 μm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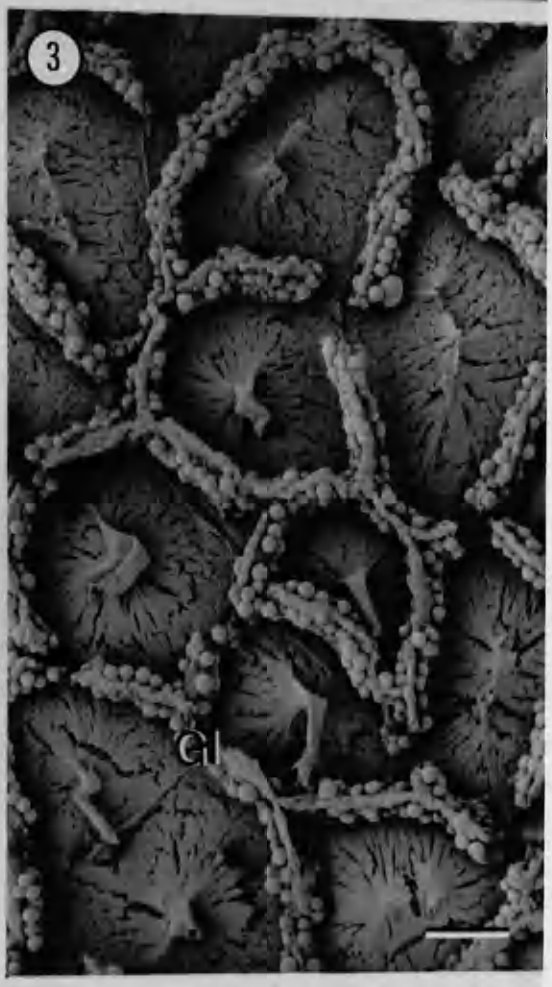
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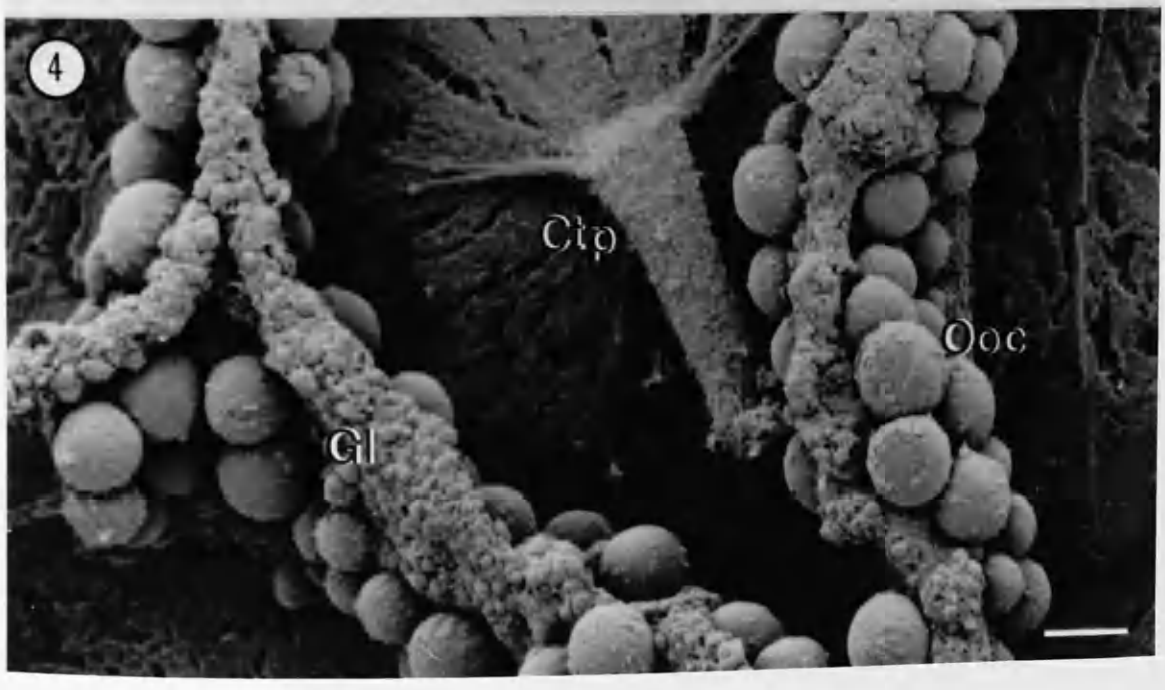
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Plate 5. Vertical section through a decalcified region of a valve, revealing the caeca. Scale bar = 20 μm

Plate 6. Surface view of the anterior part of the brachial cavity with the lophophore removed illustrating the position of the nepridiopores which are laterally displaced either side of the mouth. Scale bar = 500 μm

Plate 7. SEM showing the paired metanephridia with funnel-shaped nephrostomes. The convoluted surface of the nephrostome is densely ciliated. Scale bar = 100 μm


5
Ca

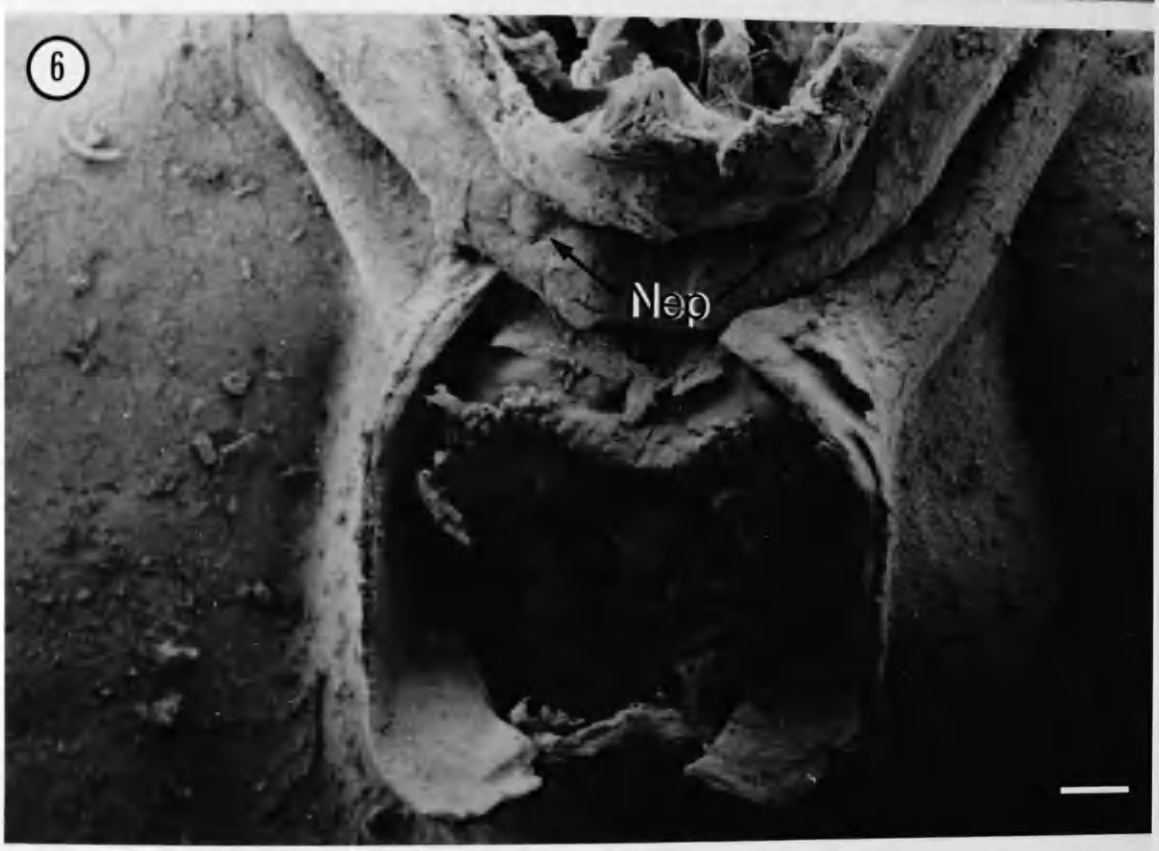
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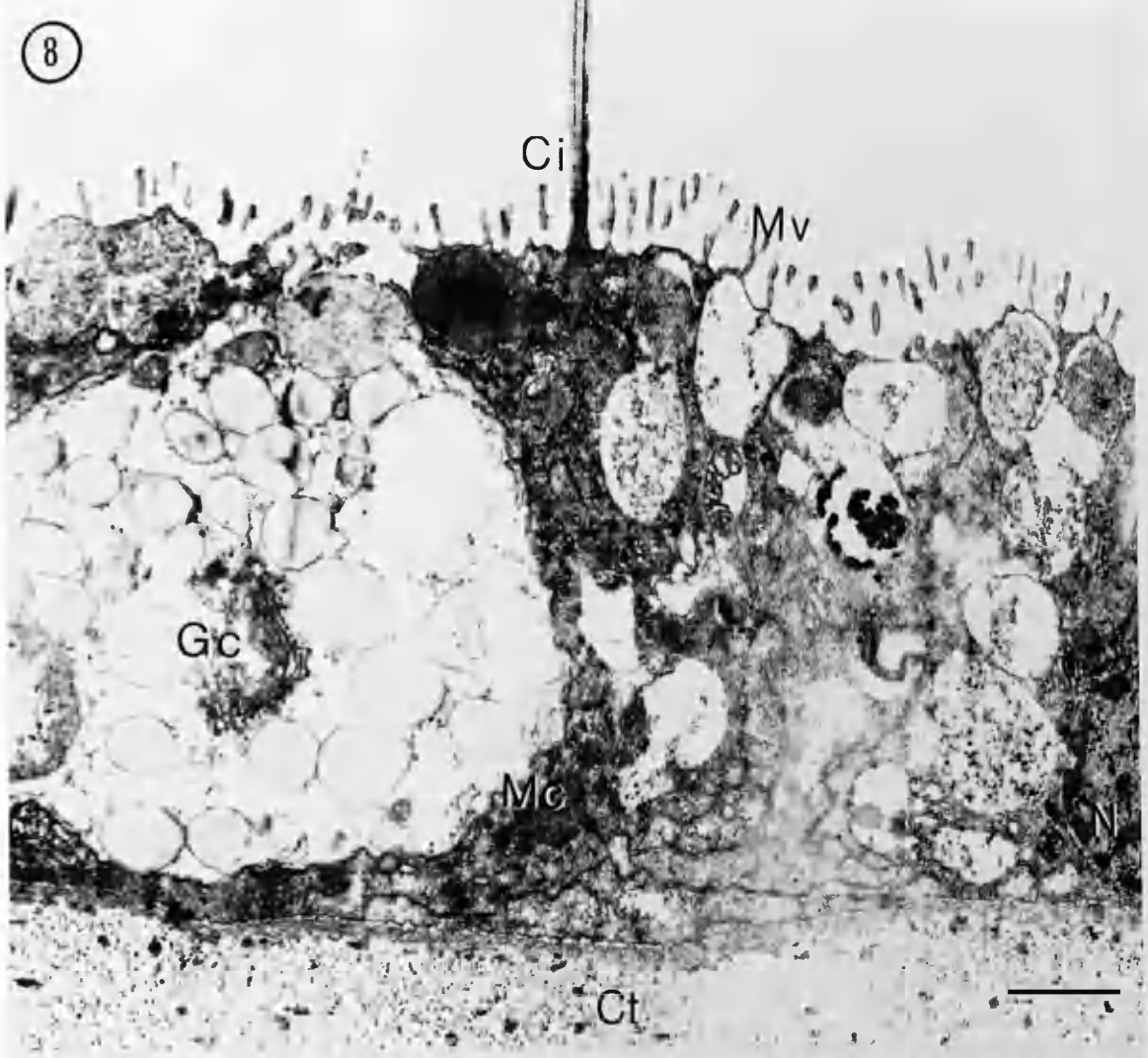
Plate 8. Transmission electron micrograph (TEM) of the proximal epithelium of the inner mantle. Flagellated columnar cells are often interspersed with mucus cells. Scale bar = 1 μm

Plate 9. The inner mantle epithelium comprises three layers. The proximal epithelium of the inner mantle (right), a layer of spiculated connective tissue (center) and the peritoneal cells (left). Scale bar = 1 μm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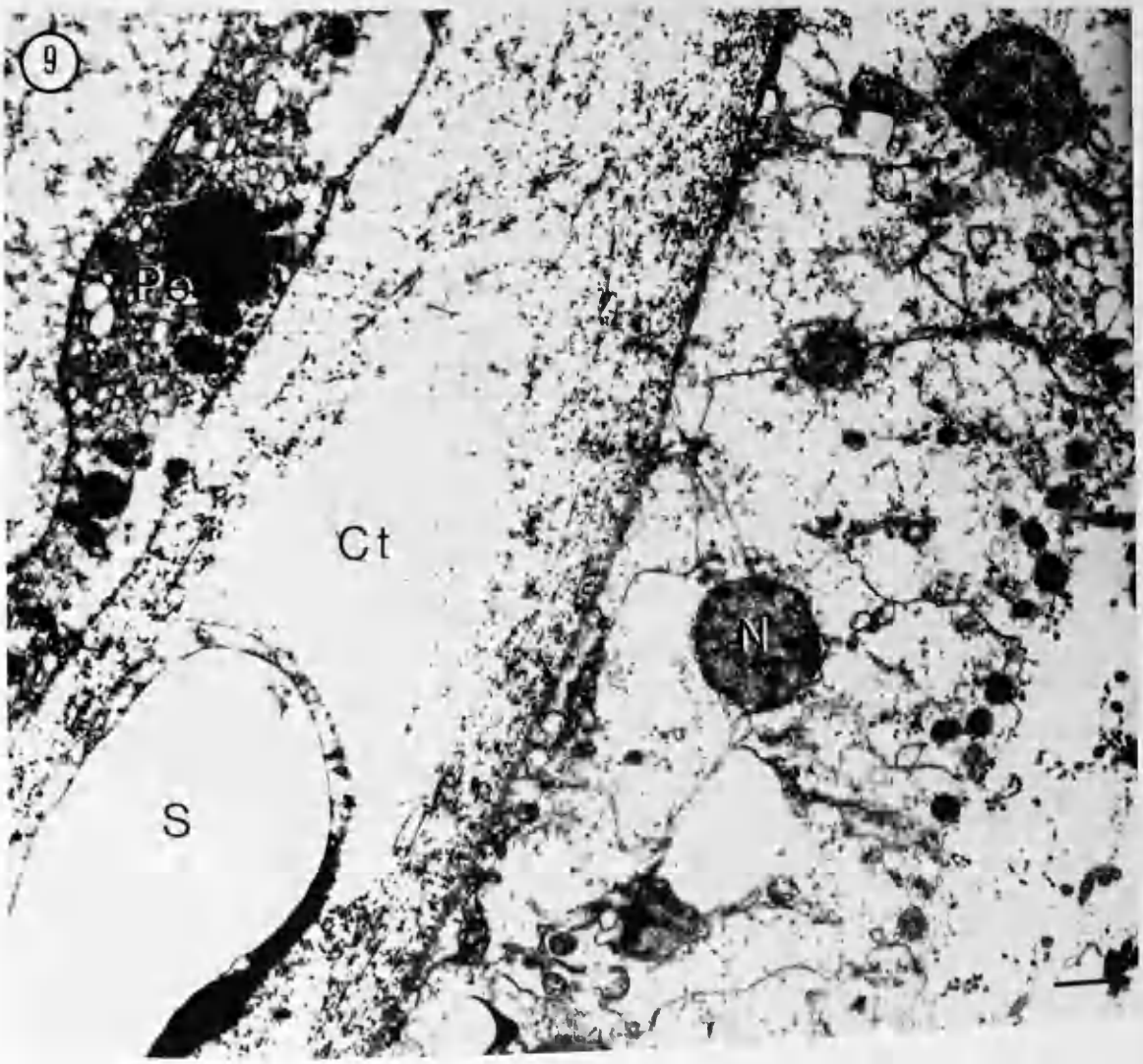
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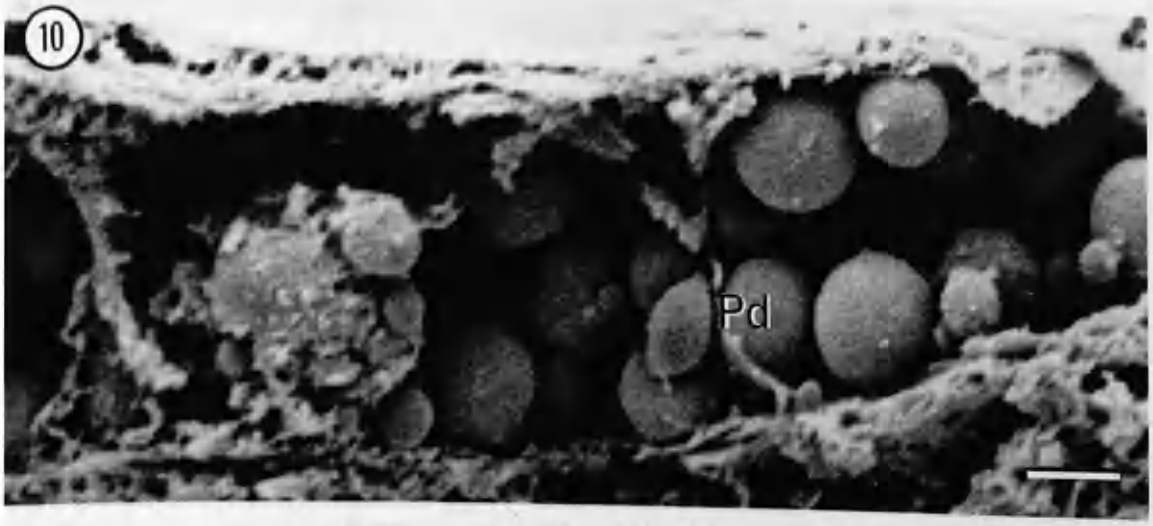
Plate 10. A vertical section of the proximal epithelium of the outer mantle revealing cells containing proteinacious droplets. Scale bar = 5 μm

Plate 11. TEM showing a vertical section through the outer mantle epithelium. Scale bar = 1 μm

Plates 12 - 13. Additional views of the outer mantle epithelium showing the variable consistency of the contents of this tissue at the ultrastructural level. Scale bars = 1 μm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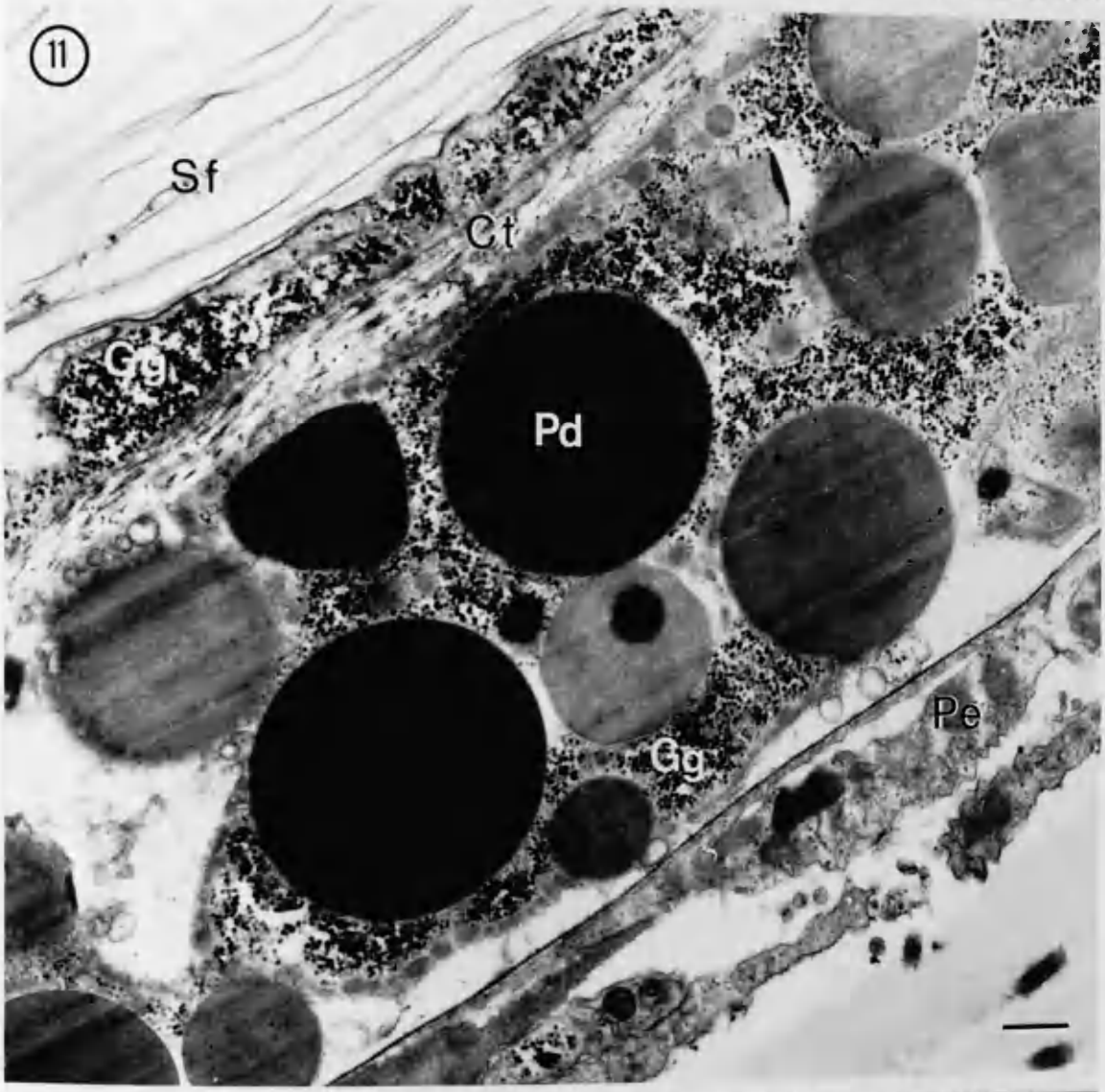
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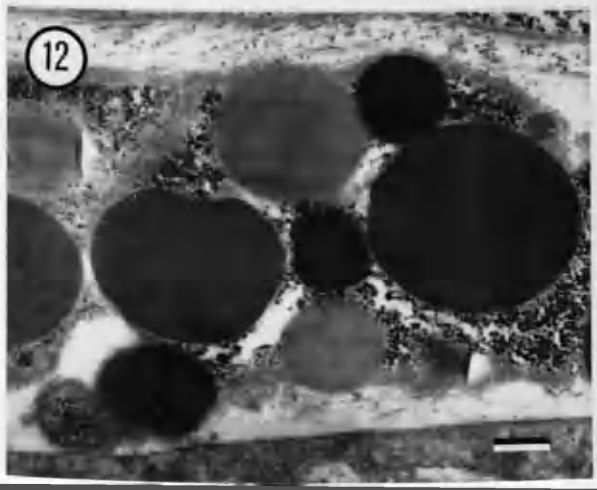
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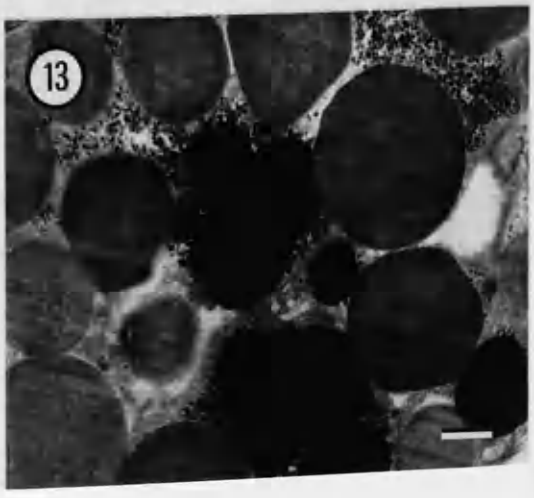
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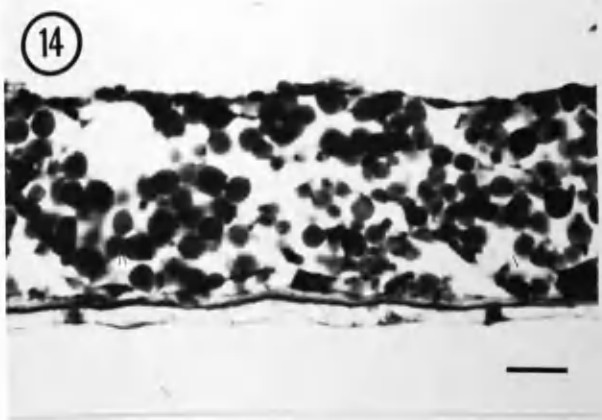
Plates 14 - 17. Light micrographs of vertical sections through the outer mantle epithelium. The contents and consistency of this tissue layer change seasonally from sparsely scattered protein droplets illustrated in 14 and 15 to the dense aggregations of glycoprotein shown in 16 and 17. Scale bars = 50 μm

Plates 18 - 19. Two vertical sections of testes taken from males from the Firth of Lorn revealing the difference in the outer mantle before (19) and after spawning (18). Scale bars = 250 μm

Plates 20 - 21. Two vertical sections of ovaries taken from females from Loch Fyne revealing the difference in the outer mantle before (21) and after spawning (20). Note the condition of the outer mantle of the post spawned male from the Firth of Lorn (18) as compared to its female counterpart from Loch Fyne (20). Scale bars = 250 μm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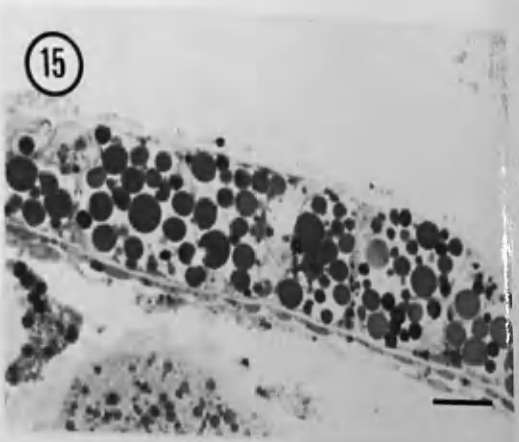
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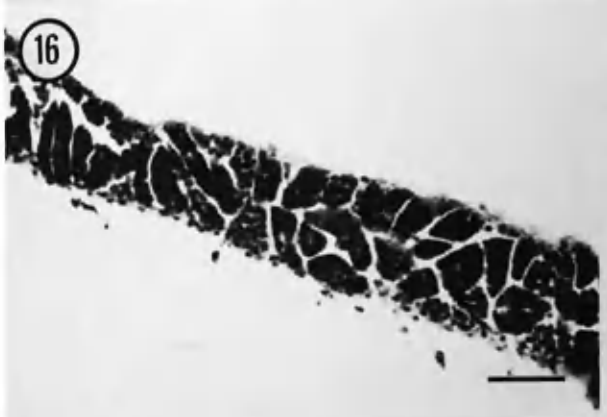
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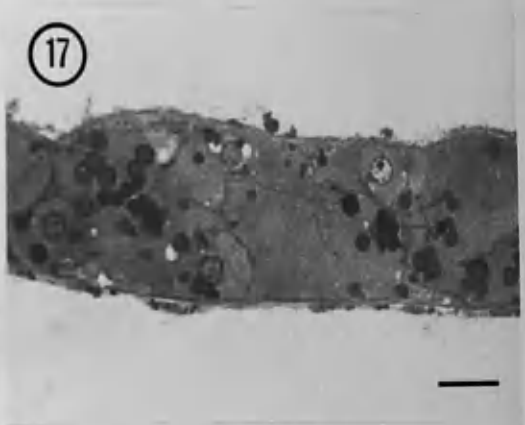
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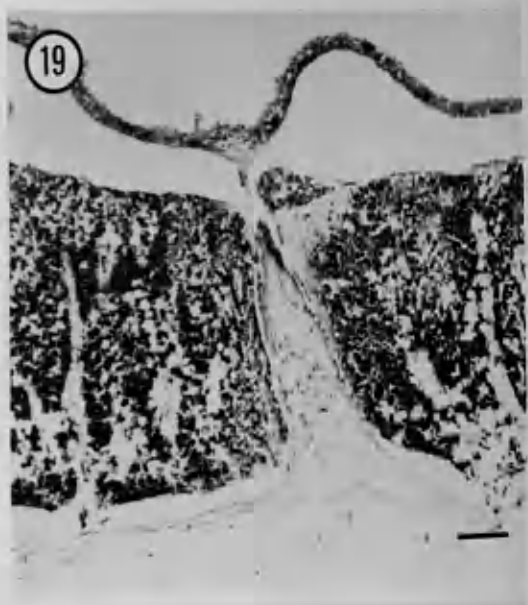
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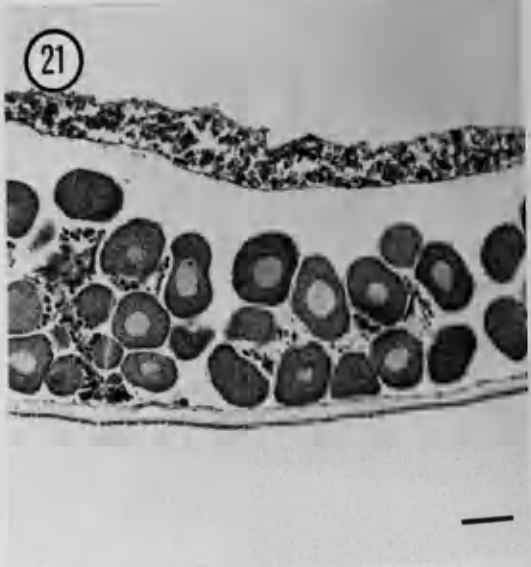
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CHAPTER 4.

GAMETOGENESIS.

CHAPTER 4.

4.0 INTRODUCTION.

An introduction to the study of gametogenesis in the Phylum Brachiopoda is necessarily brief, as information on many aspects is scarce. Few authors have contributed to this field. Most noteworthy is the review of Chuang (1983a) which draws upon the work of Senn (1934), Long (1964) and Sawada (1973) but is largely based on original ultrastructural data gathered by the author.

Inarticulate brachiopods examined by Chuang included; Glottida albida, Lingula anatina, Neocrania anomala, Crania sp.,and Discinisca sp., from Singapore; and the articulates Frenulina sanguinolenta, Neothyris lenticularis, Pumilus antiquatus, Tegulorhyncus nigricans, Terebratella sanguinea, and Waltonia inconspicua.

The first major work is that of Senn (1934), who concentrates on gametogenesis in Lingula and provides additional comparative information on Gryphus vitreus, Macandrevia cranium, and Terebratulina retusa.

Not until 1964 was the detail of Senns' work superceded by Long. Ostensibly a dissertation describing the embryology of Hemithiris psittacea, Terebratalia transversa, and Terebratulina unguicula, Long also describes oogenesis in Terebratalia transversa, splitting vitellogenesis into four arbitrary phases:-

1. Oocytes without yolk or cortical granules.
2. Oocytes with a few cortical granules.
3. Oocytes with dispersed cortical granules.
4. Mature oocytes with cortical granules at the periphery of the cell.

Chuang (1983a) distinguishes six stages in inarticulate oogenesis (Lingula anatina, Discinisca sp. and Crania sp.) and found a similar pattern in the articulate Frenulina sanguinolenta:-

1. Oocytes without lipid or cortical granule.
2. Oocytes with a few lipid droplets only.
3. Oocytes with a few lipid droplets and cortical granules.
4. Oocytes with several groups of lipid droplets, cortical granules and microsomes.
5. Oocytes with randomly distributed lipid droplets, cortical granules and microsomes.
6. Mature oocytes.

Other ultrastructural observations include those of Sawada (1973) working on Lingula unguis and a rather more specialised study conducted by Afzelius and Ferragutti (1978) who investigated the ultrastructure of the sperm of Neocrania anomala, and Terebratulina retusa, with the aim of achieving a better insight into phylogenetic relationships.

The spermatozoon of N. anomala has been sketched by Joubin (1886) and that of T. retusa was examined by Retzius (1904) and Senn (1934). Senn also depicted the spermatozoa from the genera Lingula, Argiope, Thecidium.

As no thorough synthesis of gametogenesis at the ultrastructural level exists, the purpose of the present work was to describe the ultrastructural alterations taking place in T. retusa.

Special emphasis has been placed on describing the cytological aspects of gametogenesis and interpreting these in terms of their functional significance.

4.1 OBSERVATIONS OF OOGENESIS.

4.1.1 Previtellogenesis.

Primary oogonia are located along the margins of the genital lamella proximal to the inner epithelium, usually in discrete clusters but occasionally in bands a few cells thick. They may be interspersed with lipid droplets. The germ cells are spherical or pyriform in shape

approximately 2.5 μm in maximum diameter (Plates 22, 23).

The nucleus and cytoplasm are relatively homogeneous in electron density and the organelles are indistinct. The cells are not in direct contact despite being closely associated with the connective tissue of the genital lamella. They are separated from it by the fine cytoplasmic processes of the peritoneal cells.

Morphological evidence suggests that primary oogonia divide to form secondary oogonia, which then enter the first meiotic division and become primary oocytes. Chromatin within the nucleus is notably amorphous and finely granular during the early stages of oogenesis.

4.1.2 Vitellogenesis.

The process of vitellogenesis is dynamic, but for the purposes of presentation and interpretation has been divided into six arbitrary stages. (Fig. 2.0.1-6).

(i) Stage I.

Primary oocytes are of the order of 3.0 μm in maximum diameter. The nucleus may contain regions of condensed chromatin, but is usually of a fine granular consistency and of similar electron density to the ooplasm (Plate 24).

Ooplasmic inclusions are sparse and poorly defined but clearly separated from nuclear material by a perinuclear space.

Peritoneal cells appear to be extremely pleomorphic, producing pseudopodial extensions which envelope the oocyte. These presumably modified peritoneal cells are effectively follicular cells, and form a "follicular capsule", within which the ovum grows and differentiates (Fig. 2.0.1).

(ii) Stage II.

The smallest recorded vitellogenic oocytes were in the order of

Fig. 2.0 STAGES I TO III OF VITELLOGENESIS. STAGES IV TO VI OF VITELLOGENESIS.

The whole or a portion of each Stage en enlarged to reveal prominent features of the ooplasm and det of the microvillar fringe.

Stages I to V are ato genital lamellae.

All Stages are envelfollicular cells.

Fig. 2.0.1 STAGE I

Fig. 2.0.2 STAGE II

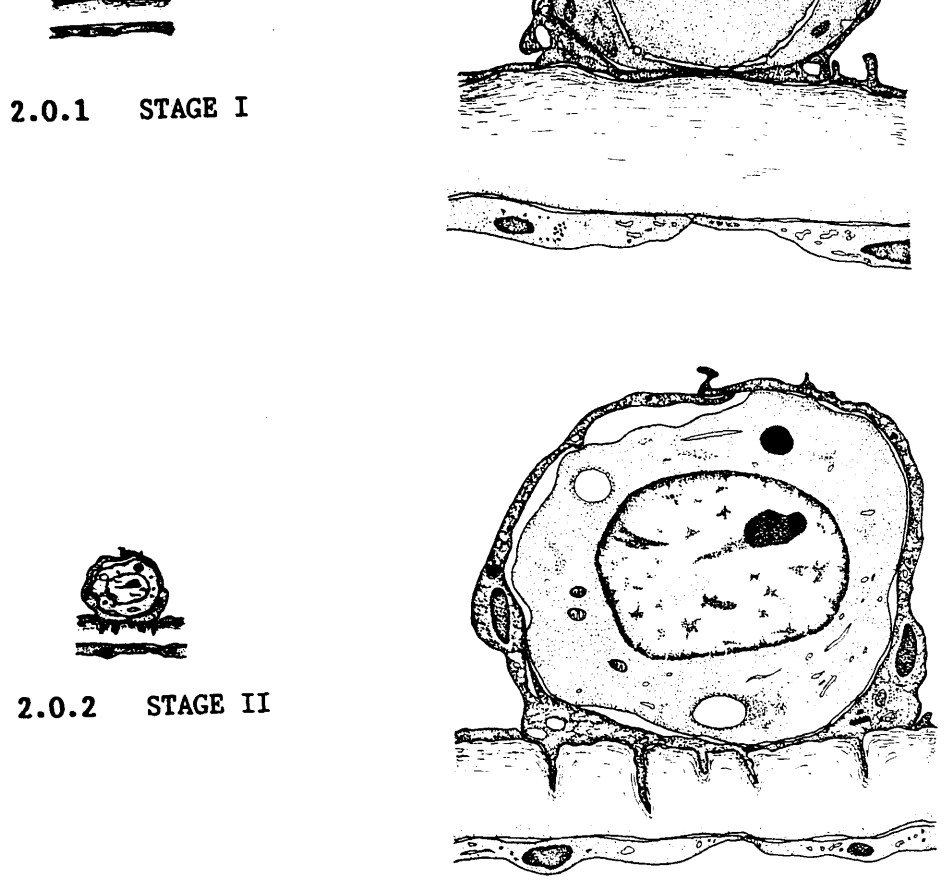
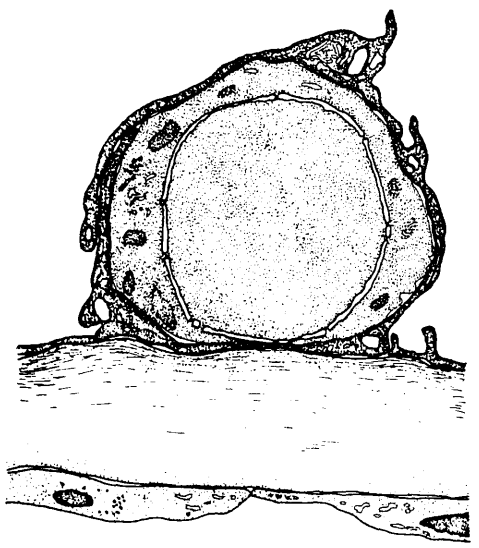


Fig. 2.0.3 STAGE III



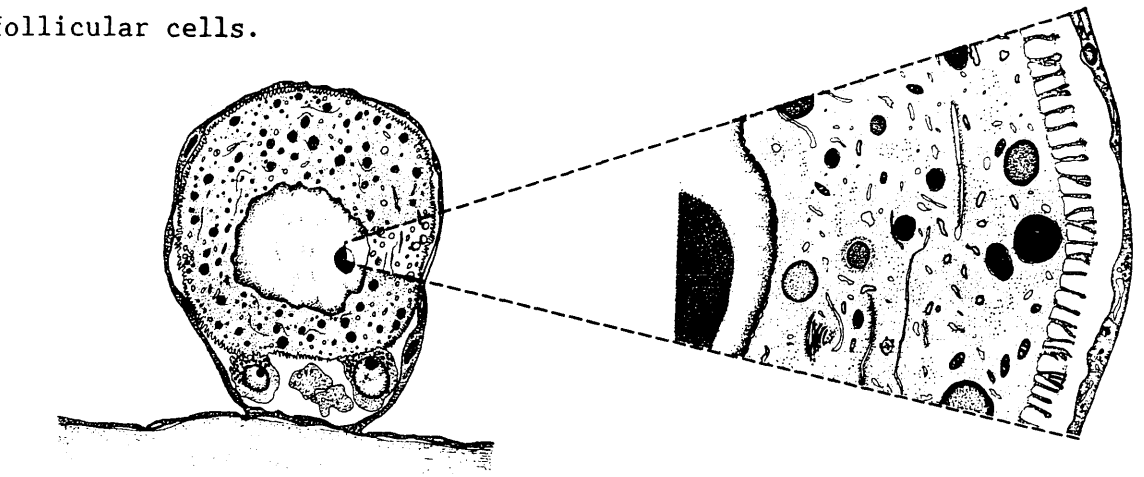


Fig. 2.0.4 STAGE IV

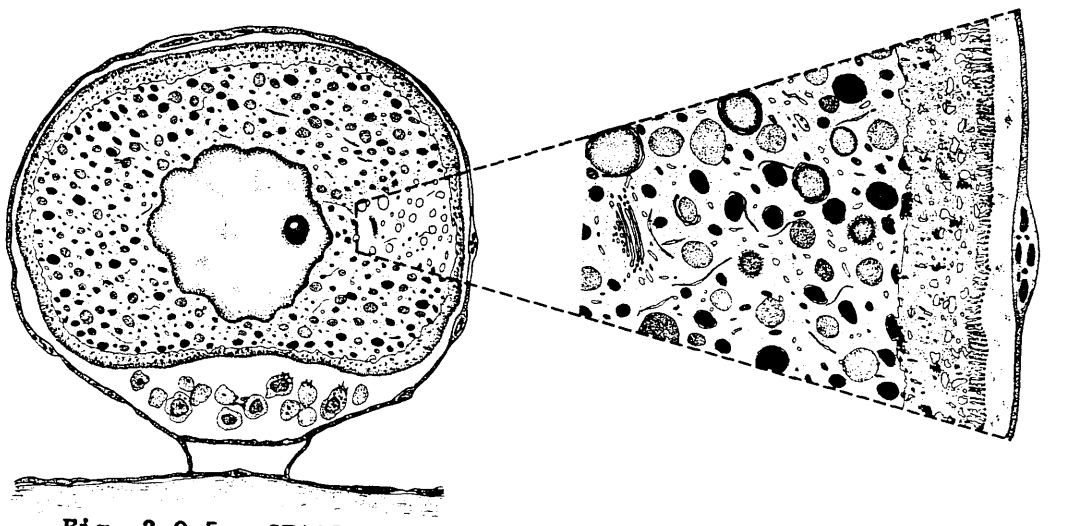


Fig. 2.0.5 STAGE V

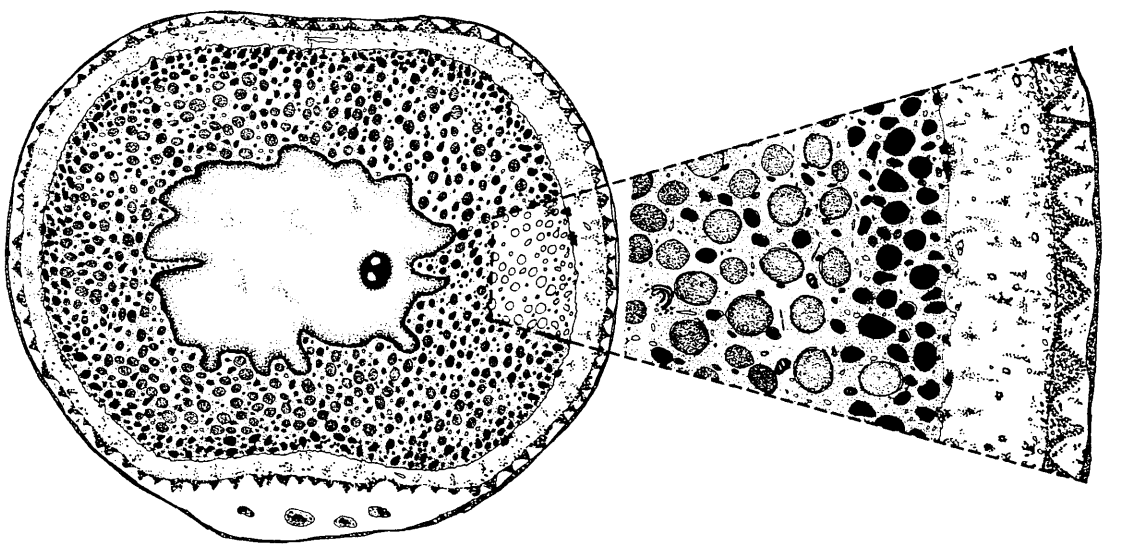


Fig. 2.0.6 STAGE VI

10um

5um

10um

5um

5 μm maximum diameter (Plates 25, 26. Fig. 2.0.2).

The oocyte within the follicular capsule is still indirectly attached to the genital lamella. Cytoplasmic processes or papilli, produced by the follicular cells in direct apposition to the genital lamella intrude into the connective tissue (Plate 25).

Perhaps the clearest indication of the initiation of vitellogenesis is the appearance of non-membrane-bound yolk granules which are composed of a fine homogeneous material which varies in electron density with size. These spherical inclusions are restricted in distribution to a few regions of ooplasm and are strongly positive to lipid-specific stain (Sudan black B).

Nuclei are about 2.5 μm in diameter, and the chromatin is condensed, particularly against the inner side of the nuclear membrane. The neucleoli are similarly condensed and also occur in close proximity to the nuclear membrane as are the nucleoli (Plates 27, 28). Nucleopores are well defined in section, and the annuli are particularly clear when the nuclear membrane is cut tangentionally (Plate 29).

At this stage of development the oolemma generally has less contact with the follicular cells, and it is more plastic and sometimes forms vacuoles (Plate 26). The ooplasm does, however, remain moderately electron dense and finely granular, containing a proliferation of more coarse granules which may be free ribosomes. This interpretation is supported by the strongly basophilic nature of the ooplasm of early vitellogenic oocytes.

Mitochondria and the occasional profiles of granular and agranular endoplamic reticulum occur throughout the ooplasm, but Golgi complexes are sparse at this stage of development.

(iii) Stage III.

Vitellogenic oocytes range in maximum dimension from approximately 7 μm to 13 μm, and are morphologically dissimilar to the preceding

stages in two important respects:- (1) the formation of microvilli and (2) the occurrence of accessory cells (Plate 30. Fig. 2.0.3).

As the oocyte increases in size, the nucleus occupies proportionally less of the total volume of the cell. Perinuclear ooplasm contains well defined groups of lipid granules and parallel layers of granular endoplasmic reticulum (Fig. 2.0.3). Mitochondria appear in a variety of forms; some are ovular or sausage-shaped in section while others are distinctly vermiform. The majority are, however, clustered into certain regions within the ooplasm, as are the other organelles. These may represent areas devoted to the generation of specific organelles. Agranular endoplasmic reticulum and free ribosomes are rather more ubiquitously distributed, and their profiles are present throughout the ooplasm (Plate 31). The oolemma is in places less closely affixed to the follicular cells, thereby creating a pericellular space, into which the oolemma is thrown into simple microvillar extensions (Plate 32).

Within a pericellular space created between the follicular cells proximal to the genital lamella and the coresponding portion of the oolemma, a cell resembling a small stage II oocyte may be seen to develop (Plates 30, 33). This addition to the ovular capsule is considered to act as an accessory cell and plays an important role in the future development of the oocyte. The origin of accessory cells was not determined but evidence provided by light micrographs suggests that the earliest recorded accessory cells are the product of an earlier unequal mitotic division.

The first information regarding the ultrastructure of early accessory cell development was derived from cells in excess of 2 μm maximum diameter. Accessory cells of this dimension have no direct cytoplasmic connection with the vitellogenic oocyte although the plasmalemma and the oolemma are closely apposed and joined by numerous desmosome-like gap junctions, thus maintaining some degree of cytoplasmic continuity (Plates 35-37).

The cytoplasm of these nucleated cells includes a few lipid granules of various sizes, as well as membrane-bound granules of fine homogeneous electron opacity which were determined to be proteinaceous

in character (Plates 33, 35). Prominant membrane bound vacuoles are common and contain a sparsely granular condensate which appears to be incorporated in numerous small vesicles (Plate 34).

Individual follicular cells are clearly discernable; the cytoplasm swelling around the elongated nucleus and incorporating mitochondria and profiles of both granular and agranular endoplasmic reticulum.

(iv) Stage IV.

A cell at this stage of development will typically exceed 15 μm diameter, and is becoming increasingly distal to the inner mantle epithelium although still attached to the genital lamella (Plate 41. Figs. 2.0.4, 2.1.1).

The ooplasm contains a variety of well distributed inclusions (Plates 44, 45) including lipid droplets with a fine, homogeneous, and evenly electron dense consistency as well as strongly electron-opaque, membrane-bound, proteinaceous granules (Plate 46). The latter appearing to fuse with some of the vesicles, which are very common in the ooplasm (Plate 47).

Precise discrimination of the type of endoplasmic reticulum present is often difficult, but cisternae are closely associated with the yolk granules, as is a finely granular material which may be glycogen. Profiles of agranular endoplasmic reticulum appear to envelope lipid yolk granules, the association becoming more intimate as the granule increases in size (Plates 48, 49).

Golgi complexes are also more numerous than in previous stages. These are formed from a characteristic assemblage of flat saccules or cisternae piled one upon the other in close parallel array, and appear to produce myriad small vesicles (Plate 50). Agranular endoplasmic reticulum is also a common associate of some Golgi complexes (Plate 51).

Simple microvilli cover the surface of the stage IV oocyte (Plate 40). Some exhibit a degree of elaboration, for example the microvilli

are larger and branched (Plate 38), while in more advanced oocytes the microvilli become thicker and the tips appear to "bud" off as membrane-bound microdroplets which are separated from the parent microvillus by a fine fibro-granular layer (Plate 39).

A group of variably-sized accessory cells, some exceeding 10 μm, now occupy the pericellular space between the oolemma and that part of the follicular envelope attached to the genital lammella. The extra volume of these accessory cells is accommodated by the formation of a slight concavity at the base of the vitellogenic oocyte and by distension of the follicular envelope (Plates 40-43).

The perinuclear cytoplasm and its inclusions are unevenly distributed, particularly in the more developed accessory cells and often displays a bias towards the oolemma (Plate 35).

Both lipid granules and proteinaceous membrane-bound granules occur within the cytoplasm. Although rather smaller than the granules found within the adjacent ooplasm, the accessory cell granules are morphologically similar in all other respects to those of the vitellogenic oocyte.

Clusters of mitochondria are important features at this stage of development (Plates 36, 37) and commonly appear in peripheral cytoplasm next to regions of plasmalemma which extend to form delicate pseudopodial processes. The latter structures interdigitate with the oolemma and the simple microvilli of early oocyte differentiation (Plates 35-37). Many desmosome-like gap junctions form whereever the cell membranes are in close proximity (Plate 37).

A single layer of follicular cells still maintains a perfect follicular capsule. The cytoplasmic extensions of the follicular cells which form the envelope may be less than 0.1 μm in section, but swell to incorporate the nucleus and other organelles, particularly mitochondria, some Golgi complexes, and the occasional lipid droplets (Plate 52).

(v) Stage V.

In oocytes with diameters in excess of 30 μm, vitellogenesis takes place within a follicular capsule which remains attached to the genital lamella (Fig. 2.0.5). As recorded in preceeding stages, the follicular capsule occurs at an increasingly distal position to the inner epithelium (Fig. 2.1.2).

The progression of the vitellogenic oocyte distally while apparently remaining fixed to the genital lamella suggests that the genital lamella is extending into the lumen of the vascula genitalia (Figs. 2.1.1-3).

This stage of development exhibits not only an increase in size and accumulation of yolk, but also a marked increase in the number of accessory cells and an escalation of the formation of microvilli separated from the main body of ooplasm (Plate 57). The nuclear membrane becomes slightly plicate (Plate 53), although the contents of the nucleus are apparently similar to those described in earlier stages of development.

Lipid and protein yolk granules are evenly disposed throughout the ooplasm. Spherical lipid granules of 1.5 to 2.0 μm diameter are common, and are often associated with mitochondria measuring 1.0 μm along the medial axis. A range of sizes and staining propensities exist within the ranks of both types of yolk granule, perhaps indicating a maturation process. The largest proteinaceous granules are strongly eosinophilic and most prevalent immediately beneath the oolemma, where some are pear shaped or subspherical and about 1.5 μm across the major axis.

Profiles of agranular endoplasmic reticulum and mitochondria are common and may be seen in association with both types of yolk granule. Occasionally microsomes or vacuoles containing vesicles occur, the contents of which vary in consistency and density. The interstitial ooplasm is still made up with what are thought to be free ribosomes; though the major component is finely granular or floccular and moderately electron dense (Plate 54).

Fig. 2.1 Series of diagrams illustrating the development of the Genital Lamella and associated Oocytes.

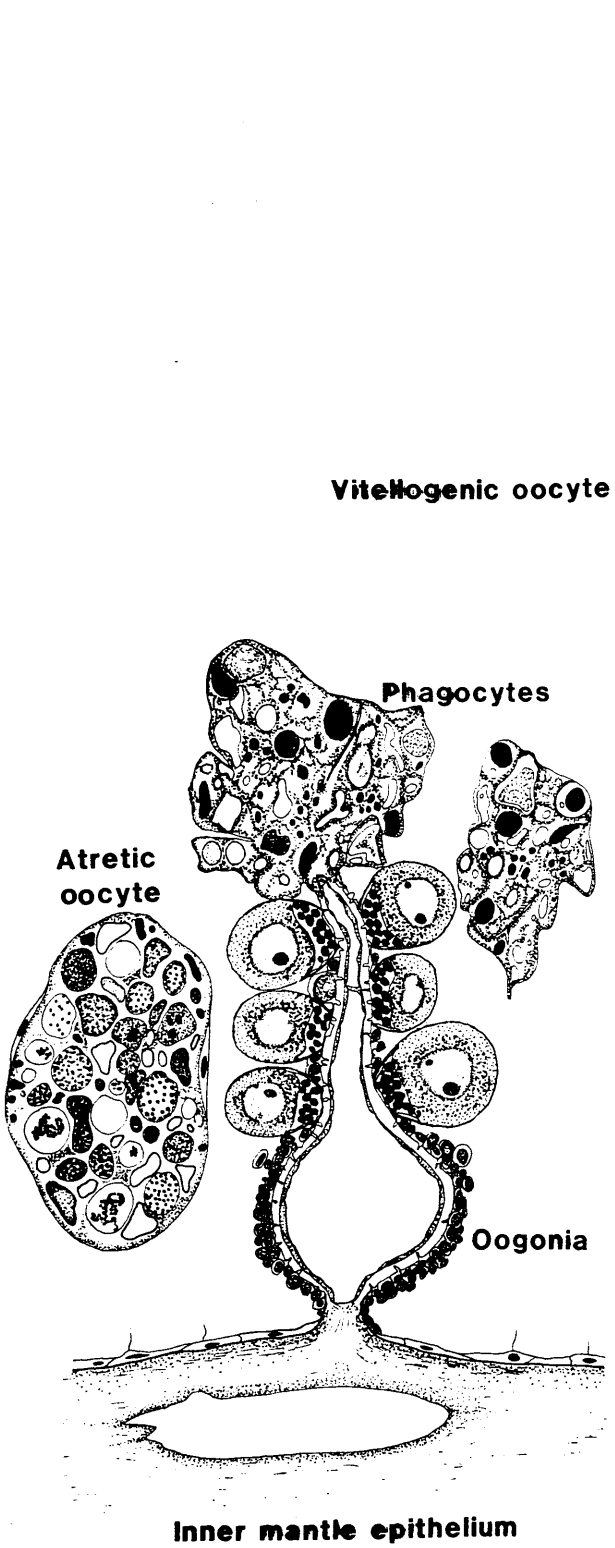


Fig. 2.1.1 POST-SPAWN

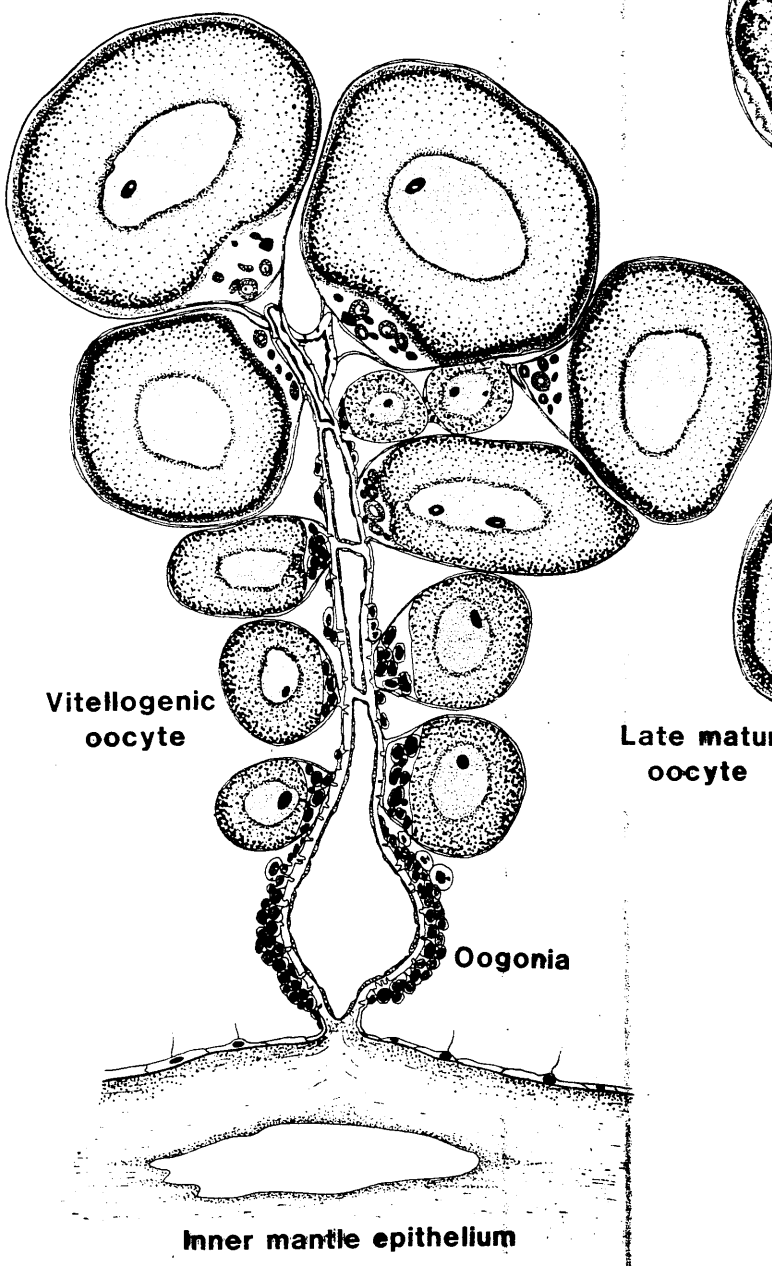


Fig. 2.1.2 DEVELOPING

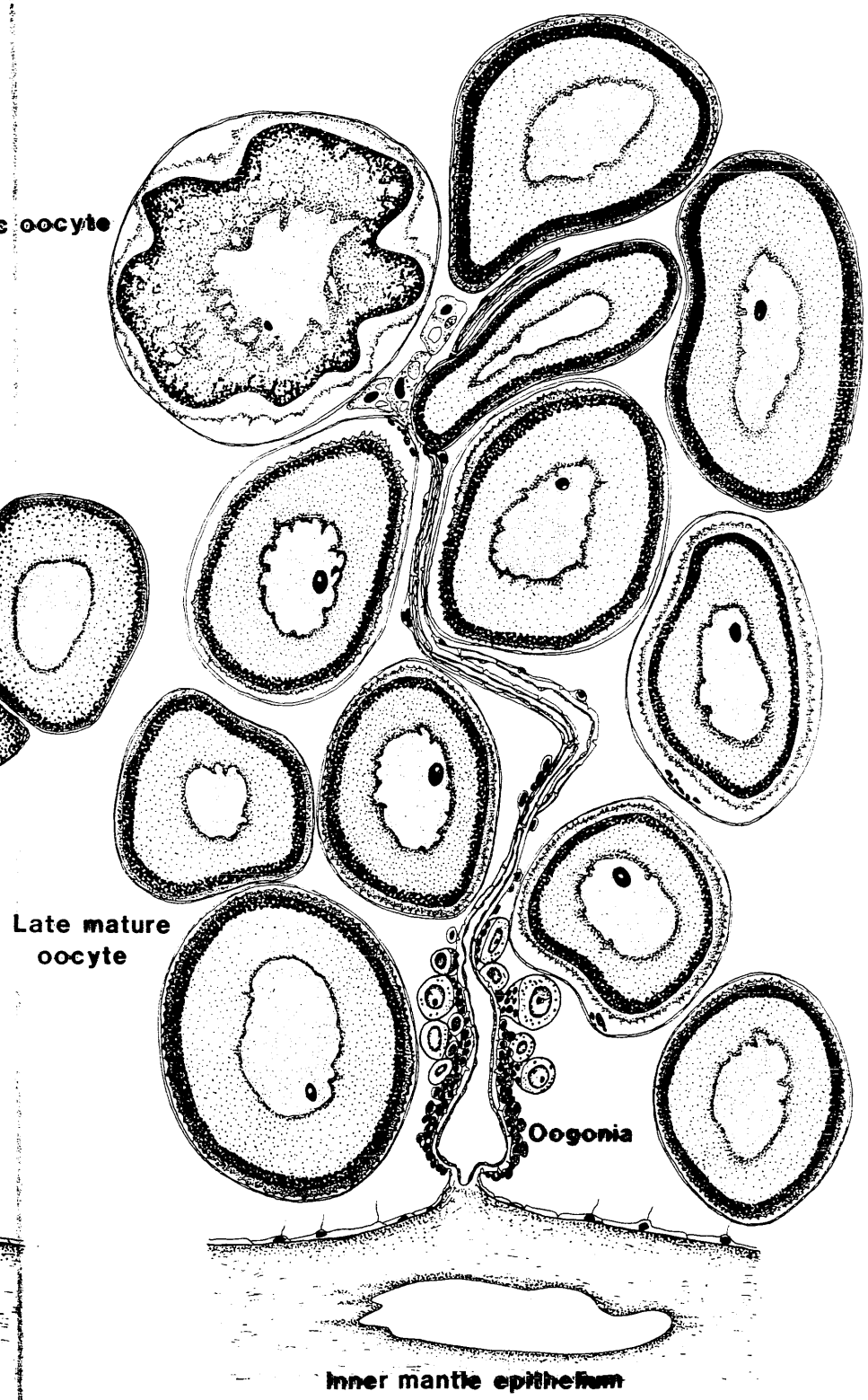


Fig. 2.1.3 PRE-SPAWN

This stage of development seems to indicate a distinct elaboration of the oolemma and microvilli. The simple digitate microvilli described so far have increased in number and form a palisade within which the individual elements of the microvilli are fused with a delicate band of fine granules to form a pseudolemma. This is effectively remote from the oolemma, separated by a peri-vitelline space. This zone containing three discernable fractions:- (1) vesicles, (2) finely granular material, and, (3) strongly electron dense coarse fibro-granular aggregations.

The vesicles are generated at the base of the microvilli (Plate 57), and are more or less electron-translucent with some containing sparse condensations of fine granules. The finely granular material between the vacuoles is quite uniformly distributed throughout the sub microvillar zone and closely resembles the material within the ooplasm.

More coarse fibro-granules, visible as electron dense aggregations would appear to have condensed next to the oolemma and have been incorporated into the ooplasm by endocytosis (Plates 57, 58). This material is enveloped by the oolemma, but the fate of the resultant vacuole or the nature of its contents was not determined. Sparse aggregations of this coarser fibro-granule have also been recorded in the coelom, in the periovular space, and also between the pseudolemma and the follicular envelope.

Accessory cells may number 15 to 20 around vitellogenic oocytes at this stage. Not all are in direct contact with the oocyte or other accessory cells. The junctions between accessory cells and oocytes are typified by those described in stage IV oocytes; however, a number of intra-accessory cell junctions were observed. In their simplest form, closely abuted cells are connected by the familiar desmosome-like gap junctions. Communication may be elaborated by the production of pseudopodial extensions which originate from one or both accessory cells which are also united with desmosomal junctions (Plate 55). Some cells have complete cytoplasmic continuity with neighbouring cells (Plate 56). Whether the latter are the result of the direct union of two mature cells or the final stages of a somatic division is unknown.

Morphologically, the accessory cells display a great deal of

plasticity, but it is of note that accessory cells do not develop microvilli.

(vi) Stage VI.

This stage of vitellogenesis marks the release of the oocyte from the genital lamella into the lumen of the vascula genitalia, prior to final yolk accumulation and ultimately spawning.

The process is initiated by the separation of the entire ovular capsule containing both oocyte and a few residual accessory cells, from the most distal regions of the genital lamella (Plates 59, 60). The smallest "free" oocytes recorded were about 35 μm maximum diameter, (Figs. 2.0.6, 2.1.3). An oocyte of this order of magnitude might be expected to accommodate a nucleus of approximately 20 μm maximum diameter, with an intensely plicate nuclear envelope (Plate 60).

Yolk granules abound throughout the ooplasm. Many of the largest or more mature membrane-bound proteinaceous droplets have accumulated at the periphery of the oocyte forming a distinctive cortex. Some of these cortical granules are pear shaped or spheroidal in section (Plate 61, 62).

Less electron dense lipid granules constitute by far the greatest proportion of the yolk. Almost all lipid granules of this stage are bounded by a single profile of endoplasmic reticulum (Plate 54). Each profile is about 0.04 μm across the cisterna, is clearly not attached to the surface of the granule although it occurs in close proximity to it. The perinucular space is approximately 0.02 μm maximum diameter. Superficially the cisternae could be described as granular but in fact the ooplasm still contains many free ribosomes some of which may lie in the space between the reticulum and the lipid granule, and it is these which give the endoplasmic reticulum the appearance of being granular.

Mitochondria measuring 0.6 μm across the longest axis are ubiquitously distributed, especially close to lipid granules. Golgi complexes and microsomes are numerous, and are interspersed with the ever present fine granular material (Plate 63). The process of endocytosis also continues unabated and may be more prevalent.

The perivitelline space is more pronuonced, resulting in a clear division between the oolemma and the pseudolemmal fringe of microvilli which have undergone more elaboration.

Initially, the fine granular matrix binding the microvilli is seen to separate into two components:- (1) an outer microvillar layer containing the membrane bound microdroplets, first noted at the tips of the earlier microvilli, and (2) an amorphous basement layer (Plates 61, 77). Portions of the outermost layer have an undulate form, and the spaces between the latter and the basement layer are filled with the membrane-bound vesicles described in stage V oocytes (Plate 76). Vesicles and granules of a fine condensate also occur in the perivitelline space, but the vesicles are less frequent.

Continuing this development, the amplitude of the undulation of the outermost microvillous layer increases to form a series of crests and troughs. The crests opposite the follicular envelope and the troughs remain attached to the basement layer. Granules and some vesicles traverse the basement layer (Plates 61, 77).

The coarse fibro-granules, condensed and endocytosed at the oolemma are also present within the perivitelline, peripseudolemmal and interstitial spaces between the cells. Concentrations of this substance are higher in the regions between the pseudolemmal-microvillar and basement layers.

Accessory cells are generally smaller and fewer in number than those recorded during earlier phases of vitellogenesis. Most residual accessory cells are devoid of yolk granules. Intra-accessory cell unions and accessory cell-oocyte communications are less common.

The margin of the oocyte is indented to accomodate the proliferation of accessory cells within the follicular envelope, and remains so, as the accessory cells are reduced in volume and number.

Oocytes achieving this state of development continue to accumulate yolk, both lipid and proteinaceous cortical granules. There appears to be no other morphologically identifiable stage; as development proceeds the oocyte merely increases in size until spawned.

The follicular envelope, still a single cell layer in thickness remains intact during the entire course of vitellogenesis and is only lost immediately prior to spawning.

4.1.3 Spawning.

Oocytes reaching the stage VI state of development increase in volume, eventually filling the vascula genitalia (Plates 64, 65, 67). Mature oocytes, or more correctly, eggs, are approximately 130 μm maximum diameter at the time of spawning, but their precise geometry is the dictate of the packing arrangement within the gonad, and it is only upon spawning that the eggs assume a more spherical profile (Plate 68).

Before the eggs are released into the brachial cavity via the nephridia, the follicular envelope is shed. Scanning electron microscopy indicates that the follicular cells retreat from the pole of the egg diametrically opposite to the slightly invaginated surface which accommodated the accessory cells (Plates 68-73).

Initially the egg bulges through the aperture created by the retreating follicular envelope (Plate 69). This process continues (Plates 70-72), revealing the reticulate surface texture of the undulating microvillar layer that has developed beneath the follicular cells (Plate 73-75). The envelope is drawn around the circumference of the egg at an even rate, so that its aperture is circular. The follicular material behind the retreating margin is globular in appearance.

Finally the remaining fragments of the follicular envelope are shed, and presumably are deposited in the vascula genitalia. The 'naked' ova pass (Plate 66), through the nephridia, probably aided by ciliary currents generated within the vascula genitalia and the nephridia. Once in the brachial cavity, the eggs are taken up in the exhalent stream from the lophophore and ejected to the exterior.

The eggs are more dense than sea water and their outer surface may be slightly agglutinous. In the static conditons of a laboratory tank

eggs are deposited in a well defined crescent close to the commissure of the parent, where fertilisation takes place.

4.1.4 Atretia of Oocytes.

Mature ova which are not spawned become atretic and are resorbed in the ovary. The dynamics of degradation were observed in a number of post-spawned females.

While still within the confines of the follicular capsule, the mature ovum is irregular in shape. The highly convoluted nuclear envelope becomes indistinct and presumably breaks down. Necrosis proceeds, producing an uneven distribution of ooplasm with substantial regions devoid of inclusions. Finally the ovum fragments into a number of anucleate spherical or subspherical vesicles which seem to contain unaltered, but condensed, fragments of ooplasm (Plates 79-81. Figs. 2.1.1, 2.1.3). It is likely that the follicular envelope degrades and the contents of the ovular capsule are phagocytosed.

4.1.5 Phagocytosis.

Examination of recently post-spawned animals reveals the presence of a host of globules and granules which appear as red to orange droplets when viewed in fresh tissue preparations.

The pigmented droplets are most obvious in post-spawned females, and are most readily observed by stripping the mantle tissue, including the gonads, from freshly sacrificed specimens. The pigmented areas are concentrated at the distal end of the genital lamella and can be traced throughout its reticulation (Plate 78. Fig. 2.1.1).

The ultrastructure of these pigmented droplets is a complex and extremely active matrix of phagocytic amoebocytes. Phagocytes are extremely pleomorphic, but two morphologically distinct types were observed. Some are flattened, forming long psuedopodial extensions of the plasmalemma, which bulge to accomodate the spherical to pyriform nucleus which measures 3 μm to 4 μm maximum diameter (Plate 84). Other

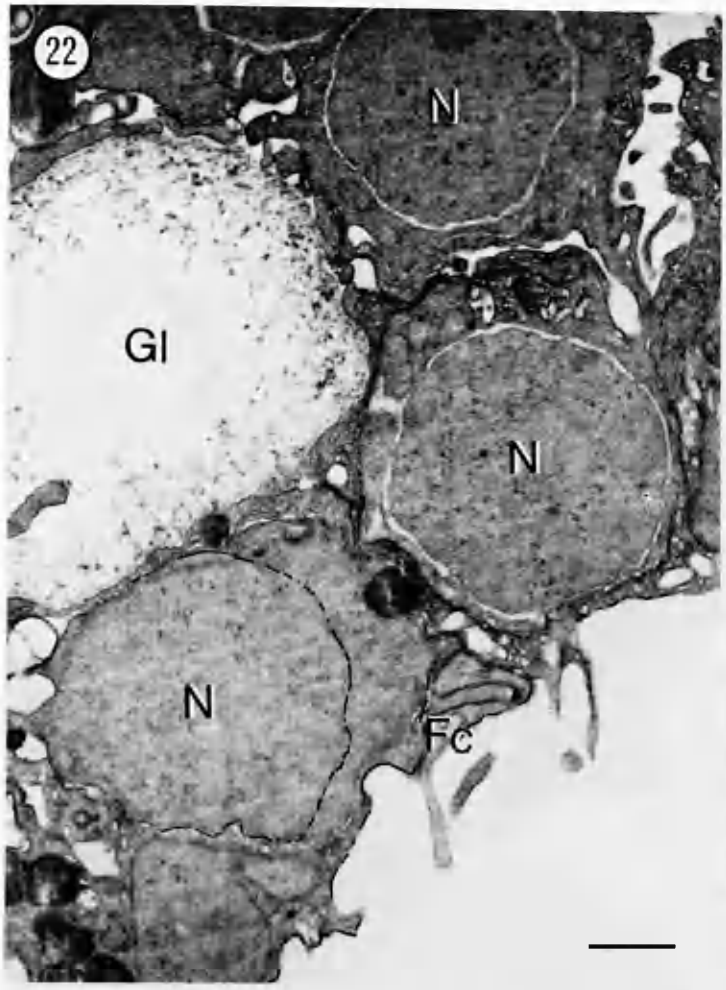
amoebocytes tend to be slightly shorter and more rotund in profile with a nucleus measuring 2 μm to 3 μm along the major axis (Plate 85).

The cytoplasm of the pigmented droplets contains a variety of well defined inclusions (Plates 82-85). Clusters of mitochondria (Plate 83), often enmeshed with profiles of granular or agranular endoplasmic reticulum, are interspersed with vacuoles and lipid granules. The latter appear similar to the lipid granules of the vitellogenic oocyte. Agranular endoplasmic reticula are distended and the cisternae are charged with fine granules which impart a greater degree of electron density than that of the surrounding cytoplasm. Golgi complexes are uncommon. Vacuoles of various sizes are profuse, and some are clearly lysosomal in function (Plate 82). Fine granular matrices as well as lipid and proteinaceous granules are abundant within the cytoplasm and are enveloped by the pseudopodial processes of the phagocytes. These matrices and granules are either incorporated into the cytoplasm intact or are apparently in stages of lysis (Plates 84, 85).

PLATES: 22–85

Plates 22 - 23. Clusters of oogonia attached to the genital lammella and enveloped by the fine cytoplasmic processes of peritoneal cells which become the follicular cells. Scale bars = 1 μm

Plate 24. The primary oocyte is surrounded by cytoplasmic extensions of peritoneal cells which form a follicular envelope or capsule within which the oocyte develops. Scale bar = 0.5 μm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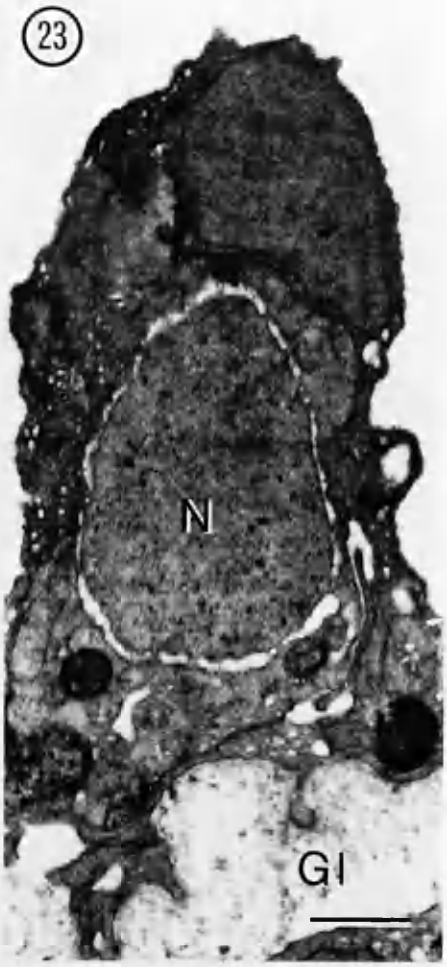
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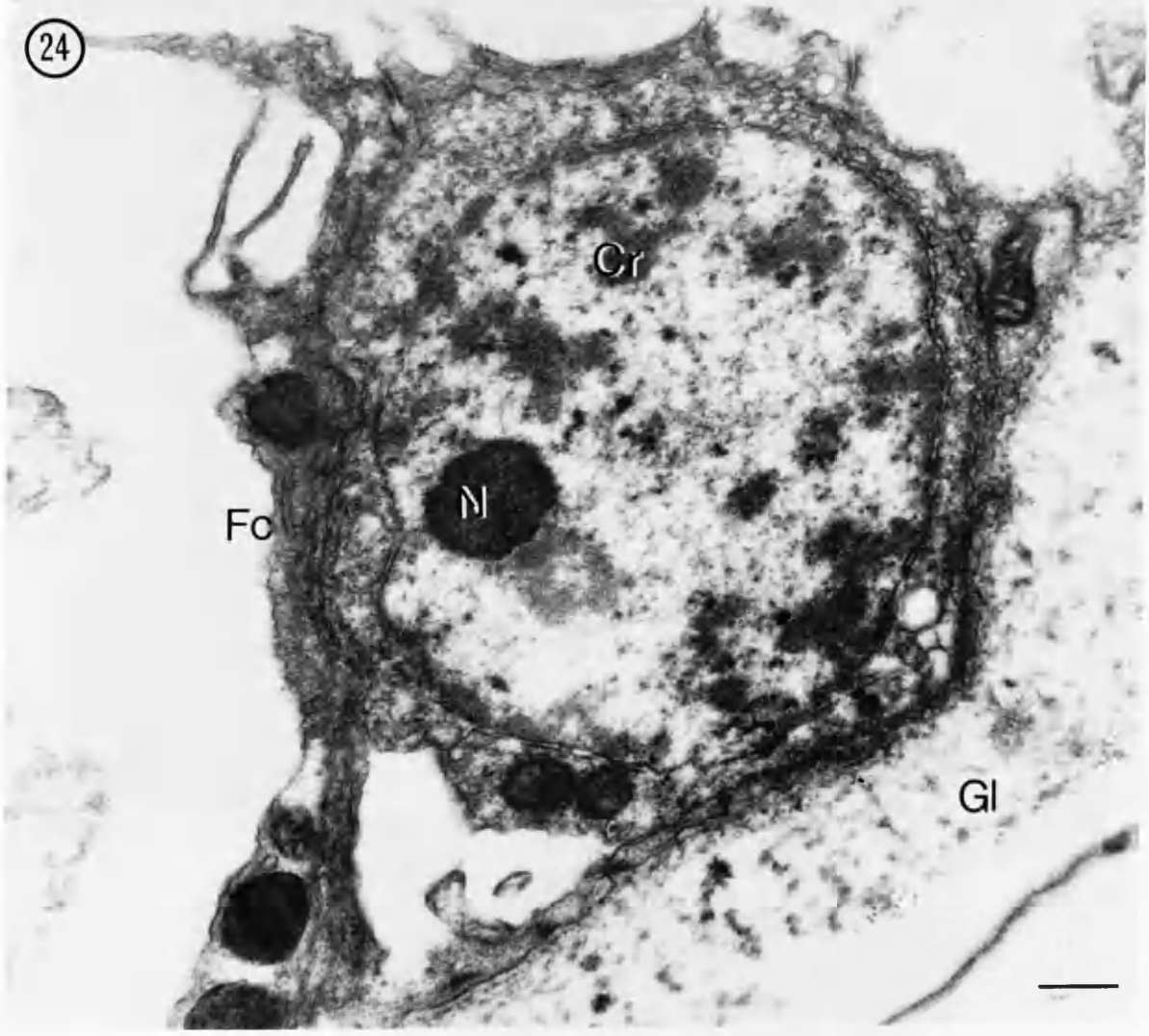
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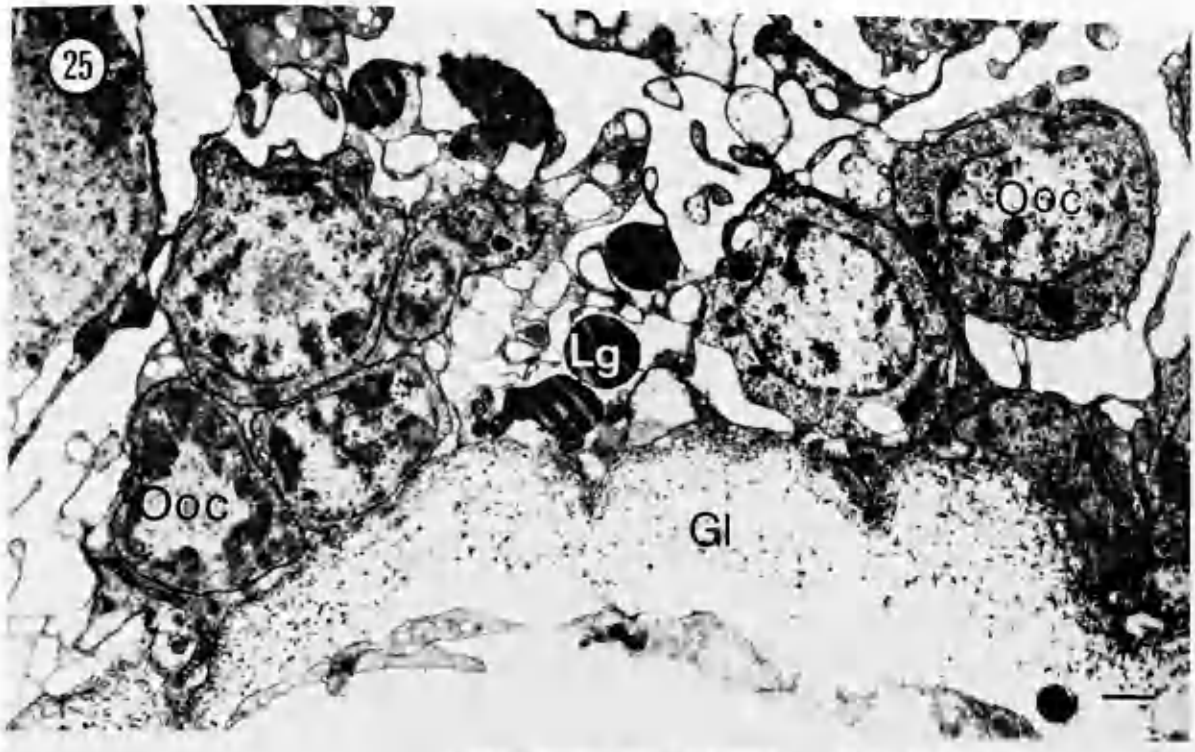
Plate 25 -26. The earliest recorded vitellogenic oocytes. The onset of vitellogenesis is marked by the appearance of non-membrane bound yolk granules, possibly lipid. Scale bars = 1 μm

Plate 27. Oocytes develop a clearly defined nucleolus during the earliest stages of vitellogenesis. Scale bar = 1 μm

Plate 28. Nucleopores. Nuclear material is often condensed against the nuclear envelope. Material appears to be exchanged between the nucleus and the ooplasm through the nucleopores. Scale bar = 0.1 μm

Plate 29. Tangential sections of the nuclear envelope reveal the annuli or spherical profile of the nucleopores. Scale bar = 0.1 μm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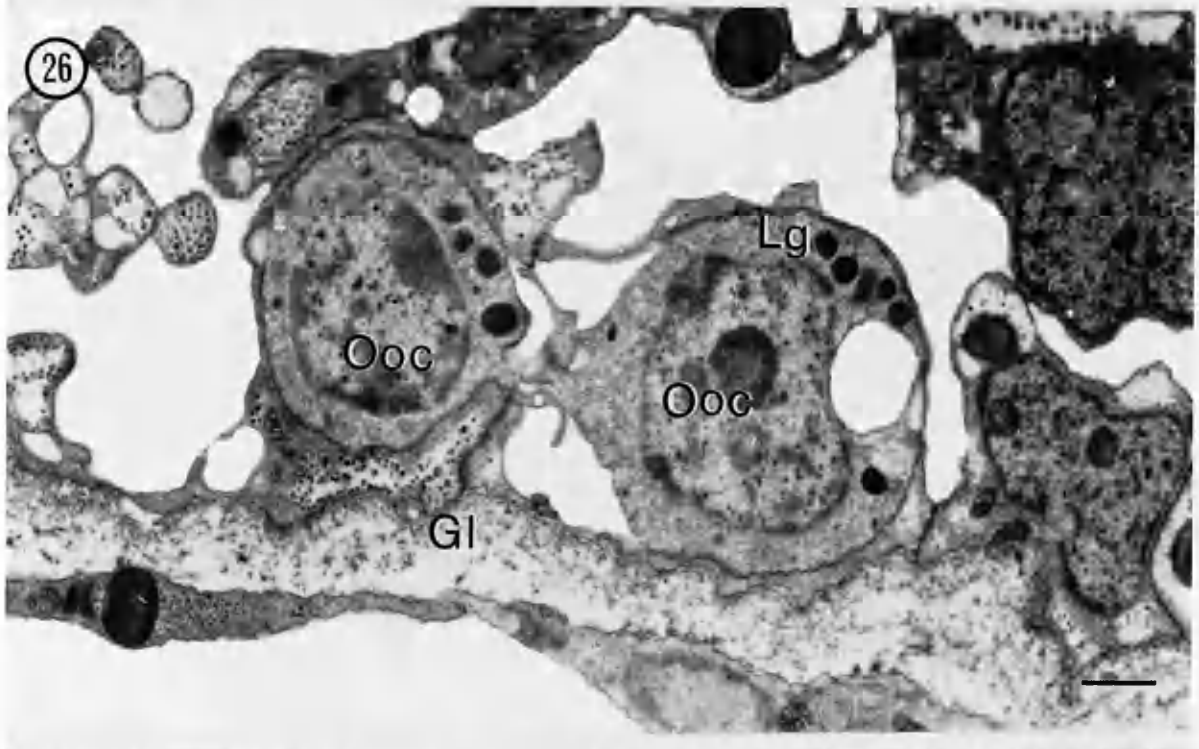
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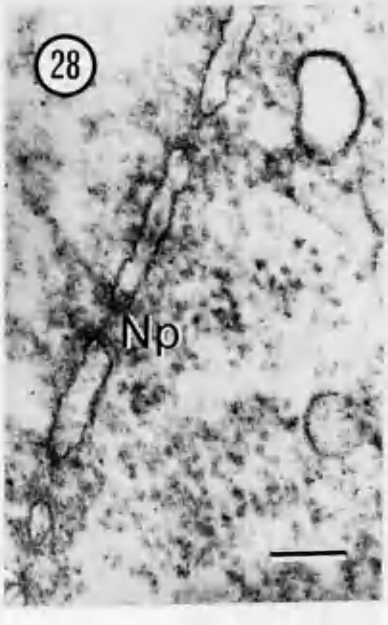
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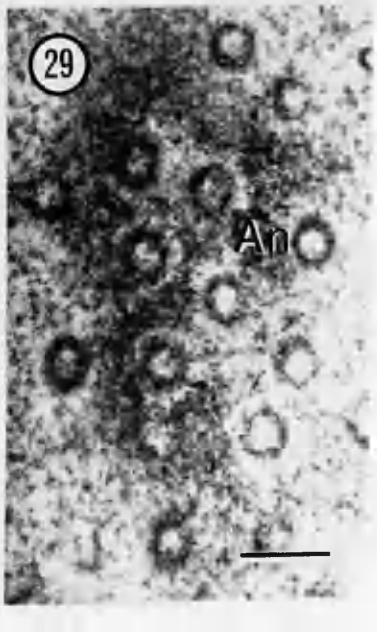
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Plate 30. As vitellogenesis proceeds an accessory cell is produced and the oolemma starts to form microvilli. Organelles are present in clusters. Follicular cells encompass the oocyte but are not closely affixed to the oolemma. Scale bars = 1 μm

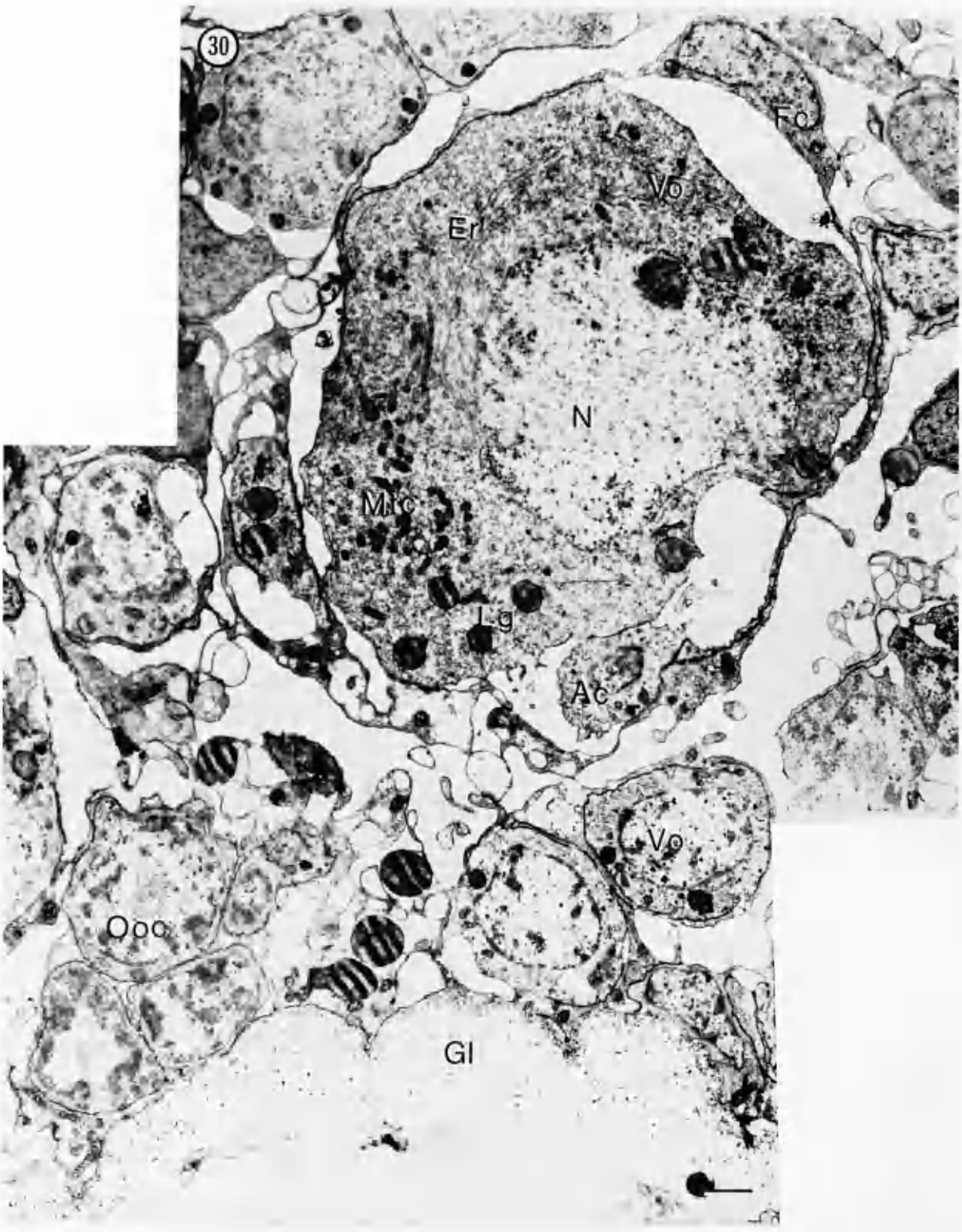
30
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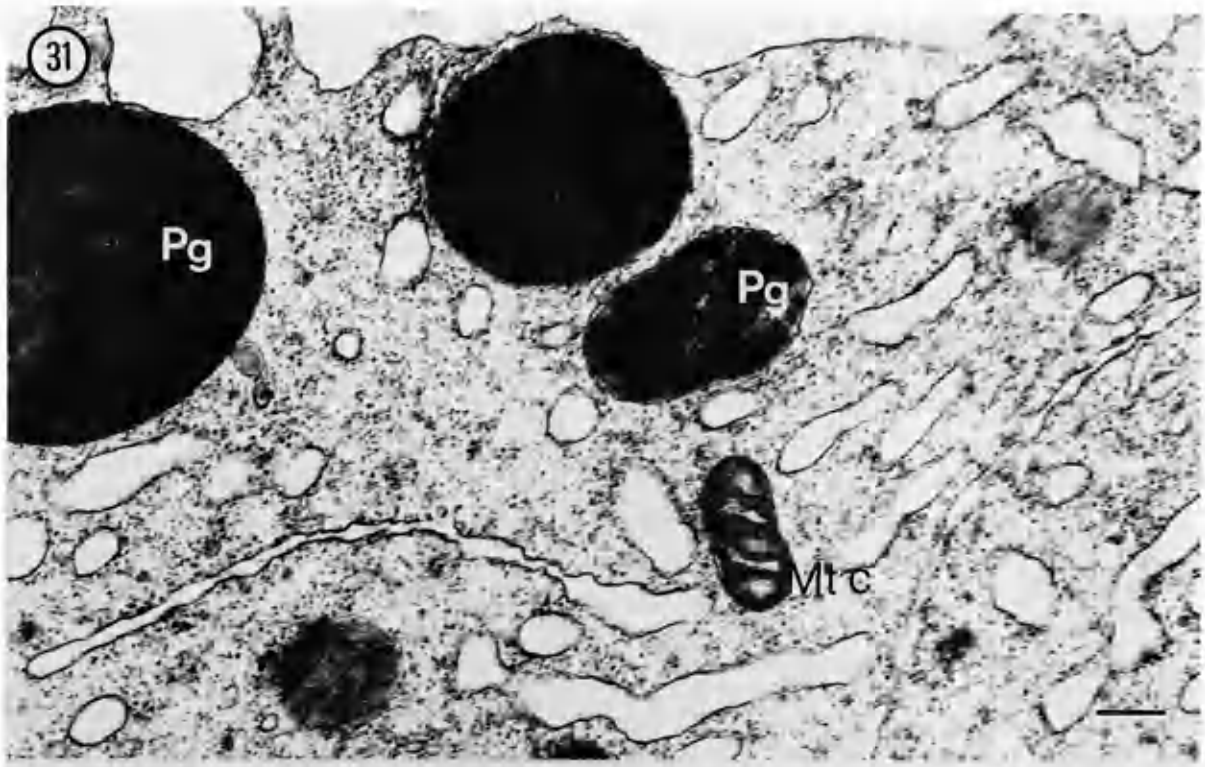
Plate 31. Mitochondria and agranular endoplasmic reticula found within the ooplasm of an oocyte during early vitellogenesis. Scale bar = 0.05 μm

Plate 32. The relationship of the follicular cell nucleus to the microvillar fringe of the early vitellogenic oocyte. Scale bar = 0.5 μm

Plate 33. An accessory cell from an oocyte during early vitellogenesis. Scale bar = 1 μm

Plate 34. A membrane bound vacuole, a common feature of accessory cells. Scale bar = 0.25 μm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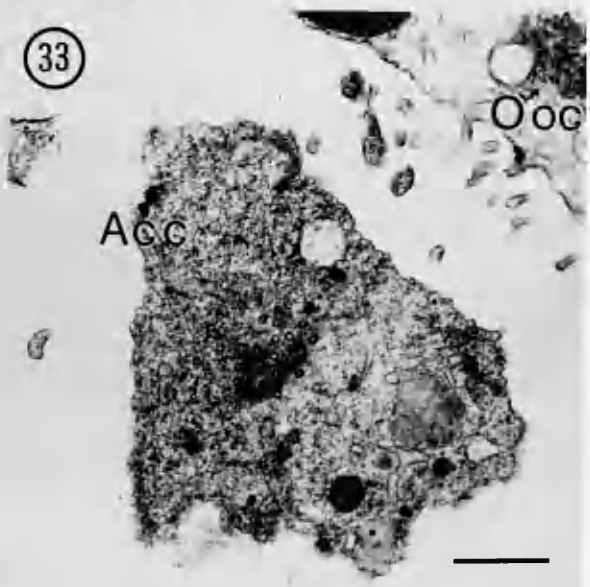
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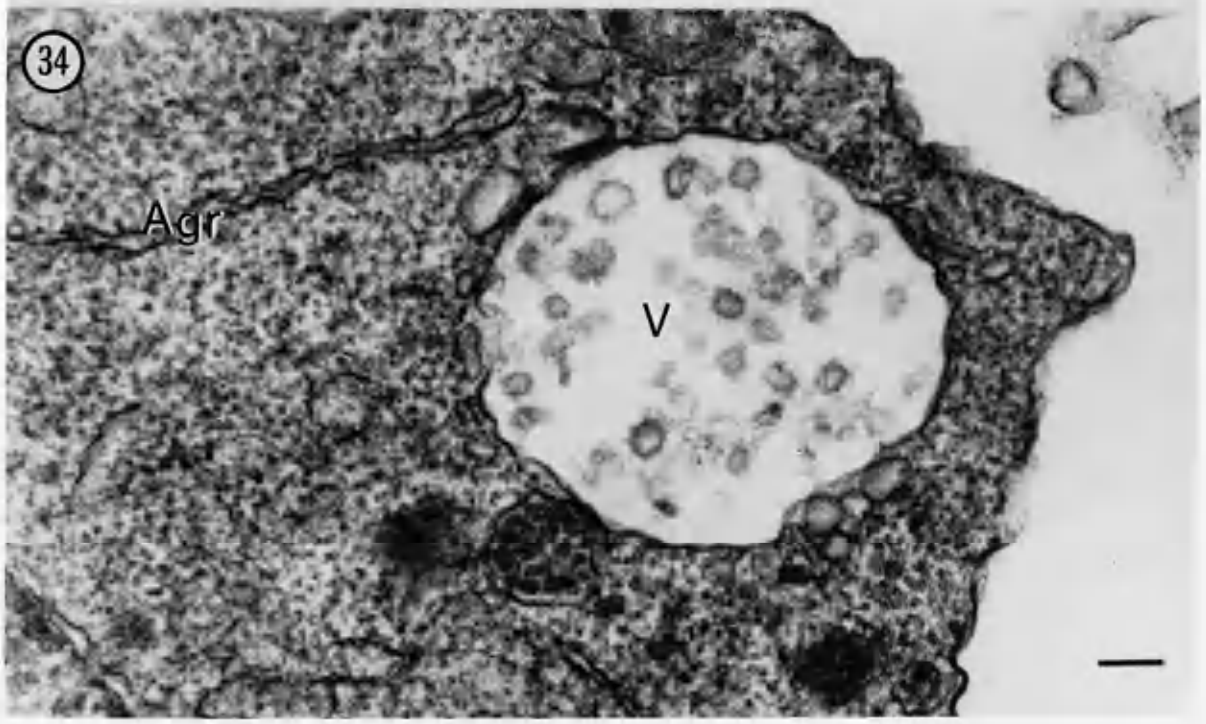
34
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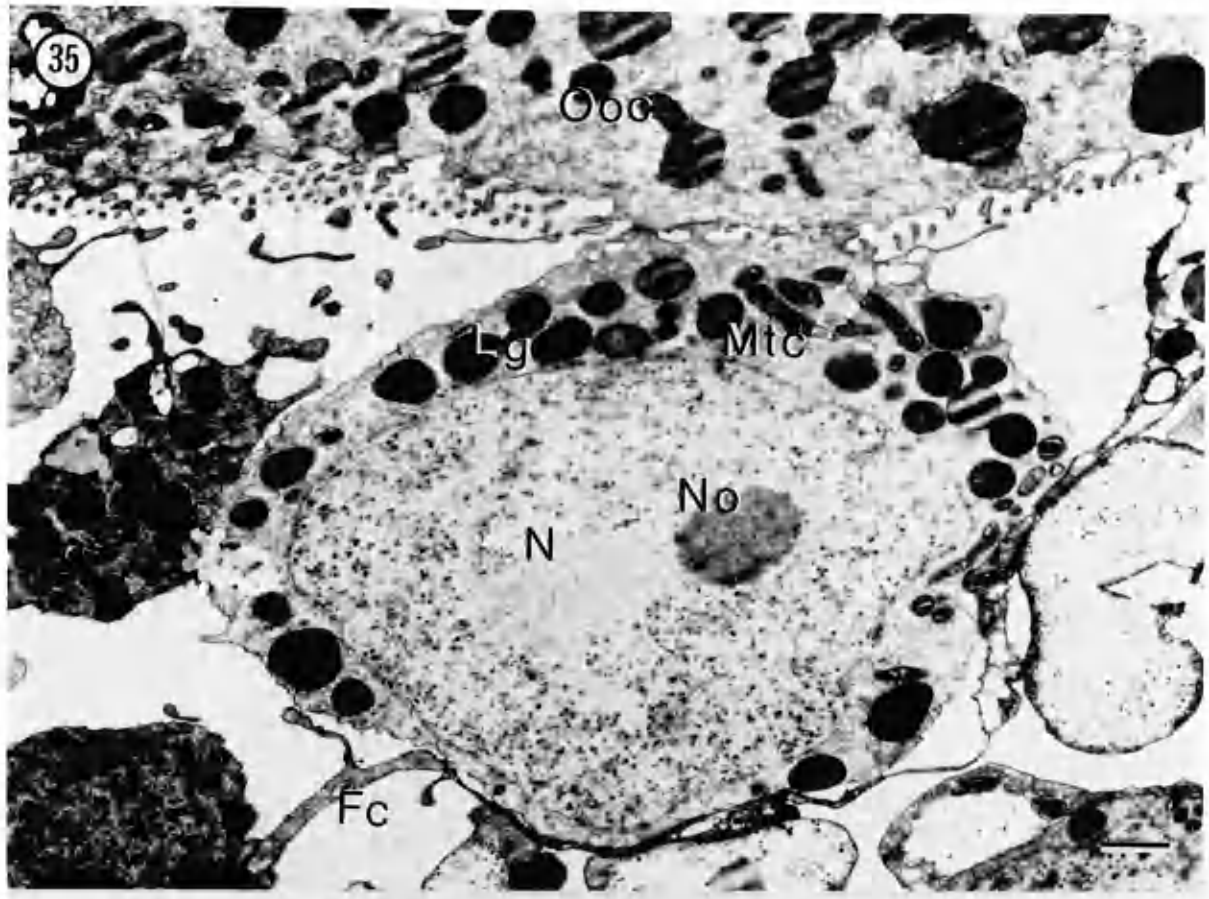
Plate 35. An accessory cell in contact with a vitellogenic oocyte. Inclusions within the cytoplasm of the accessory cell include both protein and lipid granules which are clearly displaced towards the vitellogenic oocyte. Scale bar = 1 μm

Plate 36. TEM showing the intimate relationship of the accessory cell to the vitellogenic oocyte. Note the cluster of mitochondria in close proximety to the region of contact between the two cells. Scale bar = 0.5 μm

Plate 37. Higher magnification micrograph showing the pseudopodial extensions of the accessory cell and the points of contact where desmosome-like junctions occur (circled). Scale bar = 0.25 μm

Plate 38 - 39. Microvilli become increasingly complex as vitellogenesis proceeds. Simple digitate microvilli give rise to branched structures (38), the tips of which eventually separate from the main body of the parent microvillus as a membrane-bound droplet which form a pseudolemma (39). Scale bars = 0.25 μm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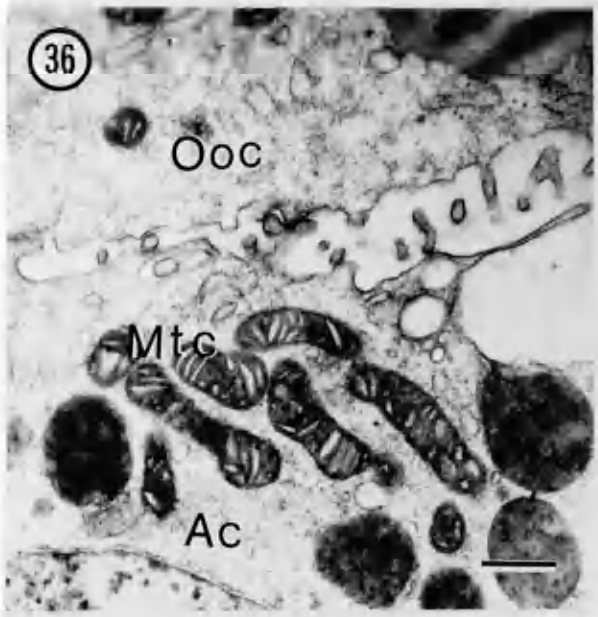
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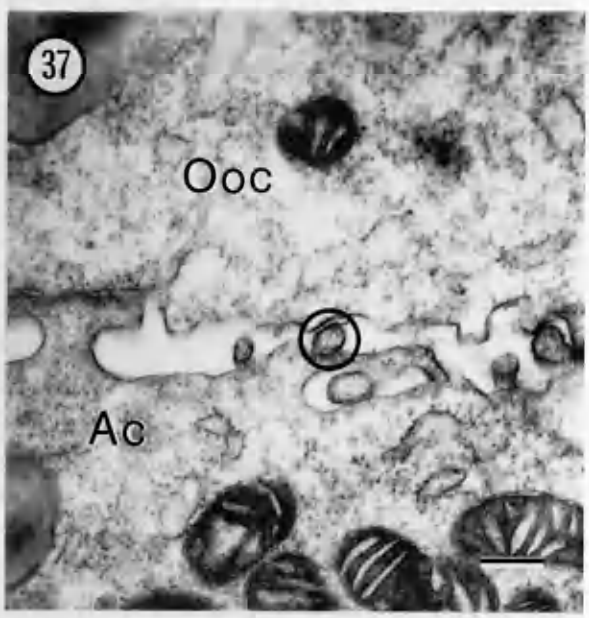
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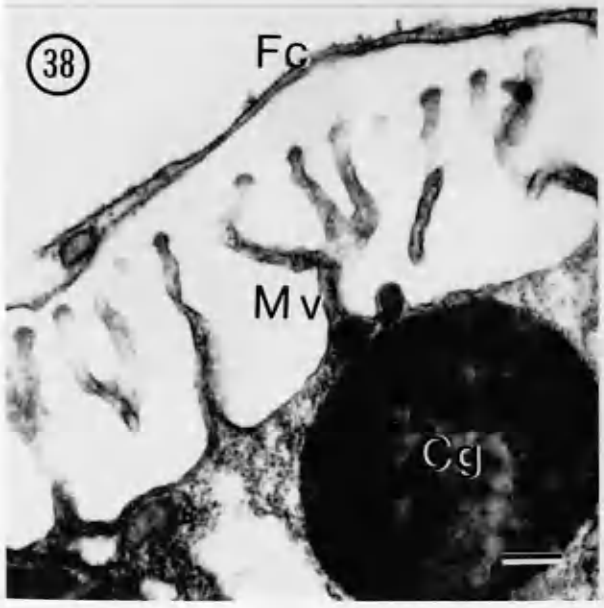
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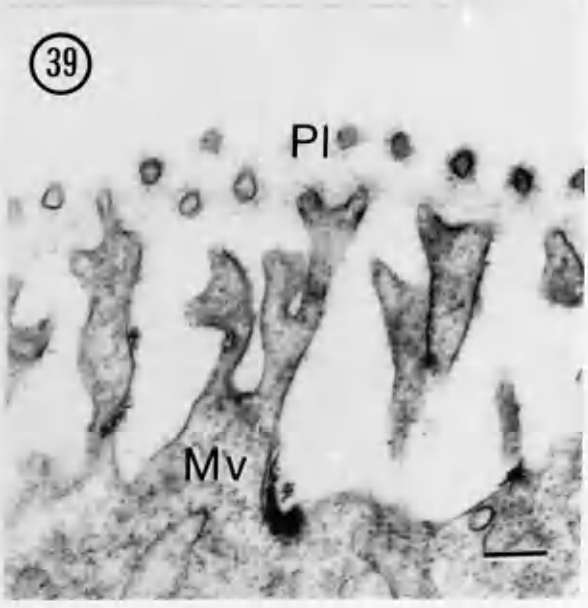
39
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Plate 40. Accessory cells proliferate during the early stages of vitellogenesis. Many of the accessory cells do not have direct contact with the vitellogenic oocyte. Scale bar = 1 μm

Plate 41 - 43. Light micrographs of semi-thin sections indicate the number of accessory cells which may be present within the follicular envelope. Scale bars = (41)50 μm, (42/43)1 μm

Plate 44. The ooplasm of a Stage IV vitellogenic oocyte contains increasing numbers of proteinacious membrane bound granules some of which are destined to become cortical granules in addition to large non-membrane bound lipid granules. Scale bar = 0.5 μm

Plate 45. The ooplasm gradually increases in complexity with the initial proliferation of organelles being followed by the production of a variety of yolk granules. The darker granules are proteinacious. Scale bar = 1 μm


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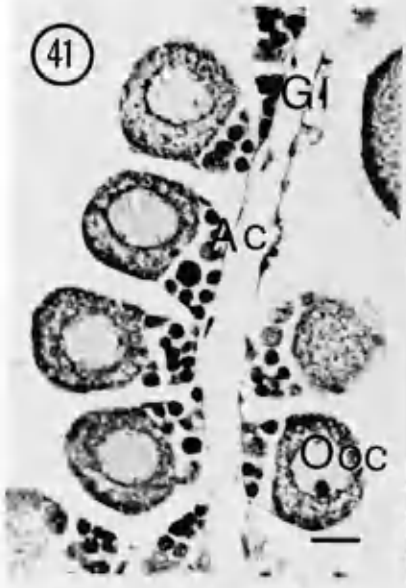
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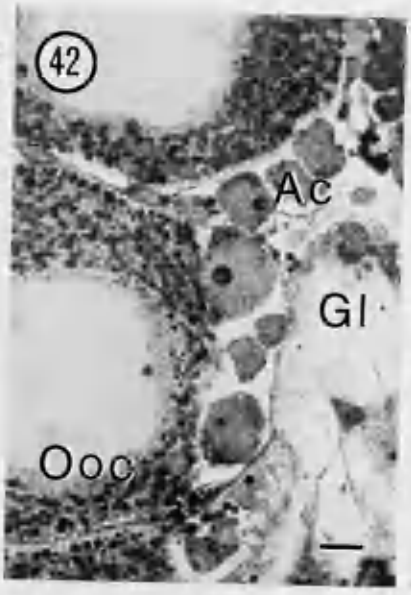
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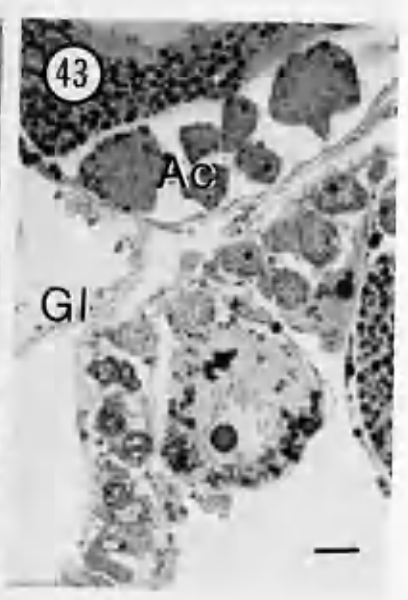
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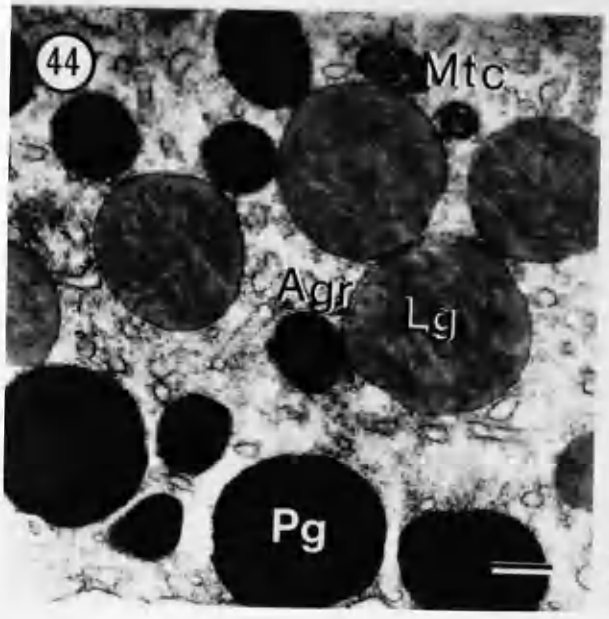
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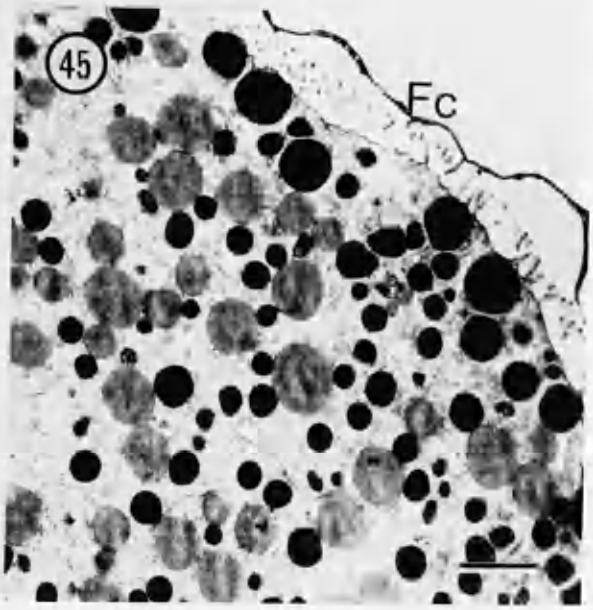
45
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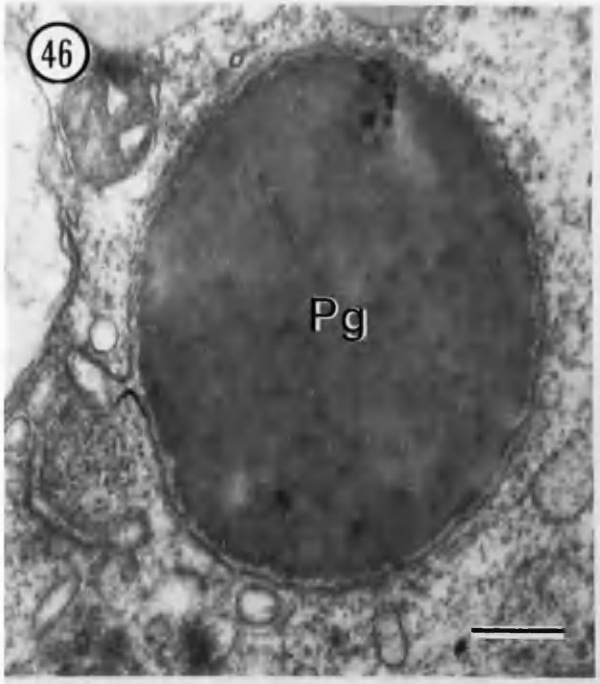
Plate 46. Proteinacious membrane bound granule. Scale bar = 0.25 μm

Plate 47. Vesicle which may be fusing with the membrane of a proteinacious granule. Scale bar = 0.1 μm

Plate 48 - 49. Lipid granules in close association with agranular endoplasmic reticula. Scale bars = 0.25 μm

Plate 50. Golgi complex. Golgi complexes are numerous in Stage IV oocytes. Scale bar = 0.1 μm

Plate 51. Agranular endoplasmic reticula are common associates of Golgi complexes. Scale bar = 0.25 μm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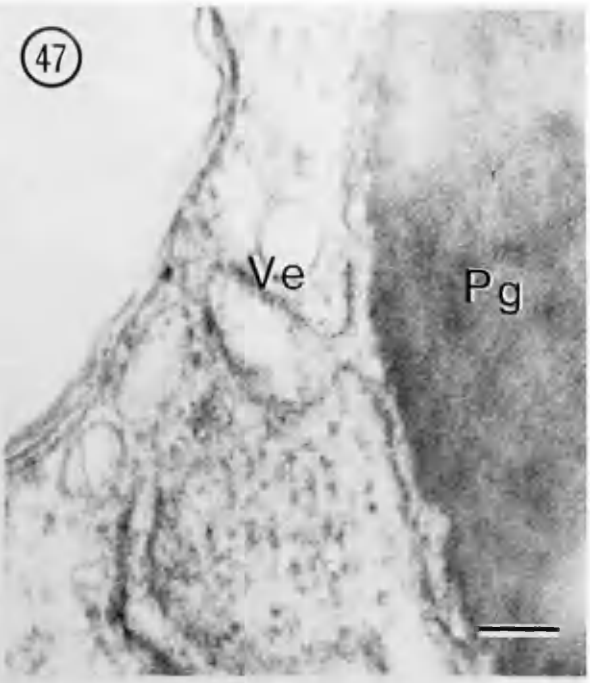
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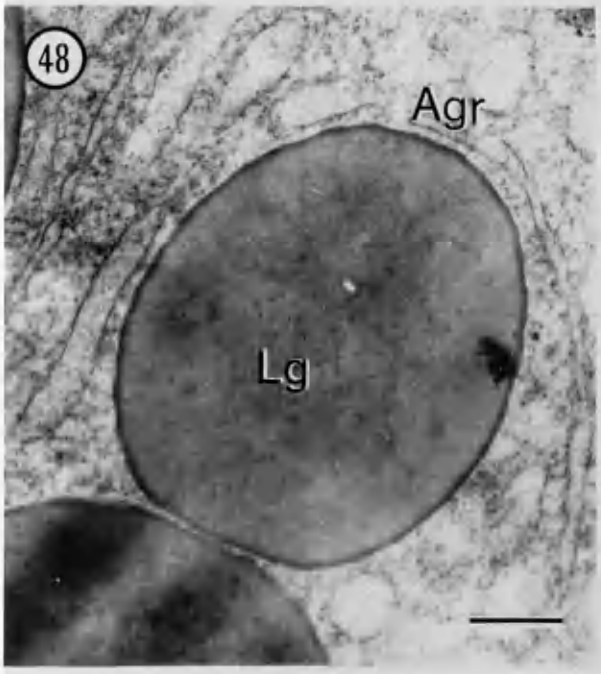
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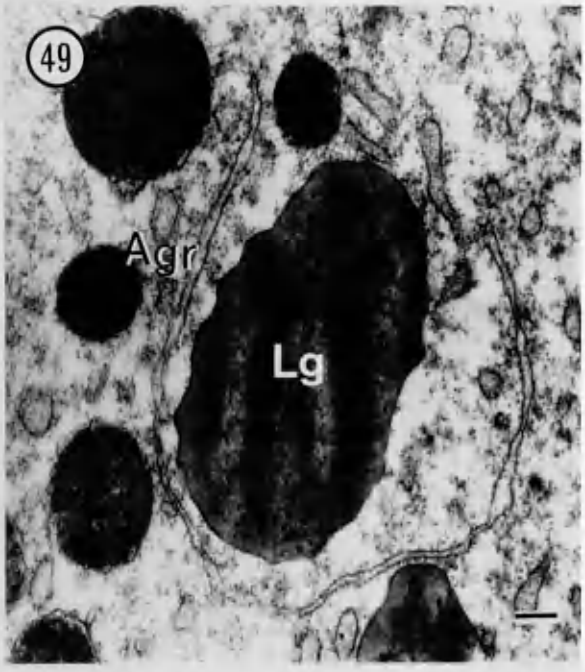
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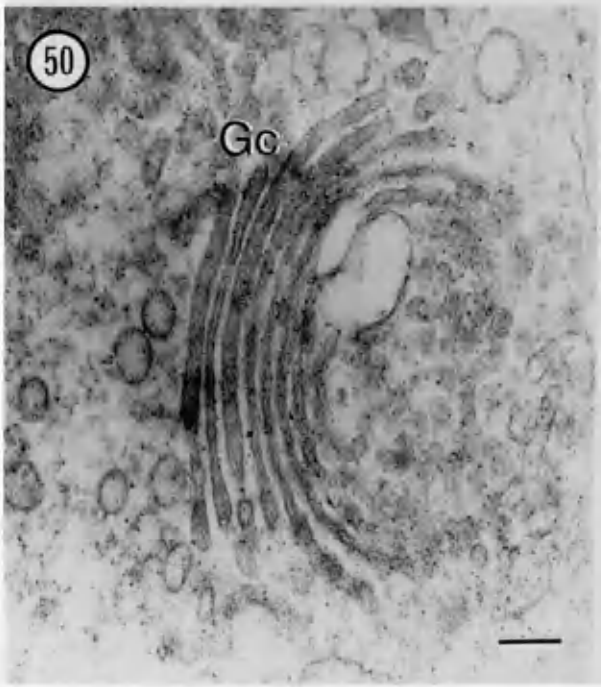
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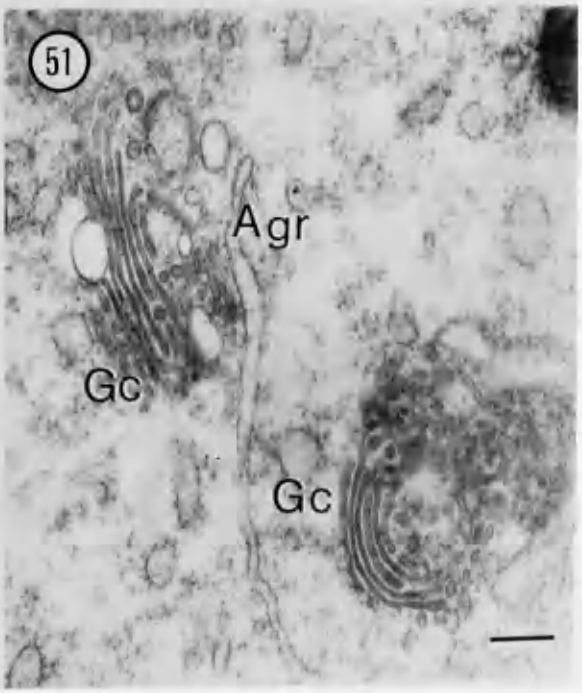
51
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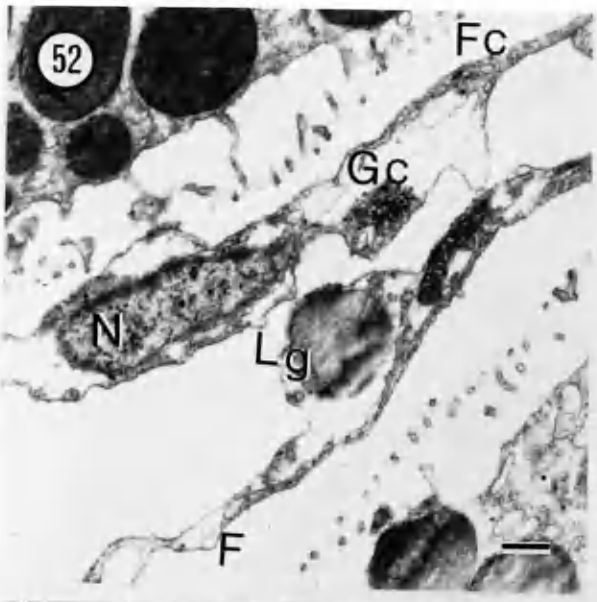
Plate 52. Two follicular cells showing the few organelles to found within their fine cytplasmic extensions which envelope the oocytes. Scale bar = 0.5 μm

Plate 53. Throughout vitellogenesis the nuclear envelope becomes increasingly plicate. Scale bar = 1 μm

Plate 54. Mitochondria and agranular endoplasmic reticula are typically found in close association with yolk granules. Profiles of agranular endoplasmic reticula envelope lipid granules. Microsomes coalesce. Scale bar = 0.25 μm

Plate 55. Intra-accessory cell communications are achieved by the formation of desmosome-like gap junctions between pseudopodia produced by the accessory cells. Scale bar = 1 μm

Plate 56. Direct communication between accessory cells may be created by the formation of cytoplasmic unions or ring canals. Scale bar = 0.5 μm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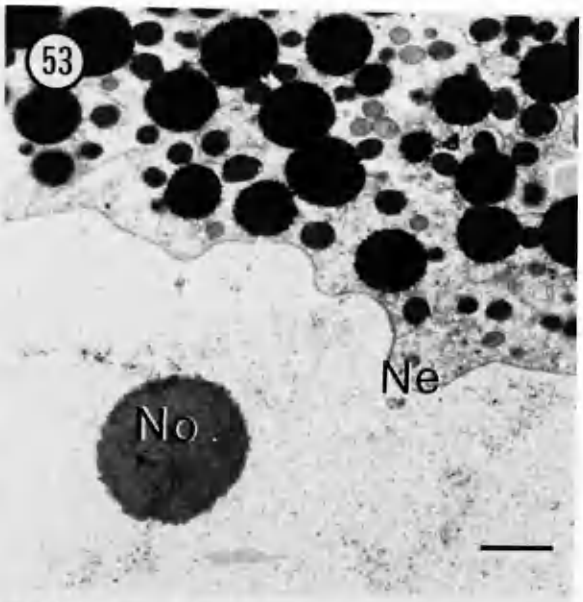
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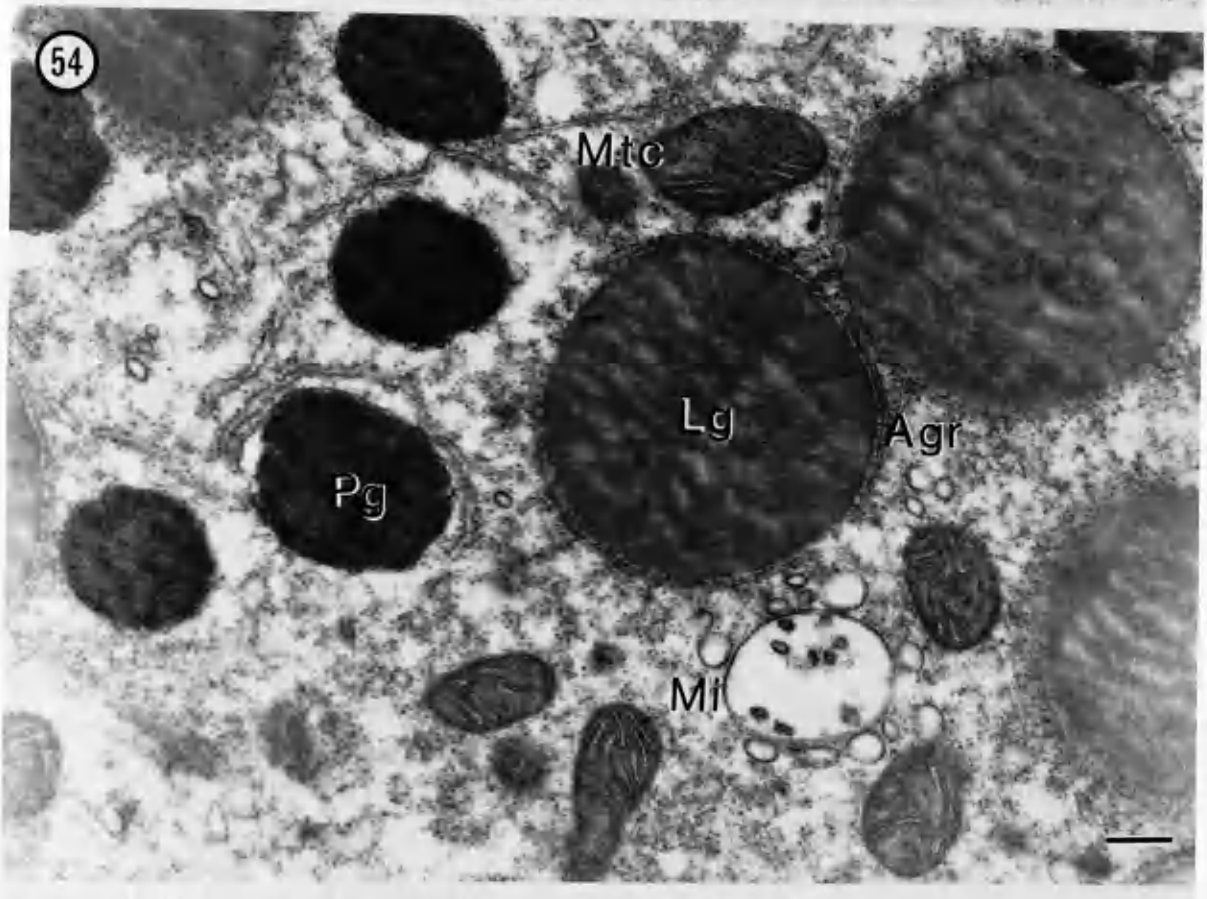
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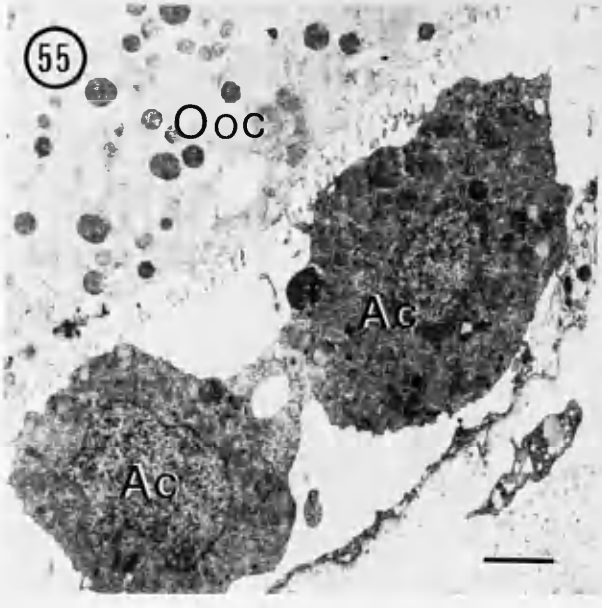
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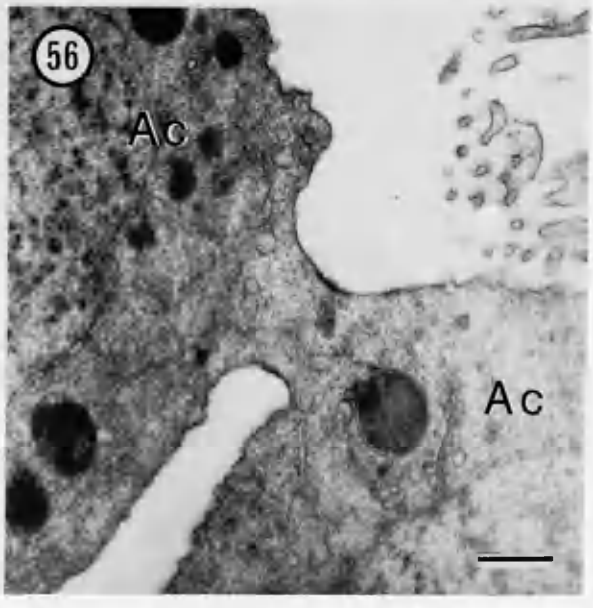
56
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Plate 57. The porifery of a Stage V vitellogenic oocyte shows a distinct elaboration of the microvilli. Indivividual elements of the microvilli are fused with a delicate band of granules forming a pseudolemma. Beneath, vacuoles carry coarse fibro-granular aggregations which appear to be incorporated into the ooplasm. Scale bar = 1 μm

Plate 58. Higher magnification micrograph of endocytotic vesicle formation by the oolemma. Scale bar = 0.25 μm

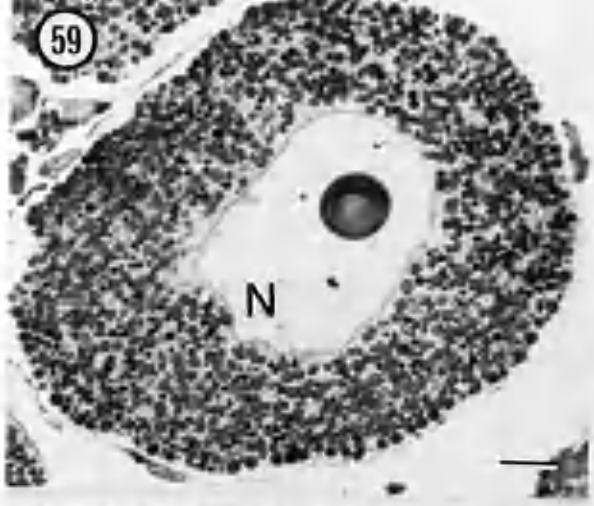
Plate 59. Semi-thin sections reveal the concentration of yolk granules visible under the light microscope in a late stage oocyte prior to liberation from the genital lamella. Scale bar = 5 μm

Plate 60. The oocyte, still within the follicular envelope is eventually released from the genital lammela (Stage VI). Scale bar = 10 μm

Plate 61. Two late mature vitellogenic oocytes in contact. The oocytes are separated by their follicular envelopes. The microvillar fringe is highly convoluted and contains numerous membrane bound microdroplets. Coarse granular material is present in both the perivitelline and periovular space. Scale bar = 1 μm

Plate 62. Section through the edge of a mature oocyte clearly showing the band of intensely stained cortical granules. Scale bar = 2 μm

Plate 63. The lipid granules of the mature oocyte are intimately associated with mitochondria and some Golgi complexes. Close examination of the circumference of the lipid granules reveals the profile of endoplasmic reticula (possibly granular endoplasmic reticula). Scale bar = 1 μm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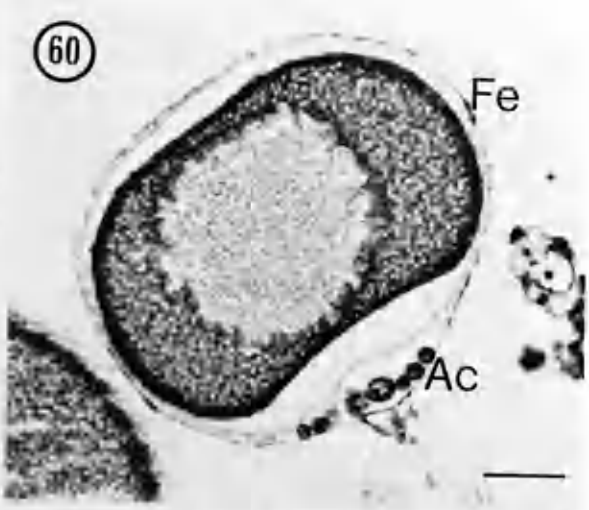
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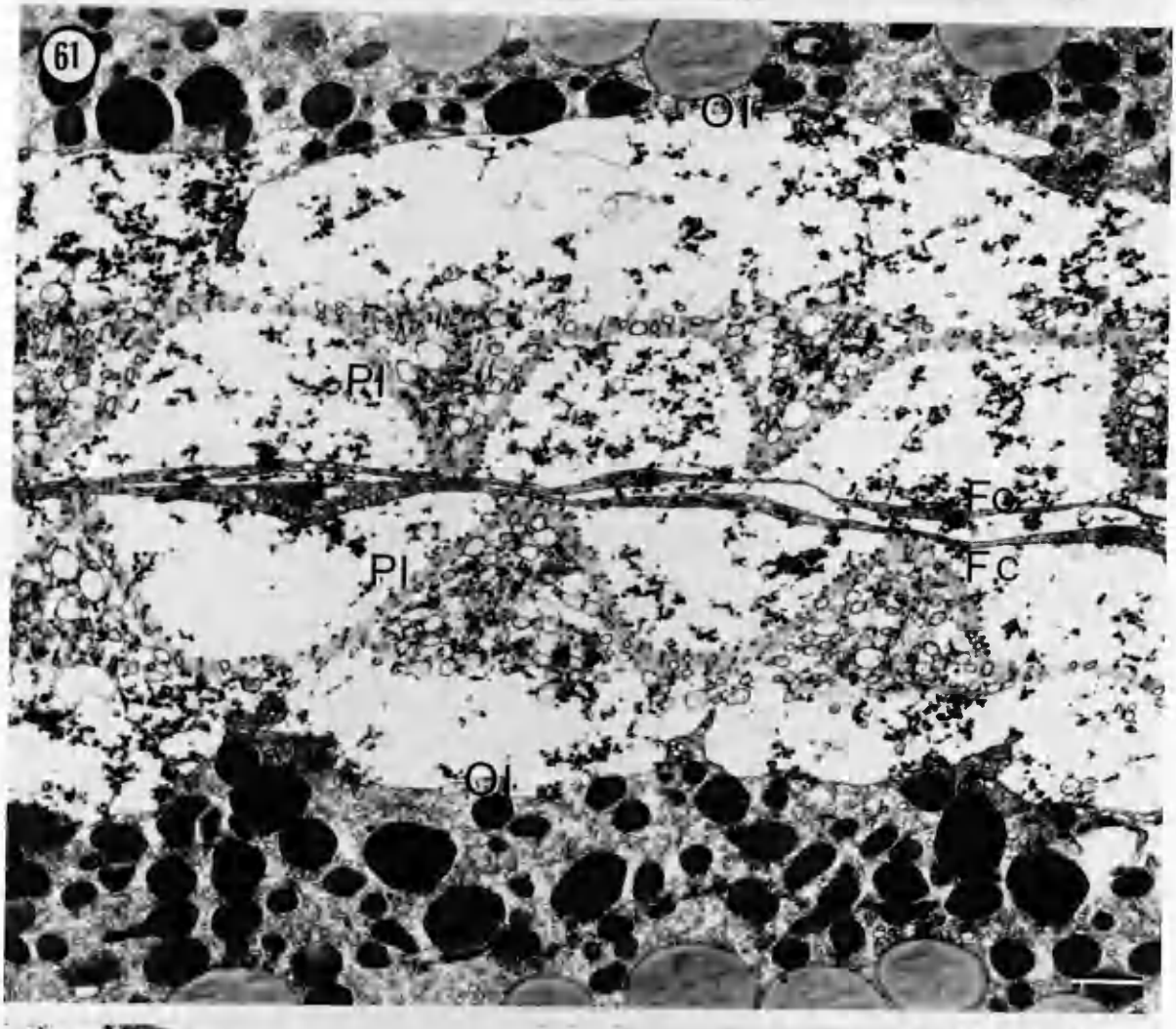
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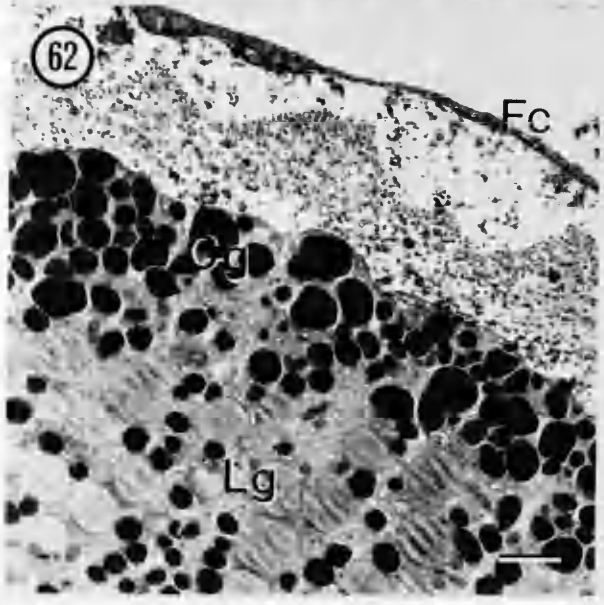
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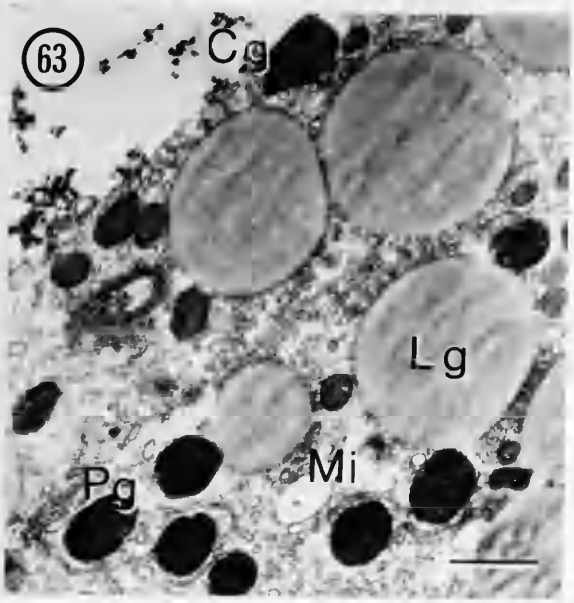
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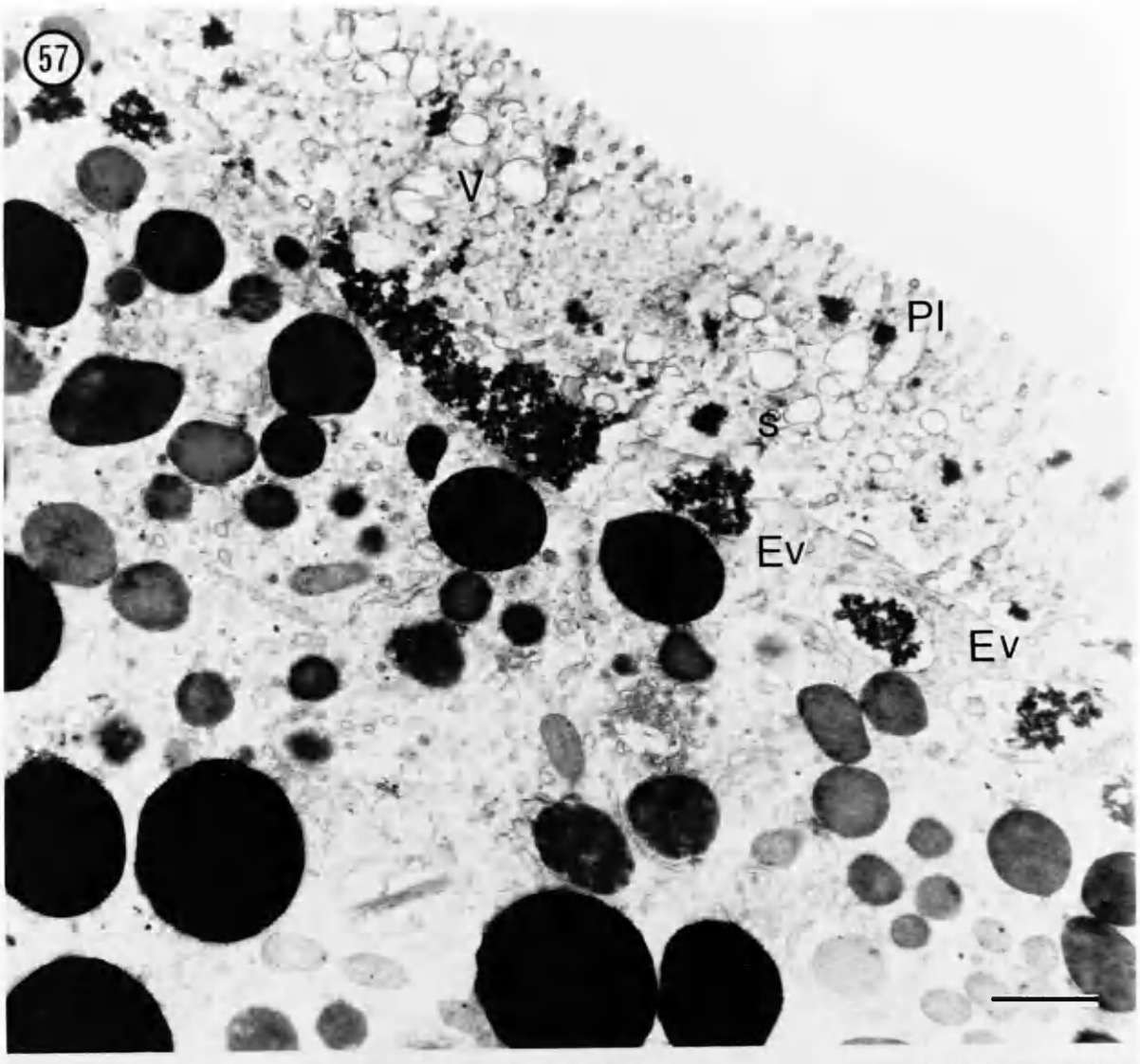
Plate 64. SEM of a vertical section through the ovary of a mature female in spawning condition. Scale bar = 100 μm

Plate 65. Higher magnification micrograph of a prespawn ovary showing part of the genital lamella. Scale bar = 25 μm

Plate 66. Surface view of a spent ovary showing an unspawned oocyte with what appears to be the remainder of shed follicular envelope. Scale bar = 50 μm

Plate 67. Light micrograph of a vertical section through the ovary of a female approaching maturity. Scale bar = 20 μm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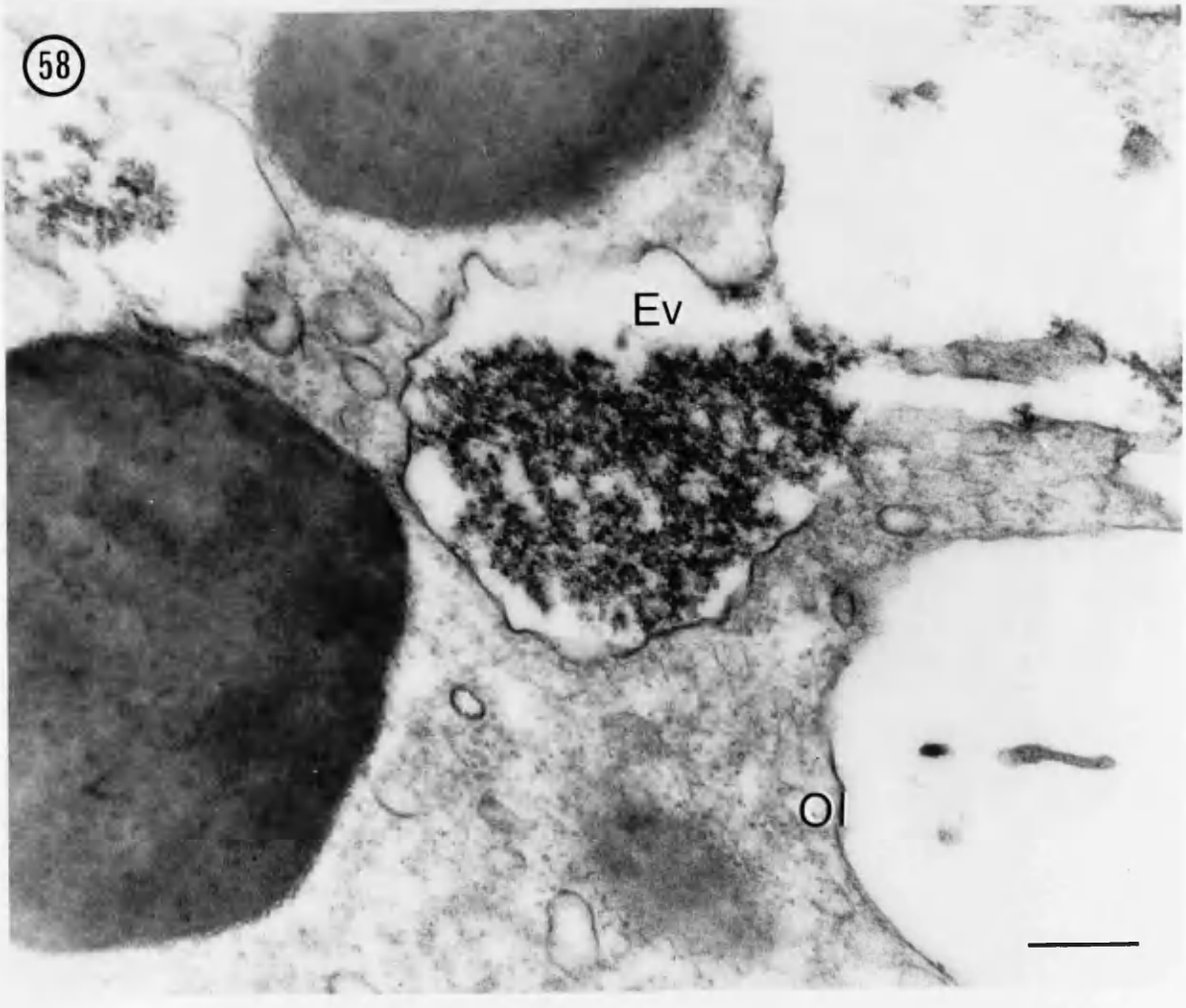
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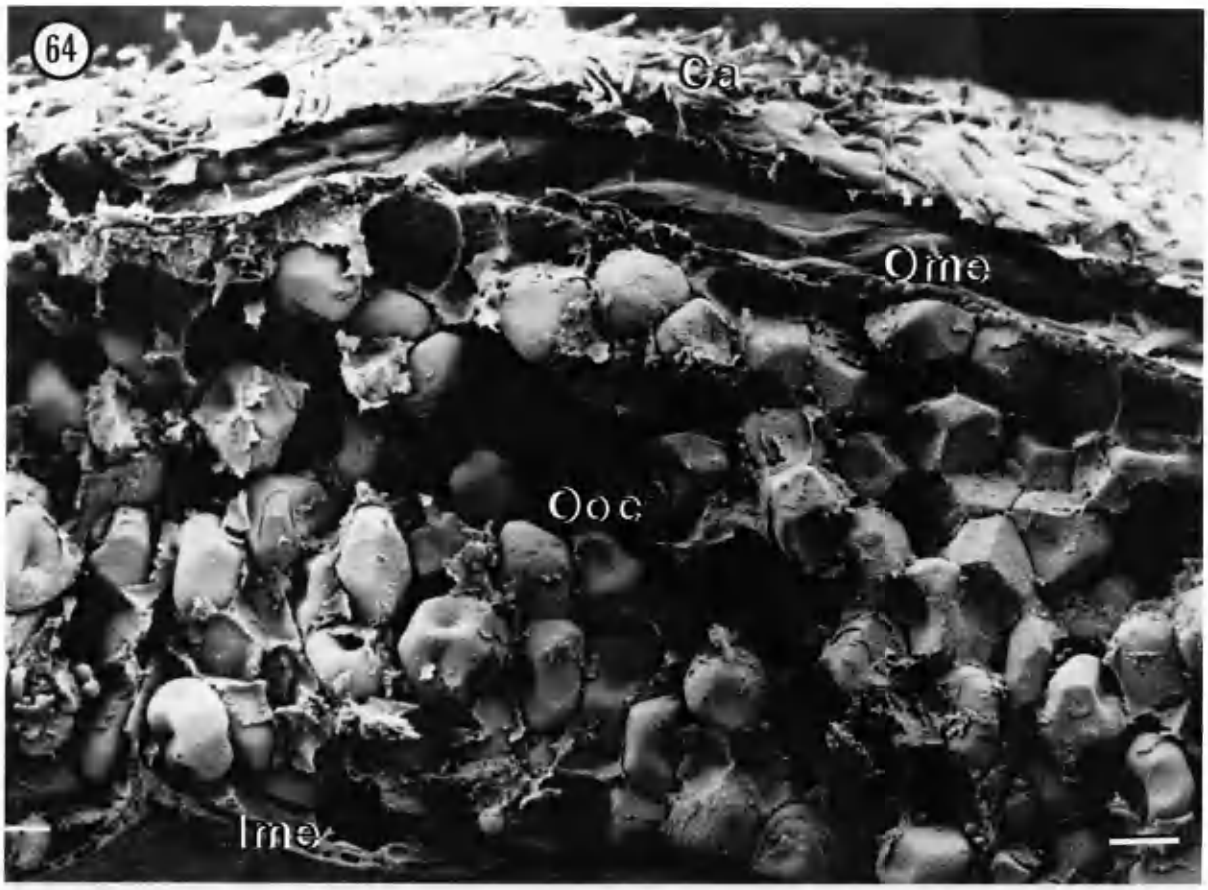
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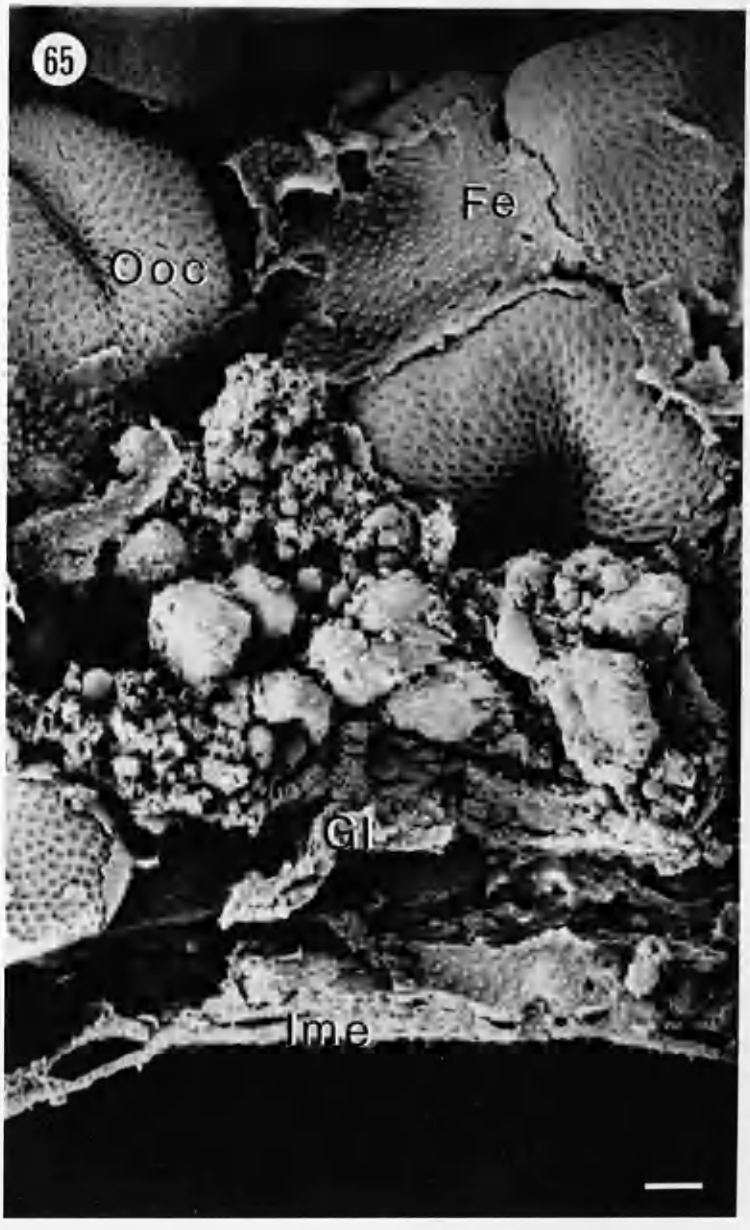
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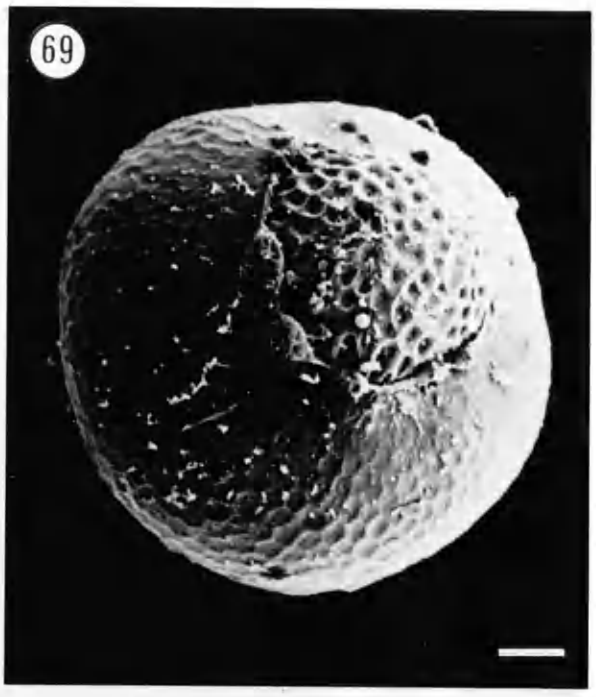
Plates 68 - 73. A series of SEM's showing the manner in which the follicular envelope retreats around the mature oocyte immediately before spawning occurs. Scale bars = 20 μm


68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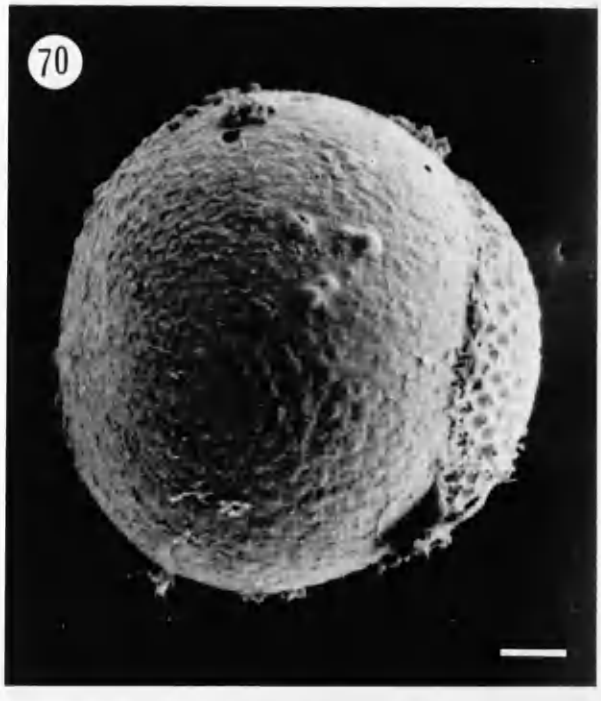
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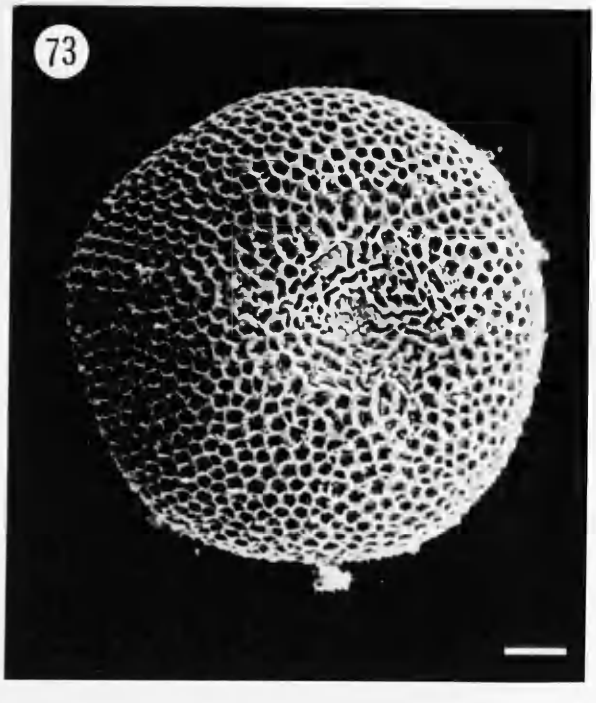
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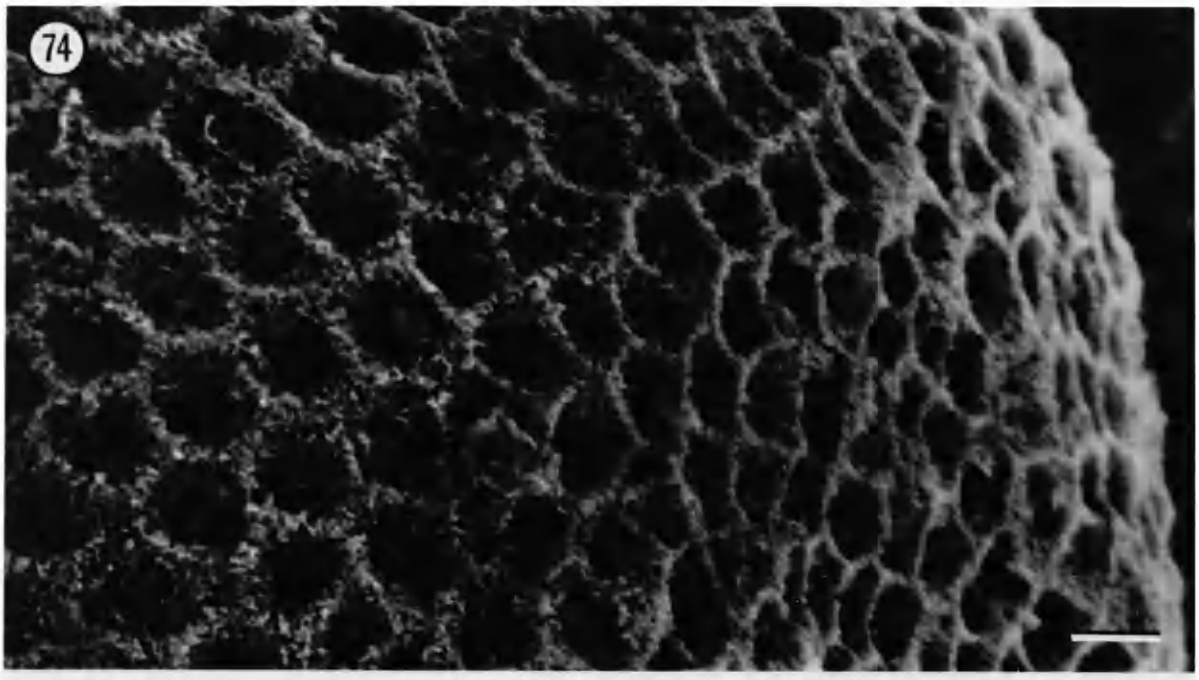
Plate 74. The highly convoluted surface of the mature oocyte is clearly visible in eggs which have been spawned. Scale bar = 10 μm

Plate 75. When highly magnified the density of the microvilli which form the convoluted surface can be observed. Scale bar = 1 μm

Plate 76. TEM of a section through the microvillar fringe taken from a mature oocyte. Membrane bound microdroplets are in the process of being formed. Scale bar = 0.25 μm

Plate 77. The margin of the mature oocyte is divisible into two regions. The perivitelline space containing granular material and a second region formed between a basement membrane and the pseudolemma. Membrane bound microdroplets are concentrated between the latter. Scale bar = 0.25 μm



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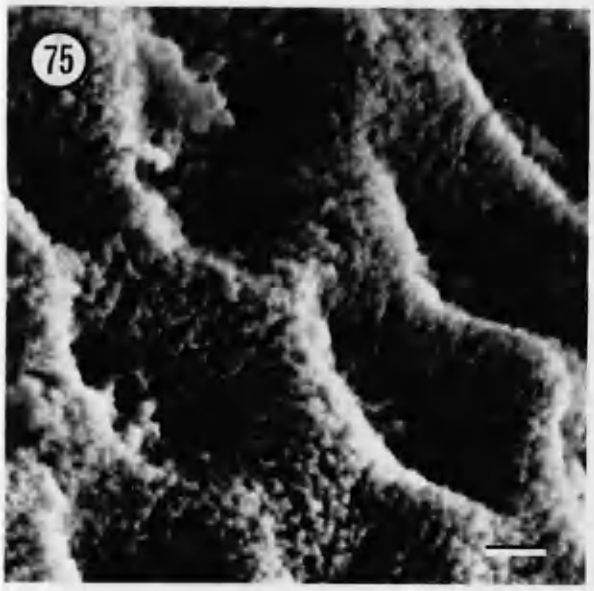

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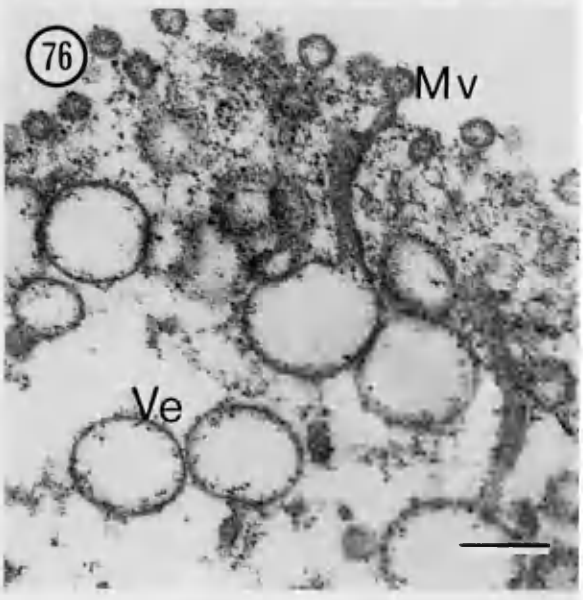

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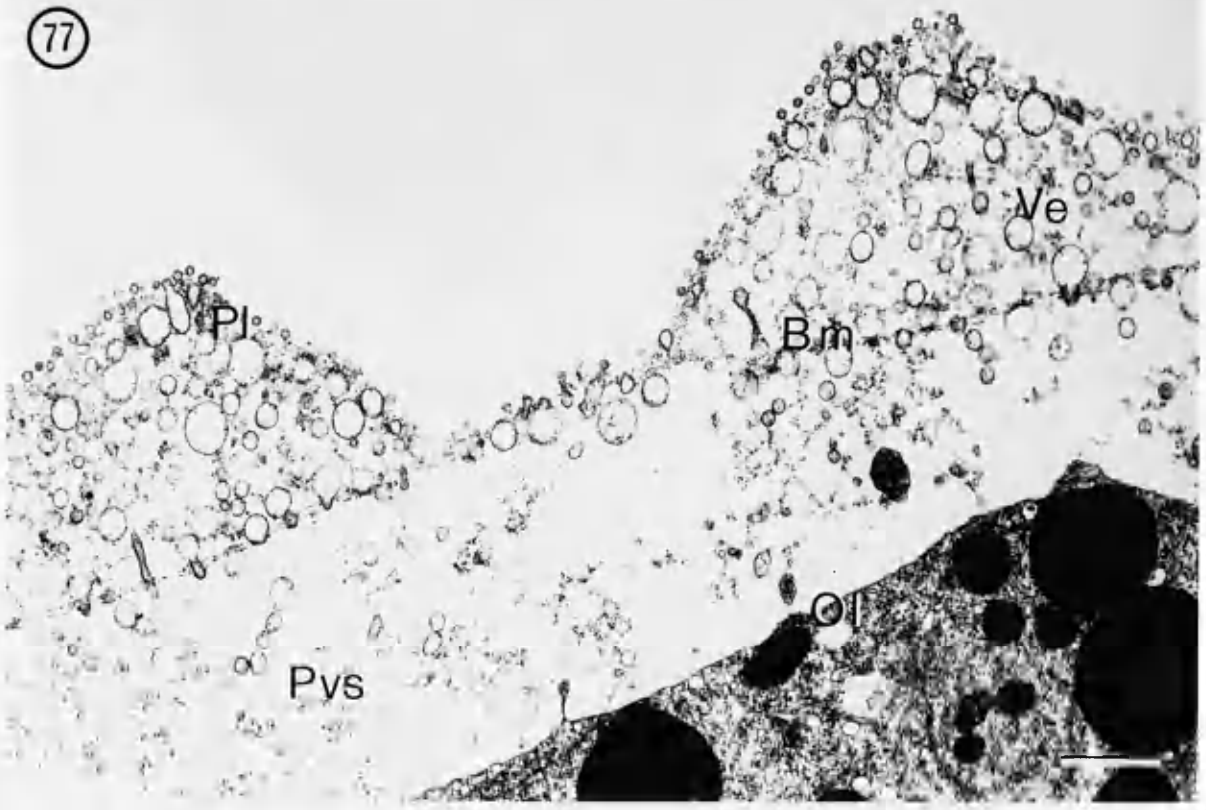

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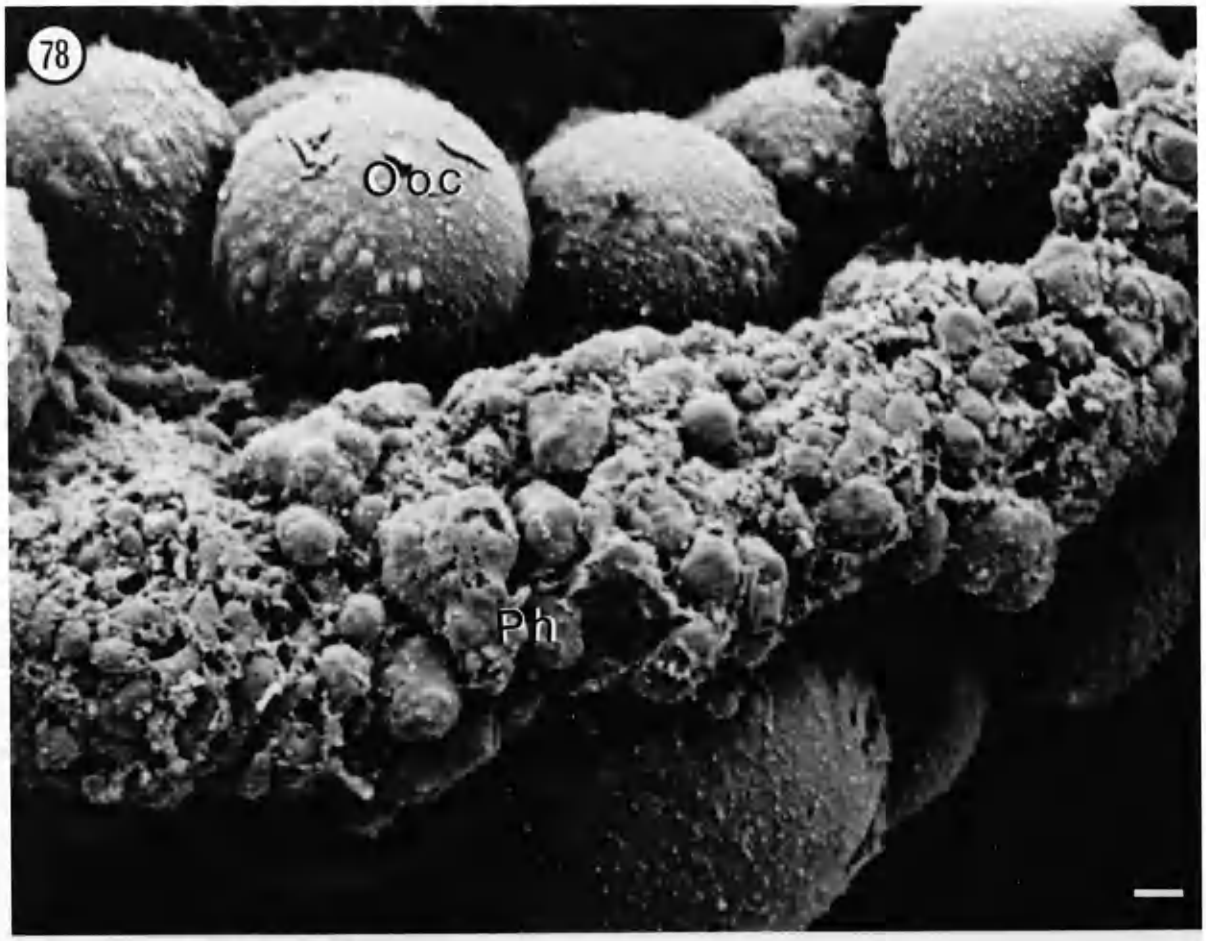
Plate 78. SEM showing the surface of the genital lamella from a recently spawned female. The band of globular material is formed by an aggregation of phagocytic cells. Scale bar = 10 μm

Plates 79 - 81. A series of light micrographs displaying various stages in the atretia of oocytes. Scale bars = (79/81)5 μm, (80)10 μm

Plate 82. Pseudopodial extensions of an amoeboid phagocyte engulfing coarse granular material. Scale bar = 1 μm

Plate 83. The cytoplasm of the phagocytes contains numerous organelles including conspicous mitochondria. Scale bar = 0.05 μm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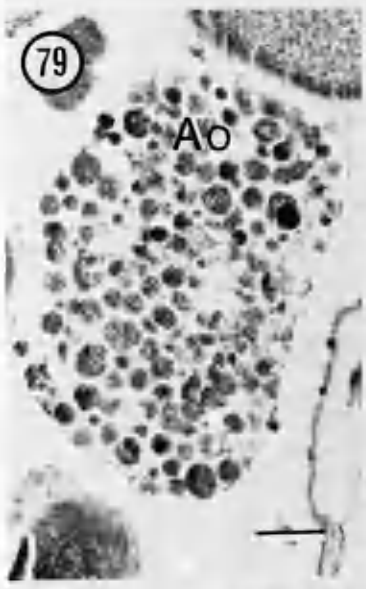
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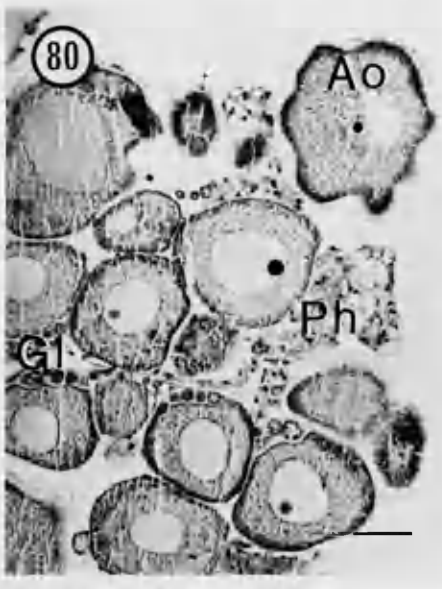
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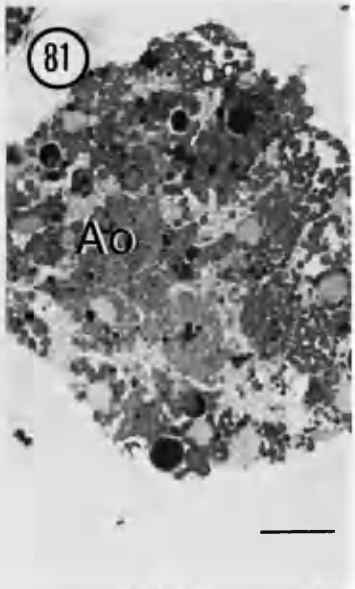
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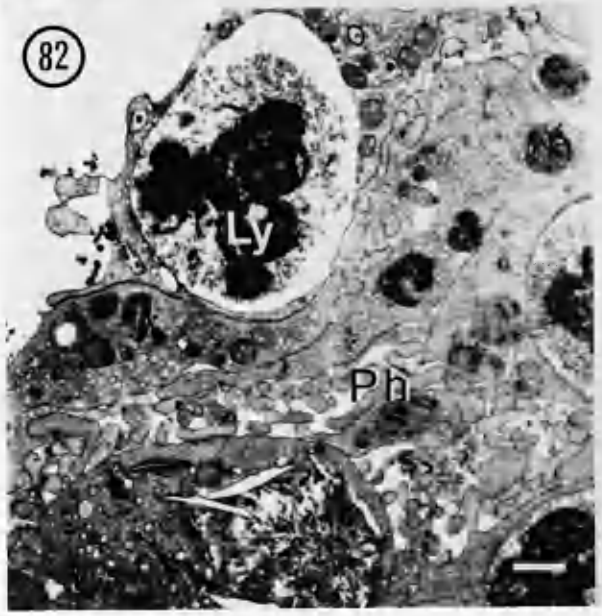
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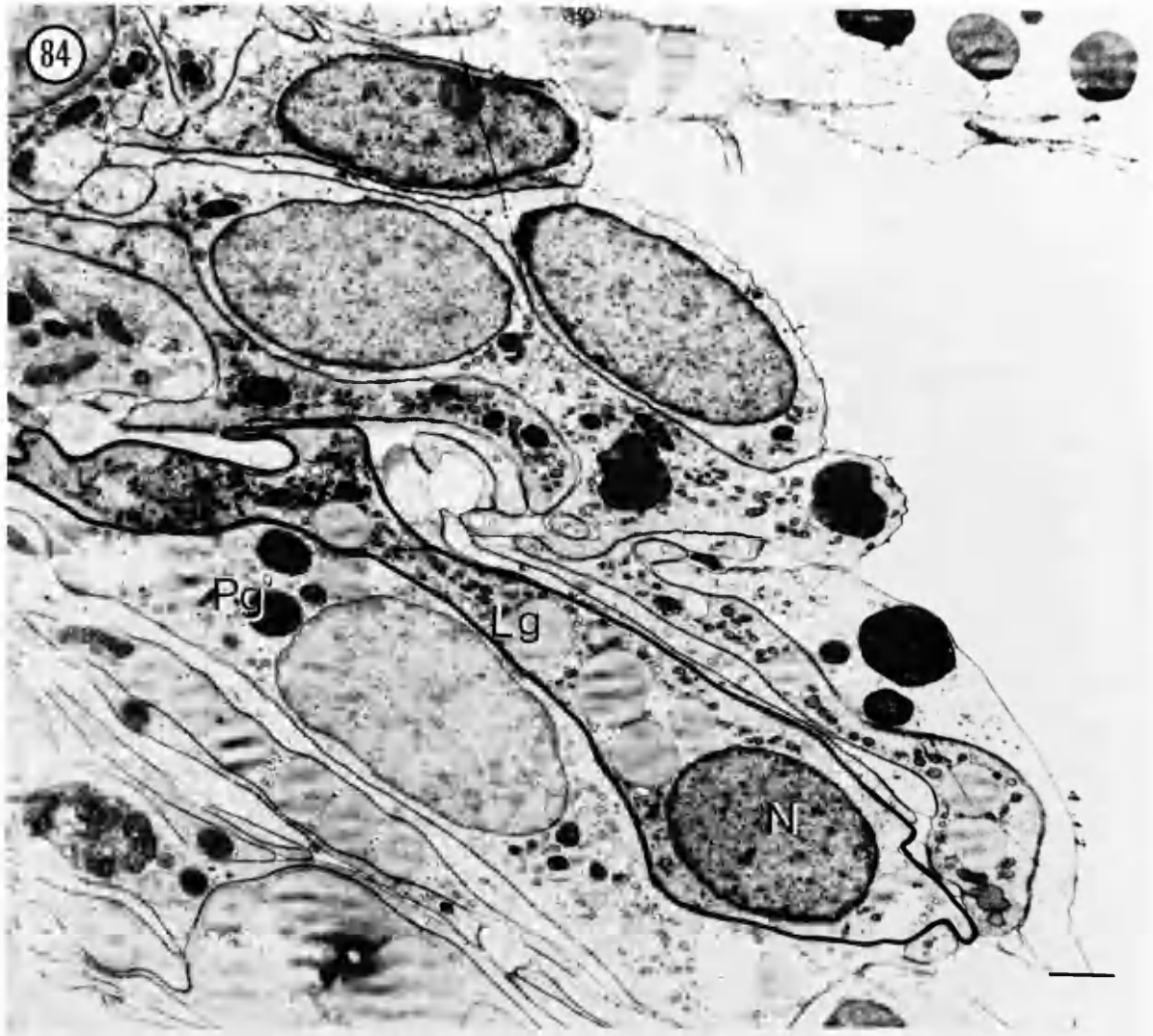
Plate 84 - 85. Two morphologically distinct types of phagocyte were observed. Some are flattened and elongated with a smooth outer profile which bulges to accomodate the well defined nucleus (84). Other amoebocytes are more rotund and appear to produce a dense pseudopodial matrix (85). Scale bars = 1 μm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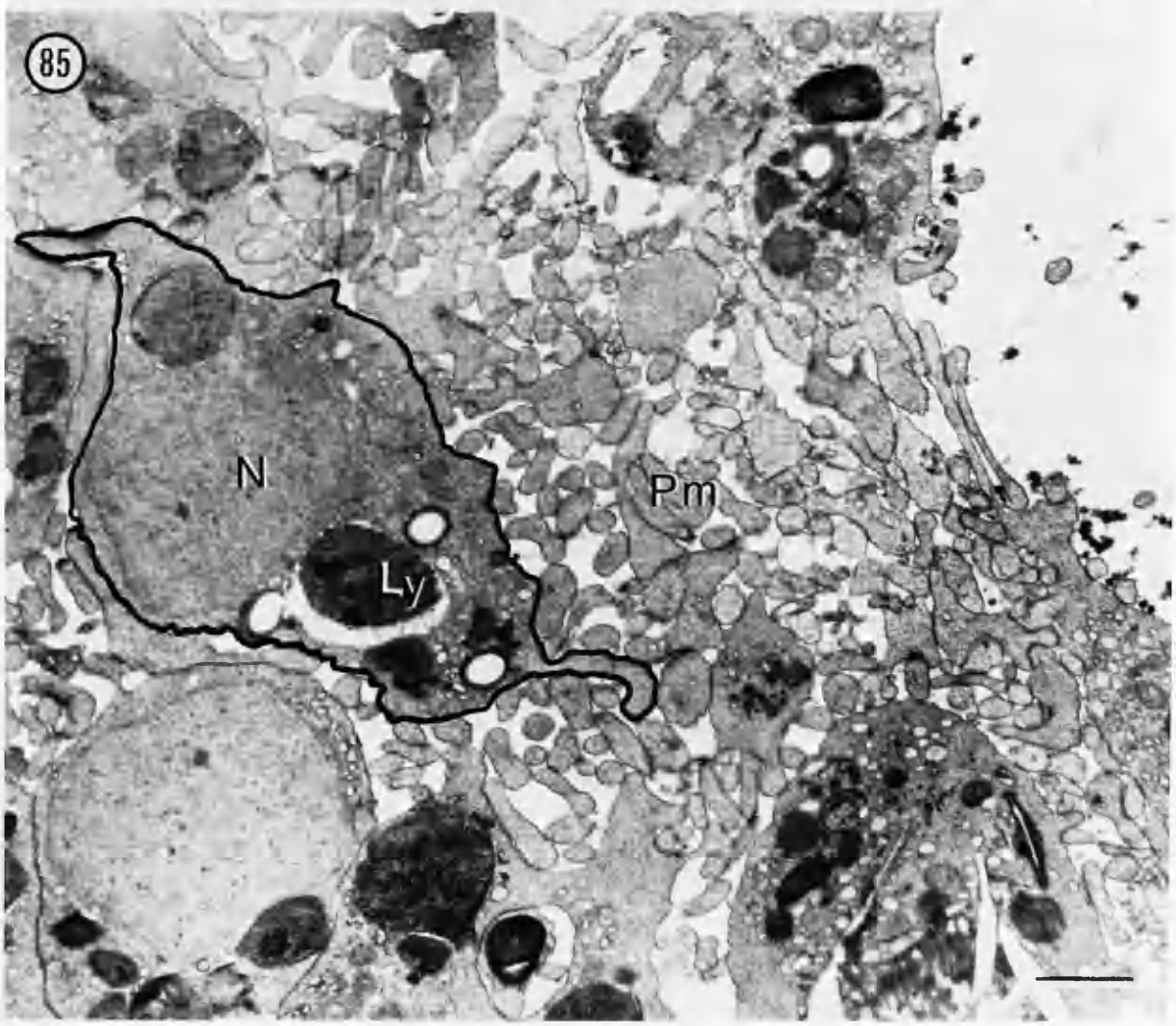
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4.2 DISCUSSION.

Oogenesis is a dynamic process which Anderson (1974) states is conveniently divisible into a proliferative and a vegetative phase. Little precise information is available about the proliferative phases of oogenesis, but much more is known about the vegetative or vitellogenic phases.

The emphasis in the present study has been directed towards understanding the ultrastructural and basic cytochemical changes occurring during vitellogenesis in the oocytes of T. retusa. As a result the vitellogenic phases of oogenesis has been divided into six stages, based primarily on key ultrastructural changes which may be indicative of the manner in which nutrients are accumulated by the differentiating oocyte.

4.2.1 Previtellogenesis.

No transitional forms between germ cells and the neighbouring coelomic epithelium were observed, suggesting that primordial germ cells are differentiated early in ontogeny. Similar findings were recorded for Lingula (Schaeffer, 1926) and by Chuang (1983a) for Glottidia albida, Lingula anatina, Neocrania anomala, Neocrania sp., Discinisca sp., Frenulina sanguinolenta, Neothyris lenticularis, Pumilus antiquatus, Tegulorhyncha nigricans, Terebratella sanguinea, and Waltonia inconspicua.

Yatsu (1902), reported that in Lingula, germ cells can be distiguished by their large size from the cells of the ileoparietal band even at the stage of eight pairs of cirri. Light microscope studies, however, of the gonads of the articulates, Gryphus vitreus, Hemithiris psittacea, Macandrevia crania, and Terebratulina retusa (van Bemmelen, 1883) and Terebratalia transversa (Long, 1964) and in the inarticulates Neocrania anomala (Joubin 1886, Blochmann, 1892) and Lingula (Senn, 1934) led some authors to suggest that germ cells are differentiated from the ordinary coelomic epithelium.

Long (1964) did not observe any meiotic divisions which could have

been interpreted as gonial divisions, and early prophase changes of the first meiotic division were not observed in sufficient detail to warrant discription. Long does, however, report that all the oocytes studied were at the diplotene stage, ie., the pause in meiotic events during which vitellogenesis occurs. It is assumed that previtellogenesis in T. retusa follows a similar course, as suggested by the arrangement of nuclear material.

Clearly, further developmental studies, are required in order to elucidate the origin, fine structure and ontogeny of germ cells in brachiopods.

4.2.2 Vitellogenesis.

Both Birbeck and Mercer (1961) and later Norrevang (1968), conluded that the oocyte could be regarded as a secretory cell which forms secretory granules, but never releases the secretion to the exterior. In order to facilitate the accumulation and synthesis of these "secretory" materials during oogenesis, the developing gametes require a substantial supply of nutrients which may be derived from either reserves laid down in storage tissue and/or directly from the food supply.

Many authors recognise three basic types of yolk formation: i) autosynthetic vitellogenesis in which the yolk is synthesised within the ooplasm of the vitellogenic oocyte; ii) heterosynthetic vitellogenesis in which the yolk components are formed in extra-ovarian locations and transported to the developing oocyte; and iii) autoheterosynthetic vitellogenesis involving a combination of the above two methods (Anderson, 1974).

During autosynthetic vitellogenesis, low molecular weight precursors may cross the oolemma by diffusion or active transport (Eckelbarger, 1984). Oocytes that utilise the heterosynthetic mode characteristically accumulate macromolecular yolk components or well developed yolk bodies by means of endocytotic processes (Eckelbarger, 1984).

According to an alternative classification (Norrevang, 1968), yolk formation is autonomous if nutrients used in vitellogenesis traverse the oolemma, or heteronomous if the yolk precursors enter the oocyte via cytoplasmic bridges that connect neighbouring cells with the developing gamete. The autonomous endocytotic form of vitellogenesis as defined by Norrevang (1968) is essentially synonymous with the more widely used term, "heterosynthetic yolk formation".

Autosynthetic yolk formation has been reported in a wide variety of animals (Anderson, 1974; Heubner and Anderson, 1976). According to Anderson (1974), relatively few groups of invertebrates exhibit a heterosynthetic type yolk formation. Recent studies, however, have indicated that at least a partial contribution is made by heterosynthetic modes of vitellogenesis, and that this occurs more frequently than previously believed (e.g., Blades-Eckelbarger and Youngbluth, 1984; Eckelbarger, 1984; Charniaux-Cotton, 1985; Pipe, 1987; Eckelbarger and Rice, 1988).

Ultrastructural observations of T. retusa indicate that vitellogenesis includes both autosynthetic and heterosynthetic processes (Fig. 2.2).

The types of nutrient accumulation recognised may be defined as;

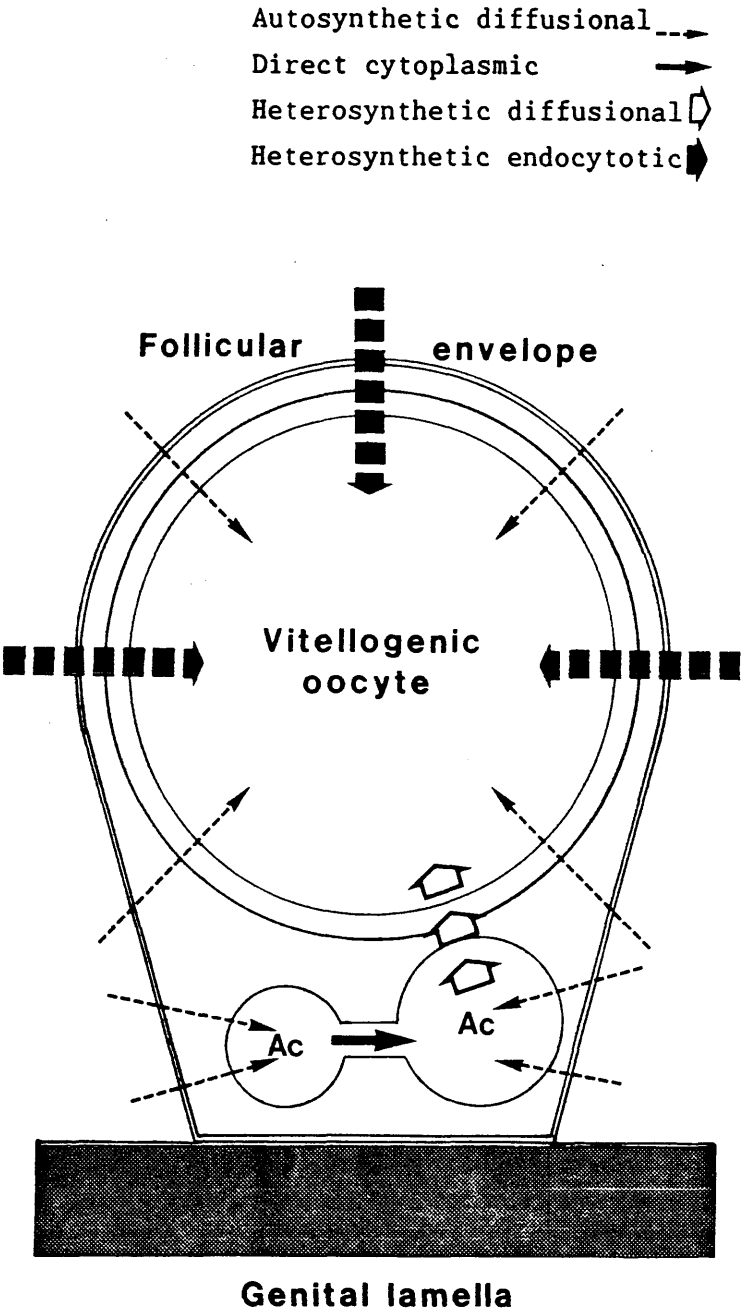
(i) Autosynthetic diffusional.
(ii) Heterosynthetic diffusional.
(iii) Heterosynthetic endocytotic.

In order to interpret the ultrastructural evidence for yolk synthesis it is necessary to visualize the follicular capsule and its contents as a functional unit.

(i) Autosynthetic diffusional nutrient accumulation.

During the early stages of vitellogenesis it appears that little of the cytological apparatus normally associated with the processes of

Fig. 2.2 Diagrammatic representation of the possible avenues by which nutrients and yolk precursors enter the vitellogenic oocyte.



synthesis (such as, endoplasmic reticulum, Golgi complexes and mitochondria) is present.

At this stage, i.e. stages I to II as defined above, it is assumed that some material passes into the oocytes by diffusion. There are structural features of the oocyte and follicular capsule which would lend themselves to such diffusion.

Presumably the follicular cells perform a dual function, in providing both mechanical support and protection. More importantly, they may serve as intermediaries between the oocytes and the surrounding coelomic fluid by engaging in the regulation of flow of materials which are subsequently to be incorporated into the oocyte. There is no evidence that material is passed from the follicle cells to the developing oocytes for yolk synthesis. Low molecular weight nutrient materials may, however, be absorbed through the oolemma in a process not rendered visible at the ultrastructural level. In the absence of experimental evidence, the definitive role of the follicular cells in oogenesis of T. retusa cannot be ascertained and it is clear that further experiments are needed to demonstrate the importance of diffusion.

(ii) Heterosynthetic diffusional nutrient accumulation.

An oocyte at the stage III phase of developement will be associated with at least one accessory cell, the origin of which is uncertain. Senn (1934) is one of the few authors to describe accessory cells in the Brachiopoda, noting their occurence in T. retusa and Gryphus vitreus. Chuang (1983a) and Sawada (1973) also observed accessory cells in the taxa they examined (see Introduction).

Ultrastructural studies conducted by Chuang (1983a) did not indicate direct contact between the nutritive cell and the oocyte. Senn (1934) is of the opinion that these "nutrient cells" are in fact aborted oocytes contained within the same follicular capsule as the developing oocyte and that a reduction in the number of these relict cells indicates that they are absorbed during vitellogenesis.

Evidence from the present study implies that accessory cells are derivatives of the oocyte. At no time during the course of these observations was more than one oocyte recorded within early follicular capsules (of stages I or II). In addition the first recorded accessory cells were rather smaller in dimension than primary oocytes.

Anderson (1974), Anderson and Huebner (1968), King (1970), Huebner and Anderson (1972), all document the incomplete cytokinesis of oogonia undergoing mitosis. This results in a differential in nuclear division which confers a certain specialisation on certain parts of the cytoplasm. The metabolism of one compartment is so specialised that it becomes the female gamete, while other compartments give rise to cytoplasmic units commonly referred to as "nurse cells". Despite the apparent absence of accessory cells during the earliest stages of oogenesis in T. retusa, it is feasible that accessory cells could also be the result of an unequal mitotic division, producing both the larger vitellogenic oocyte and the smaller accessory cell.

Ultrastructural evidence has also been presented showing the possibility of accessory cell cytokinesis, i.e. the ability of the accessory cells to divide and increase, independently of the vitellogenic oocyte.

At this point it is necessary to define the terms "nurse cell", and "accessory cell", more precisely. In terms of the function of nurse cells, morphological and experimental evidence indicates that nurse cells supply a differentiating oocyte with organelles (Wilson, 1925; Anderson and Heubner, 1968; Dapples and King, 1970; Hughes and Berry, 1970; Heubner and Anderson, 1972; King, 1972).

Accessory cells, as designated in this text, are taken to provide nutrients to the vitellogenic cell possibly in the form of yolk precursors derived from the lysis of yolk granules initially formed within the accessory cells.

All the morphological traits described in the accessory cells point towards a high level of synthetic activity, a fact borne out by the ability of the accessory cell to produce both proteinaceous and lipoid granules which closely mimic those of the vitellogenic oocyte.

Accessory cells and vitellogenic oocytes, however, appear to have only the limited cytoplasmic continuity afforded by desmosome-like gap junctions.

Gap junctions are those membrane coordinations that permit coordinated activity by providing a low resistence electrical (ionic) pathway between cells (Stryer, 1981). In connection with this Anderson (1971) wrote, "The junctions may serve a dual function a) adherence of cells b) cell to cell ion movement. Moreover, it is possible that each type of junctional complex may be involved in the transport of different ions and molecules into the oocyte".

The junctional complexes described between accessory cells and oocytes would therefore only permit the passage of low molecular weight molecules. This information, in combination with the failure of the present study to detect the migration of intact yolk bodies or organelles, suggests that yolk is first accumulated in the accessory cells. Accumulations might then be broken down into suitable precursor molecules, and transferred to the oocyte. Lysomes which could break down such molecules are prominent features of accessory cells.

The distribution of yolk granules within the cytoplasm of the accessory cells is distinctly skewed in the direction of the oolemma, as are clusters of mitochondria. The presence of the latter suggests that the passage of material to the vitellogenic oocyte is not a passive process, but one requiring energy.

Not all accessory cells are in contact with the developing oocyte. Indirect communication may also be maintained via other accessory cells, either by more desmosome-like gap junctions formed betweem interdigitating extensions of both cells, or through cytoplasmic bridges which could form as the result of incomplete cytokinesis. It seems likely that a nutrient stream of selected yolk precursors is passed between accessory cells. Such a stream may also be capable of carrying a multitude of organelles between cells united by cytoplasmic bridges. Similar cytoplasmic continuities called "ring canals" or "fusomes" have been noted in insects (Meyer, 1961).

Anderson (1974) points out an important observation by Woodruff

and Telfer, on the intercellular bridges of the saturnid moth Hyalophora cecropia. The authors concluded that movement of material in the cytoplasm of the nurse cells was unidirectional, (towards the oocyte), possibly due, at least in part, to an electrical potential gradient. Perhaps a similar mechanism directs the passage of nutrients in the accessory cells of brachiopod oocytes.

(iii) Heterosynthetic endocytotic nutrient accumulation.

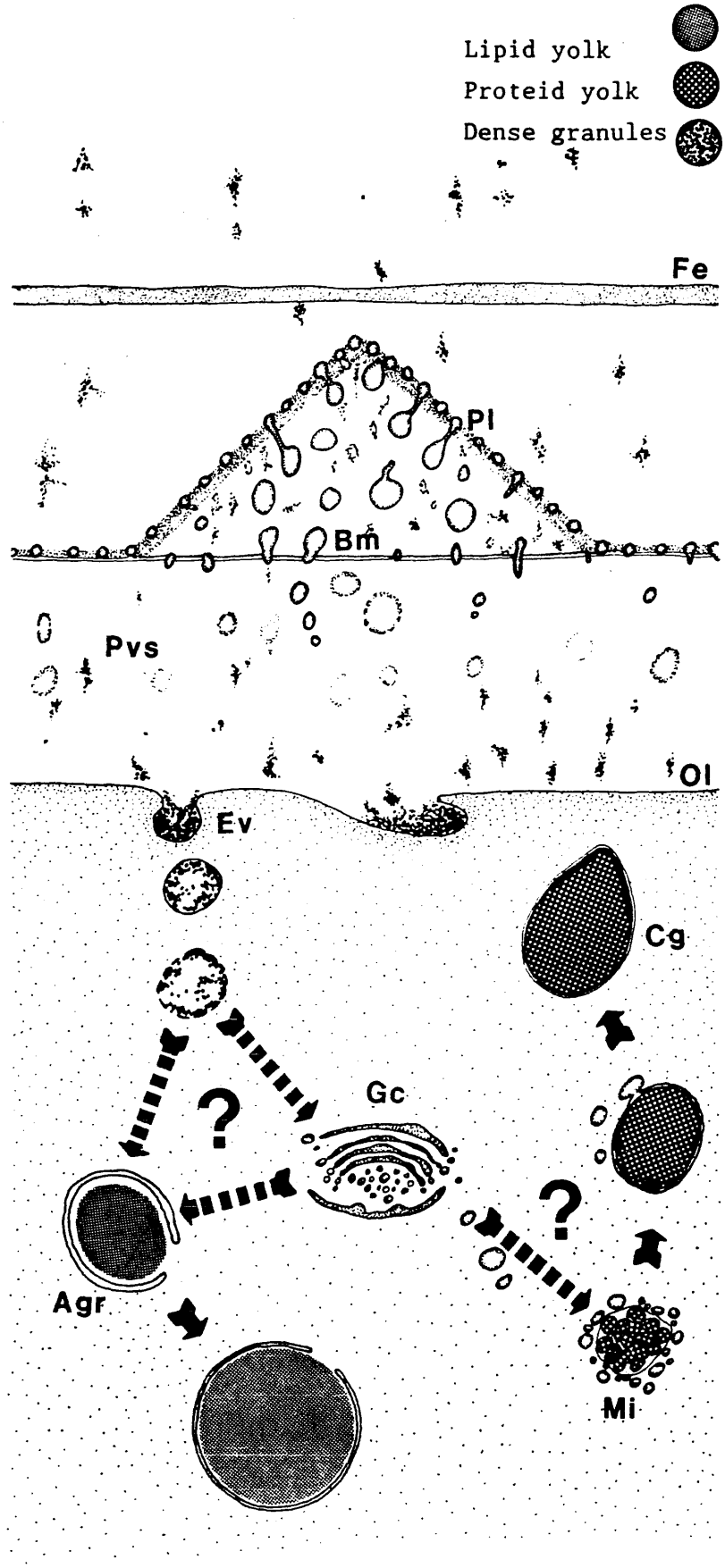
Although the functioning of the accessory cells has been discussed, the question now arises as to why the vitellogenic oocyte should require the assistance of an additional complement of cells to supplement its own efforts in yolk production ? This is perhaps best answered by examining the type of ultrastructural changes taking place in the oocyte at this stage of development. This could well affect the form in which nutrients are taken up and the efficiency with which they are acquired.

During the period of maximum accessory cell proliferation, and presumably active yolk accumulation and transport, the oolemma of the developing oocyte undergoes a distinctive process of elaboration. The increase in morphological complexity of the oolemma, (stages IV to VI), appears to serve two functions; a) it substantially increases the effective surface area to volume ratio of the oocyte, while still maintaining an overall spherical profile, and b) enhances the ability of the vitellogenic cell to endocytose a coarsely fibro-granular material, thought to be an extraovarian or heterosynthesised yolk precursor (Fig. 2.3).

The histochemistry of this ubiquitous fibro-granular material was not determined. It is present in the coelom, in the follicular capsule, in submicrovillar vacuoles and in what are assumed to be endocytotic pits in the oolemma prior to its inclusion in the ooplasm.

Although the available data suggest heterosynthetic nutrient accumulation, it should be emphasized that conventional ultrastructural studies cannot unambiguously ascertain the direction in which yolk precursors travel during vitellogenesis. To determine this it would be

Fig. 2.3 Diagrammatic representation of the possible mechanisms by which yolk granules are formed in the vitellogenic oocyte.



necessary to use labelled yolk precursors as have been employed in radioautographic and advanced ultrastructural investigations of vitellogenesis in various groups of animals (e.g., Wallace, 1978; Wallace and Selman, 1981; Telfer et al., 1982).

Despite the lack of conclusive evidence, it is thought likely that most of the detected activity is directed towards the cumulative processes of yolk formation.

The materials required for vitellogenesis may enter the vitellogenic oocyte by one of three possible avenues:- (1) through diffusion across the follicle cell and oolemma, (2) by diffusion, perhaps along an electrochemical gradient or by active transport from accessory cells as selected low molecular weight yolk precursors, or (3) by a process of endocytosis, also incorporating a heterosynthetic yolk precursor.

Stages I to VI were chosen because they identify a clear difference in the morphology of the follicular capsule and its constituents. Each stage represents an ultrastructure suited to a particular type of nutrient accumulation.

Many of the arguments regarding the process of vitellogenesis can be extended to include discussion of the economics of nutrient acquisition and the consequences of possible trade-offs in the apportionment of nutrients between the maintainance of the oocyte, the elaboration of cytological structures to facilitate the uptake of nutrients, and ultimately the synthesis and accumulation of yolk.

A scenario which might explain the different modes of nutrient accumulation during vitellogenesis in T. retusa, is that the demands of maintainance and the development of a complex microvillar envelope during the early phases of vitellogenesis exceed the ability of the oocyte to acrue sufficient nutrients to fuel these processes.

An accessory cell, which formed from an unequal division of the oocyte, during the earliest stages of oogenesis, proliferates by division. Accessory cells do not invest energy in the production of a

complex outer membrane, remain small in comparison to the oocyte, and accumulate nutrients which can be transferred to the vitellogenic cell.

The production of a small body of cells capable of supplementing the oocyte may be an inexpensive mechanism for increasing the effective surface area available for the absorption of nutrients. Moreover, by supplying materials in the form of partially synthesised yolk precursors, it is possible that the oocyte has to expend less energy to refabricate the yolk than would be expected if less refined yolk elements were acquired for autosynthesis. Indeed Gremigni and Nigro (1983) maintain that heterosynthetic yolk formation is more efficient than autosynthetic vitellogenesis. It might therefore be advantageous for the cell to adopt a morphological design capable of taking up a yolk precursor that has been partially synthesised outside the follicular capsule. This would be achieved in T. retusa by expending energy during the early stages of vitellogenesis to produce a complex elaboration of microvilli.

It is also possible that the pattern of events described in T. retusa could also be a reflection of nutrient availabilty rather than the proposed cytological development towards a more efficient mode of yolk accumulation. Alternatively, and perhaps more likely, it is conceivable that this simply reflects the inability of any one mode of nutrient acquisition to supply all the elements necessary for vitellogenesis.

4.2.3 Development of Yolk Granules.

Having established the possible mechanisms by which nutrients enter the differentiating oocyte, and gaining some knowledge of the nature of materials available for vitellogenesis, it now remains to characterise the types of yolk granule produced and to discuss the interactions of organelles which may lead to their synthesis (Fig. 2.3)

Two quite distinct forms of yolk granule are accumulated in T. retusa in addition to a host of organelles which, if not associated directly with yolk synthesis, are stored for utilization after the egg has been fertilised.

Histochemical investigations indicate that lipid droplets or granules are the major yolk component. Long (1964) used the light microscope to identify lipid droplets in Terebratalia which were presumed to be neutral and did not stain for phospholipids. Chuang (1983a) also documents the presence of lipid droplets. The spheroidal lipid granules identified in the oocytes of T. retusa are lacking in a limiting membrane and almost without exception, are partially enveloped by a single profile of what is presumed to be agranular endoplasmic reticulum. These two components are intimately associated from the earliest recognisable stages of lipid granule formation.

The occurrence of agranular endoplasmic reticulum is widespread throughout the ooplasm of T. retusa. Literature on oogenesis, however, makes little mention of this reticulum. It appears to be generally accepted that this cyto-membrane plays only a limited role in oocytes. Anderson (1974) states that oocytes in which the ooplasm contains an abudance of smooth endoplasmic reticulum have only rarely been observed.

The evidence presented here suggests that the agranular form of the endoplasic reticulum is the most conspicuous, and although morphology alone is often a poor index of function, it would seem likely that agranular endoplasmic reticulum is directly associated with the production of lipoid yolk.

Anderson (1974) reports the occurrence of lipids in agranular endoplasmic reticulum, though opinion is divided as to whether this lipid is taken in by pinocytosis, or whether it diffuses into the cell as a monoglyceride, and fatty acid micelles and is resynthesised to a triglyceride by the reticulum. Isolated microsomes, presumably derived from the agranular endoplasmic reticulum, have been shown to be capable of esterification of triglycerides with palmityl coenzyme A, suggesting that agranular endoplasmic reticulum is probably the principal intracellular site for the resynthesis of triglyceride (the sub-units from which lipid molecules are constructed (Fawcett, 1981).

Glycogen is also a prominent constituent of the ooplasm. Its mode of entry into the oocyte and the form in which it is present i.e.

Alpha, Beta or Gamma (Drochmans, 1962) was not determined, though it is thought to be represented as fine granules. Some authors have proposed that some forms of agranular endoplasmic reticulum may be involved with the synthesis and/or degradation of glycogen, and it has been suggested that agranular endoplasmic reticulum may be subdivided into several more or less distinct functional categories, on the basis of their biochemical properties (Fawcett, 1981).

The investigations of Terebratalia by Long (1964) revealed cortical granules which were positive to the histochemical tests for tyrosine, basic amino acids, and sulphidryl groups (Chevremont method), but negative to the tests for nucleic acids and PAS. Chuang (1983a) and Sawada (1973) also describe cortical granules but make no mention of their histochemistry. The cortical granules of T. retusa give a strongly positive reaction with bromophenol blue, indicating a proteinaceous component, but show no response to PAS. Comparative studies dealing with the origin of these important structures appear to agree that they are fabricated by the conjoined efforts of the rough endoplasmic reticulum and the Golgi complex (Anderson, 1974).

It is generally believed that rough endoplasmic reticulum and free ribosomes are both involved in protein synthesis within the cell (Stryer, 1981). Birbeck and Mercer (1961) found that cells secreting proteinaceous material have an elaborate rough endoplasmic reticulum while the so called "retaining cells", which do not secrete the proteid products but retain them within their cell membranes, are characterised by numerous free ribosomes. Indeed, numerous examples of these organelles are present within the ooplasm of T. retusa, and as pointed out by Chuang (1983a), and the high pyronophilia during the early stages of oocyte growth in Terebratalia (Long, 1964) and its decline in later stages may be an indicaton of the abundance of ribosomes during the early stages of vitellogenesis and their subsequent decrease. A similar pattern has been reported in Frenulina (Chuang, 1983a). It is usual for all proteid yolk granules to be surrounded by a continuous membrane (Anderson, 1972), and this is certainly the case with T. retusa.

The fusion of vesicles is taken to indicate a possible mechanism for their growth. These results are presumed to be the product of the

conjoined efforts of the Golgi complexes and either rough endoplasmic reticulum or free ribosomes with the proteid yolk granules. Many of these protein based yolk granules appear to undergo a process of both maturation and migration, during which they are transformed into cortical granules in the cortex of the vitellogenic oocyte.

Clearly not all the proteid yolk is destined to become cortical granules, but the present study provided little information on their eventual fate. These granules are no different to "immature" cortical granules in terms of their morphology.

Long (1964) identifies two other forms of yolk granule, designated 'A' and 'B'. The PAS positive 'A' granules appear during 'stage 3' in the Terebratalia oocytes and are sometimes in association with the Golgi complexes. Chuang (1983a), presumes these to correspond to oval vesicles with filamentous interiors described in Frenulina oocytes.

The 'B' granules of the Terebratalia oocytes, which are distinctly PAS positive and also positive with methods for phospholipids, are presumed by Chuang (1983a) to represent the microsomes of Frenulina oocytes because they fall within the expected size range.

The histochemical techniques employed in the present study were not sufficiently specific to make any useful comparisons with the "A" and "B" granules of Long (1964).

4.2.4 Spawning, Atretia and Phagocytosis.

Oogonia occur as a pool at the base of the genital lamella and vitellogenic cells migrate to progressively more distal regions of the lamella eventually being liberated into the vascula genitalia to complete development. Evidence suggests that the apparent distal migration is due to the elongation of the genital lamella.

Once the oocytes have been released, it is assumed that the extended lamellal region is phagocytosed along with any other necrotic material remaining within the gonad after spawning.

Recently postspawned individuals contain quantities of amoeboid cells which superficially appear as red to orange or brown pigmented globules. These are phagocytic cells which in breaking down the genital lamella and atretic oocytes, may concentrate those pigments which impart colour to the oocytes and ultimately the appearance of the gonad.

The coelomocytes of articulates were described for species of Terebratulina by Morse (1902) and Prenant (1928) and for Terebratalia coreannica and Coptothyris grayi by Ohuye (1936, 1937). Those forms noted in Terebratulina were amoeboid cells as well as pigmented and non pigmented granulocytes. The spherical or hyaline amoebocyte is phagocytic and agglutinable and appeared to be common in the coelomic fluid of the brachiopods, often being regarded as equivalents of blood corpuscles (Hyman, 1959). The granulocytes were reported to contain colourless to red, orange or brown spherules. The red globules were reported as not being lipochromes or any haemoglobin-like substance. The brown granules, however, gave positive lipoid tests. Lipid granules were also detected by Prenant (1928) in the hyaline amoeboid cells. Heller (1931) noted an amoeboid phagocytic type in Hemithiris. Oosorption in Frenulina sanguinolenta has been observed to occur in flattened cells which engulf small spheres of fragmented ovum and in large subspherical cells, 40 μm in diameter with a nucleus 5-6 μm in diameter (Chaung, 1983a).

Additional studies by the author, of other species of brachiopod have revealed the presence of similarly pigmented globules, to those detected in T. retusa, and a brief review of the literature reveals that this phenomenon may not be unique to the Brachiopoda (Nimitz, 1976). The ability to resorb and utilise the valuable reserves represented within the gonad, and not expended during spawning, would certainly prove an asset, particularly when the investment in any given propagule is high.

In order, to clarify the processes constituting vitellogenesis, it is of paramount importance to understand the biochemical nature of the oocyte, and more precisely, to be able to relate biochemical activity to function. Presumably, by employing advanced ultrastructural labelling techniques, it will in future be possible to follow the

passage of nutrients or selected yolk precursors from source. Although, the present study suffers the obvious limitations of an essentially morphological approach, the philosophy, where possible, has been to interpret form in terms of function. For this reason the six stages of vitellogenesis defined, differ from those of both Long (1964), and Chuang (1983a).

4.3 SPERMATOGENESIS.

4.3.1 Spermatogonia and spermatocytes.

In the male, light microscopy reveals the presence of a body of large faintly basophylic cells covering the base of the genital lamella, proximal to the inner mantle epithelium. These proliferating clusters of cells are assumed to be spermatogonia, presumably the derivatives of primordial germ cells. Each spermatogonium contains an irregularly mottled assemblage of chromatin and conspicuous nucleoli (Plate 86).

The spermatogonia enlarge to become spermatocytes and, assuming the normal course of events, these in turn divide through two meiotic divisions, forming spermatids which mature into spermatozoa. During maturation the developing spermatozoa are moved away from the genital lamella by repeated cell divisions beneath. The various developmental stages form bands during early gonad maturation, which are distinguished by size, density and differential staining propensity. Mature spermatozoa occur around the periphery of the masses of spermatogenic and spermiogenic cells, their tails issuing into the lumen of the vascula genitalia which is gradually occluded as maturation of the gonad proceeds (Plate 88).

Primary spermatocytes are clearly visible attached to the margins of the genital lamella, particularly in proximal regions. The base of each cell is intimately associated with the connective tissue of the genital lamella both displaying some degree of interdigitation along the crenellated boundary (Plates 86, 88).

It is assumed that the cells which intersperse the primary spermatocytes are peritoneal. Most are pleomorphic and contain prominent lipid granules. No transition stages were detected between these assumed somatic cells and spermatogonia.

The nucleus of the primary spermatocyte may be distinguished from that of neighbouring somatic cells as it is generally much larger, measuring approximately 4 μm maximum diameter (Plate 87). In later stages, the nucleus contains sparsely granular chromatin. The majority

of the chromatin is condensed against the inner side of the nuclear envelope, which is a bilayered membrane, lacking nucleopores. The finely granular cytoplasm contains several mitochondria as well as profiles of granular endoplasmic reticulum and ribosomes. These inclusion tend to be grouped into specific regions within the cytoplasm (Plate 89).

Secondary spermatocytes, the product of the first meiotic division of the primary spermatocytes, differ from the parent cell in possessing extremely condensed nuclear and cytoplasmic material. As no transition stages were observed it is possible that this meiotic division and subsequent condensation are a rapid process (Plates 89, 90).

Few inclusions can be identified due to the extreme electron density of the secondary spermatocytes, a problem compounded by the practical difficulties of cutting ultra-thin sections of sufficient quality to resolve these inclusions. Each secondary spermatocyte appears to carry a complement of two mitochondria which are larger than in the primary spermatocyte and displaced to one side of the pyriform nucleus measuring approximately 2 μm maximum diameter (Plate 90).

4.3.2 Spermatids and spermiogenesis.

Secondary spermatocytes undergo a second meiotic division to form spermatids (Plate 91). In the two spermatocyte divisions the various cytoplasmic inclusions, are usually distributed equally to the four spermatids. This seems to result from the tendency of such inclusions to be grouped, possibly near the equator of the cell or about the poles of the mitotic figure, so that cytokinesis separates them into more or less equal groups.

Spermatids possess cytoplasm which is less electron dense than secondary spermatocytes and consequently its inclusions are more easily identified. Early spermatids contain a spherical nucleus with chromatin of a coarsely granular, but slightly condensed nature typically measuring about 1.5 μm in diameter. A single pyriform

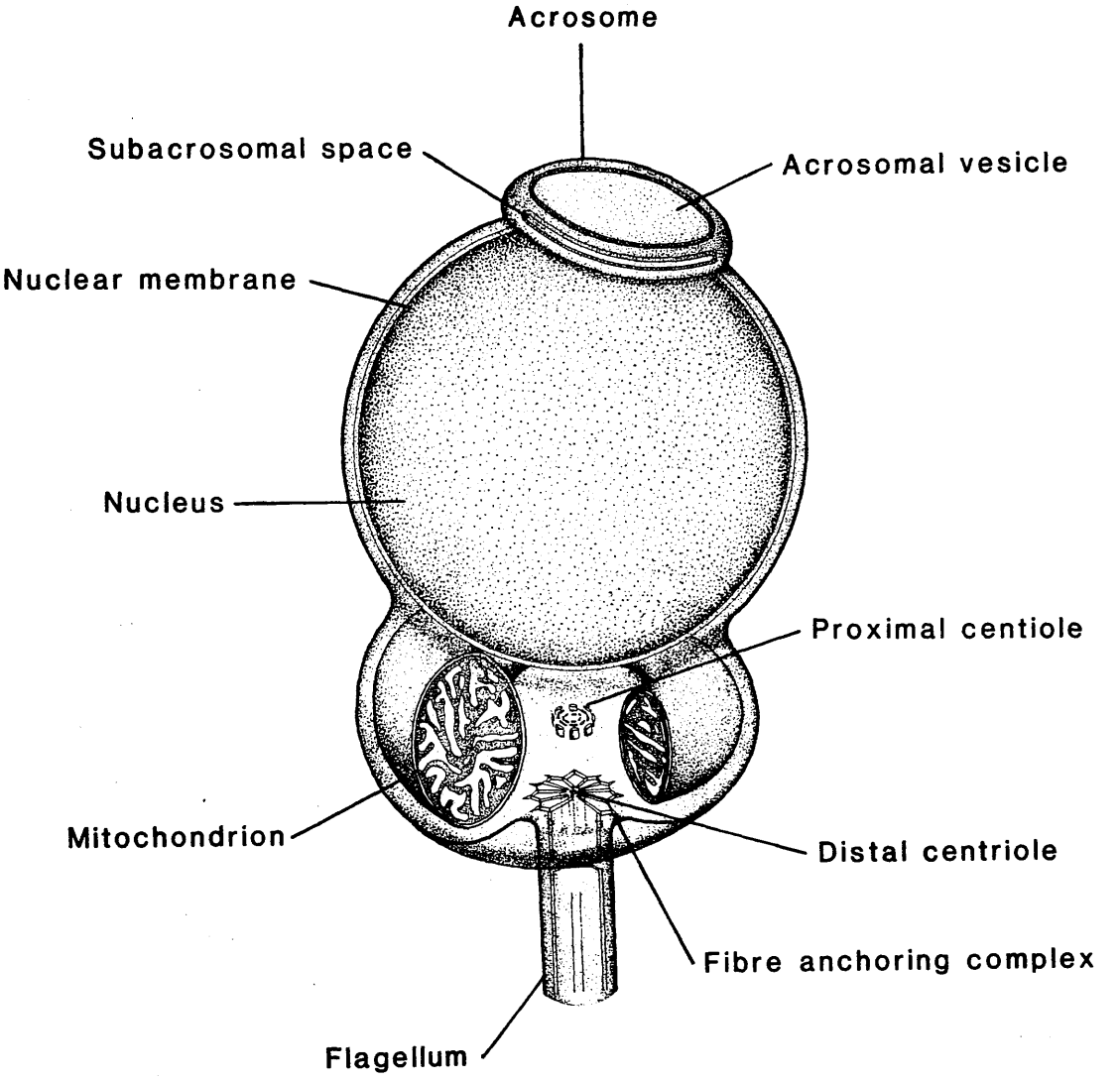
mitochondrion occupies a position which is assumed to correspond to the posterior pole of the cell (Plate 92).

Each spermatid also contains two centrioles which were observed in a variety of locations within the cytoplasm. A Golgi complex is also a feature of the cell and is seen in most spermatid stages. Since the most prominent function of the Golgi complex in most animals is the formation of the acrosome, the presence of this organelle may be taken as an indication of acrosome formation, (Franzen and Sensenbaugh, 1984).

Eventually, both centrioles come to lie at the posterior pole of the cell. It is assumed that one of these gives rise to a filament which first appears within the cytoplasm and that this is the axial filament of the future tail of the spermatozoon. The mitochondrion enlarges and assumes an assymetric ring-like form. The centrioles then migrate, by a process which was not observed, to the centre of the mitochondrial ring (Plates 93, 95). The proximal centriole is in approximately the same longitudinal axis as the distal one. This is suspended in a complex anchoring fibre apparatus with nine primary branches which bifurcate. The resulting branches fuse with the neighbouring ones to form a stellate figure as described by Afzelius and Ferraguti (1978). The sperm tail appears to issue from this anchoring complex initially as peripheral microtubules, the central doublet apparently originating from a more distal region (Plate 94). The sperm tail of T. retusa is about 50 μm long with the familiar 9x2+2 arrangement of microtubules (Plate 96).

In T. retusa, the acrosomal vesicle is positioned anteriorly and partially displaced to one side of the longitudinal axis. Initially, a flat disc about 0.05 μm thick and 0.7 μm in diameter, the acrosome is separated by a very narrow subacrosomal space from the nucleus (Plate 97). The acrosome of mature spermatozoa is rather more conical, the acrosomal vesicle swollen with a mostly electron translucenet material and a sparsely granular aggregate, thereby increasing the thickness of the acrosome to approximately 0.15 μm (Plate 98). The spherical nucleus is 1.5 μm in diameter, and has uniformly dense nucleoprotein in the spermatozoan. It's nuclear envelope has no pore complexes and no indents for the acrosome, mitochondrion or centrioles (Plate 94).

Fig. 2.4 Diagram of the head of a Speratozoon. The Mitochondrion has been cut away to reveal the Fibre Anchoring Complex.



The major ultrastructural features of the spermatozoa are summarised in a schematic diagram (Fig. 2.4).

It is assumed, as in many other animals, that excess cytoplasmic material remaining after the completion of spermiogenesis is sloughed off. This free protoplasm is then presumably phagocytosed. No ultrastructural evidence was found to substantiate the presence of phagocytes in testes. Early post-spawned males were, however, recorded through light microscopy, as showing a red-orange pigmented aggregation of cells in the testis, similar to those documented within the ovary of recently spawned females. Some of these cells could be phagocytic (Plate 102).

4.3.3 Nutritive Cells.

Clusters of nucleated cells charged with lipid are often apparent during the early stages of gonad maturation, and tend to form a band around the margin of proliferating masses of spermatocytes. In later stages of gametogenesis these cells were observed ultrastructurally.

Generally pleomorphic, the cells contain aggregations of densely staining lipid granules which may exceed 2 μm (Plate 102). The remaining cytoplasm is sparsely granular and may be punctuated by glycogen rosettes. Occasionally, secondary spermatocytes and spermatids appear to be embedded within the cytoplasm (Plate 103). These cells may act in a nutritive capacity, introducing materials directly into the dense mass of proliferating gametes (Plate 104). The origin, prior to gametogenesis, or the fate of these cells, post spawning, was not determined.

4.3.4 Spawning.

The gametes within the testis multiply, eventually filling the vascula genitalia with a mass of densely packed and inactive spermatozoa (Plate 99, 100). Spawning proceeds much as described for the female, with the spermatozoa being exuded through the nephridiopore, aided by currents created by the amply ciliated surface

of the nephridial funnel. Once in the brachial cavity, the gametes join the exhalent stream of the lophophore current and are thereby broadcast into the surrounding water, whereupon the spermatozoa achieve full motility. Individuals engaged in spawning may be readily identified by presence of a white, faintly turbid cloud around the commisure of the gaping valves.

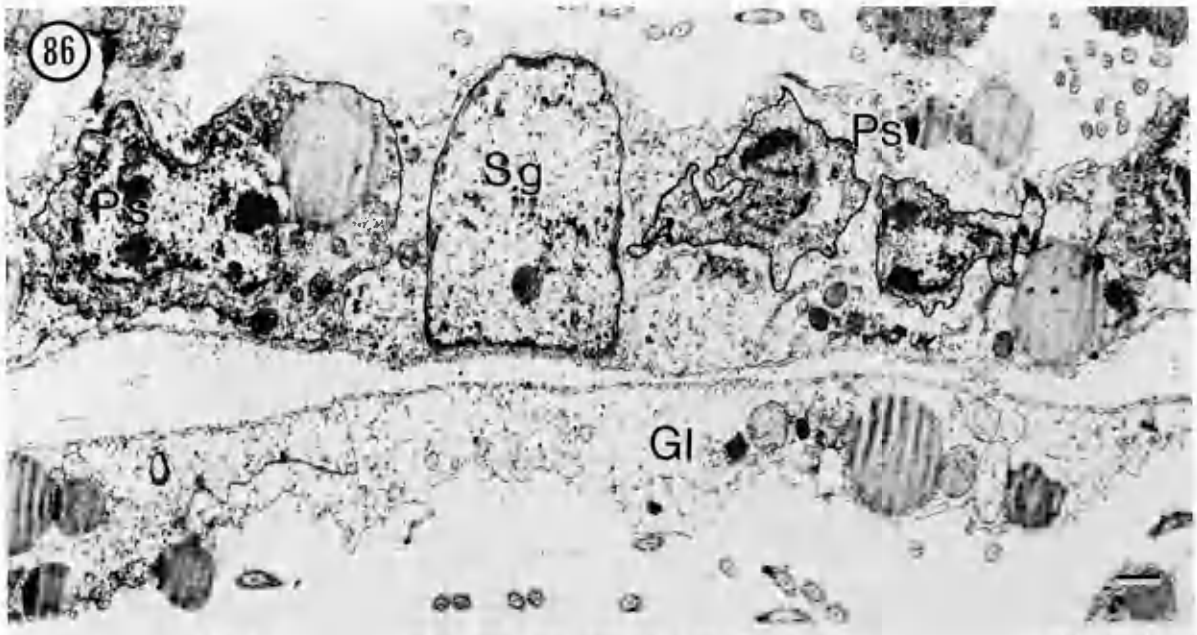
PLATES: 86–104

Plate 86. TEM of a section through part of the genital lamella. The large nucleus at the center of the plate may be that of a spermatogonium. Primary spermatocytes are outlined. Scale bar = 1 μm

Plate 87. A spermatogonium containing a well defined nucleus, nucleolus mitochondria and endoplasmic reticulum. Scale bar = 1 μm

Plate 88. Light micrograph showing the structure of the testis with a vertical section through the genital lamella. Spermatogonia generally occur at the base of the lamella as a cluster of faintly basophilic cells. The large intensely staining secondary spermatocytes are clearly visible towards the distal regions of the lamella. Scale bar = 10 μm

Plate 89. Primary spermatocytes are intimately associated with the genital lamella. The primary spermatocyte divides to produce secondary spermatocytes. Scale bar = 1 μm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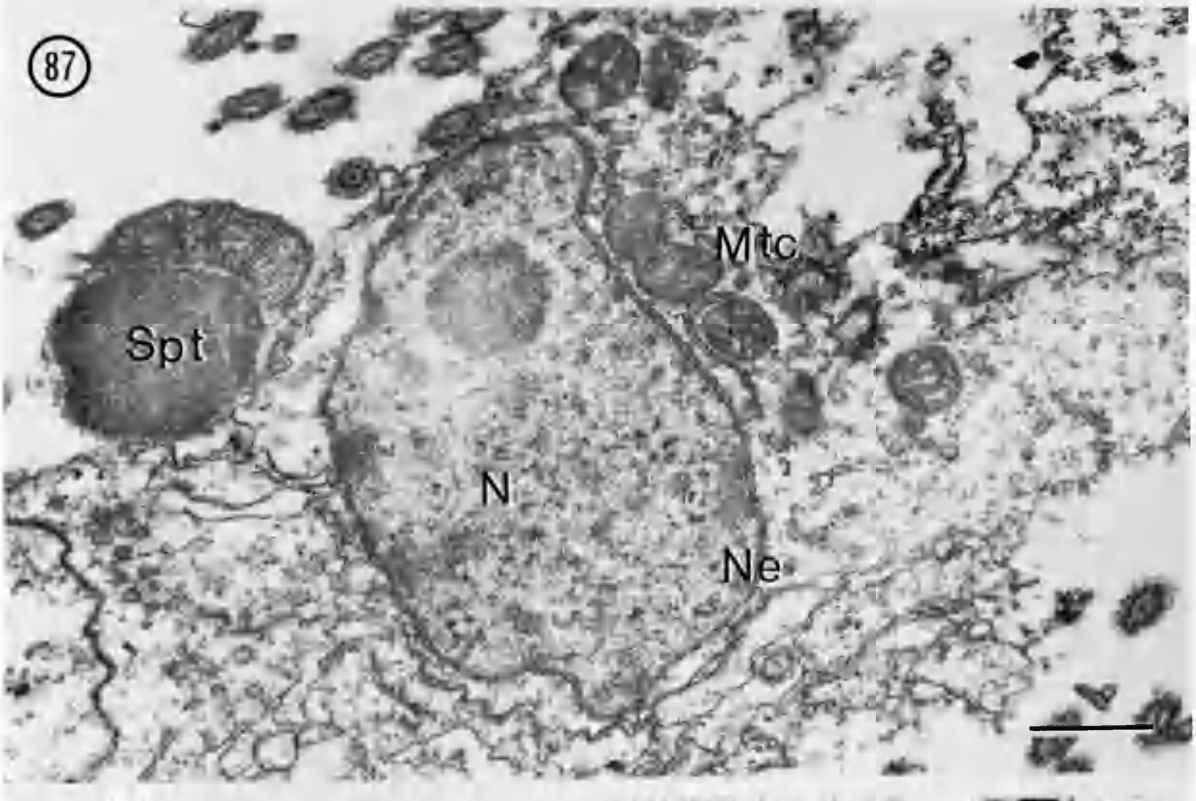
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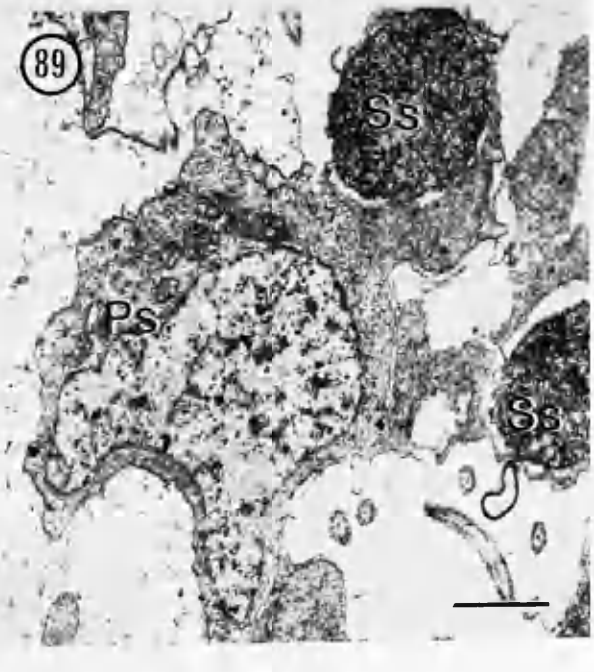
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Plate 90. Primary spermatocytes divide meiotically to produce two intensely staining secondary spermatocytes. Each secondary spermatocyte contains a number of mitochondria. Scale bar = 0.1 μm

Plate 91. Secondary spermatocytes undergo a second meiotic division to form two spermatids. Lipid granules are common. Scale bar = 0.25 μm

Plates 92 - 93. Spermatids are less electron dense than secondary spermatocytes. Each spermatid contains a nucleus and, a single, large mitochondrion, a Golgi complex. Centrioles are sometimes visible. Scale bar = (92)0.25 μm, (93)0.1 μm

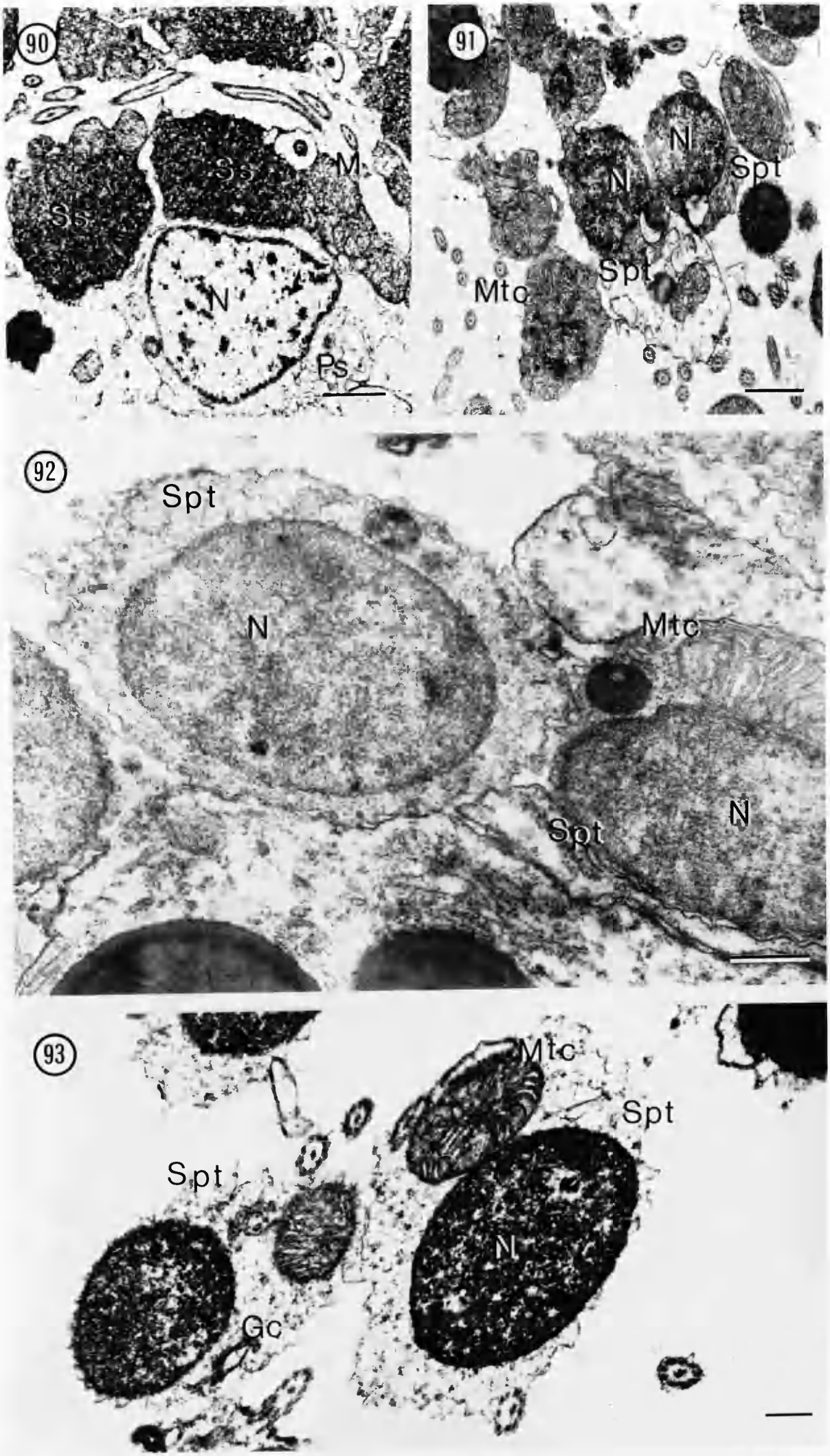
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Plate 94. Tangential section through the head of a mature spermatozoon. The acrosome was not sectioned. Scale bar = 0.1 μm

Plate 95. Transverse section through the asymetric ring of the mitochondrion showing the stellate anchoring fibre complex. Scale bar = 0.2 μm

Plate 96. Transverse section of the tail of a spermatozoon revealing the 9x2+2 system of microtubules. Scale bar = 0.05 μm

Plate 97. The acrosome is possitioned anteriorly and in vertical section appears as a flattened disc in immature spermatozoons. Scale bar = 0.1 μm

Plate 98. The acrosomal vesicle of the mature spermatozoon is conical in profile when sectioned in the vertical plane. Scale bar = 0.1 μm


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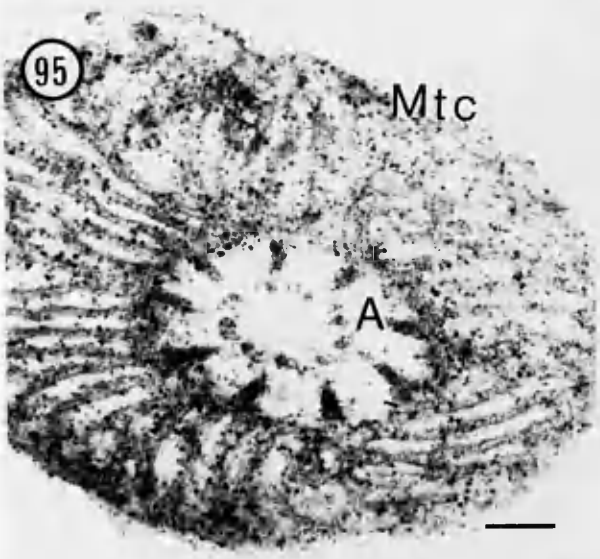
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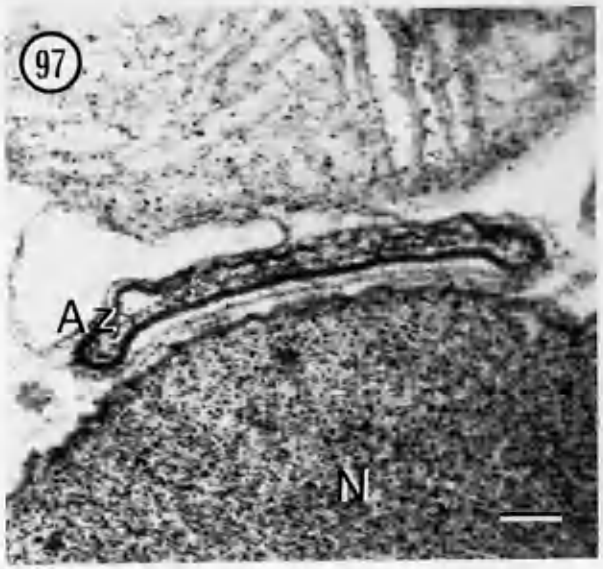
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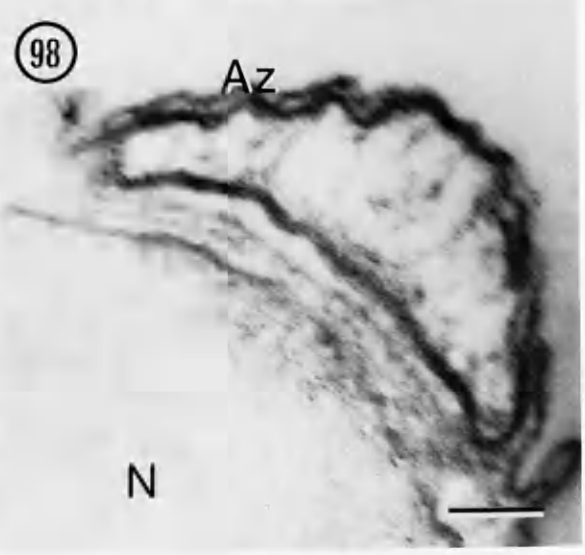
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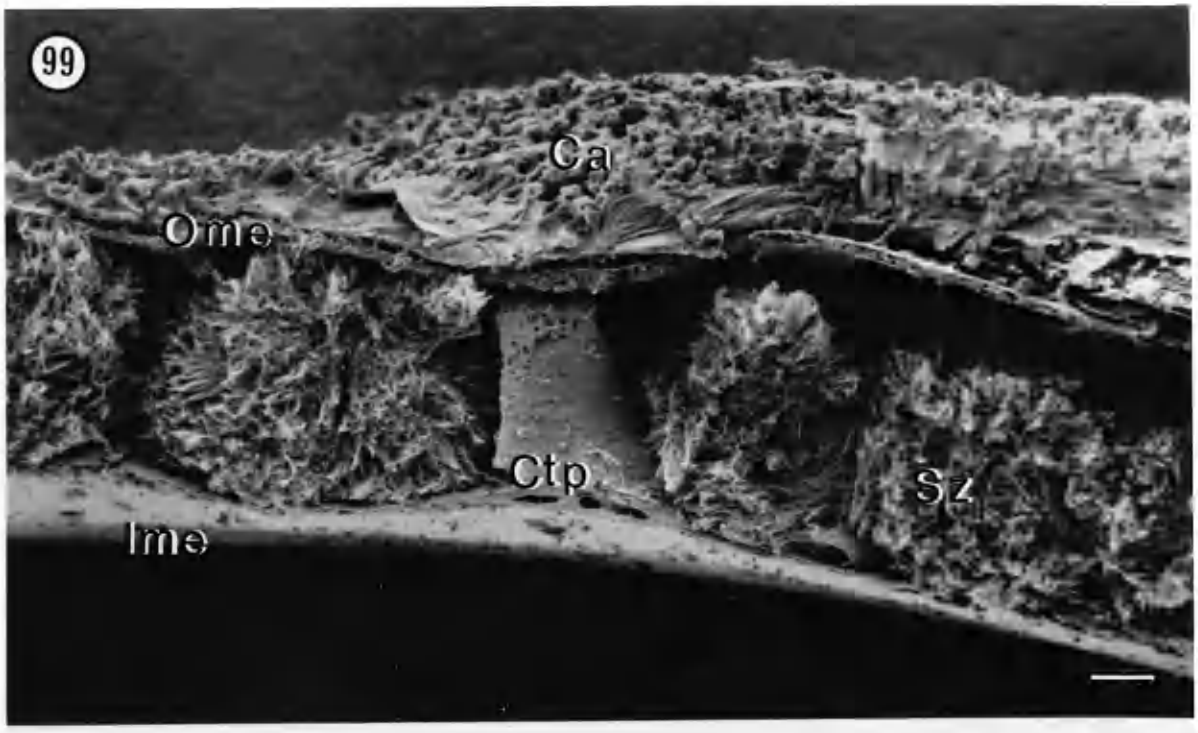
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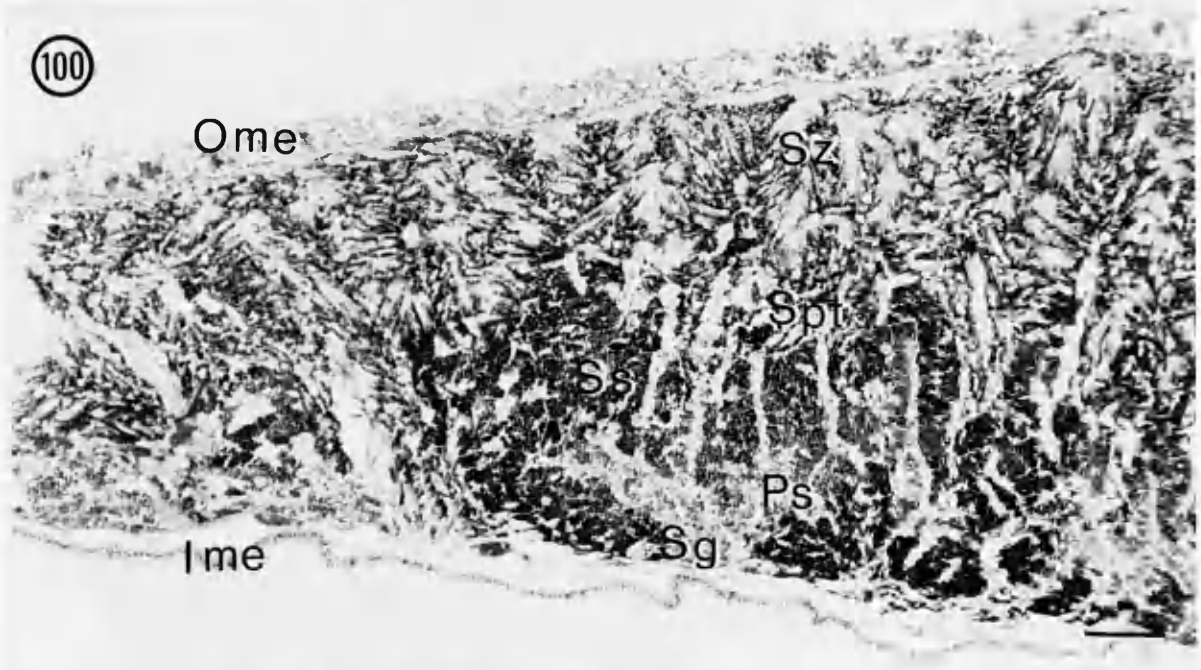
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Plate 99. SEM of a vertical section through a mature male gonad. Scale bar = 100 μm

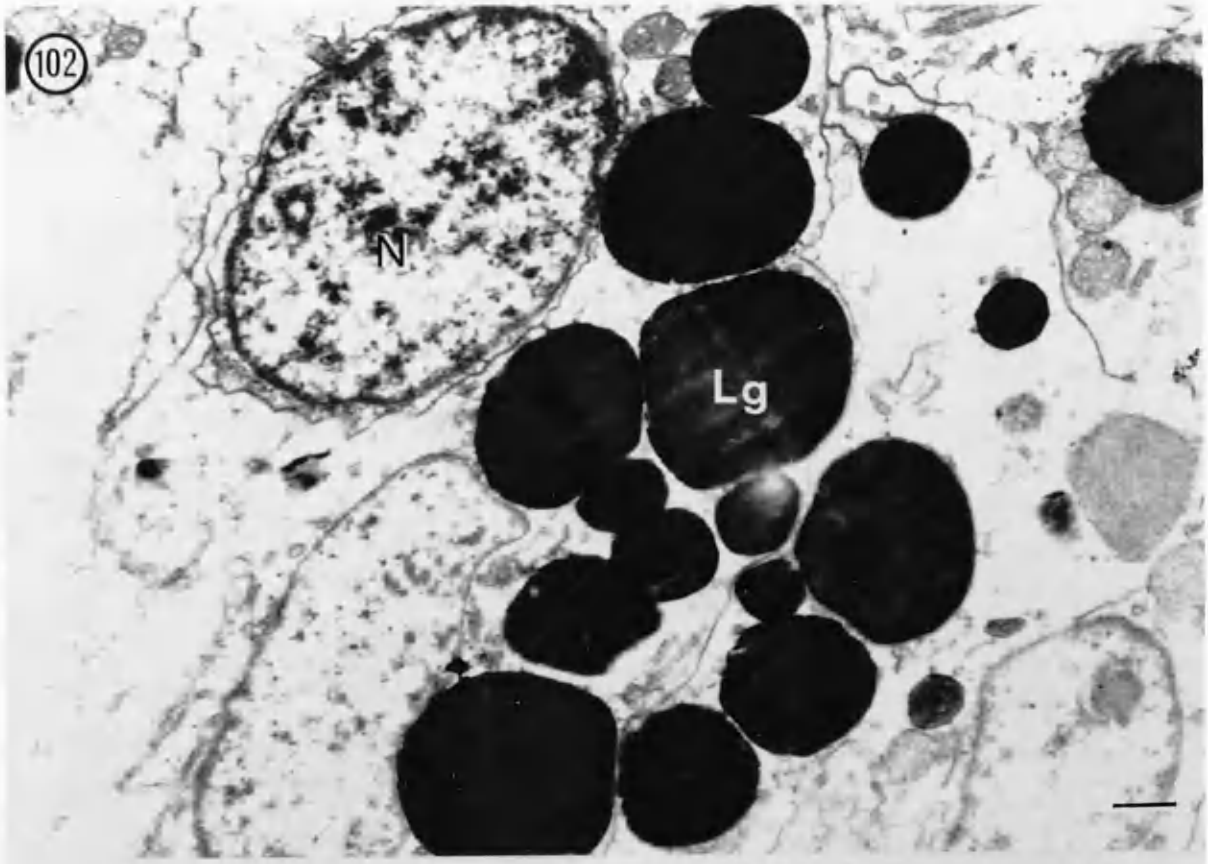
Plate 100. Light micrograph illustring the density of developing cells within maturing testes. Scale bar = 20 μm

Plate 101. A cluster of mature spermatozoa. The profile of the acrosome, nucleus and mitochondrion are clearly visible. Scale bar = 5 μm

Plate 102. Clusters of lipid granules are commonly embedded in the ting proliferamasses of cells within testes. Scale bar = 0.25 μm

Plate 103. Lipid granules were often observed within the cytoplasm of cells nucleated(outlined). Scale bar = 1 μm

Plate 104. Light micrograph of a vertical section through a testis showing lipid material. Scale bar = 5 μm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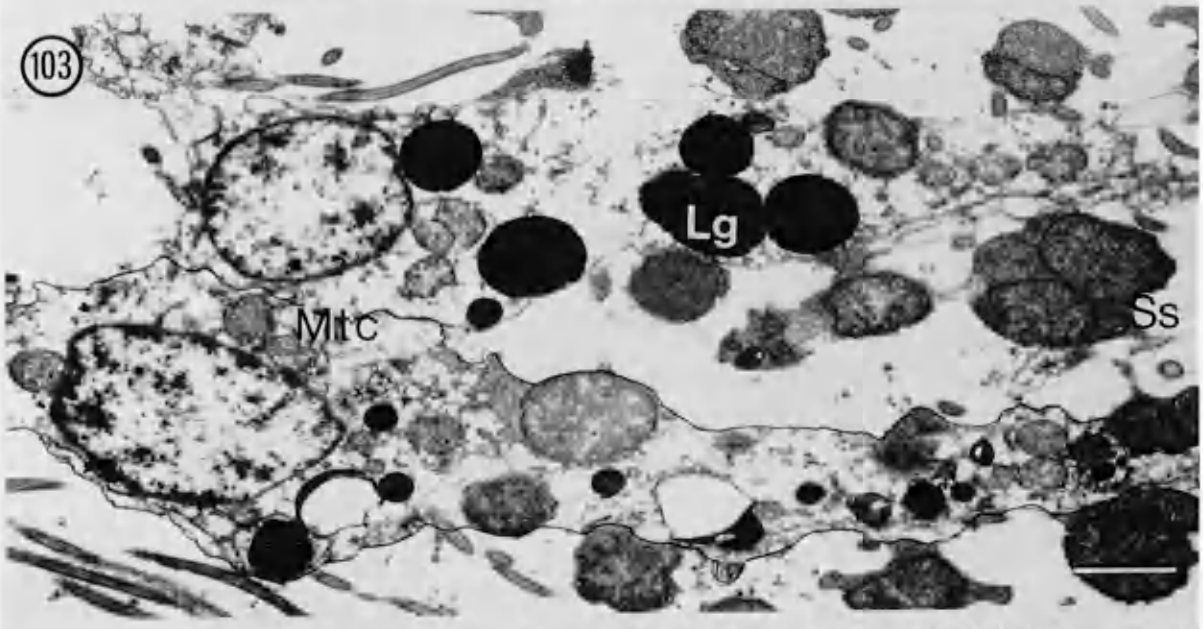
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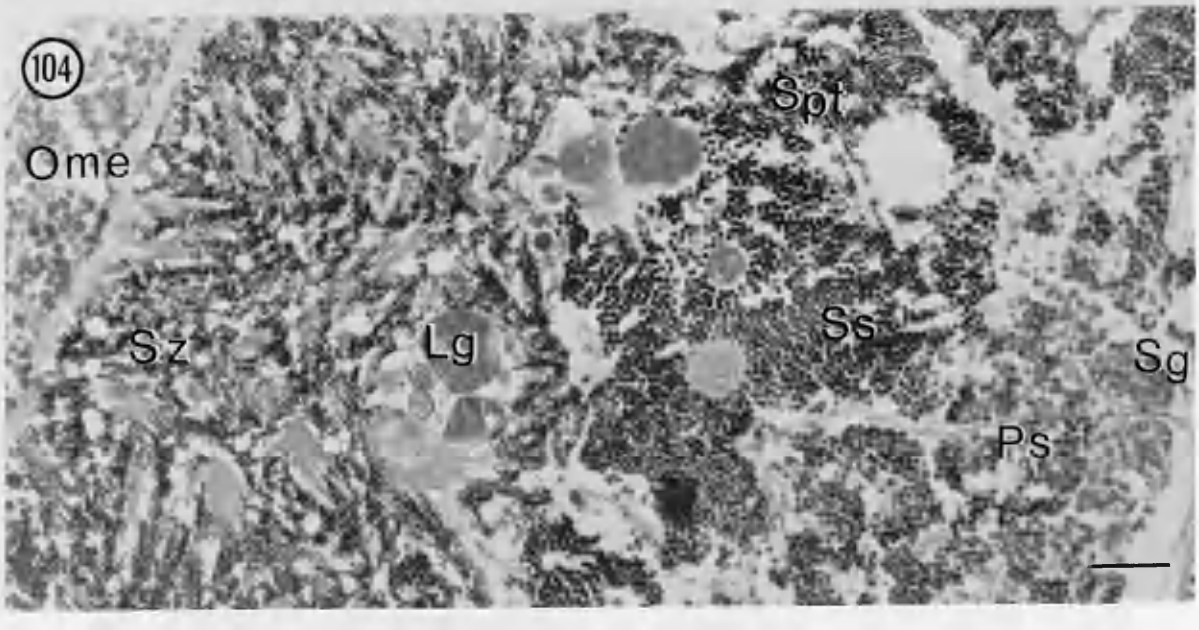
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4.4 DISCUSSION.

4.4.1 Spermatogenesis.

Sawada (1973) and Chuang (1983b) detail spermatogenesis in the inarticulate Lingula unguis and L. anatina respectively. Both conclude that primary germ cells are distinguishable by their large size from the coelomic epithelium of the ileoparietal band. Yatsu (1902) reported progenitors in Lingula larvae at the stage of 8 pairs of cirri and this is taken to indicate an early segregation of the germline from the somatic cells. Presumably these germ cells in the larval stage are tracable to a single germ cell which separates from the somatic cells to start a germ line during an early stage as in other groups of animals.

Observations made in the present study suggest that male germ cells occur as a separate population of cells in the gonad of T. retusa as no transition stages were recorded between somatic and reproductive cells.

As far as the limited evidence presented here is able to show, the course of early development is common with most other animals. The mitotic production of a large primary spermatocyte, which undergoes meiosis to produce two secondary spermatocytes, is followed by the second meiotic division, resulting in the formation of four spermatids. The latter proceeding through a series of quite distinctive morphological changes to produce a motile gamete, the spermatozoon.

4.4.2 The Spermatozoon.

Once mitosis of the spermatogonia are completed, the general stages in spermatogenesis as well as in the organelles observed in the developing sperm of T. retusa are similar to those described for other animals.

The extensive diversity of spermatozoan structure is related to the physiological demands of the fertilisation environment (Franzen, 1956; Afzelius, 1972). The sperm described here follow a common

structural pattern in having a flattened cap-like acrosome and a spherical nucleus, a cristate mitochondrion and two centrioles, the distal of which forms the basal body of the axoneme which has a 9x2+2 arrangement of microtubules. This morphology is generally seen in animals where the gametes are released into the surrounding water for fertilisation to take place (Franzen, 1982).

Spermatozoa deposited in an aqueous prefertilisation milleu and which fertilise externally have rounded or bullet-like forms and vigorous locomotor powers. They demonstrate radial symmetry and have been called "primitive" sperm because of their simple morphological form and because they are characteristic of animals which discharge sperm directly in water (Franzen, 1956; Franzen, 1970; Afzelius, 1972). Rouse and Jameson (1987) named the sperm of externally fertilising animals "ecto-aquasperm", removing the phylogenetic implications of the term "primitive sperm" (Retzius, 1904; Franzen, 1956).

Whereas brachiopods have spermatozoa of the ecto-aquasperm type, those of the other lophophorate phyla (Phoronida, Bryozoa,ectoprocta) have not (Franzen, 1956).

The comparative work of Afzelius and Ferraguti (1978), reflects the desire of many ultrastructural workers of that era to attribute phylogenetic interpretations using morphological differences as indices. In documenting the structure of two quite distinctive groups from the phylum, namely; Neocrania anomala, and Terebratulina retusa the authors conclude that although, both belong to the "primitive type of spermatozoon," they are very different. The T. retusa sperm has many of the features attributed to spermatozoa from the lower deuterostomes, and that the N. anomala spermatozoon seems slightly closer to those of the lower protostomes. This is taken to indicate that protobrachiopods were close to the branching point between the protostome and deuterostome lines, with the ancestors of the articulates closer to the latter branch.

Ultrastructurally, the spermermatozoa display a number of interesting features. Afzelius and Ferraguti (1978), first reported the existence of branched stellate rays emenating from the distal

centriole, forming a complex anchoring apparatus. The spermatozoon of T. retusa has the unusual feature of a single mitochondrion around the anchoring apparatus. This also occurs in the ecto-aquasperm of species in several other invertebrate phyla eg. the anthozoa Metridium sp. (Afzelius, 1979), the echiurid Ikedosoma gogoshimense (Sawada et al., 1975), the polychaeta with Owenia fusiformis (Rouse, 1988) Chaetopterus pergamentaceus (Anderson and Ekberg, 1983) and Nereis virens (Bass and Brafield, 1972) and is a feature of the echinoderm sperm (see Jamieson, 1985 for a review). Therefore it would seem that little phylogenetic significance can be attached to this feature as it has been evolved independently and in parallel several times.

Since they are deposited into an aqueous prefertilisation environment in which metabolisable nutrients are absent, the sperm of T. retusa presumably depend upon intracellular reserves for energy production. The utilisation of glycogen stores, when available, has been well documented in some species (Anderson and Personne, 1975). As glycogen deposits appear to be absent in the sperm of T. retusa, it is possible that energy could be derived from the oxidation of mitochondrial phospholipids, as has been demonstrated in the sea urchins (Afzelius and Mohri, 1966).

4.4.3 Nutritive Cells.

Nutritive cells occur among the germ cells of Lingula. They are distinguished by the presence of two types of granules, namely lipid droplets of low electron density and granules of greater density, about 3μm in diameter (Sawada, 1973). Chuang (1983b) reported similar observations for L. anatina in which the granules measured up to 1 μm in diameter.

Although the nutritive cells were abundant in immature gonads, they degenerated as maturation progressed in the L. unguis (Sawada, 1973), while the mature testis with fully formed spermatozoa in L. anatina were found to be abundant in nutritive cells still charged with the two types of granules (Chuang, 1983b). This anomaly may be explicable in terms of the quantity of nutrients available and the frequency with which the animal reproduces. An animal reproducing

several times a year, or perhaps continuously, may harbour a number of generations of gametes within the gonad. Should there be a specific requirement for nutritive or phagocytic cells during a particular phase of the reproductive cycle, it would not seem unusual for these cells to be present within the gonad at different times.

A similar phenomenon appears to occur in the recently spawned T. retusa. Ultrastructural evidence indicates the presence of cells containing lipid deposits, which are assumed to be nutritive in function. Recently spawned individuals also display the red to orange pigmented globular material, similar to that documented within the female. It was not possible to directly identify this material with the nutritive cells, nor were the phagocytic cells, which constituted the globular material in the female, observed.

The ability to selectively resorb and to deliver specific nutrients, to the gametes occurs in a number of animals. Nimitz (1976), documented irregularly shaped cells containing basophylic granules and lipid globules in the ripe and recently spawned testes of the sea stars Pisaster ocraceous and Patiria miniata. Nutritive phagocytes have also been reported in sea urchins (Holland and Geise, 1965).

CHAPTER 5.

THE REPRODUCTIVE CYCLE.

CHAPTER 5.

5.0 INTRODUCTION.

Collins (pers. comm.) compiled a discussion document containing a bibliography of works referring to brachiopod reproductive cycles; this is included, with few amendments (Table 2). By no means exhaustive, this work quotes from all the major sources available, and serves to illustrate that information regarding the breeding cycles of brachiopods is far from complete.

General discussion of the topic can be found in Hyman (1959), Williams and Rowell (1965) and Rudwick (1970). These reviewers highlight the inadequacies of the literature and demand that a structured and systematic review of reproductive strategies of brachiopods be conducted. This point was echoed by Jablonski and Lutz (1983).

Most of the current data are derived from the readily accessible intertidal inarticulates. Yatsu (1902) and Kume (1956) for example, studied the breeding season of Lingula inguis from Japan. Chuang (1959) and Paine (1963) worked on the same species in Singapore and southern Florida respectively. Hammond (1982) examined the breeding season, larval development and dispersal of Lingula anatina.

The only complete studies of the Articulata emanate from New Zealand and one from the west coast of the United States. The latter by Rokop (1977), monitored the spacial and temporal variation of gonad quality of the shelf species, Frielea halli. Percival (1944, 1960) and Rickwood (1968) contributed to the life histories of the shallow water species Terebratella inconspicua, Tegulorhynchia nigricans, and Pumilus antiquatus. All exhibit well defined two and three monthly breeding seasons as indicated by brooding behaviour.

All extant species of articulate brachiopod for which development is known, are nonplanktotrophic, with the larval stages lasting from hours to a few days. In many of these species the larvae are brooded (Senn, 1934; Percival, 1944, 1960; Long, 1964; Rickwood, 1968;

Rudwick, 1970; McCammon, 1973; Logan, 1975; Webb et al., 1976; Thayer, 1975).

In contrast to the articulates, the inarticulate forms undergo planktotrophic development, with a shell-bearing larval stage (Chuang, 1977).

Works related to the reproductive behaviour of the genus Terebratulina are rare. Thayer (1975) made some preliminary observations of T. unguicula. Wait et al. (1976) recorded brooding behaviour in T. septentrionalis.

In order to establish the population structure and dynamics of T. retusa from the Firth of Lorn, Curry (1982) carried out some pioneering examinations of the reproductive cycle.

After establishing that the male to female ratio was approximately equal, Curry concluded that T. retusa from this locality spawn in both the late spring and late autumn, that the spawning event was likely to be highly synchronised and settlement of larvae indicated a larval stage lasting no more than three weeks. Temperature was surmised as being the cue for the precise timing of spawning. Curry also induced spawning and successfully reared larvae to an advanced stage of development. The larvae were considered competent to settle within five days.

Attempts to sample the larvae in the field proved fruitless. Early post-settled individuals have, however, been studied by Curry (1982) and Collins (1986). Collins' results suggested that there is only one period of settlement corresponding to Curry's autumn settlement, and a sigmoidal growth curve, characterised by an early post settlement lag phase.

The aim of this investigation was to resolve these discrepancies in observed settlement by examining the gonads of animals sampled each month for approximately a year. A stereological methodology was adopted in order that quantitative estimates of gamete production could be achieved.

Although the population in the Firth of Lorn was of primary concern it was decided that a second shallow water site in Loch Fyne, offering very different environmental conditions, should also be studied.

Conducting parallel investigations of two geographically and probably genetically isolated populations (Collins et al., 1988), would yield a wealth of information about the reproductive strategy and the way in which this has been adapted in the exploitation of different habitats.

The inherent flexibilty of a given mode of reproduction has important paeleobiological implications where the reproductive strategy may have restricted the dispersion and ultimately the rate of mutation.

TABLE 2.

SPAWNING PERIODS OF BRACHIOPODS.

Northern Hemisphere.

Species	Author	Year	J F M A M J J A S O N D
Inarticulata			
Lingula ungica	Yatsu	1902	<---->
	Sewell	1912	----> <------> <---
	Kume	1956	
	Chuang	1959	----------
	Chuang	1973	----------
	Hammond	1982	---------> <-----
Glottida pyrimidata	Paine	1963	(1)<----------> - <-------->
Neocrania anomala	Rowell	1960	<---------->
Articulata			
Terebratulida			
Terebratalia transversa	Long	1964	-->- - - - - - - - - - - - - -<----
	Paine	1969	
Terebratulina retusa	Curry	1982	<---> <--->
Terebratulina septentrionalis	Noble & Logan	1971	----------
Terebratulina unguicula	Thayer	1975	
Argyrotheca jacksonii	Jackson et al.	1971	----------
Argyrotheca bermudana	Jackson et al.	1971	----------
Laqueous vancouverensis	Thayer	1975	
Frielea halli	Rokop	1977	<- - - - - ->
Theceidina			
Theceidellina barretti	Jackson et al.	1971	(single spawning)
Theceidellina congregata	Jackson et al.	1971	(single spawning)

Southern Hemisphere.

Species	Author	Year	J	A	S	O	N	D	J	F	M	A	M	J
Terebratulida														
Pumilus antiquatus	Rickwood	1968		<---	---	---	---	>						
Terebratella dorsata	McGammon et al.	1968												
Terebratella inconspicua	Percival	1944										<---	---	>
	Doherty	1979		<---	---	---	>							
Terebratella sanguinea	Tortell	1981								(2)				
Megellania venosa	Mcammon	1973	-	-	-	-	-	-	<---	---	>	-	-	-
Neothyris lenticularis	Tortell	1981								(3)				
Rhynconellida														
Hemithiris pssitacea	Thayer	1975												
Notosaria nigricans	Percival	1944										<---	---	>
Neorhyncia strebeli	Mcgammon et al.	1968	-	-	-	-	-	-	-	-	-	-	-	-

Notes;

(1) Latitudinally displaced populations are characterised by different durations of spawning season; brief in the north, but occurring throughout most of the year in the south.

(2) Specimens collected between January and March; gonads were ripe but the individuals could not be induced to spawn.

(3) Specimens collected between January and March; males released sperm when the water temperature was slightly increased.

5.1 STEREOLOGY: PRINCIPLES AND PRACTICE.

Most reproductive studies lack quantitative detail. This is primarily the result of employing subjective indices of gonad condition. To address this problem it was decided from the outset that

a critique of the reproductive strategy of T. retusa should be conducted in such a manner as to permit the maximum degree of practical objectivity, and provide quantitative information with respect to gametogenesis and spawning. Efforts have been directed towards providing estimates of the actual amounts of reproductive material, and to ensure more precise recognition of spawning, while measuring the quantities of gametes released, and following the development of germinal cells.

5.1.1 Stereology.

Recently a body of mathematical methods which permit the quantitative estimation of three dimensional structure from two dimensional measurements, (Wiebel, 1979) have been adopted by a number of workers as a suitable vehicle for the examination of the reproductive cycles of some bivalve molluscs (Bayne et al., 1975; Lowe et al., 1970; Sundet and Lee, 1984, Mackie, 1986; Lee, 1985; Morvan and Ansell, 1988; Newell and Bayne, 1980; Yankson, 1983).

Stereological theory implies that it is possible to relate volumes of a biological structure, e.g. cells within a gonad, to area measurements made on histological sections.

Histological preparations of thin sections of genital tissue provide an excellent method for investigating cellular organisation. This makes it possible to view germinal cells in detail and in the positions they would normally occupy within the reproductive structure.

Lee (1985) drew from the work of Briarty (1975), Bayne et al. (1978), Elias et al. (1971) and Lowe et al. (1982), to provide a lucid account of a stereological method. This methodology has been adapted within the context of the present study to cope with a variety of technical problems afforded by the unique character of the brachiopod reproductive system.

5.1.2 Prerequisites of a Stereological Investigation.

In order to construct a reproductive index based on stereological principles it is neccesary to define four major factors:

(i) Gonad Volume.

The volume of any tissue block under stereological examination is by convention termed the reference space. The reference space, i.e. the volume of the structure to be sectioned, must be defined in order that all subsequent measurements be related to it.

(ii) A Scale of Measurement.

Quantitative stereology is carried out by superimposing grids of lines and points over a two dimensional image, e.g. The image of a histological section as viewed through a microscope. Various stereological methods favour the use of different grids or 'test systems'.

(iii) Sampling Protocol.

To estimate the volumes of various tissue phases present within the reference space, in this instance the gonad, it is of paramount importance to devise a sampling program. More precisely, a tissue sectioning and cell phase counting protocol, which gives an unbiased estimate of the volume of a given tissue.

(iv) A Standard for Comparison.

Once an estimate of the proportions of the phases present within the reference space has been achieved, it is necessary to standardise these measurements, to allow meaningful interpretation of such a reproductive index between animals of different sizes. This requires a

measure of some fixed parameter which either increases or remains stable throughout the life of the organism.

5.2 METHODS.

5.2.1 Experiment to Determine the Gonad Volume.

Anatomical investigations (Chapter 1) reveal, that the gonad consists of two flattened palmate lobes in each valve, each of which is composed of a complex reticulate lattice. Determining the volume of such a structure directly is extremely difficult and involves very delicate dissection. Therefore a method of gonad volume estimation was devised to circumvent these problems.

(i) Method.

Preliminary investigations revealed that the onset of maturity occurred in specimens exceeding approximately 5.5 mm in shell length. To avoid the inclusion of juveniles, specimens usually greater than 10 mm were used for analysis.

Forty specimens of T. retusa, including both sexes, a range of sizes and differing states of maturity, were decalcified and the gonad within the mantle envelope removed intact.

Either the pedicle or brachial mantle portion from each individual was selected and the two discrete lobes of the gonad separated. Half of the gonad was used for direct measurement of gonad volume and half for an estimate of gonad volume calculated from area and average cross sectional depth.

(a) Direct Determination of Gonad Volume.

Each gonad portion was carefully dissected from the mantle envelope. The wet weight was recorded by allowing the gonad to drain

on a piece of absorbent tissue for a few seconds prior to introduction into the balance chamber, where the mass was recorded after 30 seconds. A simple gravimetric technique was employed to determine the density of the gonad.

A saline solution of known density was prepared. Preliminary trials were conducted to ensure that the density of the solution would exceed the expected density of the gonad.

Gonad portions were washed in distilled water, drained and introduced into the saline solution. Measured aliquots of distilled water were added to the solution which was stirred periodically to prevent stratification.

The point at which the gonad started to sink was determined as that at which the density of the gonad was equal to the solution. The density of the solution was therefore recalculated as follows :-

Density of Gonad = Density of Solution =

Mass of Salt /Initial volume of salt solution + Volume of distilled water added

Repeated experiments showed that the density of both the male and female gonads could be measured to +/- 0.002 grams mm^{-3}.

Using the principle that volume is a function of mass and density it is possible to calculate the volume of the gonad portion as :-

Volume of Gonad = Mass of Gonad / Density of Gonad

(b) Estimation of Gonad Volume.

With the gonads spread in a petri dish so that the gonad portions were in plan view, their outline was traced using a camera lucida attached to a Wild binocular microscope. The area of each scaled tracing was calculated using a M.O.P.-Kontron image analyser.

Each corresponding gonad portion was then dehydrated, embedded in wax and sectioned according to the schedules described in chapter 2. Sections were cut vertically along the anterio-posterior axis. The length of the resultant profile was divided visually into 30 approximately evenly spaced portions. The vertical profiles or 'gonad depths' were recorded using a graduated eyepiece graticule mounted in a Watson Microsystem 70 microscope. Average gonad depths were multiplied by the corresponding area measurement for a given gonad portion to give the estimated gonad portion volume.

(ii) Results.

A least squares regression of experimentally derived gonad portion volume with estimated gonad portion volume reveals a significant relationship.

$$\text{Gonad Volume} = 0.492(\text{Estimated Gonad Volume}) + 0.016$$

$$P>0.0001;\ \text{rsquare}=0.94;\ \text{d.f.}=1,40;\ F=294.8$$

Using the above relationship it was possible to predict the volume of the gonad by simply combining the area of gonad with the corresponding average depth measurements taken from histological sections. The equation for the line was then applied to the result to make a suitable correction.

5.2.2 Measurement.

The correct choice of stereological test system is to a large extent dictated by the type of material being sampled. A lattice of points represents the simplest system and is used for volumetric work, test lines for surface measurements and a test area for particle counting (Freere and Wiebel, 1966).

Estimates are made by counting the coincidence of some particular feature of the image or histological section with the test points or lines. Wiebel et al. (1966) developed a multipurpose test system

combining many of these requirements to permit collection of a variety of data. This is the 'Wiebel 42' test system as illustrated by Lee (1985) and consists of a square field containing a series of truncated lines, arranged in alternately spaced parallel rows. In all there are 21 lines and therefore 42 truncation marks or crosses. Housed within a Watson Microsystem 70 microscope as an eyepiece graticule, this system was employed for the stereological analysis of reproduction in T. retusa.

Measuring the proportional area of each phase requires that when a histological slide preparation is viewed, only those tissue phases superimposed immediately beneath truncation marks are scored. It is from a series of such 'point' counts, that the proportional area of each phase is estimated.

5.2.3 Sampling Protocol.

Stereological analysis is by definition expensive of both time and resources which makes the development of a strict and statistically robust sampling protocol a fundamental prerequisite.

The order of priority for the determination of the necessary parameters for T. retusa, was as follows :-

(i) Selection of a plane or planes of histological section which gives all the cell phases present within the gonad of an individual an equal chance of being sampled.

(ii) Comparison of all four portions of the gonad within an individual to determine the degree of synchrony between and within portions.

(iii) Estimation of the number of subsamples required from an individual to accurately assess reproductive condition.

(iv) Analysis of the variability between individuals within samples in order to extrapolate to the reproductive strategy of the parent population.

5.2.3.1 Orientation of Sections.

Details of the reproductive anatomy of T. retusa have already been documented in the preceeding chapters, and are pertinent to any regimen of sampling.

T. retusa is dioecious with a reproductive system comprising paired gonads (see Chapter 1.). One pair of gonads is present in each valve, developing within the vascula genitalia which extend anteriorly from the body cavity. Gametes develop along the margins of the reticulate genital lamella, which is fused to the outer mantle epithelium.

The distribution of reproductive material within the gonad is not random. A vertical section is the only plane in which the gonad is likely to contain a representative spectrum of phases. An anterio-posteriorly orientated section of a portion of gonad taken in the vertical plane has the added advantage of allowing the gonad to be sampled throughout its length, while also facilitating measurement of the profile width or 'gonad depth', the value of which has already been discussed.

5.2.3.2 Comparison of the Gonad Portions Within an Individual.

As the gonad exists as four discrete portions within an individual, it was important to establish the level of reproductive synchrony between portions. This was necessary to determine the legitimacy of considering one portion of gonad as representative of that individual's reproductive state.

(i) Method.

Six specimens of T. retusa, three of each sex, at varying states of maturity, subjectively described as spent, filling, and full, were selected.

After partial decalcification the genitalia were removed,

dehydrated, embedded in wax and sectioned in the prescribed manner at various depths. Three sections were taken from each portion of the gonad, i.e. 12 from each animal.

Using the stereological graticule, ten fields of 42 point counts were made, using a stratified random sampling pattern (Lee, 1985) of oocytes and spermatocytes in females and males respectively. These cells types were chosen as they are present at all states of maturity.

(ii) Results.

Point counts are proportional and must therefore be subject to an appropriate arcsine transformation before normality can be assumed.

Analysis of Variance reveals that at the 99% level of significance the differences in cell area density between portions of gonad within an individual are not significantly different for males or females.

State of Maturity	Males	Females
Spent	F>1.09	F>0.94
Filling	F>3.99	F>1.08
Full	F>1.05	F>3.03

This implies synchrony between portions of gonad within an individual and in practical terms means that one portion of the gonad may be considered as representative of the animal's reproductive state.

As the sections of gonad were taken from different positions within the gonad, this result also indicates that one section of the tissue is sufficient to offer a reasonable estimate of the specimen's reproductive state. In practice at least two sections per animal were used.

5.2.3.3 Estimate of the Number of Subsamples Required.

In order to predict the proportions of different cell phases

present within the gonad, with a specified level of statistical accuracy, it is necessary to determine the number of point count fields required.

The number of point count fields per individual is a function of the required sample accuracy in terms of probability and the resolution required in detecting the minimum difference of cell area density. This is in turn affected by the field size i.e. the magnification used to view the tissue. Changes in magnification affect the field size covered by the point counting grid and therefore affect sampling accuracy. The magnification also affects the ability to resolve and identify different tissue phases.

A series of experiments were therefore devised to define the optimum number of fields required for a given range of magnifications.

(i) Determination of Optimal Field Size and Sampling Precision.

The histological examinations presented in earlier chapters serve to indicate that the various tissue phases within the gonad are divisible into an hierarchy which, although introducing an element of subjectivity, is based upon a detailed preliminary investigation of the real differences between cellular components.

The hierarchical definition of those cell phases to be measured was as follows :-

Male Gonad	**Abreviation**
Spermatogonia	SPG
Primary spermatocytes	SPC
Secondary spermatocytes	SSC
Spermatids and spermatozoa	SPT
Non reproductive tissue	NRPD
Coelomic space (cell free)	EMT

Female Gonad	Abreviation
Oogonia	OOG
Oocytes	OOC
Late mature oocytes	LMT
Non reproductive tissue	NRPD
Coelomic space (cell free)	EMT

The categories of reproductive material listed above are largely self explanatory and reference to the sections describing gametogenesis will clarify the identifiable differences between the cell types chosen (See Chapter 4).

Late mature oocytes (LMT) are identifiable as having a densely staining circumference of proteinacious cortical granules which appear red when stained with Papanicolaou's and Harris haematoxylin.

Tissue defined as 'Non reproductive' constitutes all those parts of the gonad which may for example, have a structural role. This category might also include follicular material shed prior to spawning and/or atretic tissue which may be associated with phagocytes as well as accessory cells.

(i) Method.

Lee (1985) and Mackie (1986) offer a complete method for the analysis of optimum field size and number of samples.

Resolving all the tissue phases given above required a magnification of at least one thousand times, corresponding to a field size of 0.014 mm^2. This was the highest magnification available and the choice of field size was thus fixed. The primary concern was therefore to determine the number of fields to be counted.

Using the same sections generated for the experiment to determine the uniformity of the tissues in the gonad, a series of systematic field counts were made (Lee 1985). The tissue phases defined above were scored for animals that were 'filling' and 'full'. In total 50

fields of 42 points were counted for each individual and the mean and standard deviation of these counts calculated for each tissue phase.

(ii) Results.

Example:

Male filling:	**SPG**	**SSC**	**SPT**	**EMT**	**NRPD**
Mean	0.32	5.08	10.6	25.9	0.059
St.dev.	1.24	4.39	5.56	4.41	0.308
Male full:					
Mean	0.28	17.84	0.84	22.86	0.28
St.dev.	1.25	4.24	3.29	4.09	0.96

Female filling:	**OOG**	**OOC**	**LMT**	**EMT**	**NRPD**
Mean	0.14	0.3	25.18	14.62	1.54
St.dev.	0.4	0.64	7.38	7.52	2.16
Female full:					
Mean	0.09	0.49	25.36	14.81	1.25
St.dev.	0.31	1.04	8.49	6.42	2.00

The highest standard deviation was that of a full female late mature oocyte (LMT) phase. The magnitude of deviation was much the same within all those animals investigated.

Statistically, it was demonstrated that 50 counts of 42 points of 0.014 mm^2 field size will provide a mean which is greater than 95% certain of showing a 5% difference in cell phase at the 1% level of significance, (Sokal and Rohlf, 1981). A count of 50 fields was

therefore adopted as being sufficient to gain an accurate picture of the reproductive state of individuals.

5.2.4 Experiment to Determine the Total Shell Volume.

To establish a reproductive index to compare animals of different sizes, it is necessary to isolate some measurable feature of an organisms anatomy which either increases or remains stable throughout life. Aspects of shell morphometry are often used in bivalve molluscs.

Shell length would appear to be a suitable character for T. retusa. A more searching examination of shell growth in some specimens reveals that shell material is deposited at the mantle edge in such a way that growth or size might more accurately be described, in these specimens, as a measure of height. It was considered therefore, that some measure of shell volume might be more appropriate than a one dimensional parameter.

(i) Method.

Viewed as a whole the valves of T. retusa describe an ellipsoid profile. The volume of an ellipse can be calculated as :-

$$4/3\pi r^3 \quad \text{where} \quad r^3 = \text{length}/2 \times \text{width}/2 \times \text{height}/2$$

Specimens of T. retusa were killed, the valves scrubbed, and tissues removed with sodium hypochlorite. A small pellet of 'Plasticene' was introduced into the cavity between the valves, which were then hermetically sealed with the minimum of silicon rubber.

The length, width and height of each specimen was measured to within 0.1 mm with vernier calipers.

Sealed specimens, weighted with 'Plasticene' were then delivered into an apparatus similar to that used by Comely and Ansell (1988) and the equivalent displacement of water measured.

(ii) Results.

A least squares regression of shell volume derived from displacement with corresponding calculated volumes is statistically significant.

$$\text{Shell Volume} = 0.836(\text{Estimated Shell Volume}) + 16.452$$

$$P>0.001;\ \text{rsquare}=0.939;\ \text{d.f.}=1,94;\ F=1454.97$$

The equation of the line was therefore used to apply an appropriate correction to shell volume estimates, made from measurements of length, width and height.

5.2.5 Summary of Stereological Procedure.

Stereological theory implies that the area density of a fraction of a tissue phase, principally the proportion of the 42 points of the grid count of a given cell type, is equivalent to the volume fraction of that tissue phase in a 3-dimensional structure, (Freer and Wiebel, 1976).

In practical terms this translates as follows; five male and five female specimens were selected from each monthly sample from both sample sites. The length, width and height were recorded for each individual. With the valves separated and partially decalcified the gentalia were removed intact. A scaled tracing was then produced of all portions of the dissected gonad in plan view and the total gonad area calculated.

One portion of the gonad from each individual was then dehydrated and embedded in wax. Four posterio-anteriorly orientated vertical sections were taken and stained. All histological procedures were conducted according to the schedules given in the first chapter.

Fifty fields of 42 points were counted using a stratified random sampling pattern. Finally, 30 approximately evenly spaced measurements of gonad depth were recorded along the length of the section.

The requisite parameters were then calculated as follows for each specimen :-

ESTIMATED SHELL VOLUME = $4/3\pi$(LENGTH/2 x WIDTH/2 x HEIGHT/2)

CORRECTED SHELL VOLUME = 0.8362(ESTIMATED SHELL VOLUME)+16.4522

ESTIMATED GONAD VOLUME = TOTAL AREA OF GONAD x AVERAGE DEPTH OF GONAD

CORRECTED GONAD VOLUME = 0.4918(ESTIMATED GONAD VOLUME)+0.0159

5.2.6 Analysis of Results.

Data were analysed with a Micro-Vax computer using the SAS statistical package. Two-way Analysis of Variance was employed to examine the significance of variation within and between samples from both localites. All proportional data were normalised using an arcsine transformation. Wherever 'dummy' records were generated, due to poor section quality for example, the number of degrees of freedom was reduced by one for every value created. Dummy values were calculated as the mean of the product of the row and column for a given cell type from the data recorded for the other individuals in that sample. In practice, however, this makes little difference to the result as the sample size is large.

5.3 RESULTS.

5.3.1 Presentation.

The results of the stereological analysis of T. retusa are presented graphically as :-

a. Gonad volume fraction of a cell phase.

b. Mass of cell phase.

c. Standard mass of cell phase.

d. Variance to mean ratio of a cell phase.

The gonad volume fraction (GVF) of each cell phase was calculated as both a percentage and as an estimate of the mass of each cell type. A reproductive index of standard mass was then constructed to permit the comparison of animals of different size. Finally, the variance to mean ratios of gonad volume fractions were also calculated as a measure of within sample synchrony.

The estimated mass of a given cell phase was computed as follows :-

Estimated Mass of Cell Phase =

Volume Fraction x Estimated Gonad Volume x Density of Gonad

(The density of the gonad was assumed to be $0.02 mg mm^{-3}$ for both males and females).

Calculation of the mass of each cell phase can only provide approximate relative masses as no infomation directly pertinent to the volume to weight relationship of each tissue type was determined.

The reproductive index or more precisely the standard mass of a cell phase was calculated as a function of total shell volume.

Standardised Mass of Cell Fraction =

(Volume Fraction x 0.02 x Total Gonad Volume x 1000) / Shell Volume

When the standard mass of cell fraction was regressed against shell volume there was no significant relationship at the 99% level of testing. With a coefficient of variation of -0.00008, the result indicated that the standard mass could be used as an index which was independant of the size of the animal.

P>0.0201; rsquare=0.0270; d.o.f.=1,198; F=5.488

Utilising the above methods increases the information which may be obtained from stereological analysis. Percentage gonad volume fraction provides an insight into the relative percentages of different cell fractions independent of size, but quantities presented in this fashion are liable to reciprocal variation with other cell phases.

The calculation of the mass of each cell phase can be used to estimate the reproductive effort and spawning efficiency. In this instance the quantities of reproductive material produced will be considered. GVF and standard mass are plotted as mean values +/- one standard deviation with crosses representing maximum and minimum values.

Variance to mean ratio of the GVF are presented, as this is a sensitive measure of the degree of reproductive synchrony within the population for a particular cell fraction at a given time of the year. As the product of the ratio approaches zero the greater the degree of synchrony within the population.

5.4 STEREOLOGICAL EXAMINATION OF GONAD TISSUE.

The results are representative of monthly samples taken between October 1985 and September 1986 inclusive. The data relating to individual cell phases will be presented for both sexes and localities, followed by comparisons between sexes and sites.

5.4.1 Examination of the Female Gonad Tissue from the Firth of Lorn.

Oogonia. Table 3. Figs. 3.0.1-4.

Oogonia represent a small percentage of the GVF, rarely exceeding 2%. Oogonia are present at all states of the reproductive cycle, increasing in number during the autumn and declining thereafter to a December minimum. Quantities increase until late February, fall gradually to May and the process is then repeated, resulting in the autumn peak. Synchrony of development of this cell type within samples is relatively high. Analysis of Variance however, indicates that there are significant differences between and within samples in the amounts of oogonia present.

Oocytes. Table 3. Figs. 3.1.1-4.

These too represent a small fraction of the total GVF, on average approximately 1%. The mass of oocytes is greatest between August and November, declining thereafter to a minimum in early spring. Samples display least synchrony between September and December, with least variation occuring in April. Variances are significantly different within samples, but are not significant between samples.

Late Mature Oocytes. Table 3. Figs. 3.2.1-4.

Stereological data for late mature oocytes reveals a clear seasonal pattern. This cell phase constitutes approximately 50% of the GVF between September and December, values have fallen sharply by January. May appears to mark the start of an increase in the GVF, though it is of note that a result for one individual in April is responsible for the apparent increase in mean GVF between February and May. As might be expected, at least over the range of sizes exploited in this study, the larger the individual, the greater the mass of late mature oocytes produced.

The graph of standard mass indicates a rapid rise in late mature oocytes in August. On average a mature female will produce

approximately 0.15 mg of late mature oocytes per unit of total shell volume. The variance to mean ratio is highest in October falling to minimal values in November and December. There are significant differences in the GVF variation, both within and between samples.

Coelomic Space. Table 3. Figs. 3.3.1-4.

Changes in the GVF of coelomic or cell free space show reciprocity with that of the late mature oocytes. The GVF generally exceeds 50%. When expressed as a mass, however, this phase displays maximal values between September and December, corresponding to the maxima of late mature oocytes. This indicates an increase in the overall volume of the gonad. The variance to mean ratio exhibits greatest variation between October and November, with a fairly constant level of variation over the rest of the year. Differences between and within samples are significant.

Nonreproductive Tissue. Table 3. Figs. 3.4.1-4.

Non reproductive material also represents a minor volume fraction, averaging less than 5% of the total. Values are maximal between January and April. This cell phase shows a slight increase between August and October, when the population is also most synchronous. Variation is such that there are significant differences between and within samples.

Summary of Female Reproductive Tissue. Figs. 3.5.1-4.

A composite graph of all the gonad volume fractions reveals cell free or coelomic space to represent the largest proportion of the volume, followed by late mature oocytes. As the volume of the latter increases there is a corresponding reduction in the amount of coelomic space, to the extent that at maximal values, late mature oocytes occupy the greater volume fraction. Generally, coelomic space constitutes more than 50% indicating that reproductive material never entirely occludes the vascula genitalia.

The reproductive cycle is displayed as an overlay plot of the standardised masses of all the cell fractions. The major spawning period occurs between late October and the beginning of January. Late mature oocytes increase in volume rapidly in August achieving a peak in November with spawning occurring throughout December and January.

Over the size range of animals used, the mass of the gonad increased with the size of the individual. The exact nature of this relationship was not determined.

TABLE 3.

Analysis of Variance.

Females from the Firth of Lorn.

Cell Phase	Source	DF	F Value	PR > F
OOG	Within	10	7.47	0.0001
	Between	44	2.79	0.0001
OOC	Within	10	1.17	0.3037
	Between	44	1.92	0.0003
LMT	Within	10	133.28	0.0000
	Between	44	30.99	0.0000
EMT	Within	10	95.45	0.0000
	Between	44	18.46	0.0000
NRPD	Within	10	6.21	0.0001
	Between	44	3.44	0.0001

Fig. 3.0.1 Mean, Standard deviation and maximum and minimum values for the percentage GVF of Oogonia (% OOG).

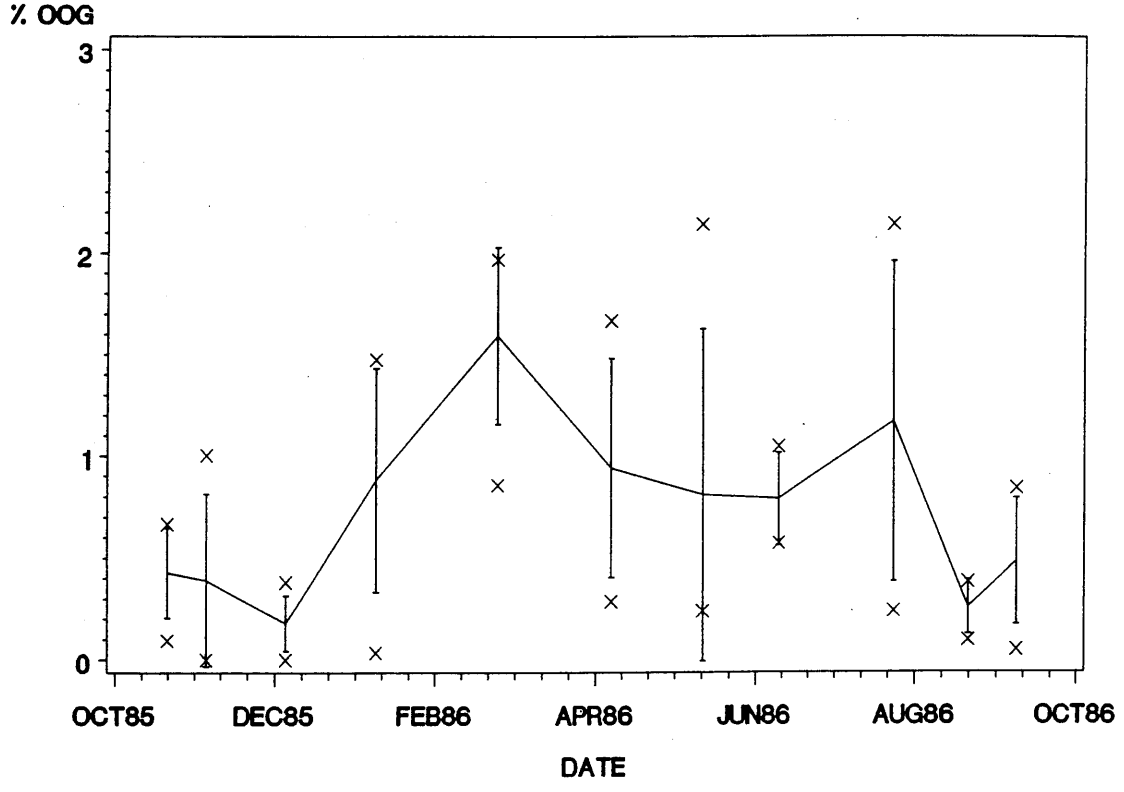
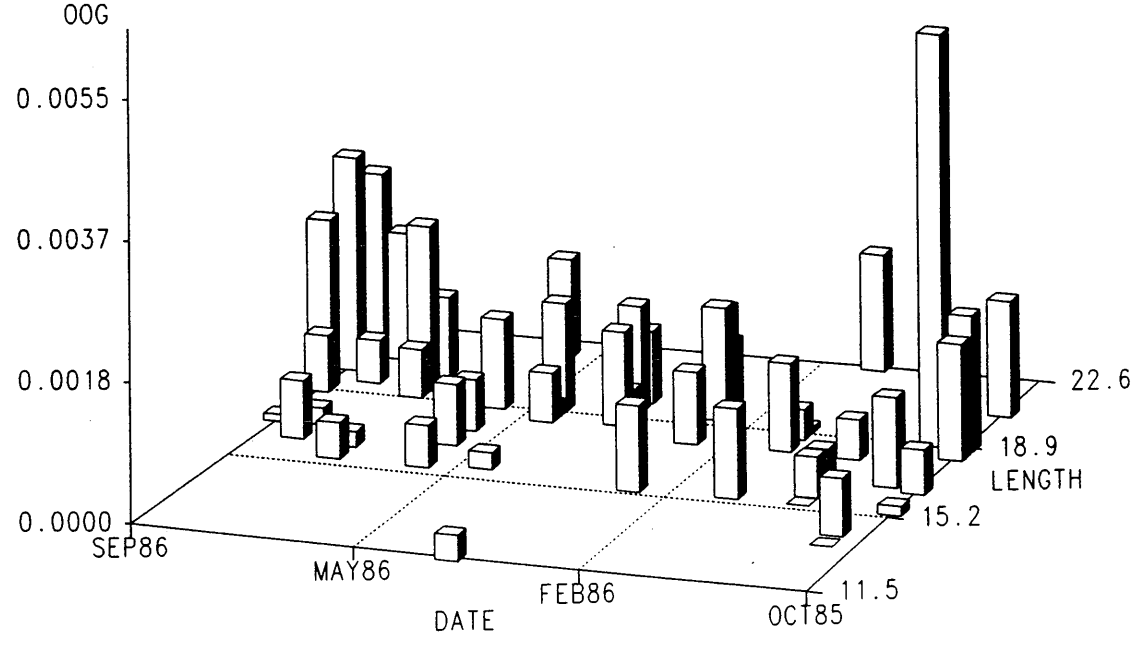


Fig. 3.0.2 Mass of Oogonia in individuals against length and sample date.



OOG: Mass of oogonia in grams
LENGTH: Length in mm.

Fig. 3.0.3 Mean, Standard deviation and maximum and minimum values for the Standard Mass of Oogonia (OOG mg/mm^3).

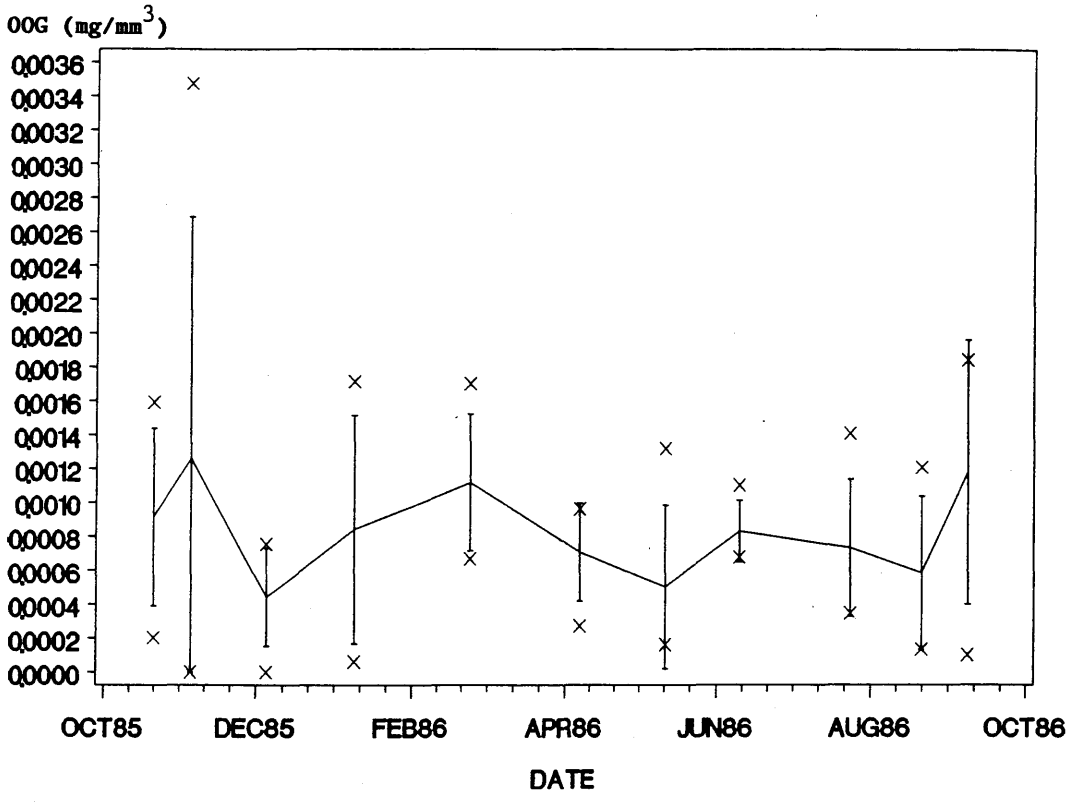


Fig. 3.0.4 Variance to Mean Ratio of the GVF of Oogonia (OOG).

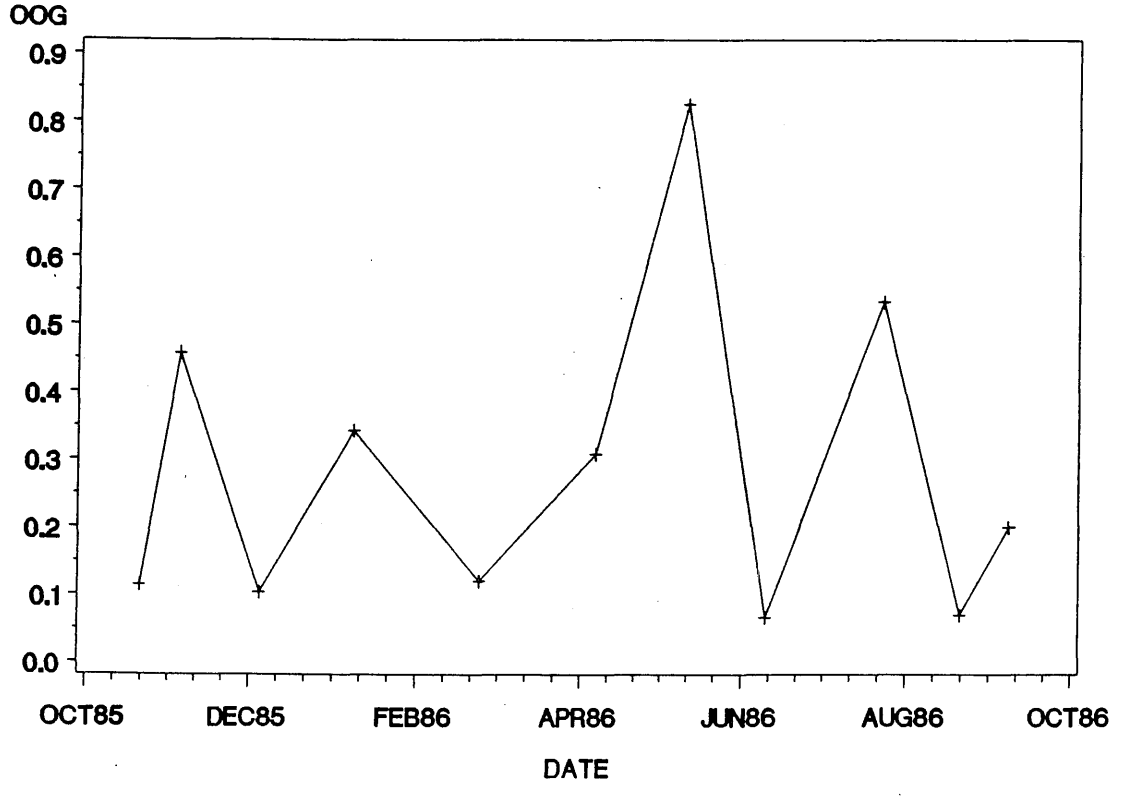


Fig. 3.1.1 Mean, Standard deviation and maximum and minimum values for the percentage GVF of Oocytes (% OOC).

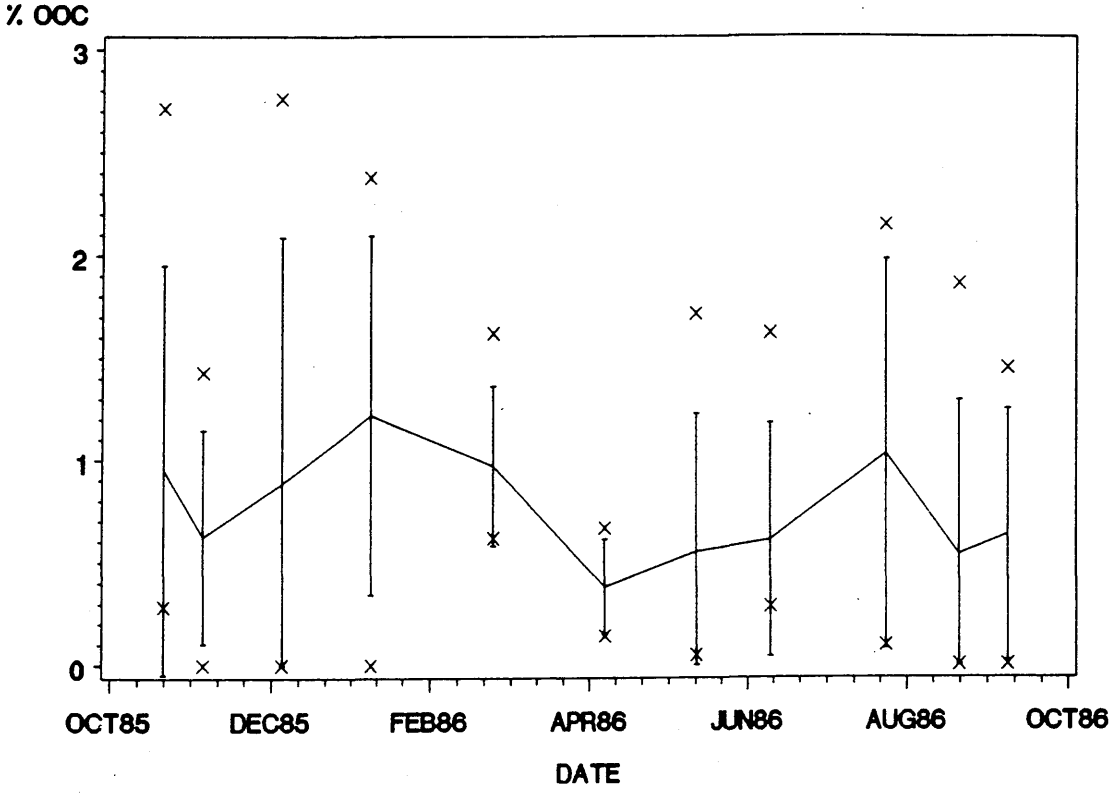
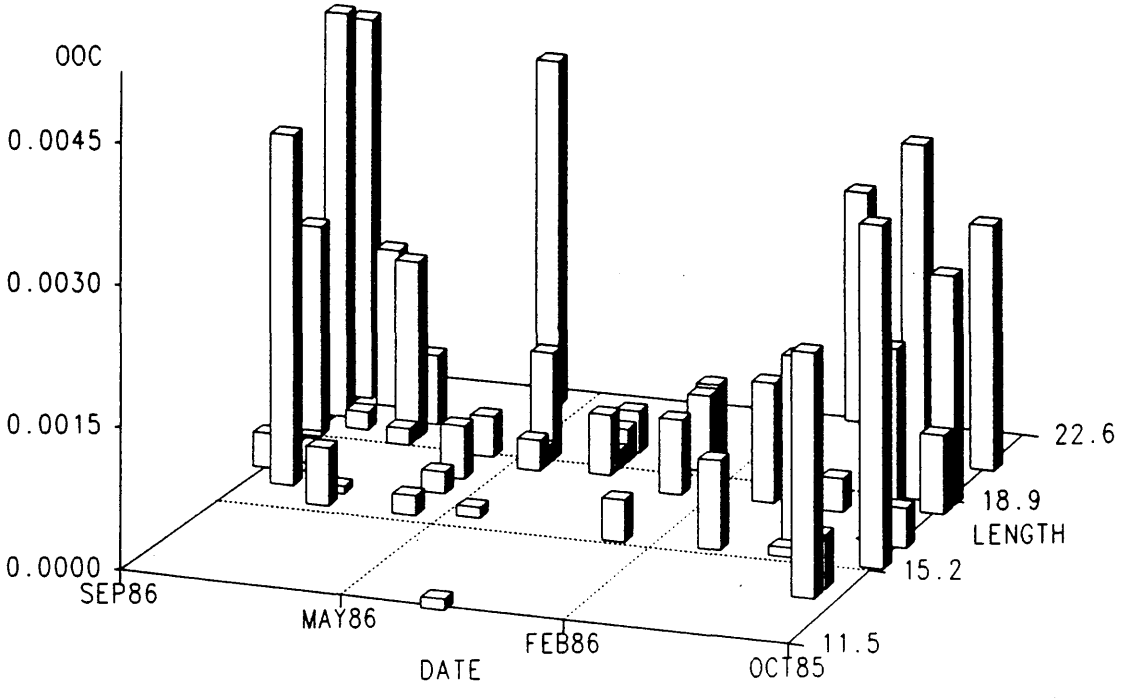


Fig. 3.1.2 Mass of Oocytes in individuals against length and sample date.



OOC: Mass of oocytes in grams
LENGTH: Length in mm.

Fig. 3.1.3 Mean, Standard deviation and maximum and minimum values for the Standard Mass of Oocytes (OOC mg/mm^3).

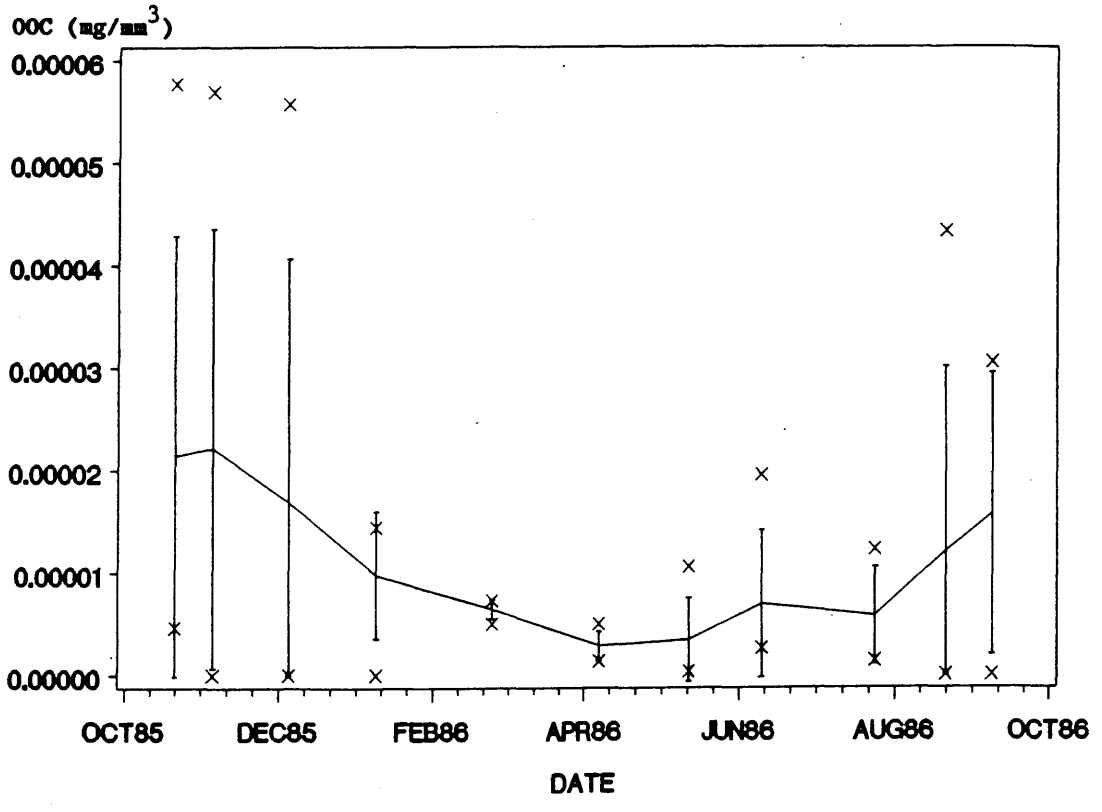


Fig. 3.1.4 Variance to Mean Ratio of the GVF of Oocytes (OOC).

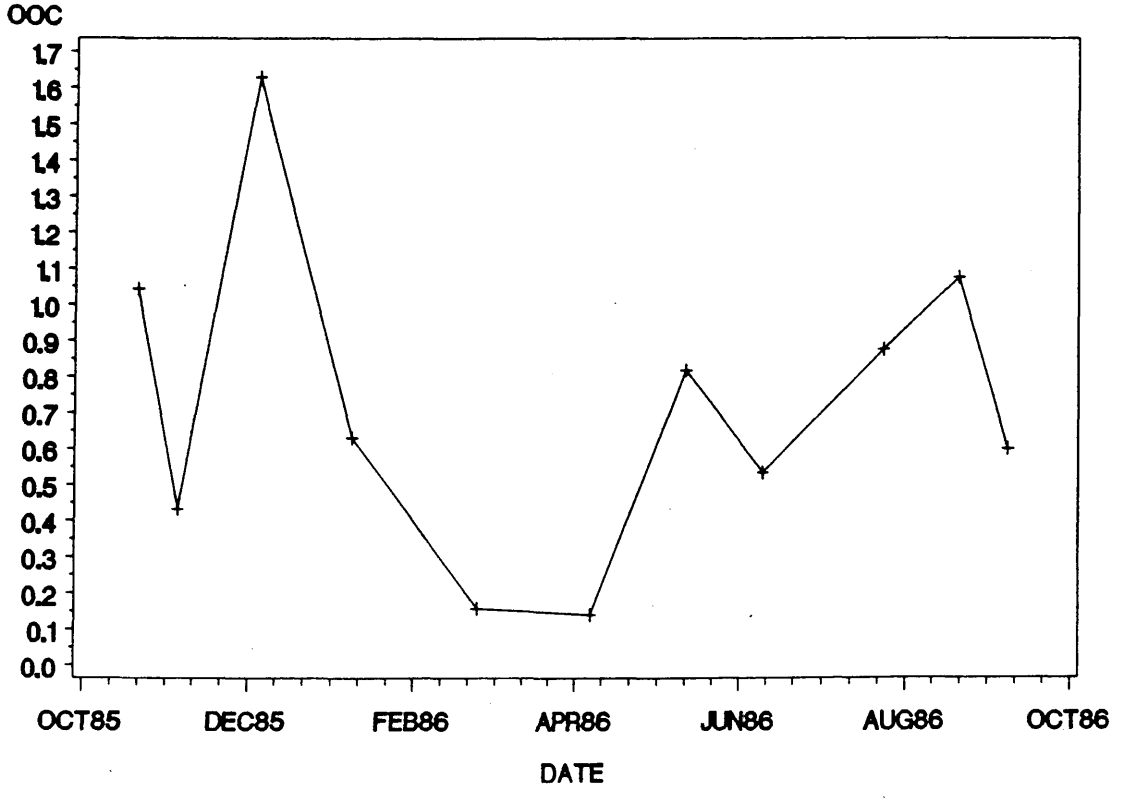


Fig. 3.2.1 Mean, Standard deviation and maximum and minimum values for the percentage GVF of Late Mature Oocytes (%LMT).

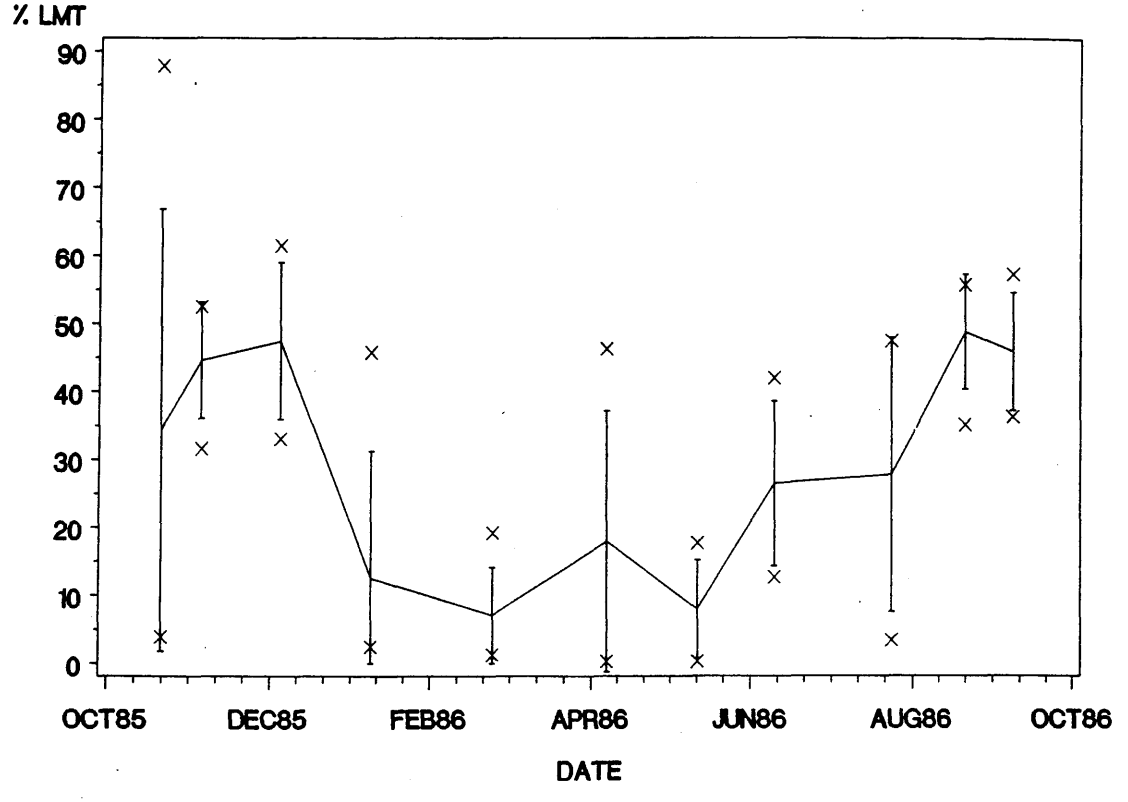
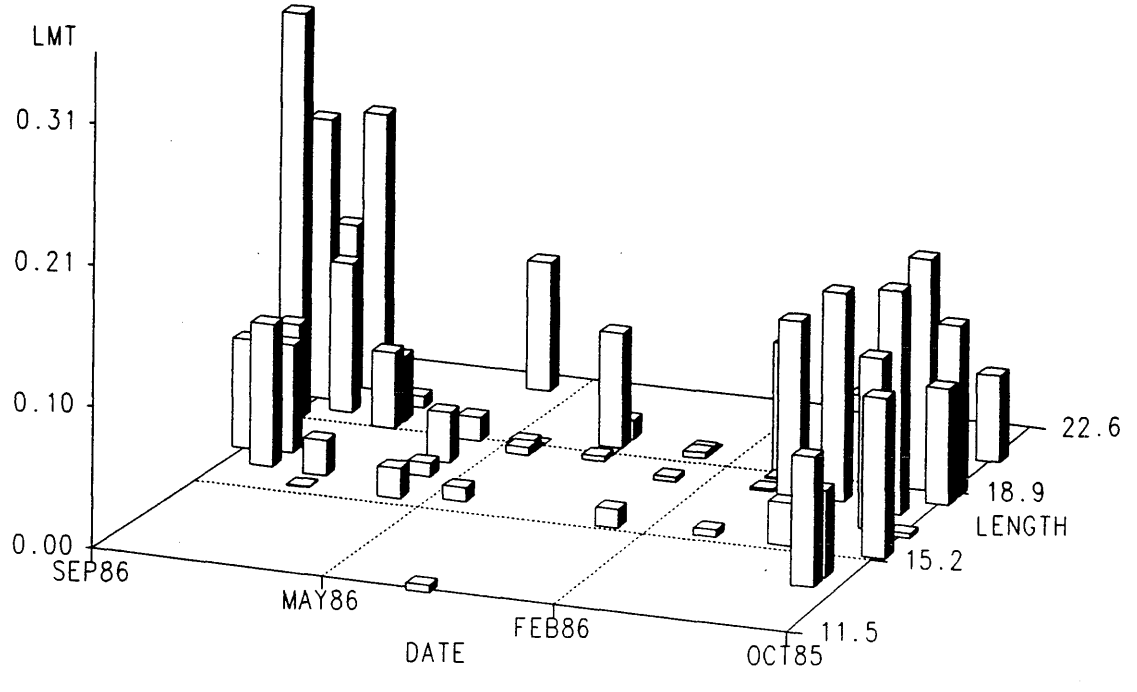


Fig. 3.2.2 Mass of Late Mature Oocytes in individuals against length and sample date.



LMT: Mass of late mature oocytes in grams
LENGTH: Length in mm.

FEMALES: FIRTH OF LORN.

Fig. 3.2.3 Mean, Standard deviation and maximum and minimum values for the Standard Mass of Late Mature Oocytes (LMT mg/mm^3).

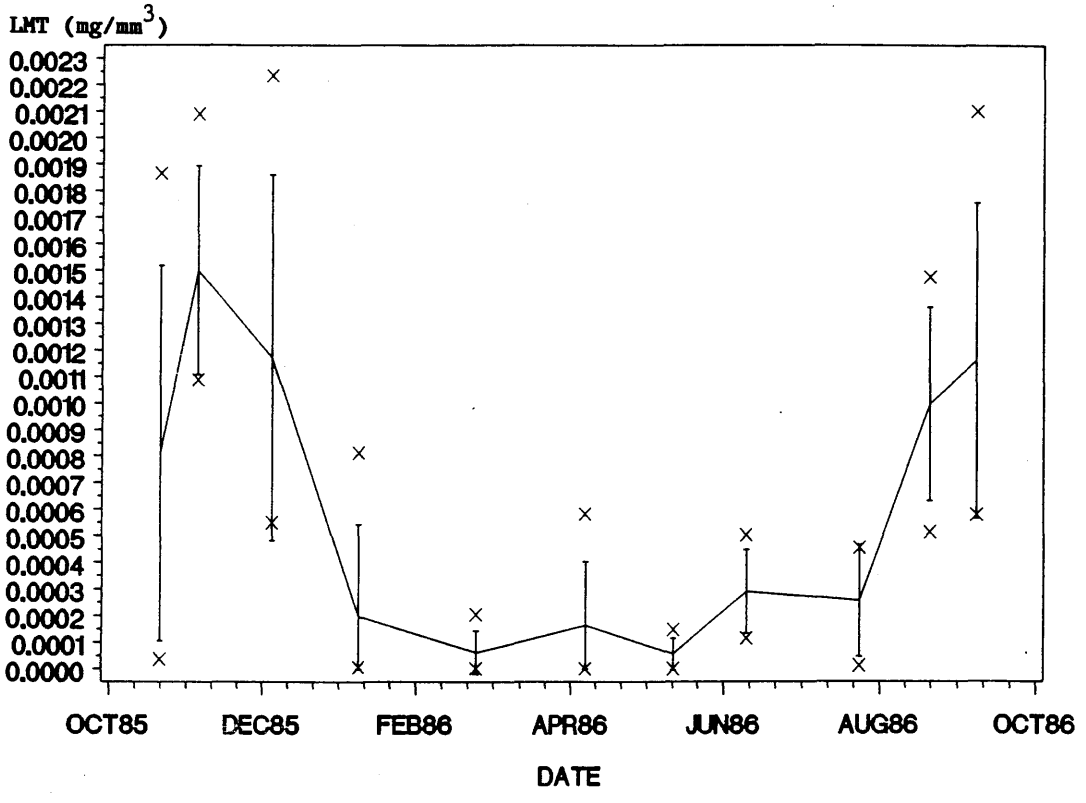


Fig. 3.2.4 Variance to Mean Ratio of the GVF of Late Mature Oocytes (LMT).

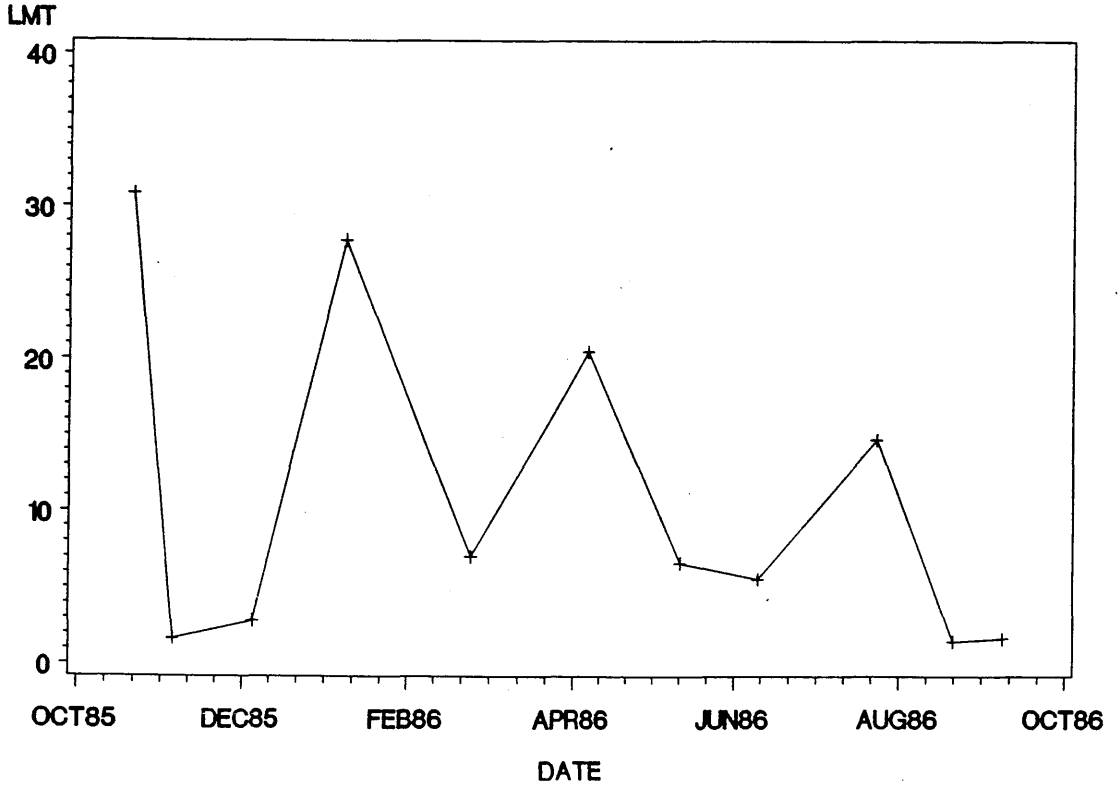


Fig. 3.3.1 Mean, Standard deviation and maximum and minimum values for the percentage GVF of Coelomic Space (% EMT).

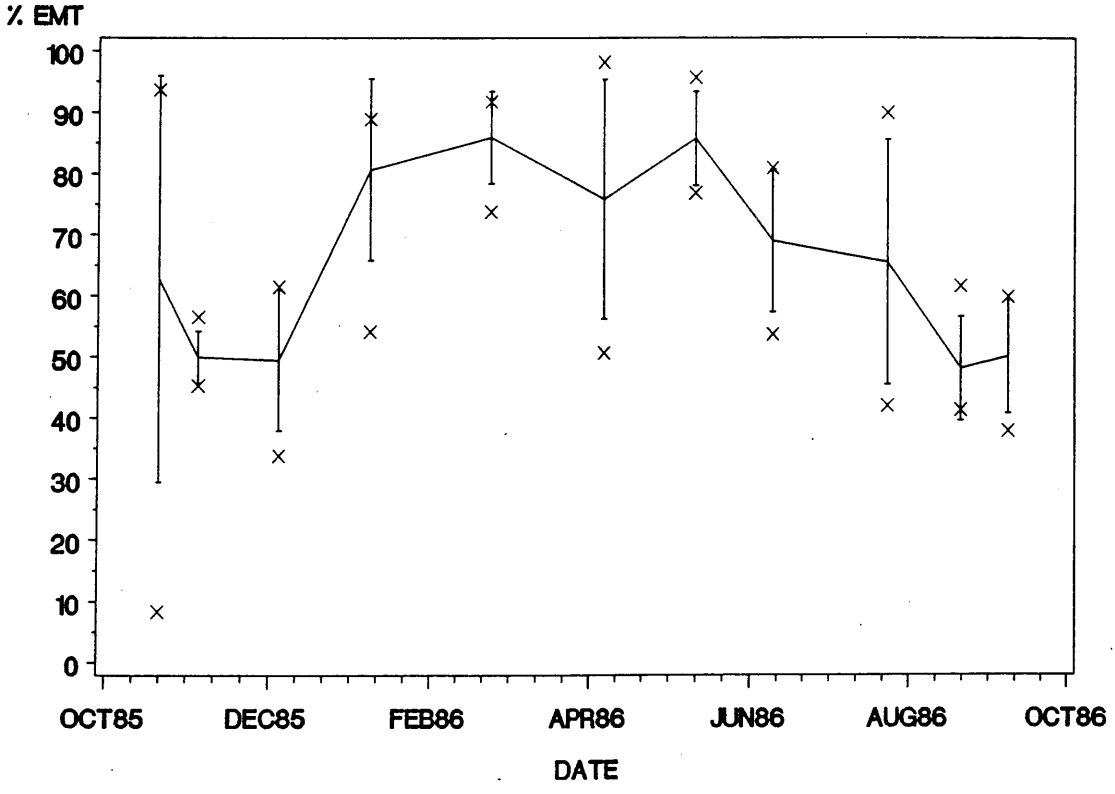
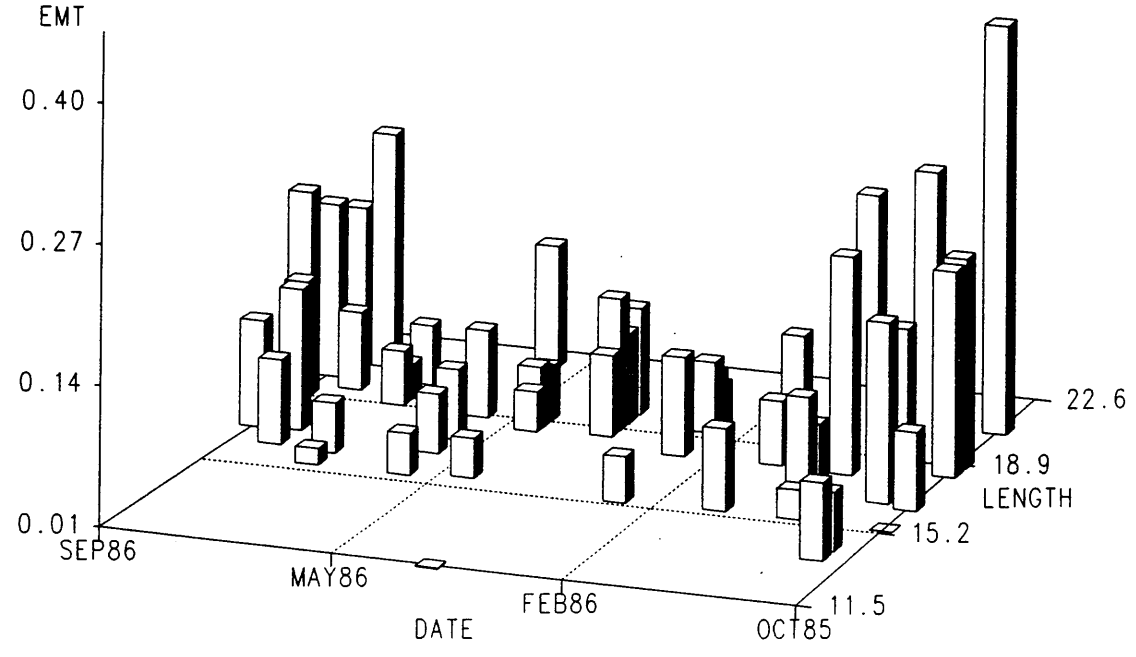


Fig. 3.3.2 Mass of Coelomic Space in individuals against length and sample date.



EMT: Mass of "empty space" in grams
LENGTH: Length in mm.

Fig. 3.3.3 Mean, Standard deviation and maximum and minimum values for the Standard Mass of Coelomic Space (EMT mg/mm^3).

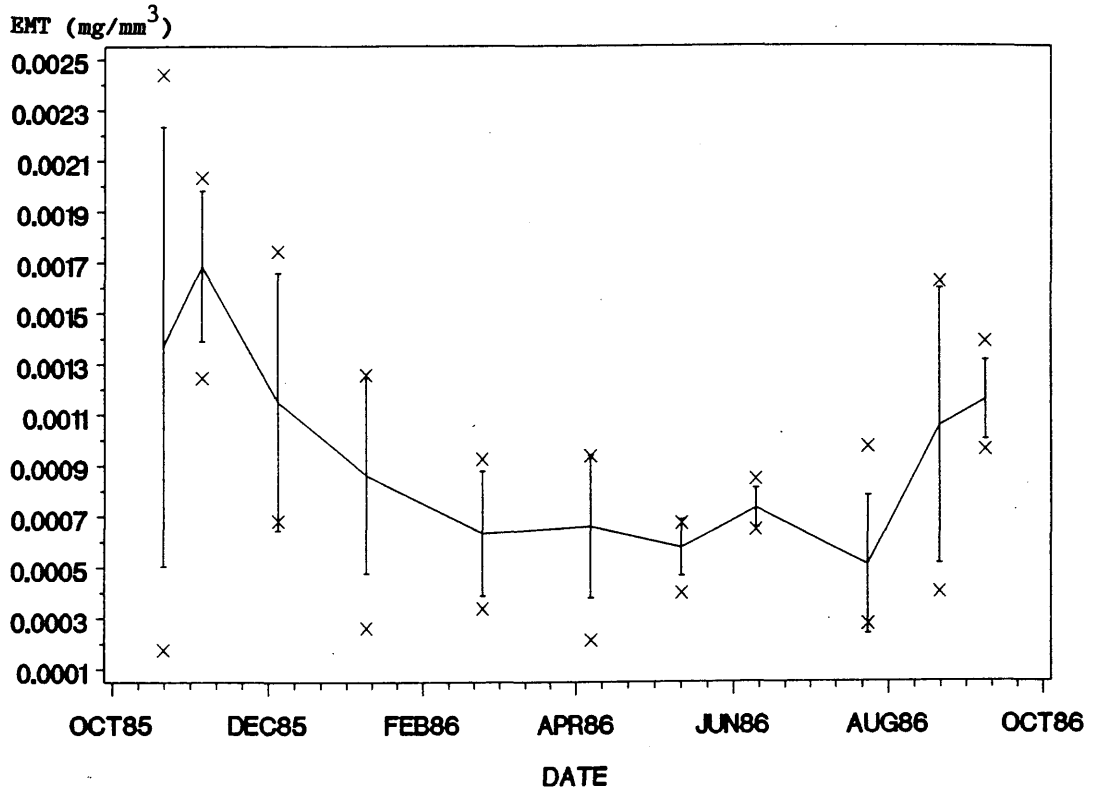


Fig. 3.3.4 Variance to Mean Ratio of the GVF of Coelomic Space (EMT).

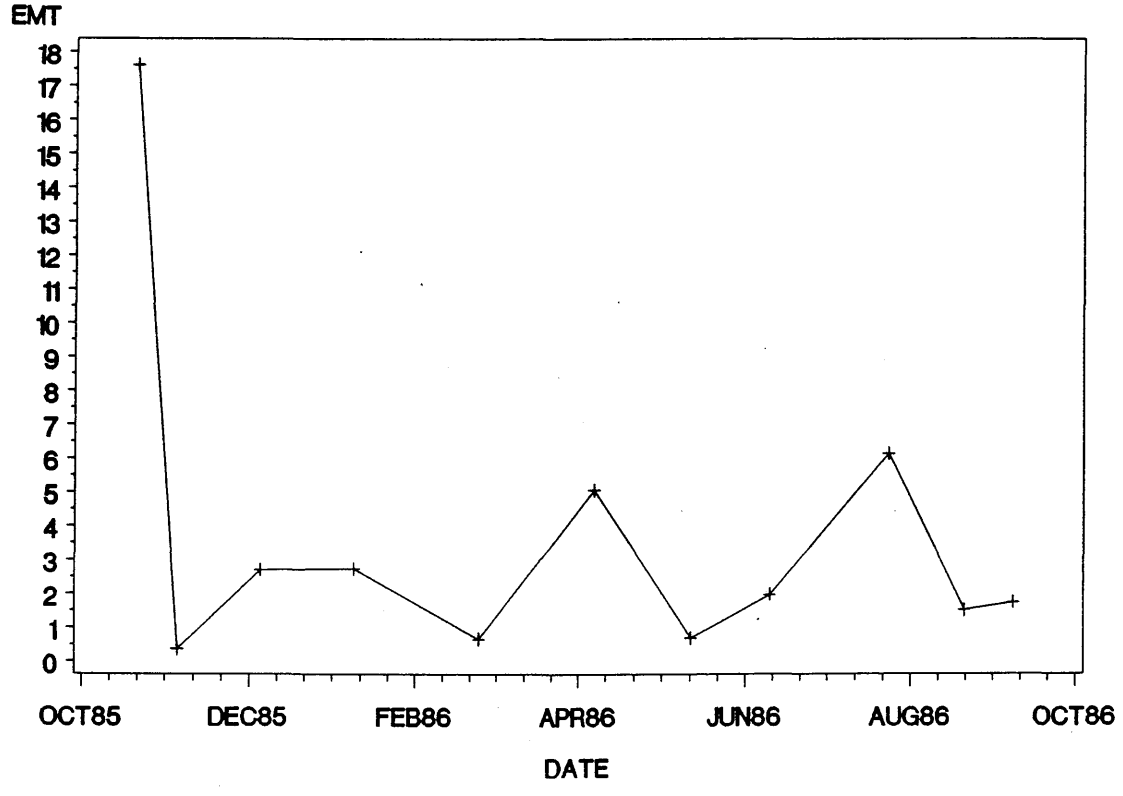


Fig. 3.4.1 Mean, Standard deviation and maximum and minimum values for the percentage GVF of Non Reproductive Tissue (% NRPD).

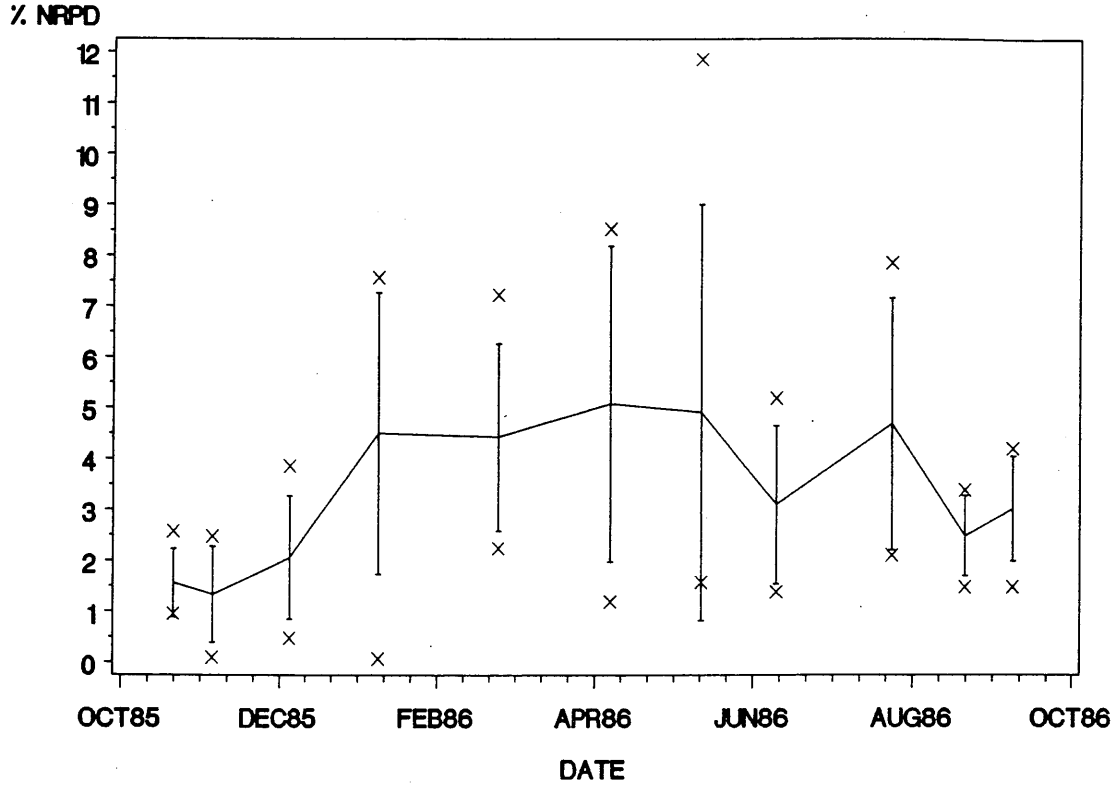
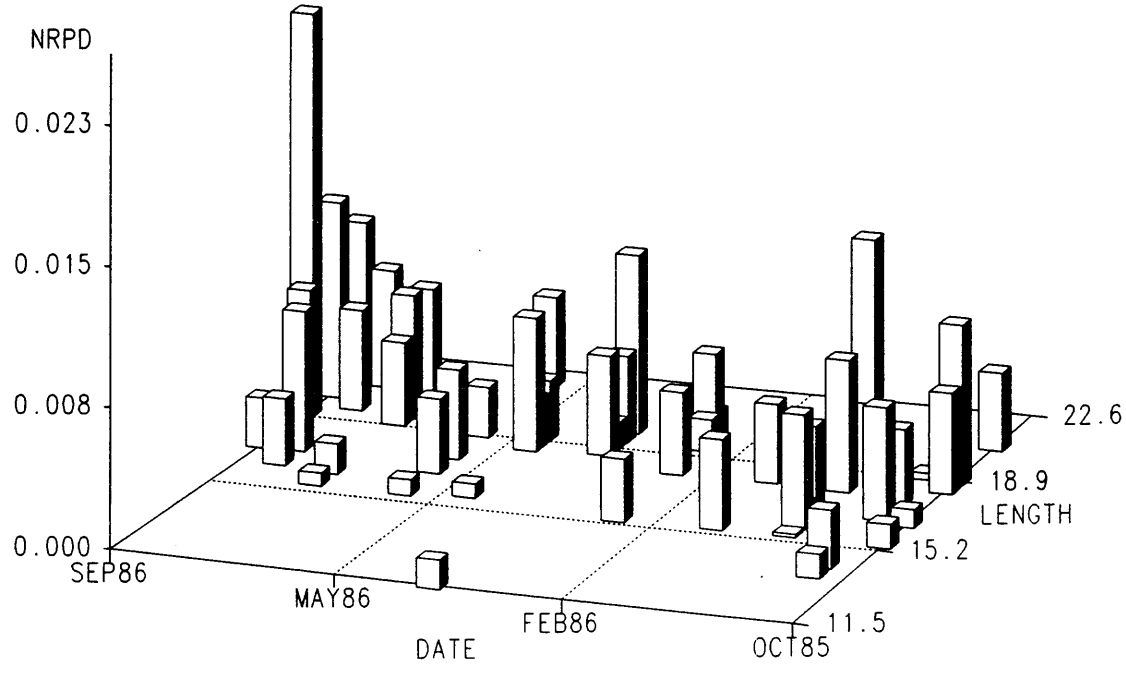


Fig. 3.4.2 Mass of Non Reproductive Tissue in individuals against length and sample date.



NRPD: Mass of nonreproductive tissue in grams
LENGTH: Length in mm.

Fig. 3.4.3 Mean, Standard deviation and maximum and minimum values for the Standard Mass of Non Reproductive Tissue (NRPD mg/mm^3).

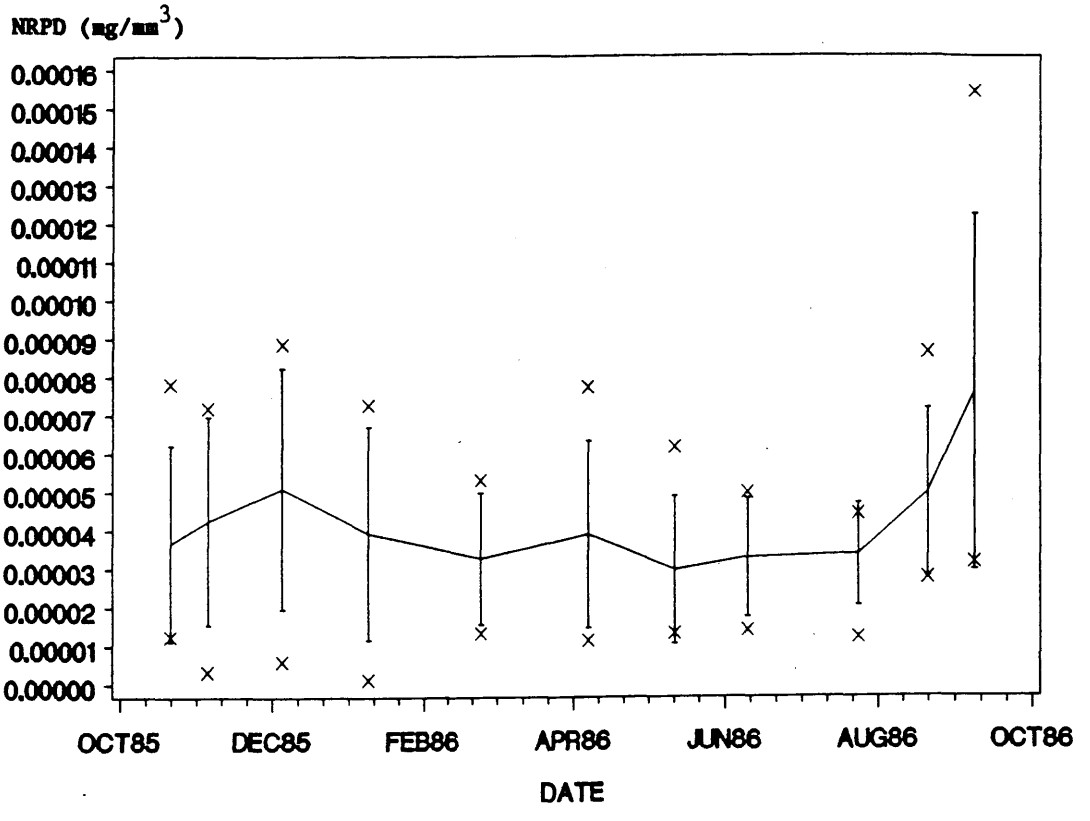
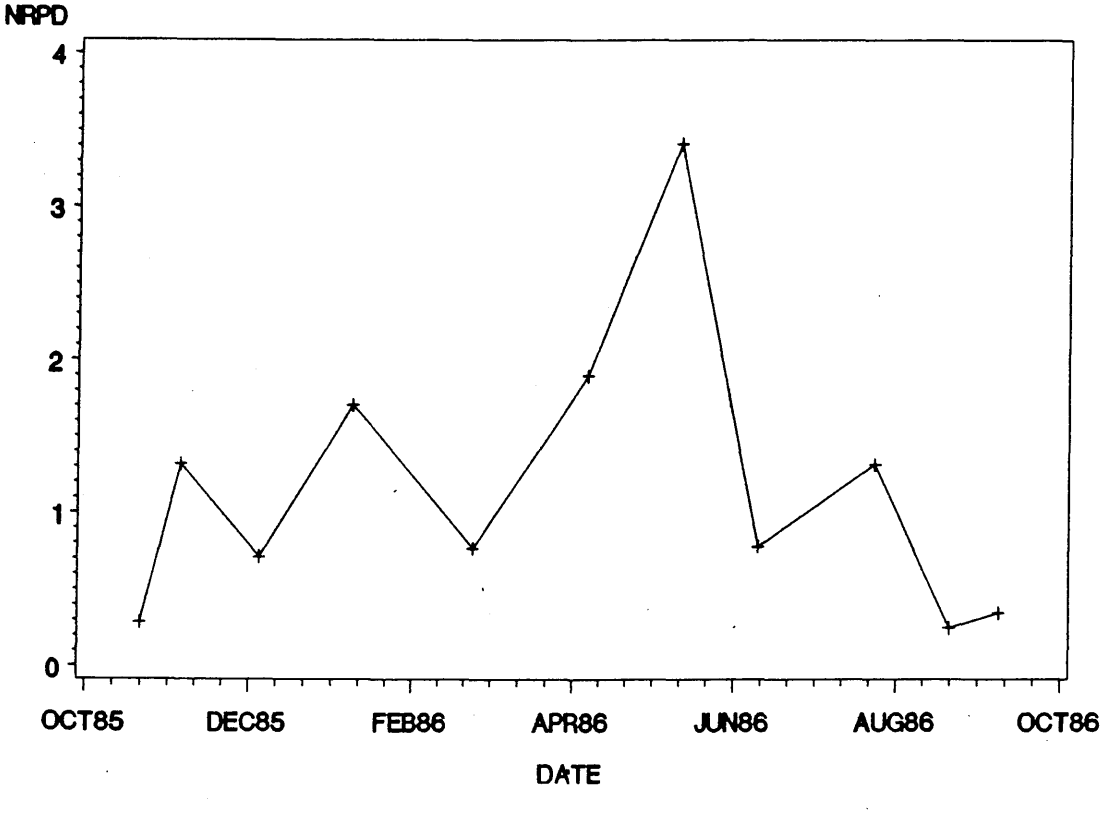


Fig. 3.4.4 Variance to Mean Ratio of the GVF of Non Reproductive Tissue (NRPD).



FEMALES: FIRTH OF LORN.

Fig. 3.5.1 Combined Plot of the Mean GVF of all the female gonad tissue phases (% VF).

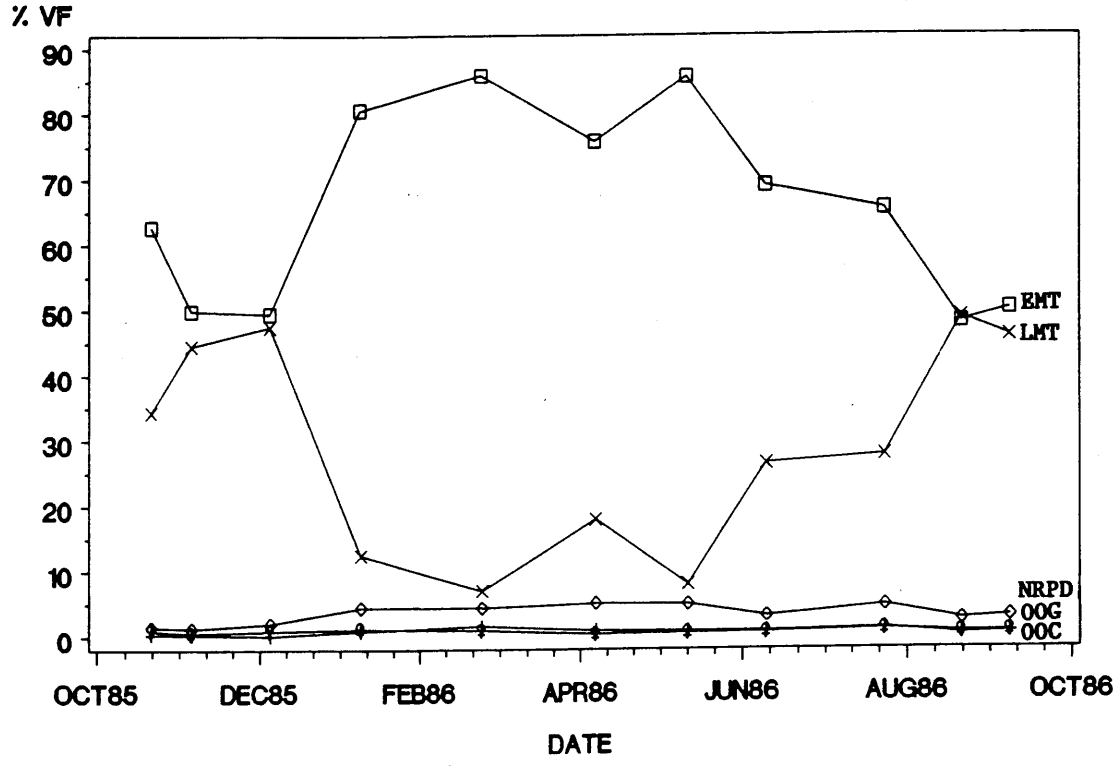
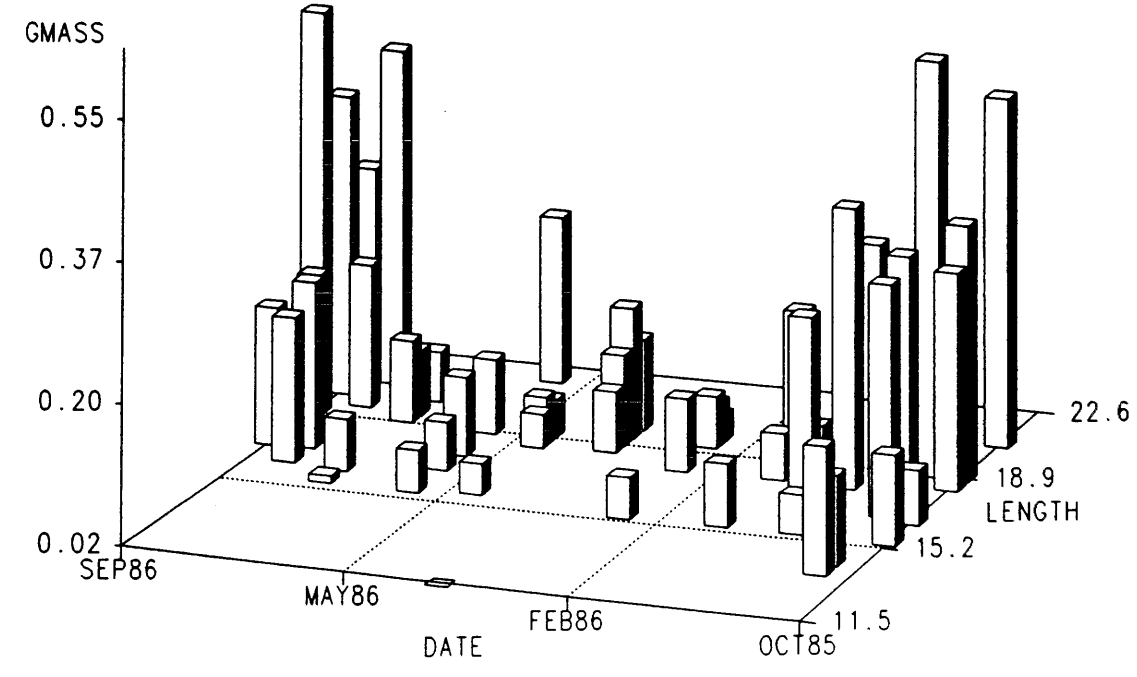


Fig. 3.5.2 Total mass of gonad in individuals against length and sample date.



GMASS: Mass of gonad in grams
LENGTH: Length in mm.

FEMALES: FIRTH OF LORN.

Fig. 3.5.3 Combined Plot of the Mean Standard Mass of all the female gonad tissue phases (ST. MASS mg/mm^3).

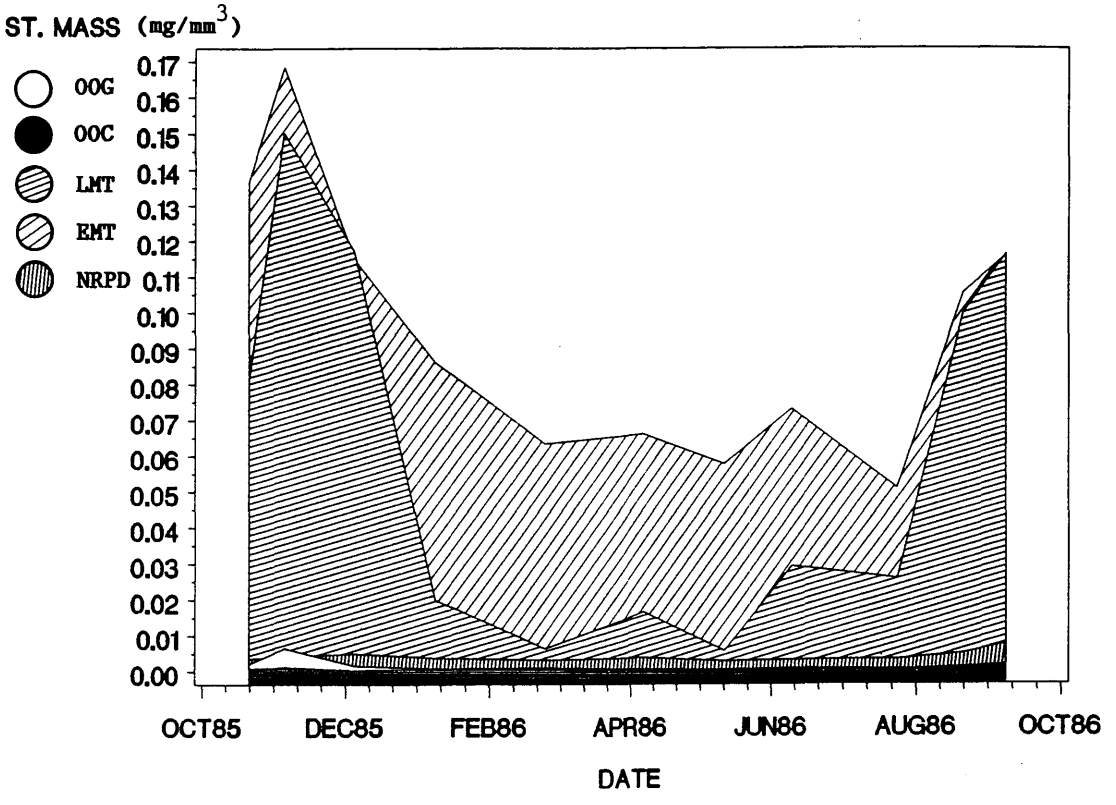
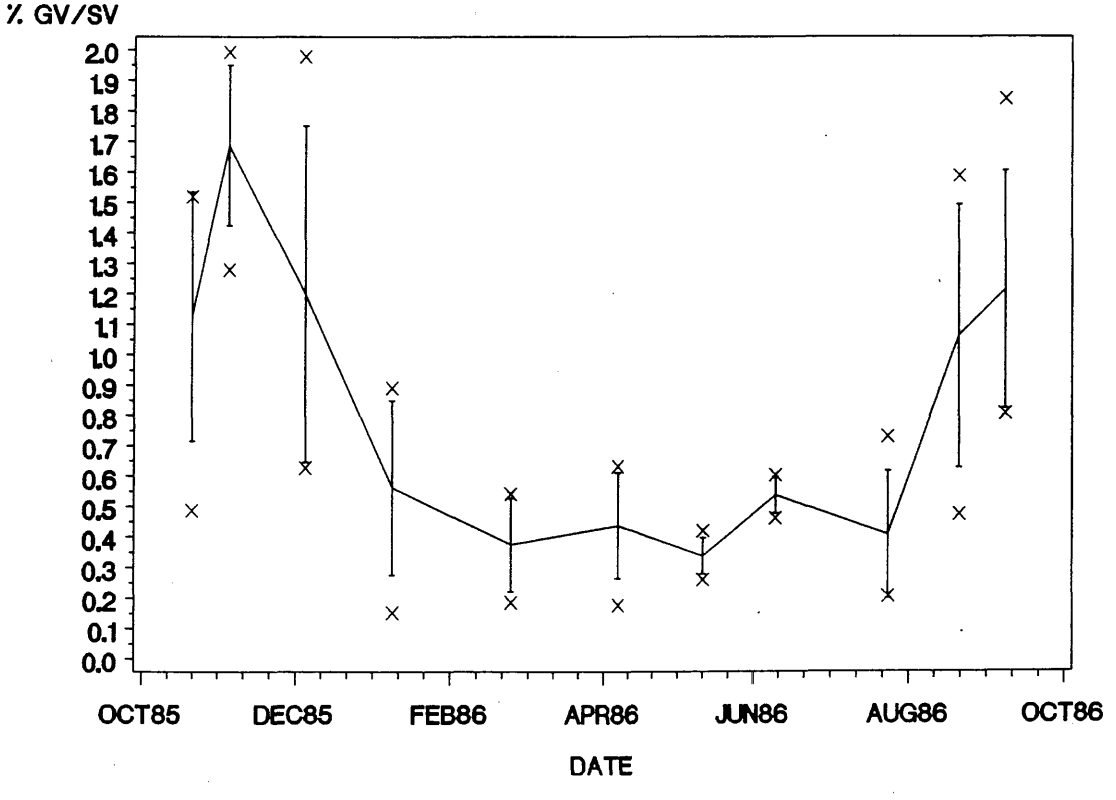


Fig. 3.5.4 Mean, Standard deviation and maximum and minimum values of percentage gonad volume per unit shell volume (% GV/SV).



5.4.2 Examination of Male Gonad Tissue from the Firth of Lorn.

Spermatogonia. Table 4. Figs. 4.0.1-4.

Spermatogonia reach their largest volume fraction of approximately 4% during May, with values rising steadily from an October/November minimum. The masses of this cell fraction within individuals indicate that some spermatogonia are present throughout the year. Greatest within sample synchrony was observed between September and December and during May. The corresponding GVF values were lowest in September and December but highest in May. Analysis of Variance indicates significant differences within and between samples.

Primary Spermatocytes. Table 4. Figs. 4.1.1-4.

Primary spermatocytes increase steadily in volume fraction from a minimum in December, peaking in July at approximately 25%. This pattern is reflected in the estimated masses of individuals and when expressed as a standardised mass. Mass per unit volume increases rapidly between May and June achieving the highest values in July and declining thereafter to a period of consistently low values from December until May. During this time there is a high degree of synchrony between both individuals and samples, a feature which is repeated in July. Significant differences exist within and between samples.

Secondary Spermatocytes. Table 4. Figs. 4.2.1-4.

As might be expected the development of secondary spermatocytes follows a similar phase to both spermatogonia and primary spermatocytes. It is of note however, that this cell phase occupies less than half the GVF of primary spermatocytes. With the smallest values for GVF between February and April there is an increase until July followed by a gradual decline towards the late autumn and throughout the winter. The degree of synchrony between individuals is highly variable and this is reflected in significant differences between and within samples.

Spermatozoa and Spermatids. Table 4. Figs. 4.3.1-4.

The spermatid/sperm cell phase constitutes the largest GVF of any reproductive component of the gonad. Maximal values achieve a plateau from October until January. During subsequent months values decrease to the lowest average in April. Spermatids/sperm occur in relatively small quantities between February and May, which hails the start of a slight increase throughout the summer months. Commencing in August there is a rapid escalation in the numbers of cells of this phase reaching a peak in November. Marked seasonality of production combined with the lack of synchrony between individuals of the same sample gives rise to significant differences.

Coelomic Space. Table 4. Figs. 4.4.1-4.

Coelomic or cell free space within the male not only represents the largest proportion but consistently occupies greater than 40% and up to 80% of the GVF during early spring. A similar pattern emerges with respect to the mass within individuals and when standardised. This shows higher values between a period spanning the late summer until early winter. Variance to mean ratios are close to zero between spring and mid summer indicating a relatively high degree of synchrony within the population at this time. Variation displays a dramatic increase between July and September and again between November and March. This is reflected in significant differences both between and within samples.

Nonreproductive Tissue. Table 4. Figs. 4.5.1-4.

Nonreproductive materials are a minor proportion of the GVF, and rarely exceed 3%. Although the actual amounts of this phase are small, it appears to represent a fairly constant proportion of the genital tissues in the male. When expressed as a mass there is a marginal increase from June until July, but a single large value generated by an individual tends to detract from the significance of this increase. Such a result is however, of interest and will be discussed. Greatest

synchrony exists between individuals in December and September, though differences between and within samples are significant.

Summary of Male Reproductive Tissue. Figs. 4.6.1-4.

Considered as an entity the male gonad increases in size in concert with the increase in mass thereby maintaining the same overall density. The gonad is largest in November and gradually decreases in volume to a minimum in May. Throughout the summer the gonads increase in volume until September and then more rapidly, peaking in November. A composite plot of GVF illustrates the maintainance within the gonad of coelomic or cell free space throughout the reproductive cycle. It is evident that the GVF of spermatid/sperm falls as that of the primary spermatocytes starts to rise surpassing the spermatid/sperm fraction between February and April. The GVF of the primary spermatocyte achieves a peak in July and declines thereafter. The situation is thereby reversed as the level of the spermatid/sperm displays a reciprocal increase. The GVF of secondary spermatocytes is always lower than the spermatid/sperm and primary spermatocytes, but the production of secondary cells mirrors their development.

TABLE 4.

Analysis of Variance.
Males from the Firth of Lorn.

Cell Phase	Source	DF	F Value	PR > F
SPG	Within	10	27.07	0.0000
	Between	44	6.06	0.0001
SPC	Within	10	168.17	0.0000
	Between	44	10.91	0.0000
SSC	Within	10	99.75	0.0000
	Between	44	9.88	0.0000
SPT	Within	10	111.38	0.0000
	Between	44	33.45	0.0000
EMT	Within	10	104.25	0.0000
	Between	44	18.24	0.0000
NRPD	Within	10	13.32	0.0001
	Between	44	5.07	0.0001

Fig. 4.0.1 Mean, Standard deviation and maximum and minimum values for the percentage GVF of Spermatogonia (% SPG).

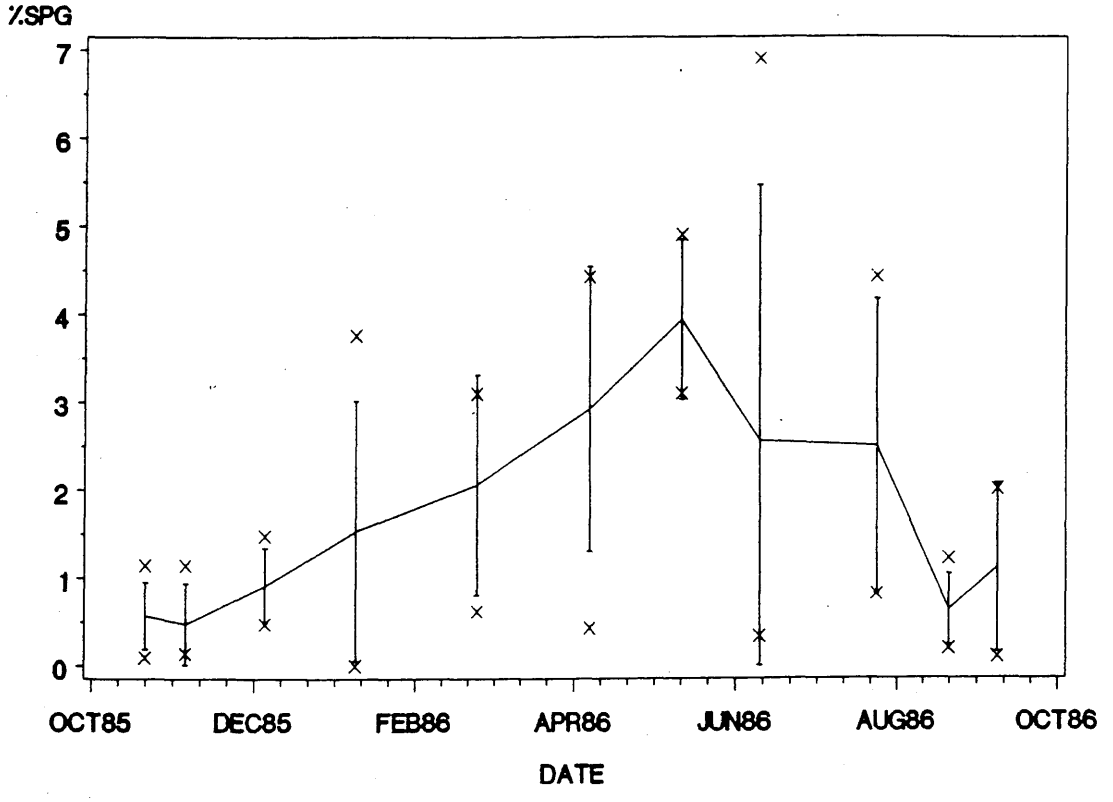
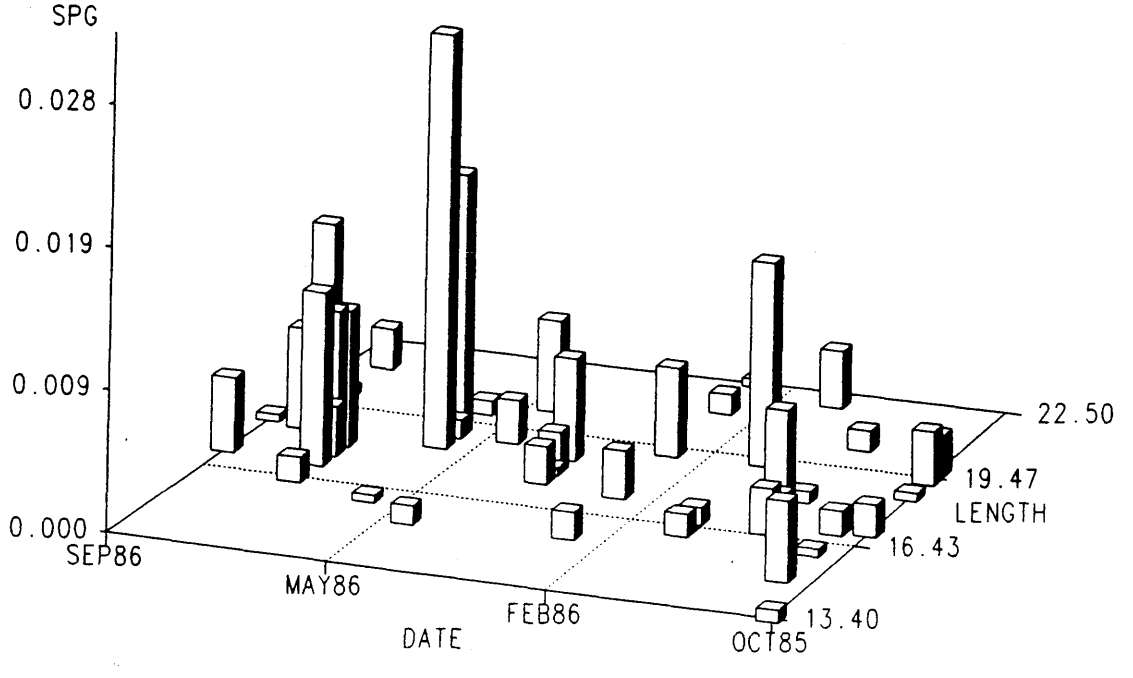


Fig. 4.0.2 Mass of Spermatogonia in individuals against length and sample date.



SPG: Mass of gonad in grams
LENGTH: Length in mm.

Fig. 4.0.3 Mean, Standard deviation and maximum and minimum values for the Standard Mass of Spermatogonia (SPG mg/mm^3).

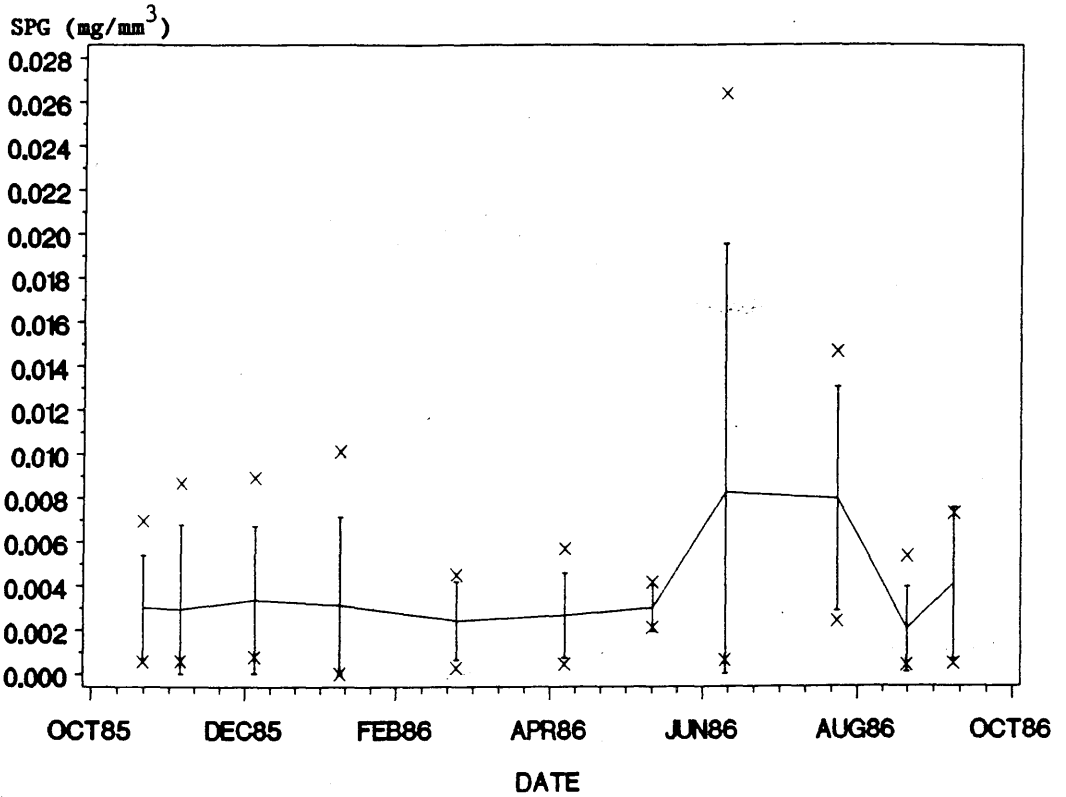


Fig. 4.0.4 Variance to Mean Ratio of the GVF of Spermatogonia (SPG).

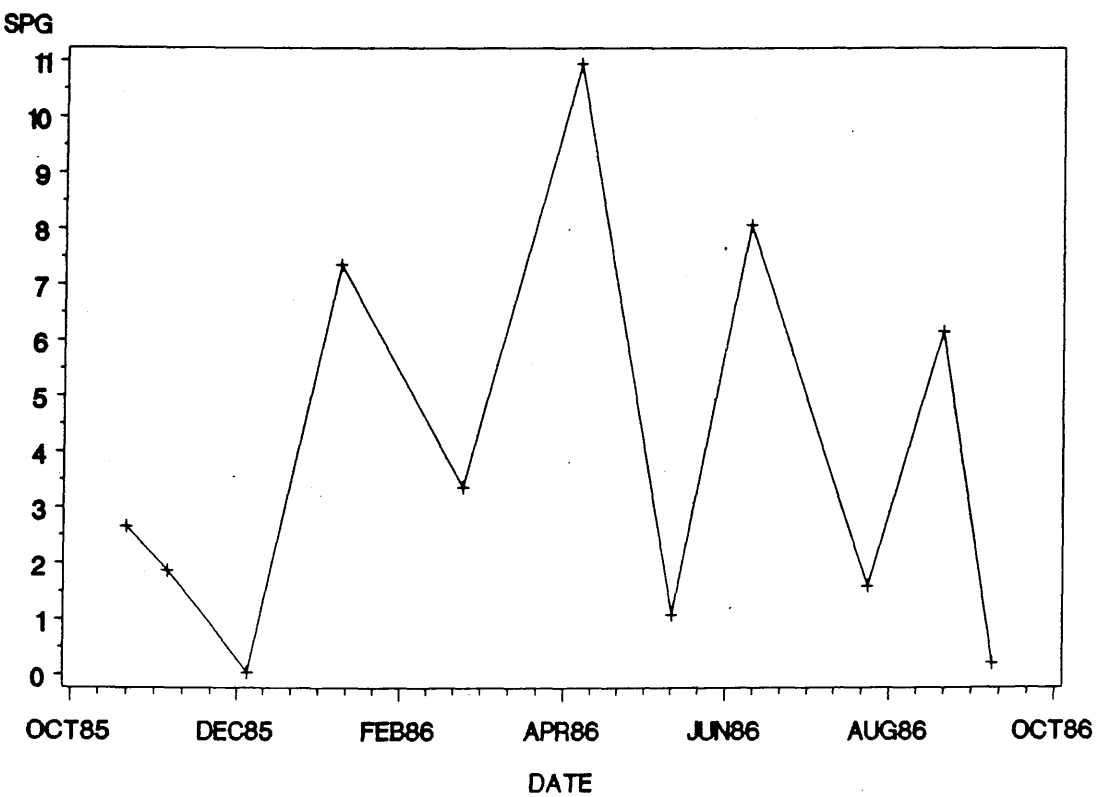


Fig. 4.1.1 Mean, Standard deviation and maximum and minimum values for the percentage GVF of Primary Spermatocytes (% SPC).

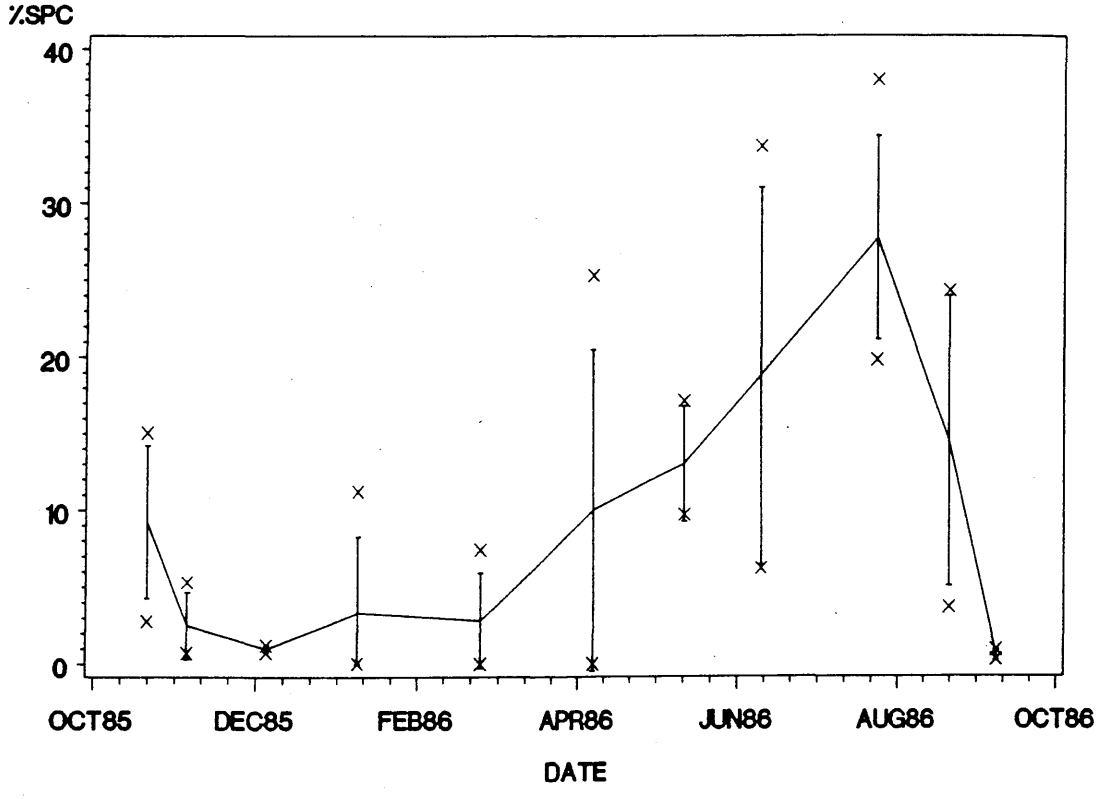
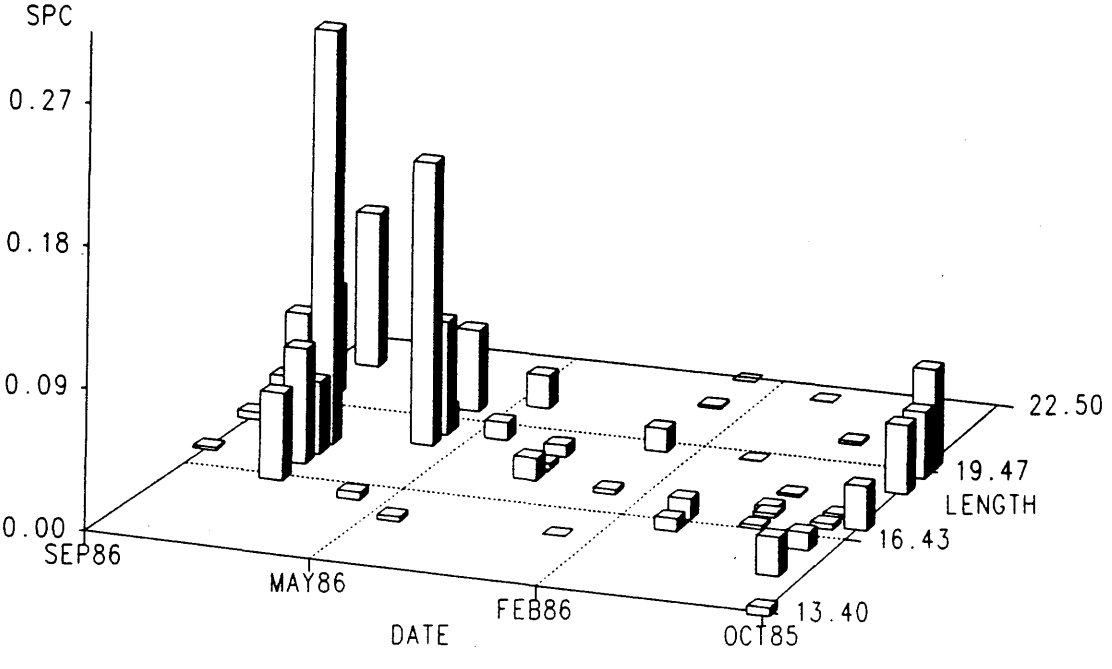


Fig. 4.1.2 Mass of Primary Spermatocytes in individuals against length and sample date.



SPC: Mass of gonad in grams
LENGTH: Length in mm.

Fig. 4.1.3 Mean, Standard deviation and maximum and minimum values for the Standard Mass of Primary Spermatocytes (SPC mg/mm^3).

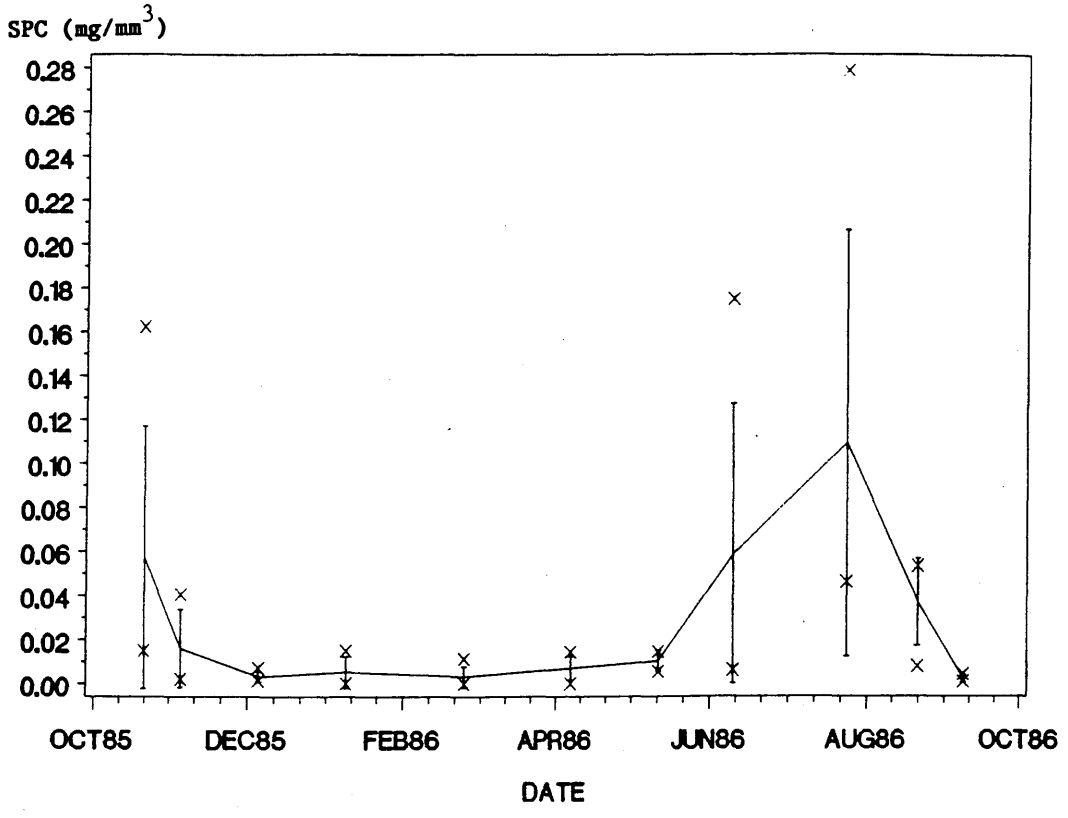


Fig. 4.1.4 Variance to Mean Ratio of the GVF of Primary Spermatocytes (SPC)

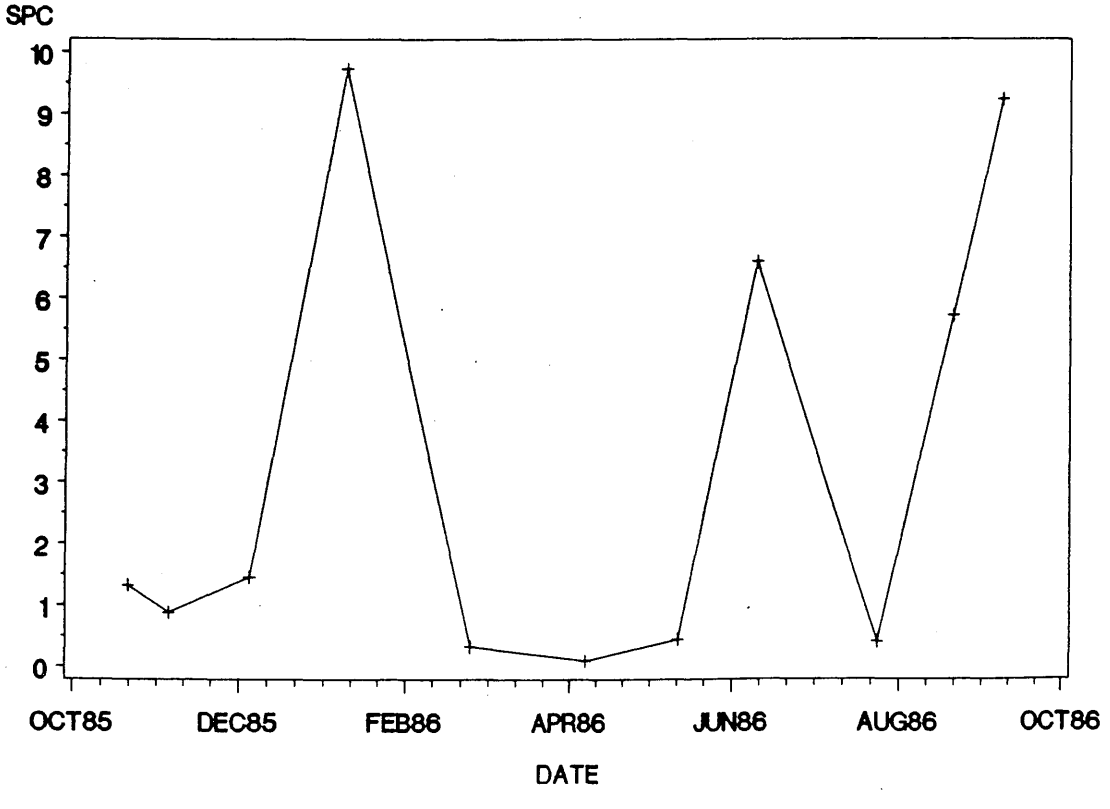


Fig. 4.2.1 Mean, Standard deviation and maximum and minimum values for the percentage GVF of Secondary Spermatocytes (% SSC).

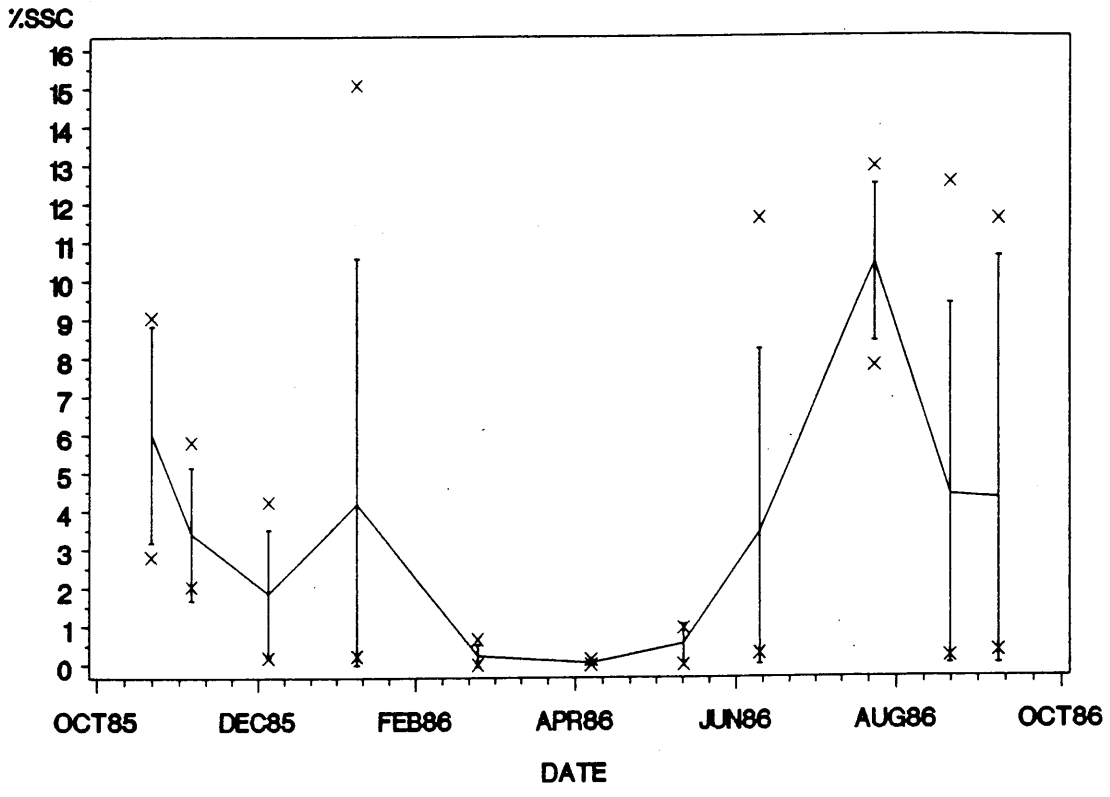
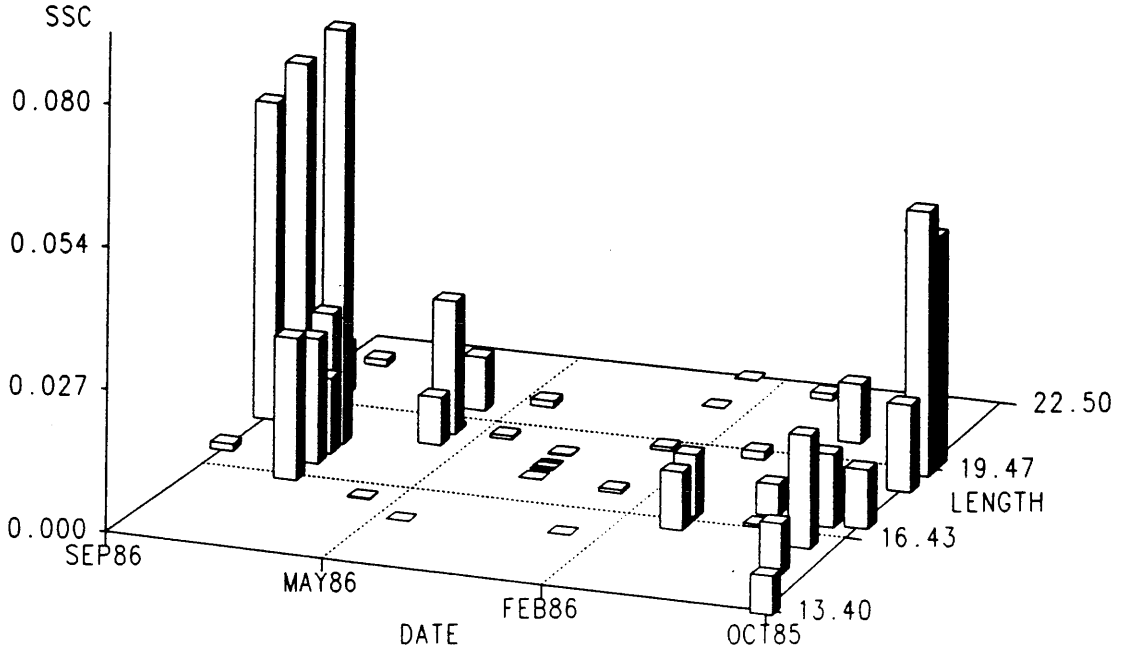


Fig. 4.2.2 Mass of Secondary Spermatocytes in individuals against length and sample date.



SSC: Mass of gonad in grams
LENGTH: Length in mm.

Fig. 4.2.3 Mean, Standard deviation and maximum and minimum values for the Standard Mass of Secondary Spermatocytes (SSC mg/mm^3).

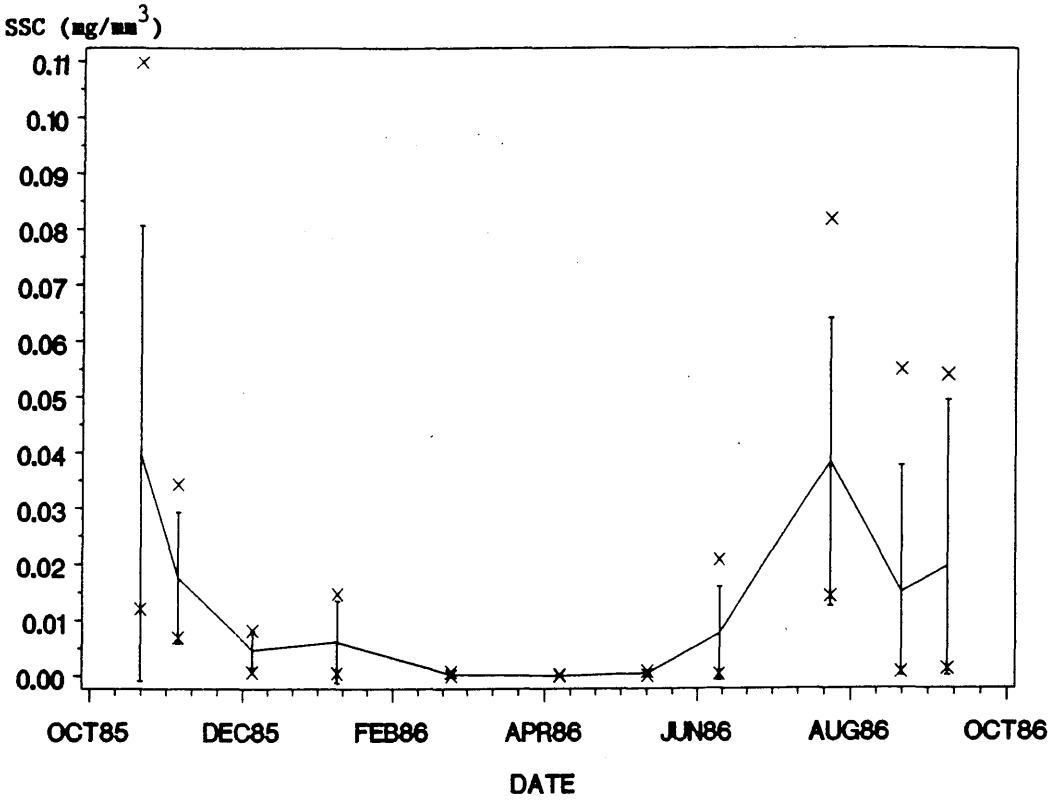


Fig. 4.2.4 Variance to Mean Ratio of the GVF of Secondary Spermatocytes (SSC).

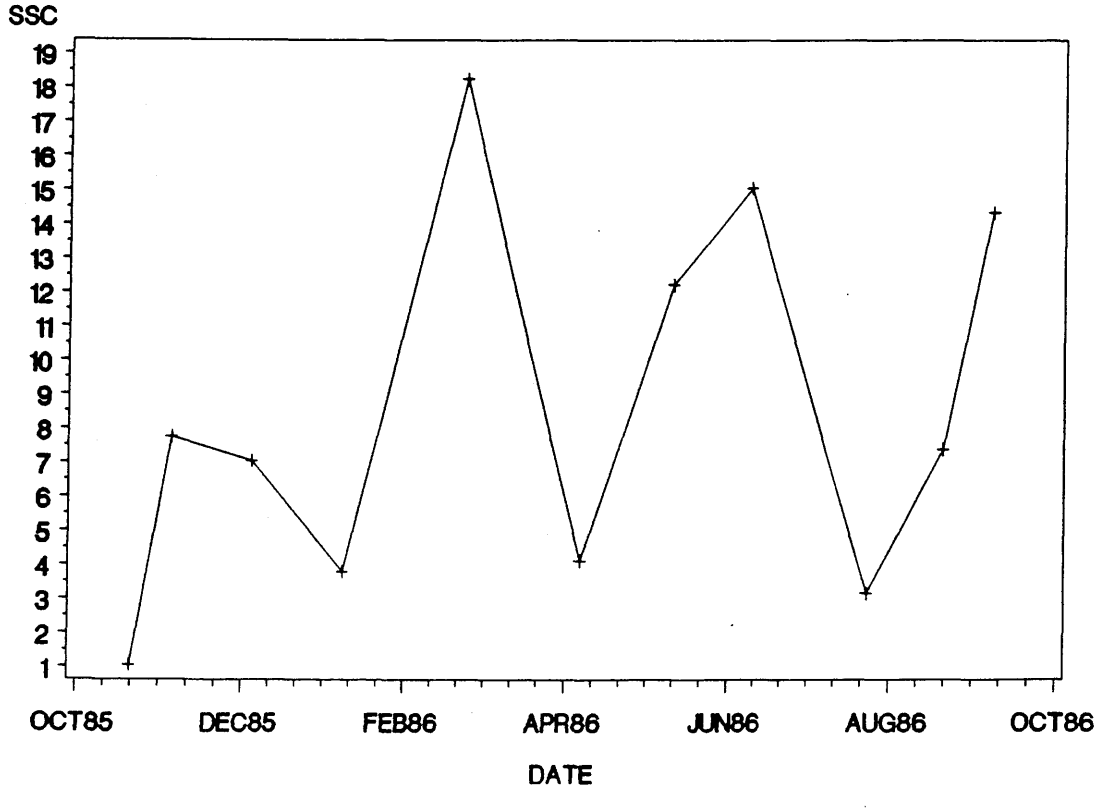


Fig. 4.3.1 Mean, Standard deviation and maximum and minimum values for the percentage GVF of Spermatids and Spermatozoa (% SPT).

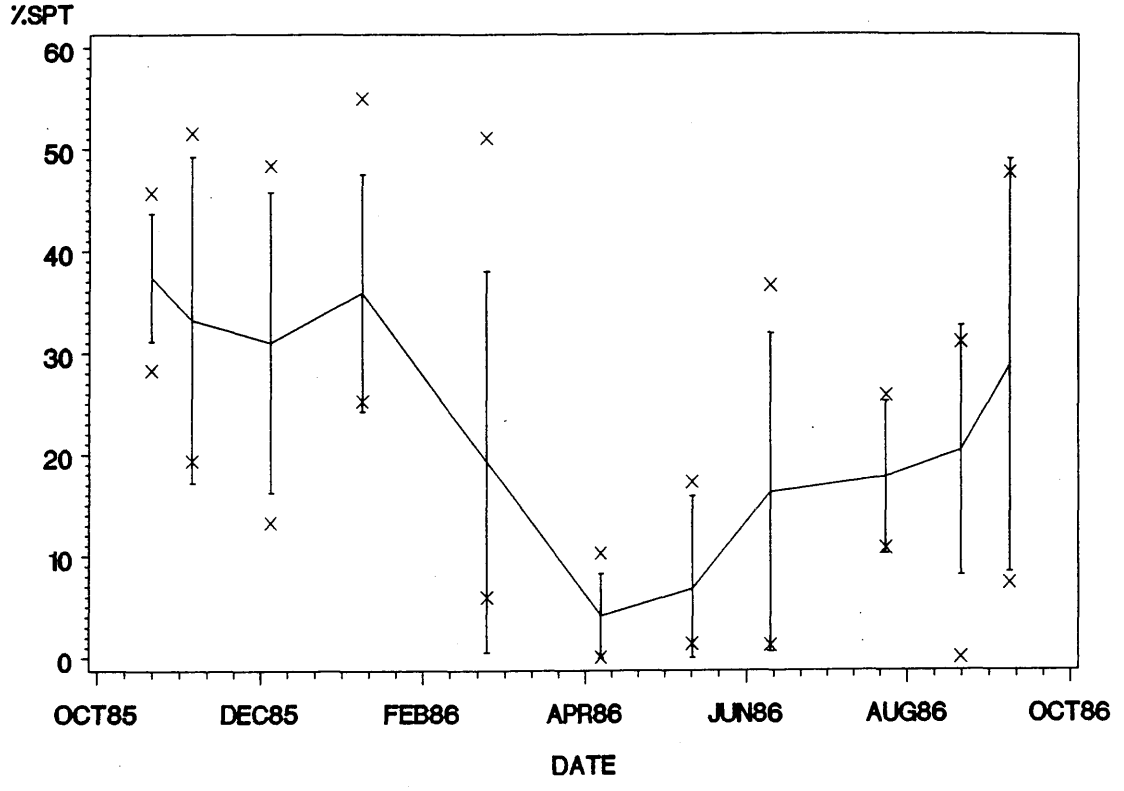
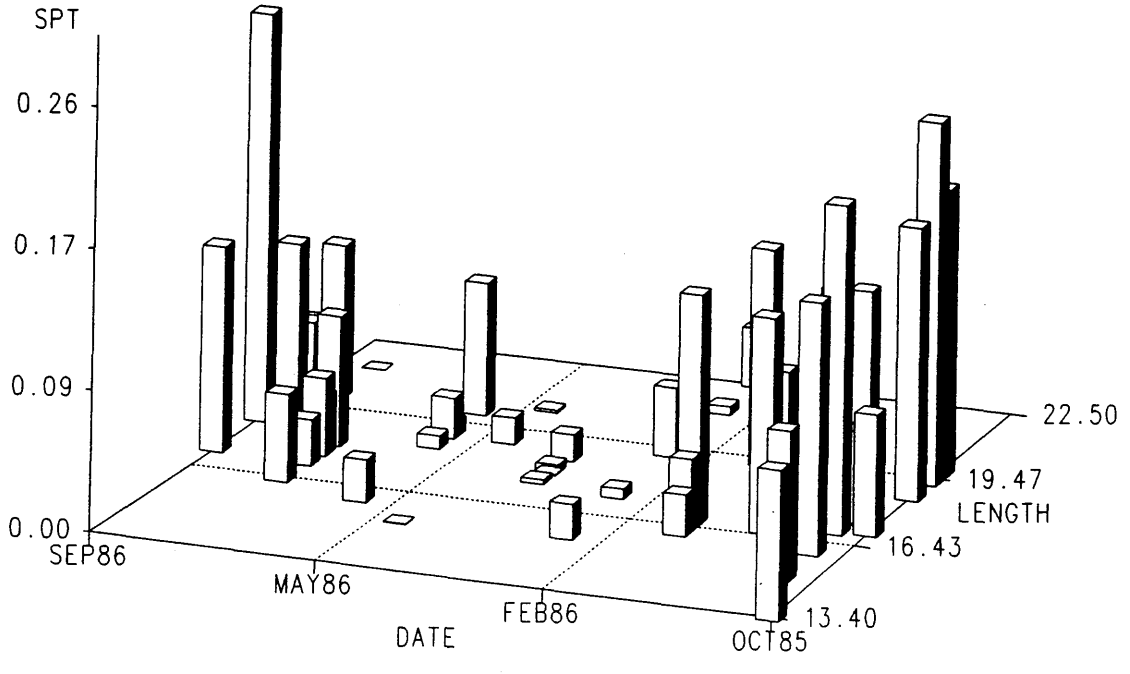


Fig. 4.3.2 Mass of Spermatids and Spermatozoa in individuals against length and sample date.



SPT: Mass of gonad in grams.
LENGTH: Length in mm.

Fig. 4.3.3 Mean, Standard deviation and maximum and minimum values for Standard Mass of Spermatids and Spermatozoa (SPT mg/mm^3).

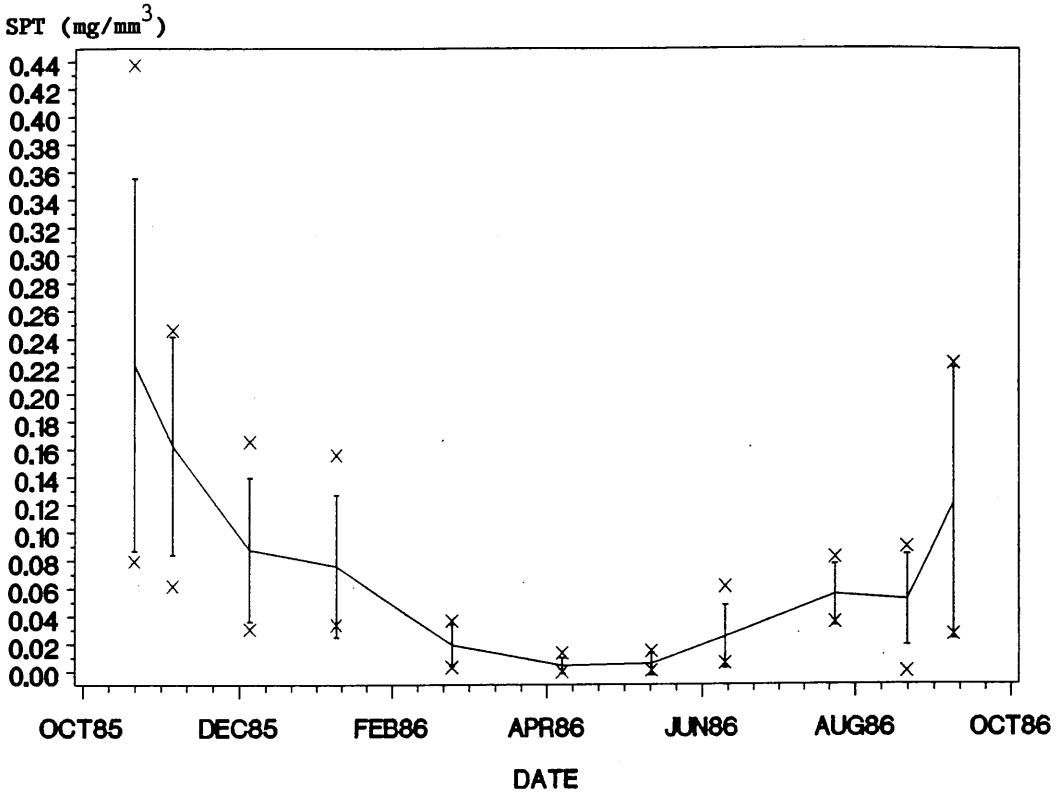


Fig. 4.3.4 Variance to Mean Ratio of the GVF of Spermatids and Spermatozoa (SPT).

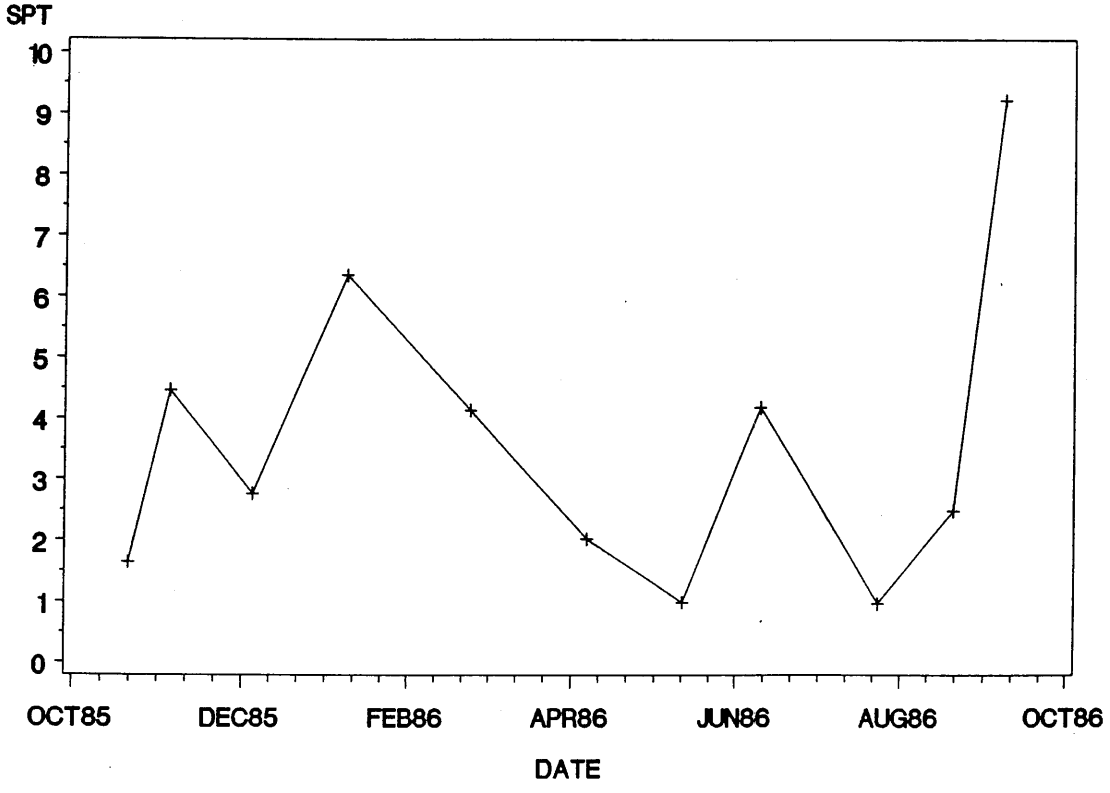


Fig. 4.4.1 Mean, Standard deviation and maximum and minimum values for the percentage GVF of Coelomic Space (% EMT).

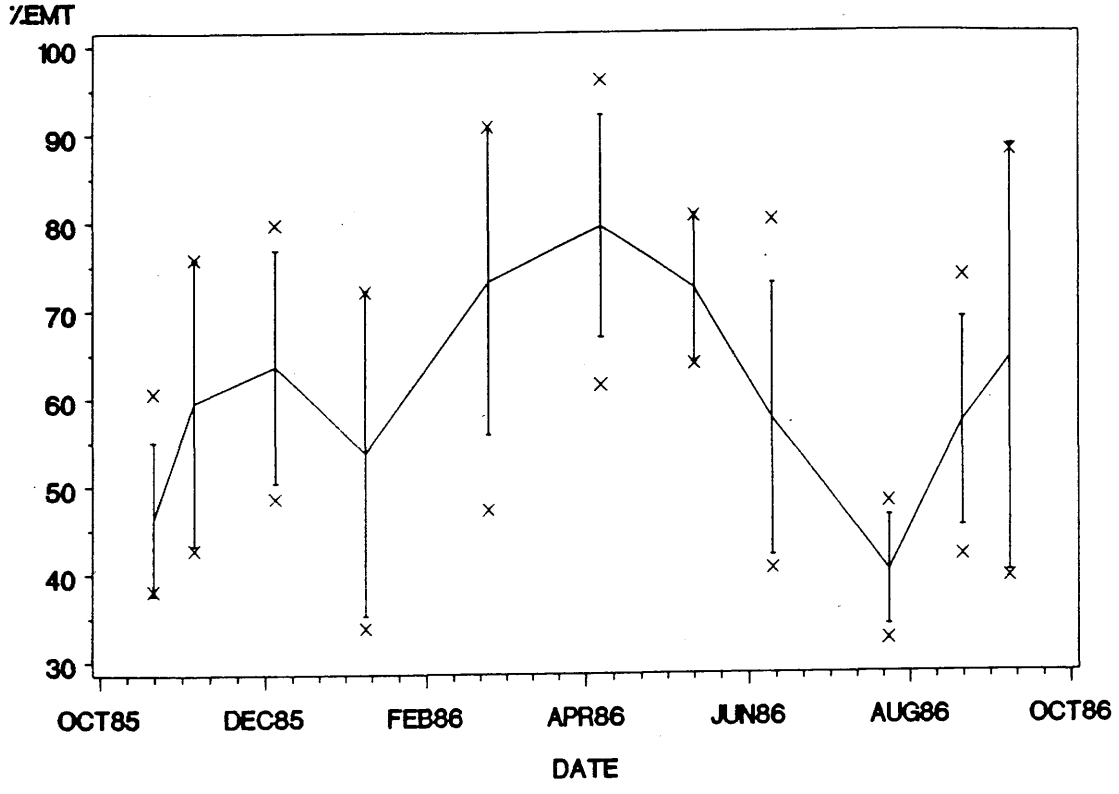
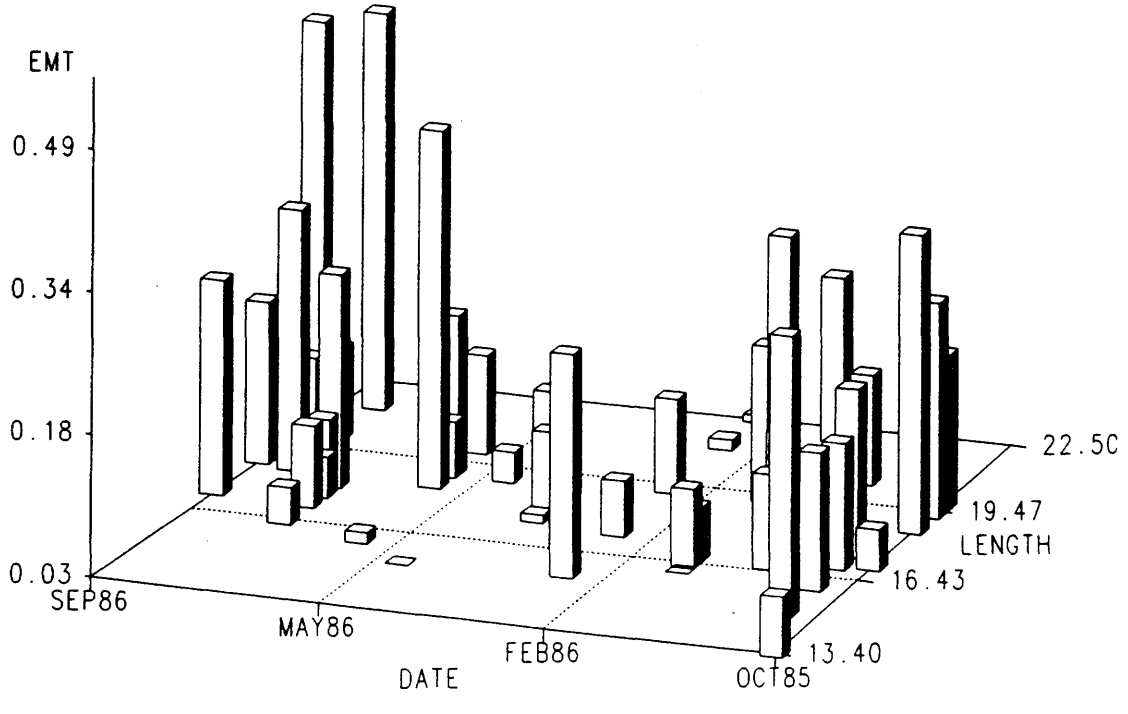


Fig. 4.4.2 Mass of Coelomic Space in individuals against length and sample date.



EMT: Mass of gonad in grams
LENGTH: Length in mm.

Fig. 4.4.3 Mean, Standard deviation and maximum and minimum values for the Standard Mass of Coelomic Space (EMT mg/mm^3).

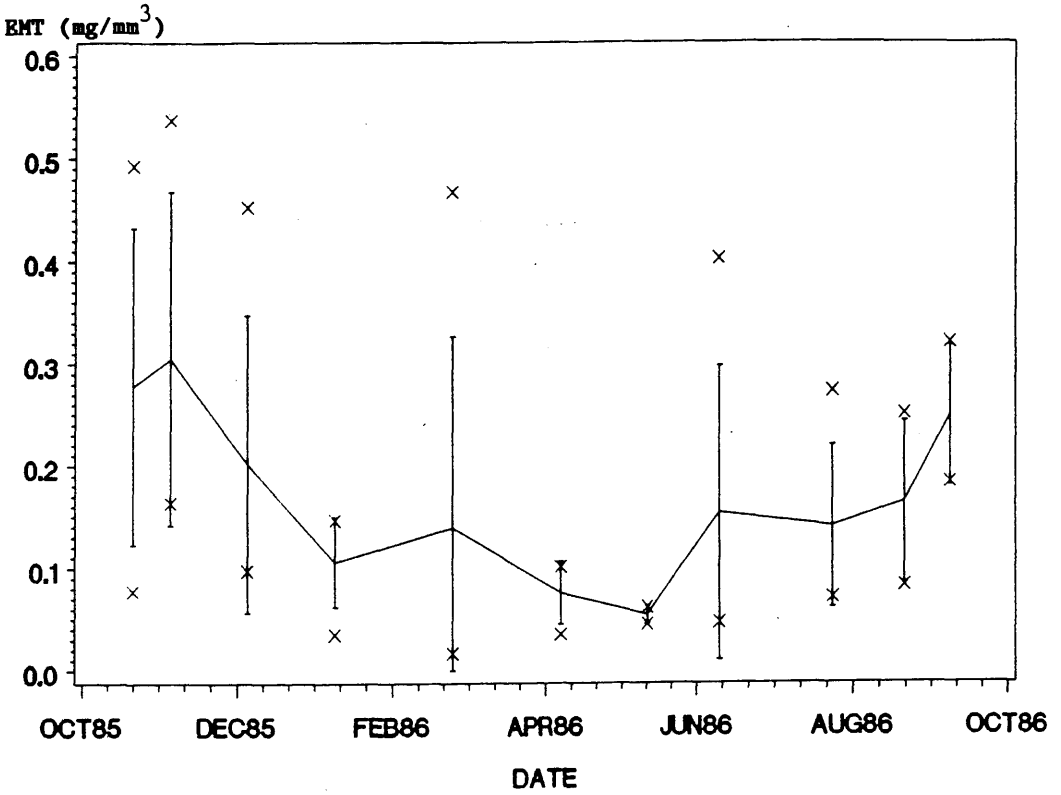


Fig. 4.4.4 Variance to Mean Ratio of the GVF of Coelomic Space (EMT).

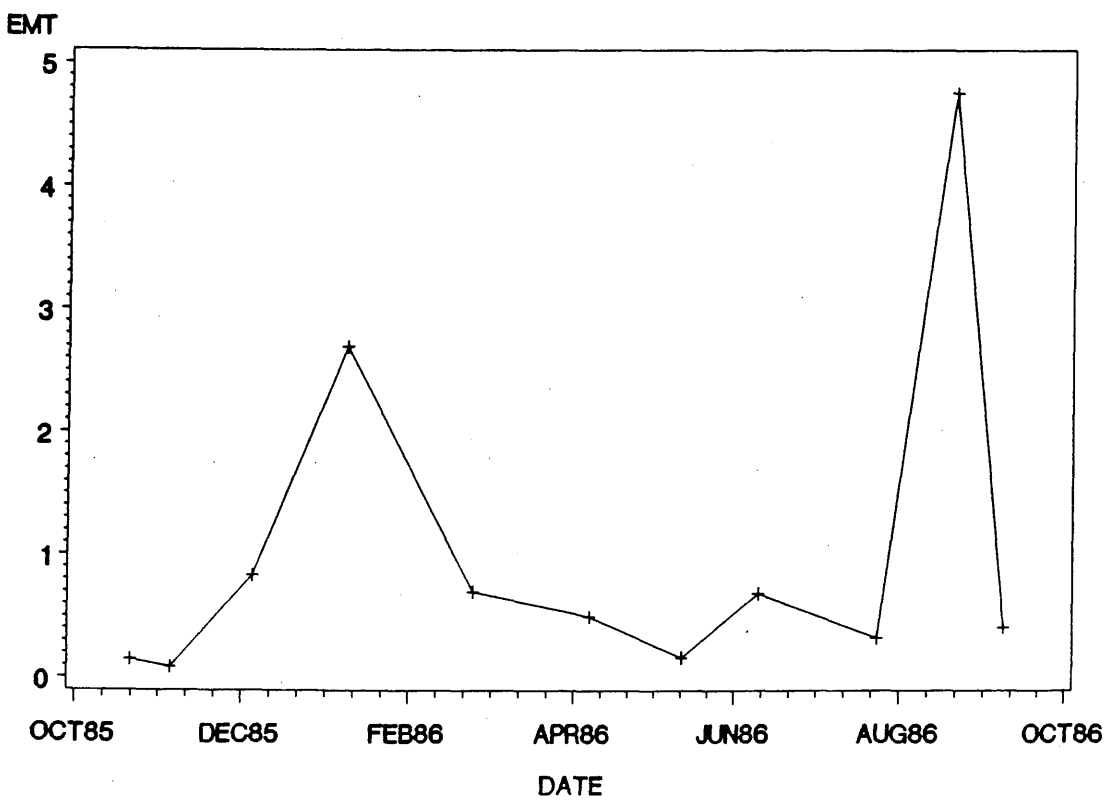


Fig. 4.5.1 Mean, Standard deviation and maximum and minimum values for the percentage GVF of Non Reproductive Tissue (% NRPD).

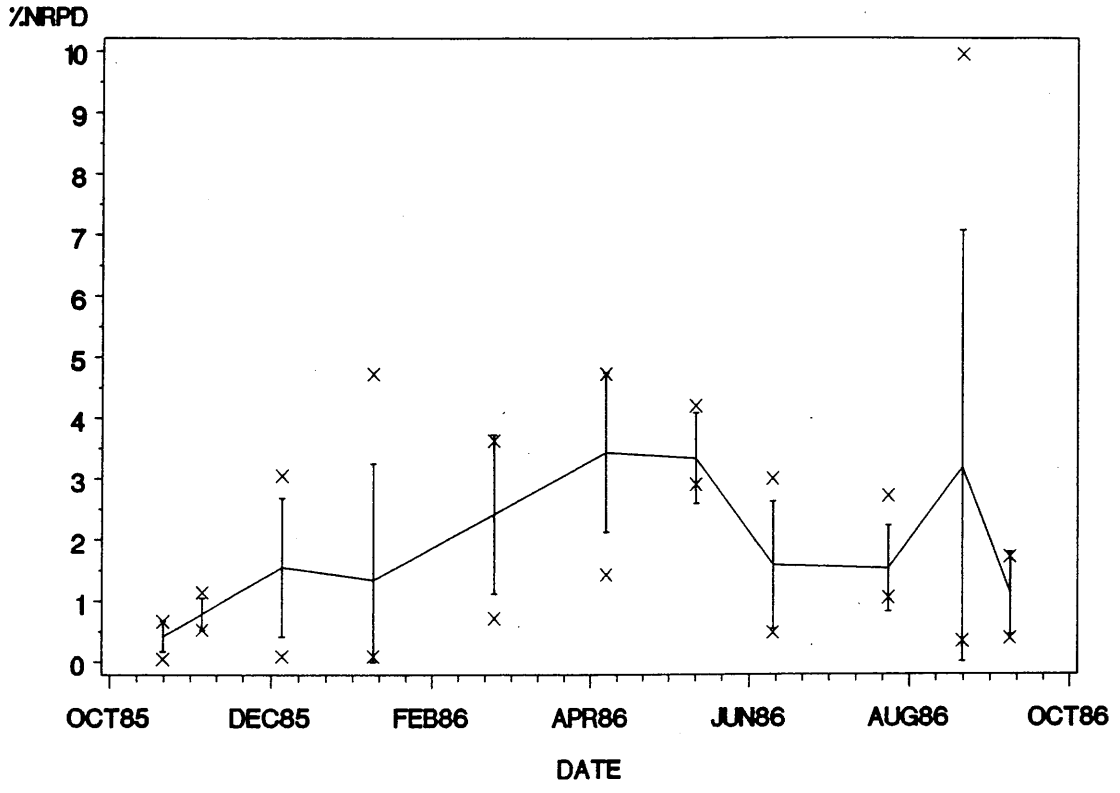
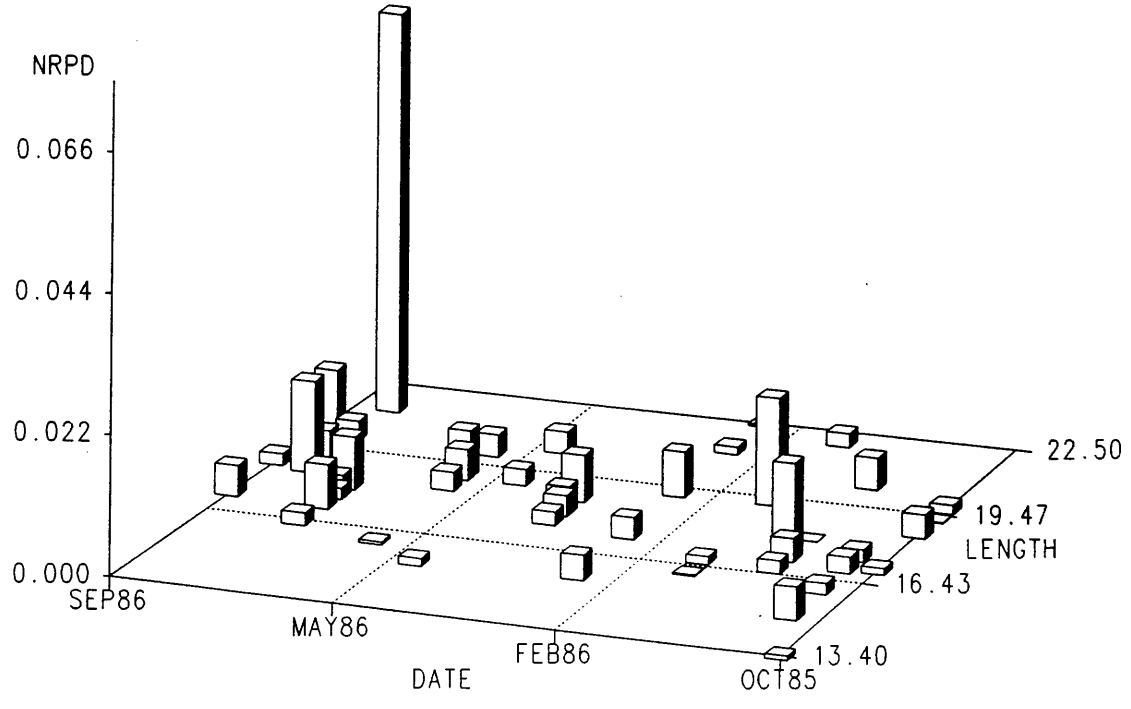


Fig. 4.5.2 Mass of Non Reproductive Tissue in individuals against length and sample date.



NRPD: Mass of gonad in grams
LENGTH: Length in mm.

MALES: FIRTH OF LORN.

Fig. 4.5.3 Mean, Standard deviation and maximum and minimum values for the Standard Mass of Non Reproductive Tissue (NRPD mg/mm^3).

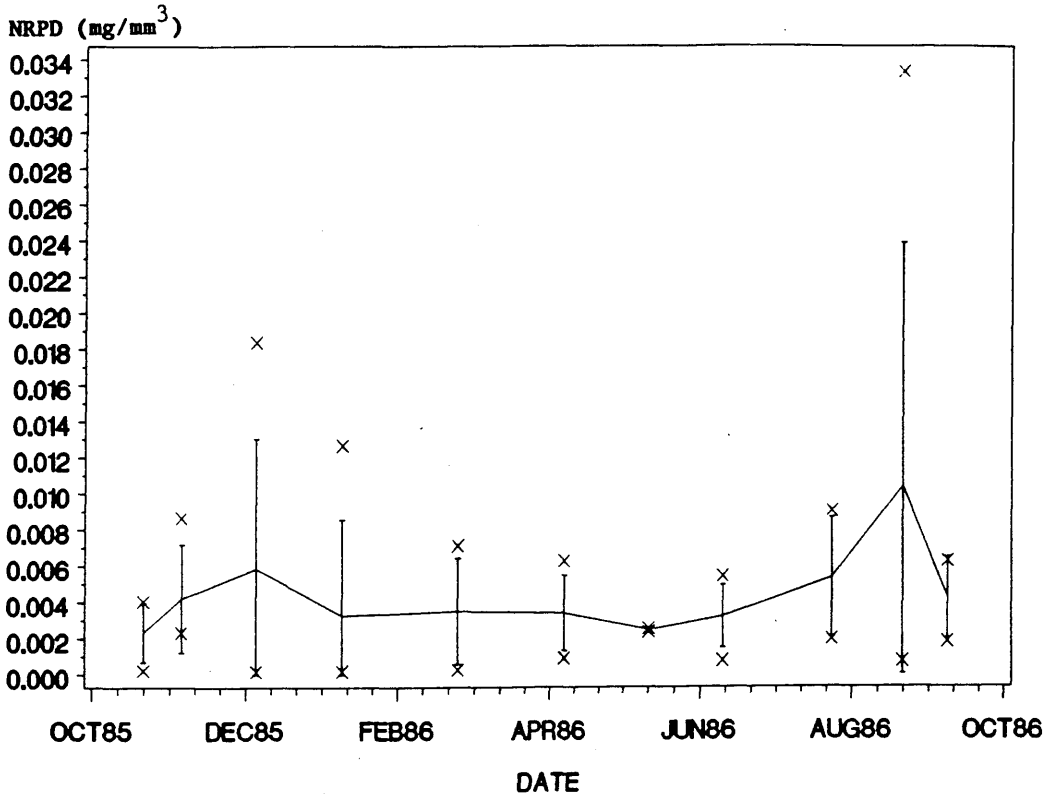


Fig. 4.5.4 Variance to Mean Ratio of the GVF of Non Reproductive Tissue (NRPD).

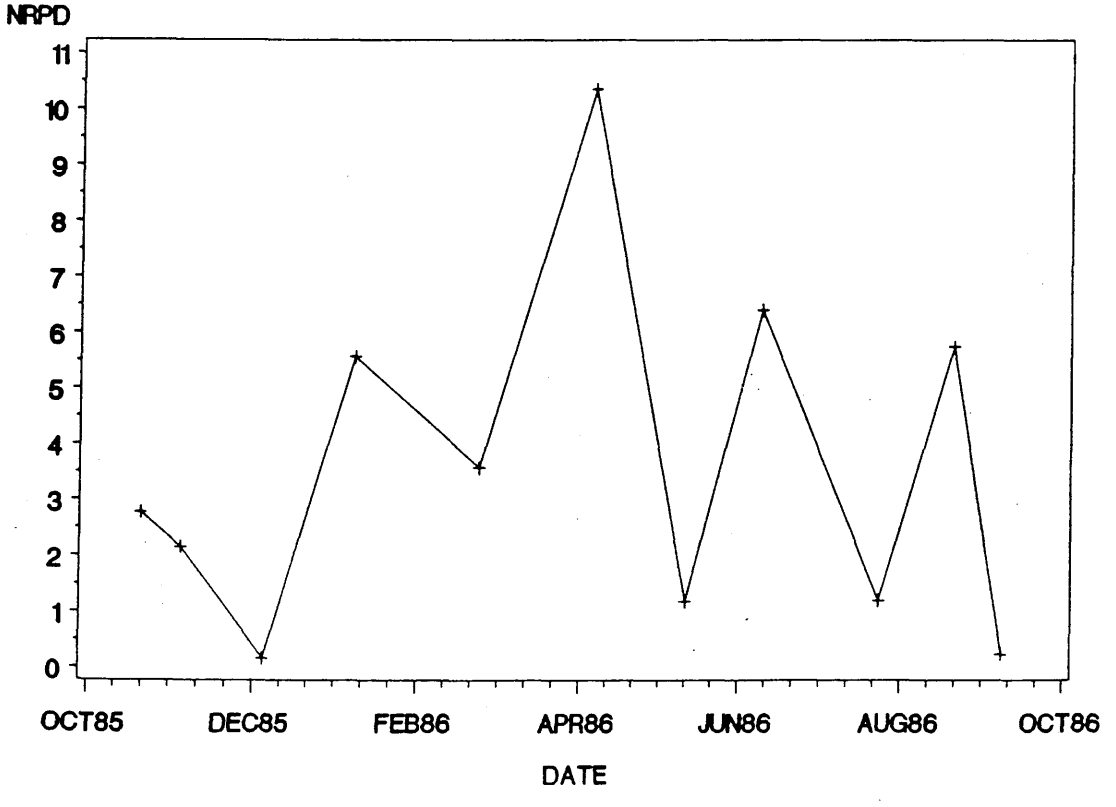


Fig. 4.6.1 Combined Plot of the Mean GVF of all the male gonad tissue phases (% VF).

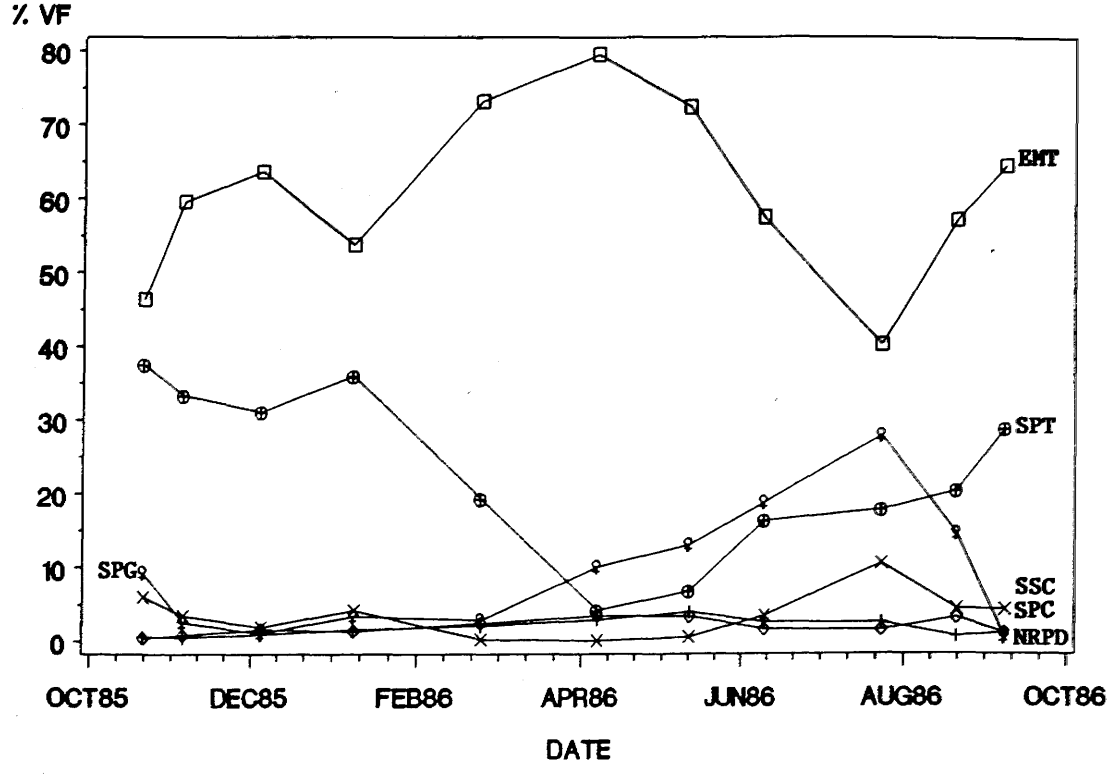
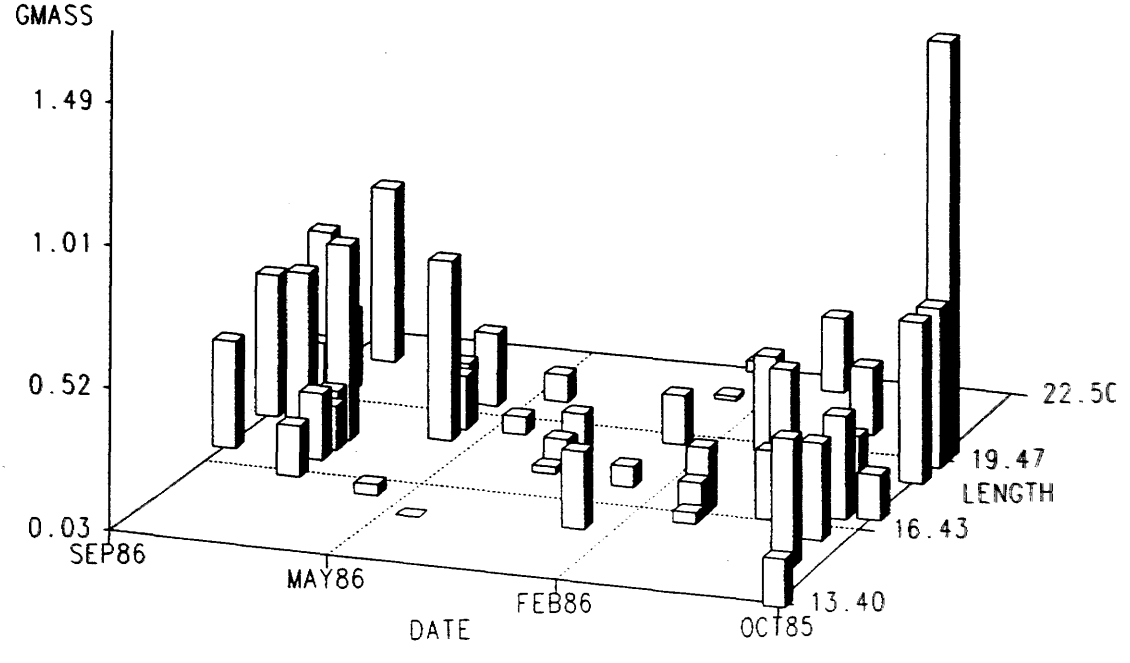


Fig. 4.6.2 Total mass of gonad in individuals against length and sample date.



GMASS: Mass of gonad in grams
LENGTH: Length in mm.

Fig. 4.6.3 Combined Plot of the Mean Standard Mass of all the male gonad tissue phases (ST. MASS mg/mm^3).

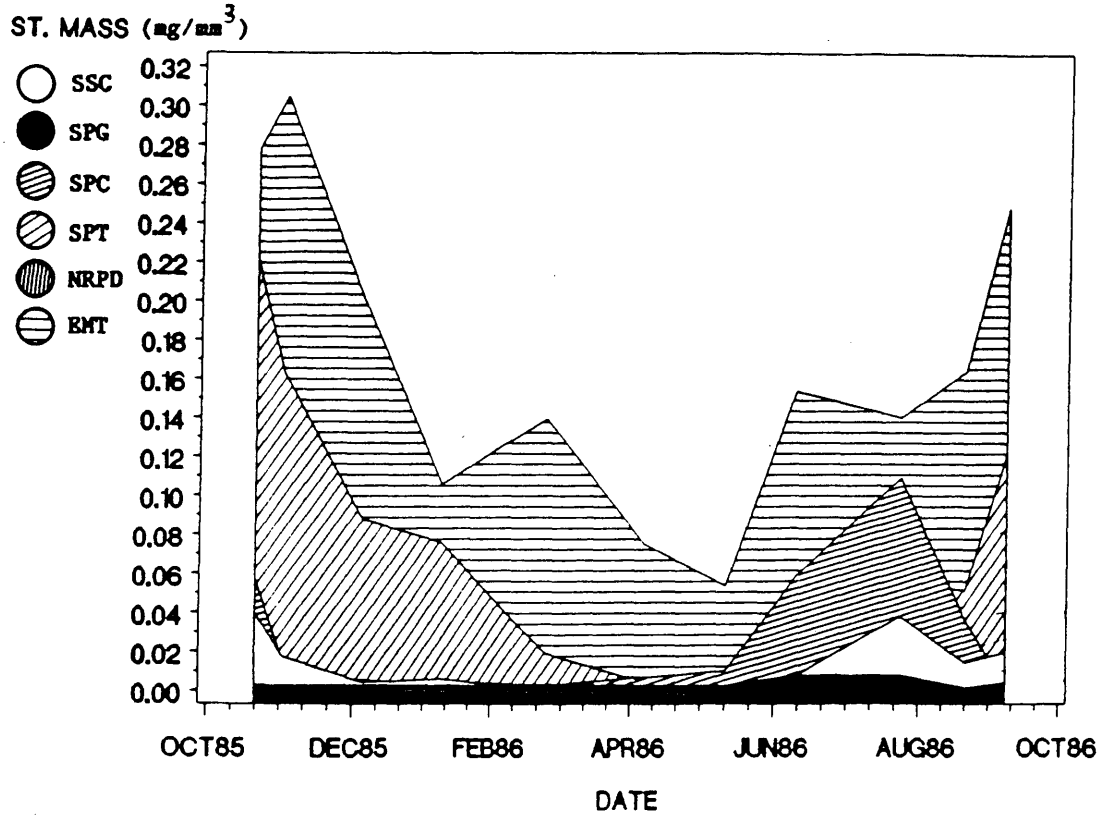
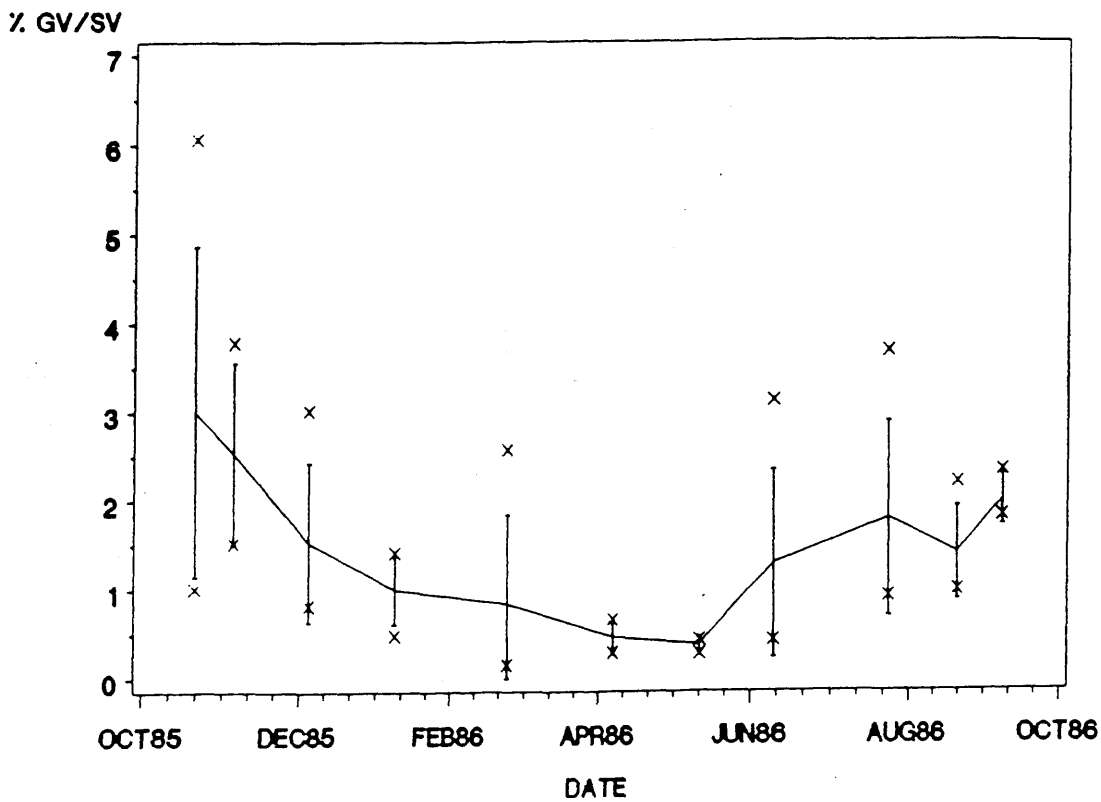


Fig. 4.6.4 Mean, Standard deviation and maximum and minimum values of percentage gonad volume per unit shell volume (% GV/SV).



5.4.3 Examination of the Female Gonad Tissue from Loch Fyne.

Oogonia. Table 5. Figs. 5.0.1-4.

Oogonia constitute less than 1% of the GVF. Their occurrence shows a notable increase in October and March, with minimum values in November and April. As this cell fraction varies little in the size of individual cells, an increase in mass or standard mass is indicative of greater numbers. The evidence points towards these cells becoming more numerous in January and late March. Oogonial production, is on the whole quite synchronised within the population with variance to mean ratios of the order of less than 0.55. Differences are significant, both between and within samples.

Oocytes. Table 5. Figs. 5.1.1-4.

Oocytes gradually increase as a proportion of the gonad tissue from minimal values in late autumn, until February. This is immediately followed by a rapid increase in late March, declining thereafter. The summer months are characterised by more minor peaks and troughs which diminish during the autumn and winter. The population lacks synchrony and this is reflected in significant differences between and within samples.

Late Mature Oocytes. Table 5. Figs. 5.2.1-4.

The pattern of accumulation and subsequent reduction due mainly to the spawning of late mature oocytes indicates that the Loch Fyne population has one major spawning period and a number of minor spawning events. The GVF of this cell phase is maximal between September and November with values decreasing during the winter, and ranges from 30 to 65%. Remarkably, from February onwards there are a series of three peaks and troughs, each succeeding peak being slightly higher than its predecessor. Peaks occur on alternate months from March until November. Immediately prior to spawning ripe female gonads may contain approximately 0.3 mg mm^{-3} shell volume of late mature oocytes. The

population is somewhat asynchronous, resulting in high variance to mean ratios which are maximal in April and lowest in July. As might be expected the variations within and between samples are significant.

Coelomic Space. Table 5. Figs. 5.3.1-4.

Coelomic space appears to form a significant proportion of the GVF during all stages of the reproductive cycle. Although the values of GVF are somewhat reciprocal with changes in late mature oocytes, it is clear that increases in late mature oocytes are mirrored by corresponding, though lesser increases in coelomic space. During April coelomic space represents 60% of the GVF and always exceeds 30%. The population is least synchronous between September and November and between February and March. Variations within and between samples are significant.

Nonreproductive Tissue. Table 5. Figs. 5.4.1-4.

Nonreproductive material forms a small proportion of the GVF, rarely exceeding 5%. The material is most abundant during July and November exhibiting minor fluctuations between the minima of December and June. For most of the year the population is synchronised with low levels of nonreproductive material with the gonad becoming asynchronous in June and between August and September. There are significant differences between and within samples.

Summary of Female Reproductive Cycle. Figs. 5.5.1-4.

Oogonia, oocytes and nonreproductive tissue generally constitute less than 5% of the GVF. Late mature oocytes and coelomic space alter reciprocally and range between approximately 30% and 70% of the volume fraction. It is of note that late mature oocytes never oocupy less than 30%. The gonad clearly varies in size with state of the reproductive cycle, and is largest between September and November. This is followed by a period of contraction until February. Thereafter, the gonads of some individuals appear to increase and decrease in

volume on alternate months, corresponding to the rise in late mature oocytes.

TABLE 5.

Analysis of Variance.
Females from Loch Fyne.

Cell Phase	Source	DF	F Value	PR > F
OOG	Within	9	2.89	0.0021
	Between	40	2.00	0.0002
OOC	Within	9	2.22	0.0185
	Between	40	1.60	0.0097
LMT	Within	9	61.35	0.0000
	Between	40	16.20	0.0000
EMT	Within	9	44.68	0.0000
	Between	40	12.20	0.0000
NRPD	Within	9	4.28	0.0001
	Between	40	4.30	0.0001

Fig. 5.0.1 Mean, Standard deviation and maximum and minimum values for the percentage GVF of Oogonia (% OOG).

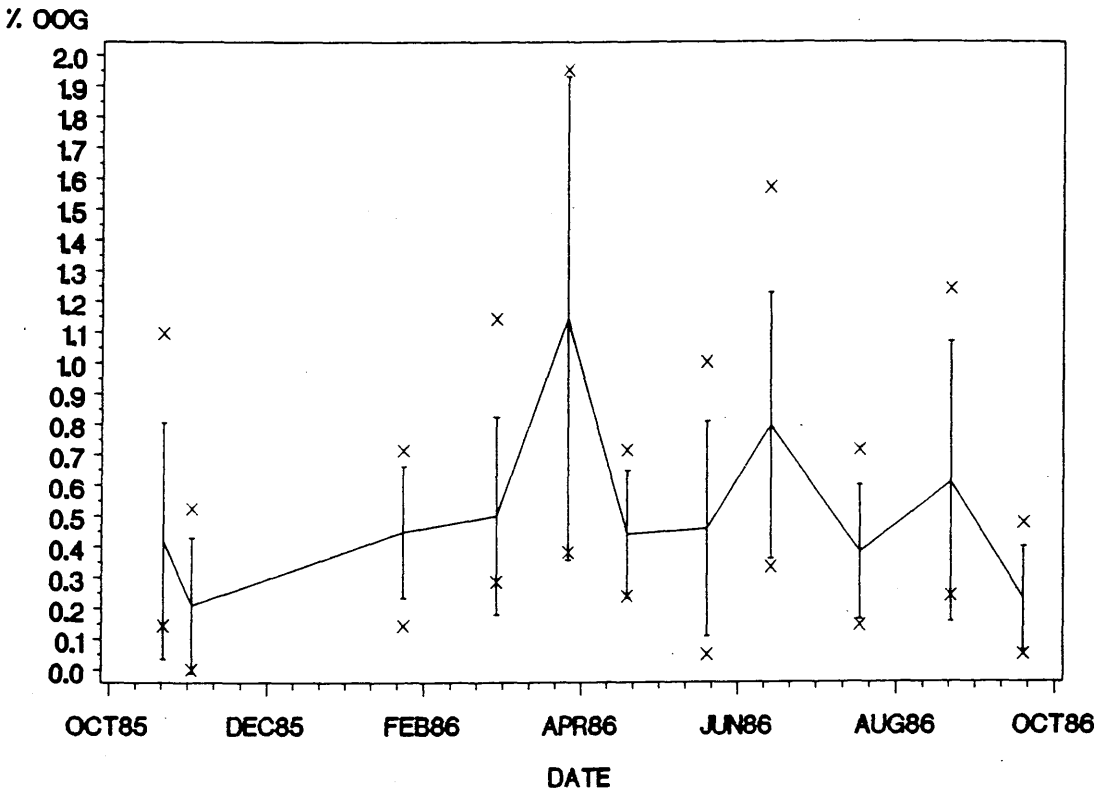
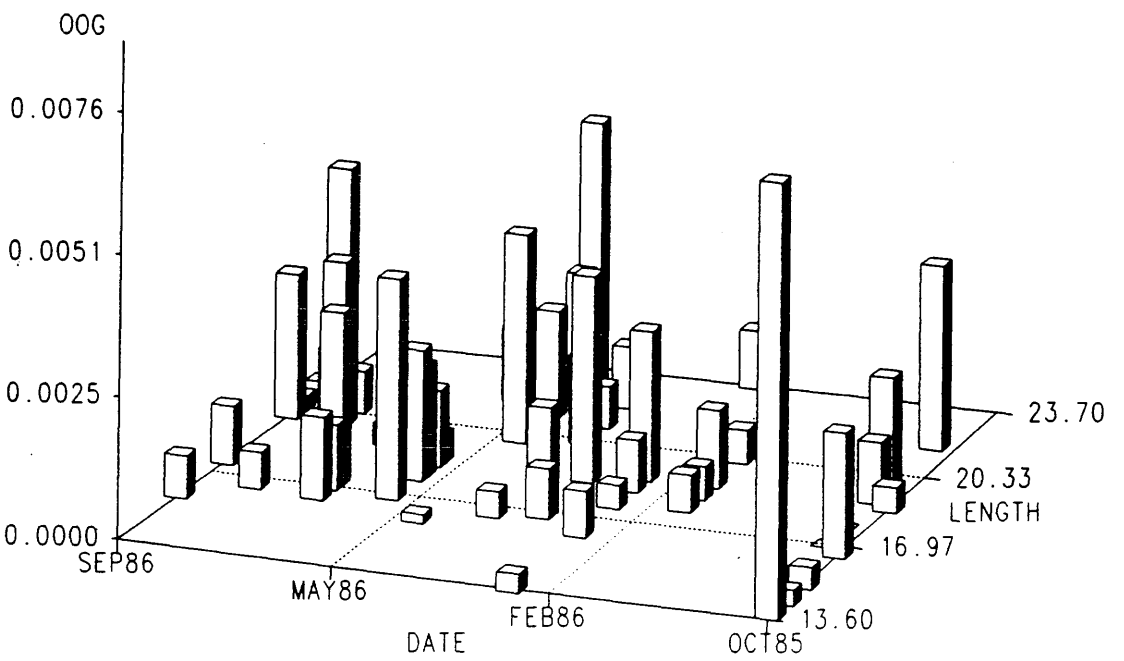


Fig. 5.0.2 Mass of Oogonia in individuals against length and sample date.



OOG: Mass of oogonia in grams
LENGTH: Length in mm.

FEMALES: LOCH FYNE.

Fig. 5.0.3 Mean, Standard deviation and maximum and minimum values for the Standard Mass of Oogonia (OOG mg/mm^3).

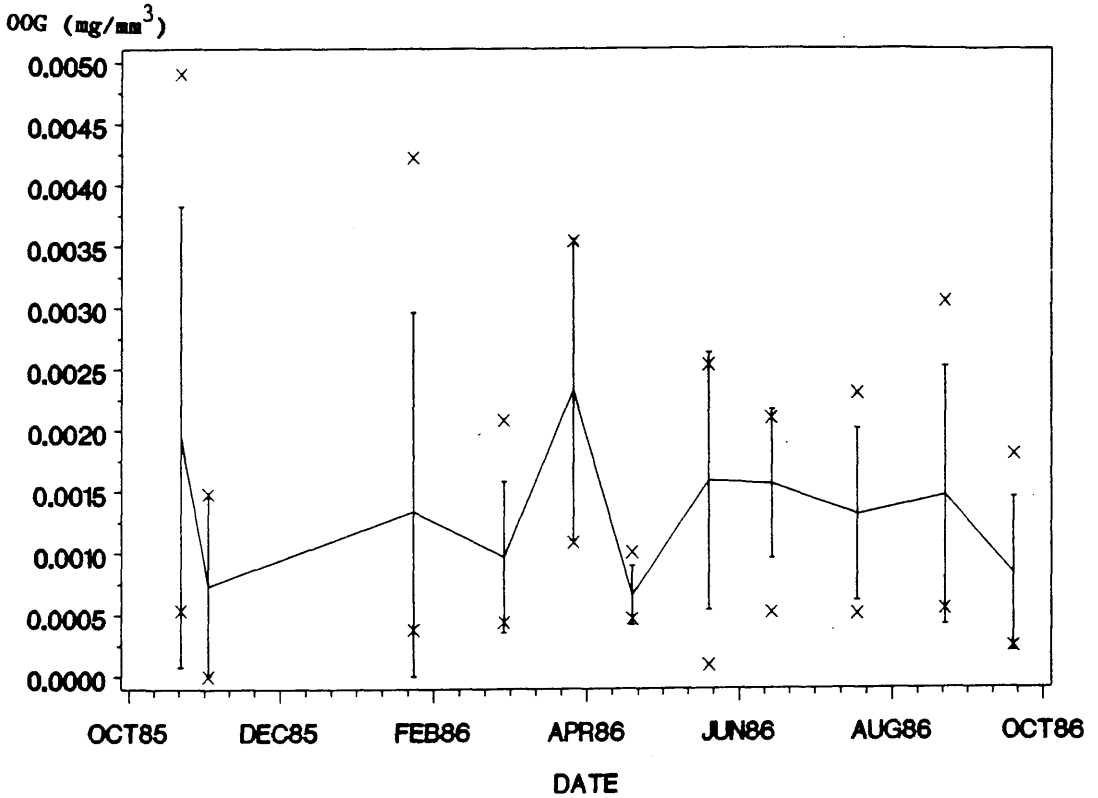
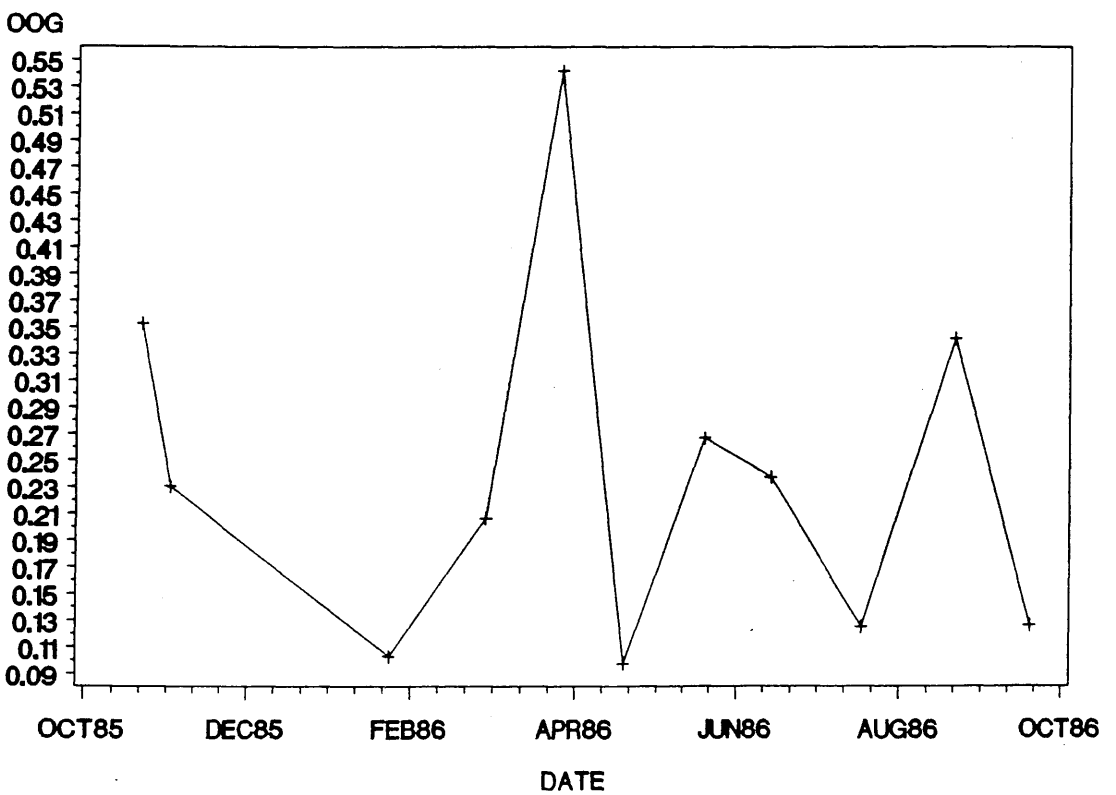


Fig. 5.0.4 Variance to Mean Ratio of the GVF of Oogonia (OOG).



FEMALES: LOCH FYNE.

Fig. 5.1.1 Mean, Standard deviation and maximum and minimum values for the percentage GVF of Oocytes (% OOC).

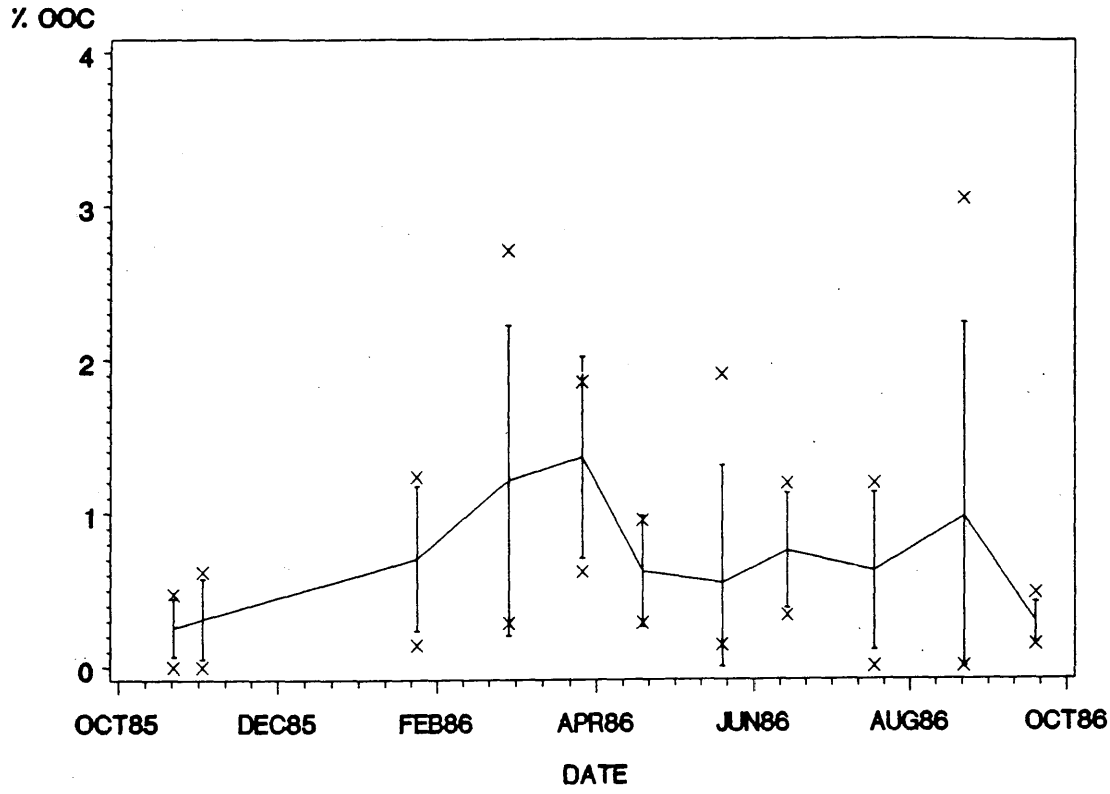
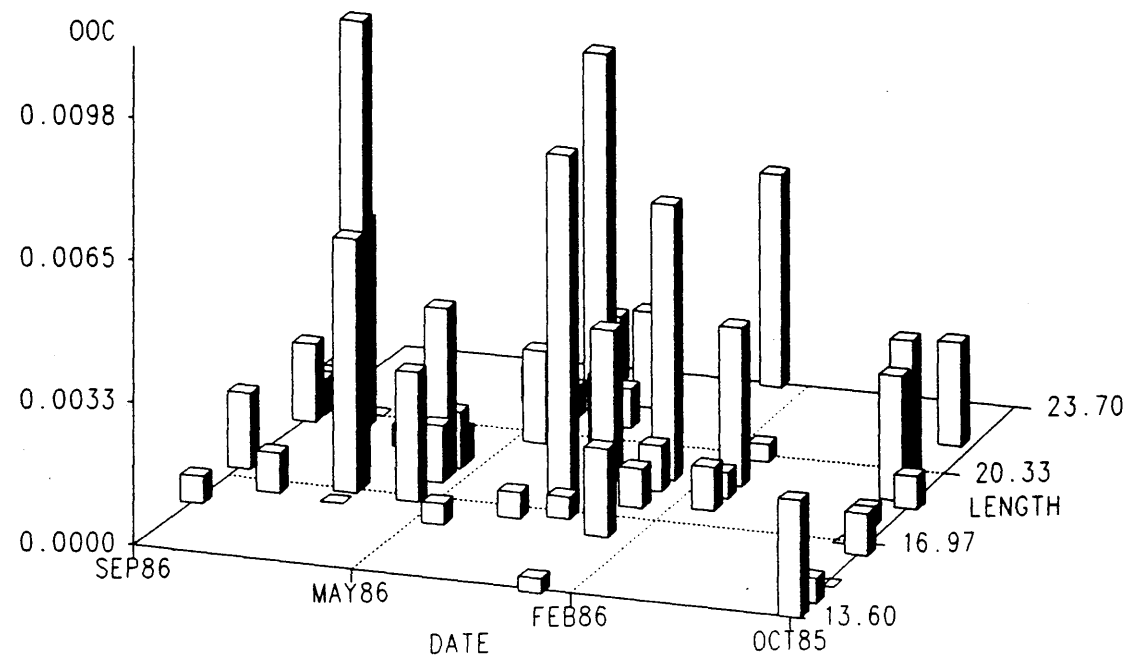


Fig. 5.1.2 Mass of Oocytes in individuals against length and sample date.



OOC: Mass of oocytes in grams
LENGTH: Length in mm.

Fig. 5.1.3 Mean, Standard deviation and maximum and minimum values for the Standard Mass of Oocytes (OOC mg/mm^3).

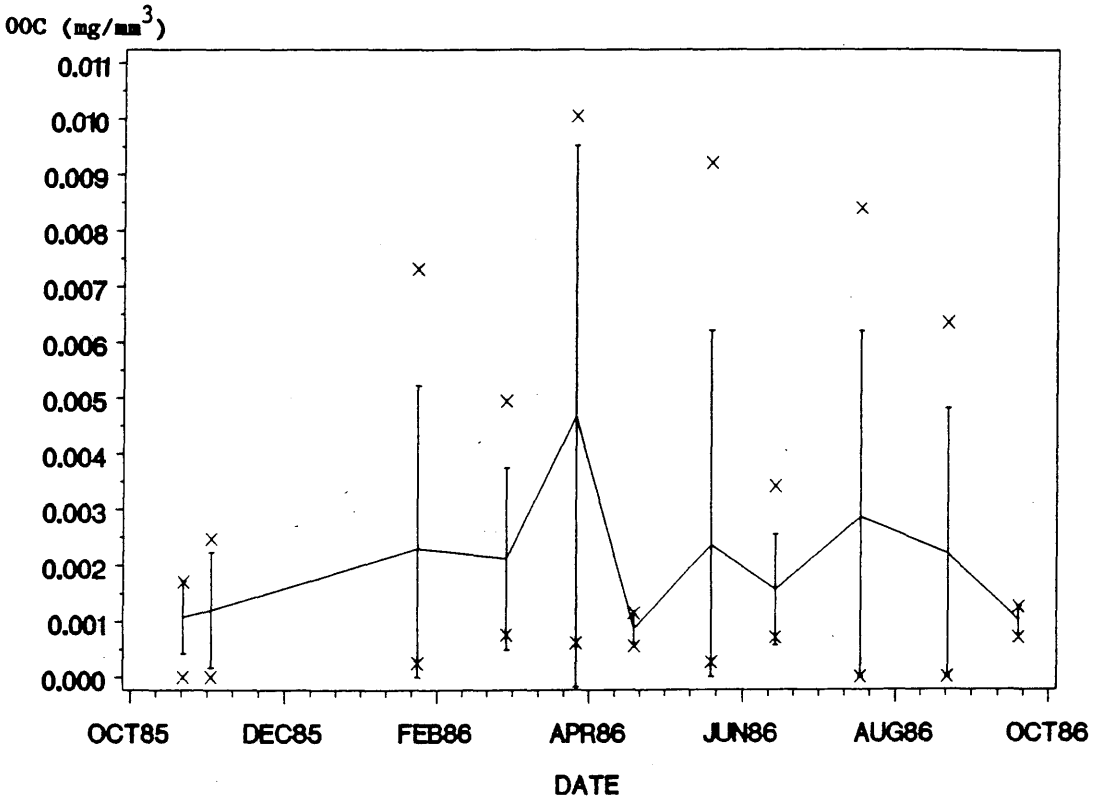
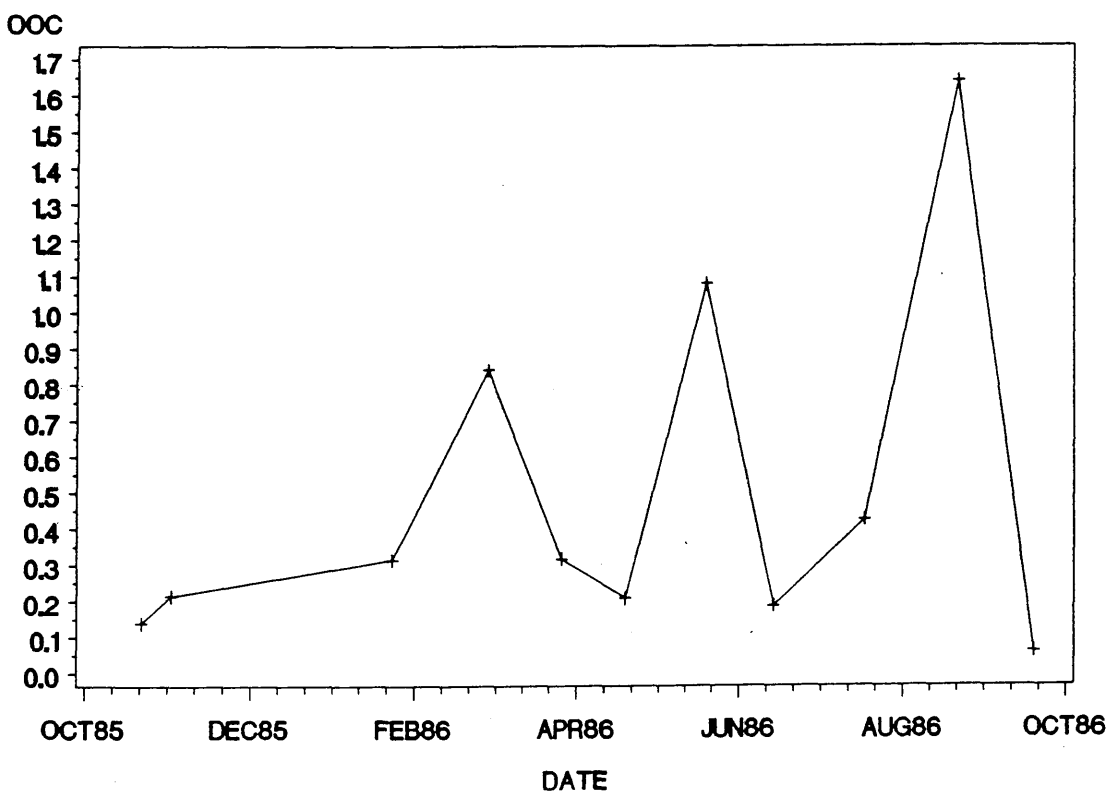


Fig. 5.1.4 Variance to Mean Ratio of the GVF of Oocytes (OOC).



FEMALES: LOCH FYNE.

Fig. 5.2.1 Mean, Standard deviation and maximum and minimum values for the percentage GVF of Late Mature Oocytes (%LMT).

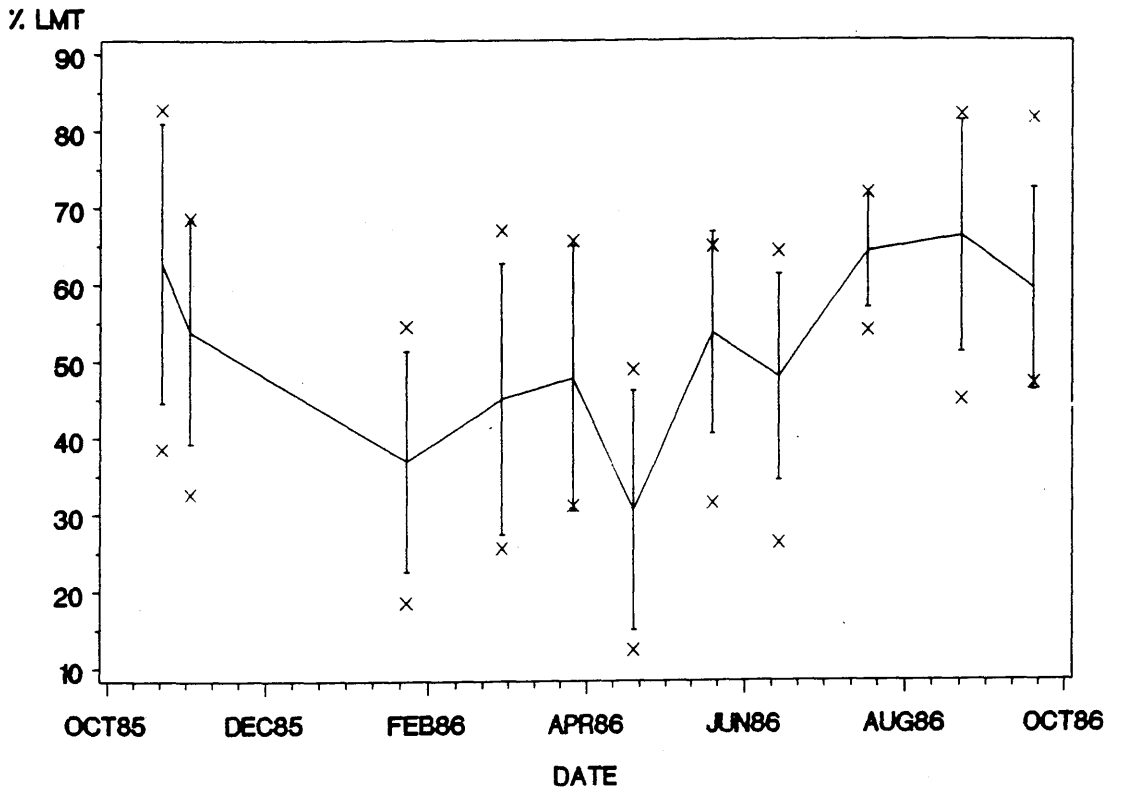
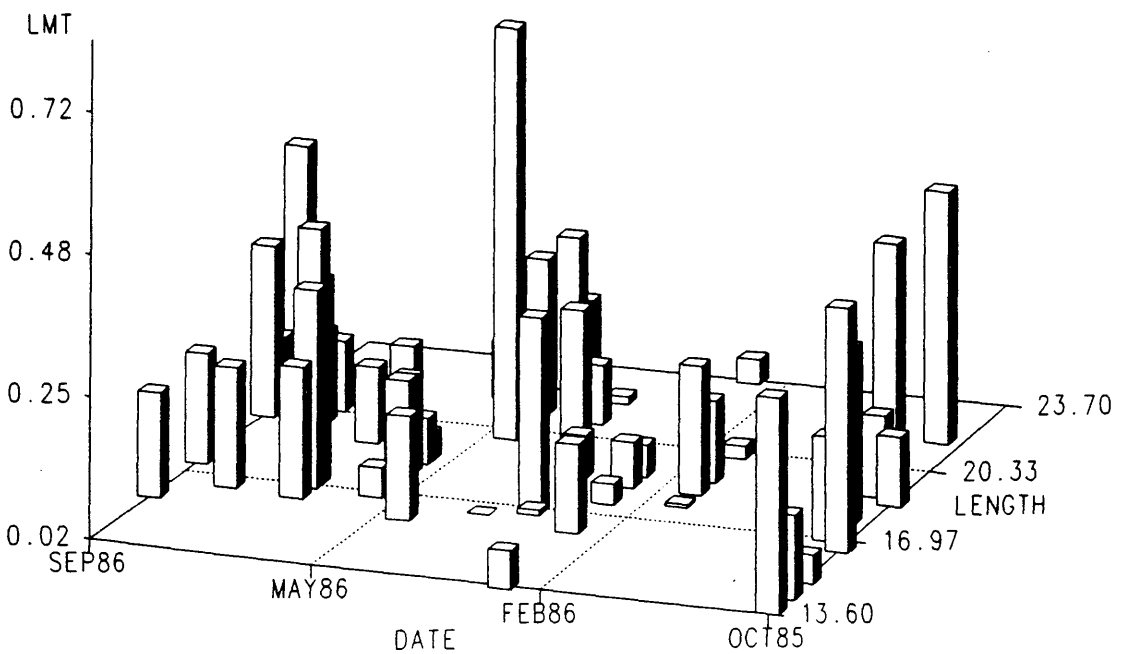


Fig. 5.2.2 Mass of Late Mature Oocytes in individuals against length and sample date.



LMT: Mass of late mature oocytes in grams
LENGTH: Length in mm.

Fig. 5.2.3 Mean, Standard deviation and maximum and minimum values for the Standard Mass of Late Mature Oocytes (LMT mg/mm^3).

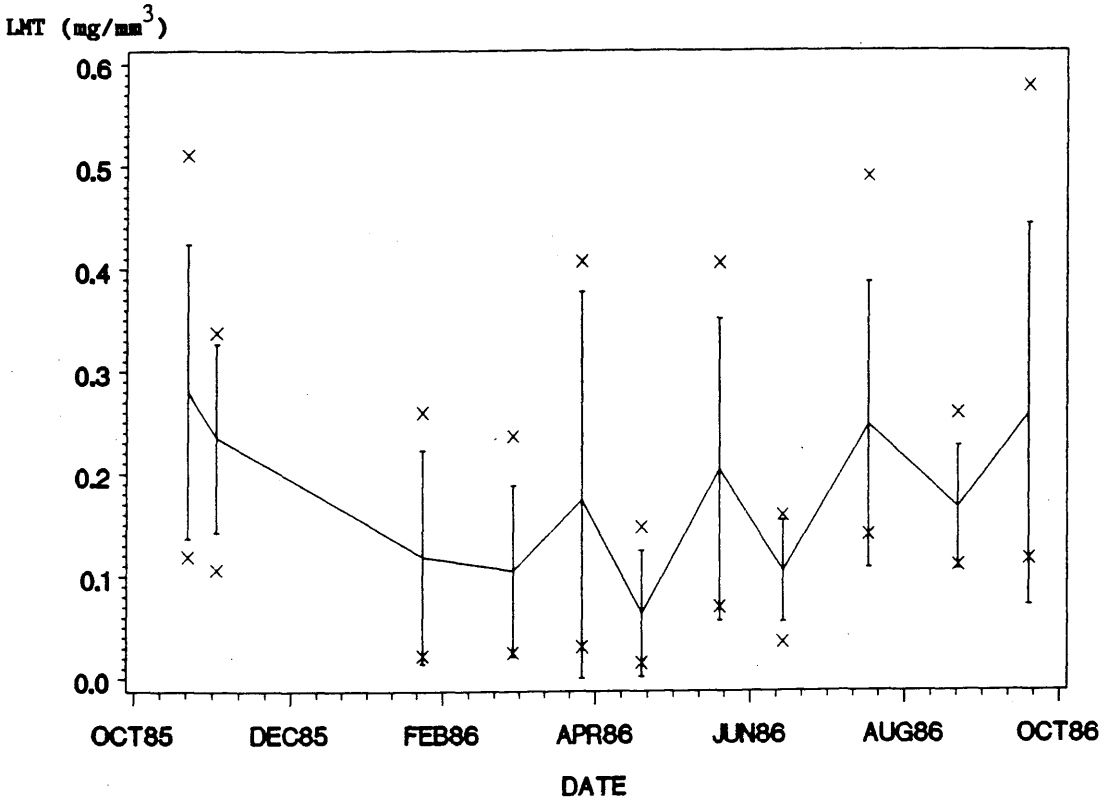


Fig. 5.2.4 Variance to Mean Ratio of the GVF of Late Mature Oocytes (LMT).

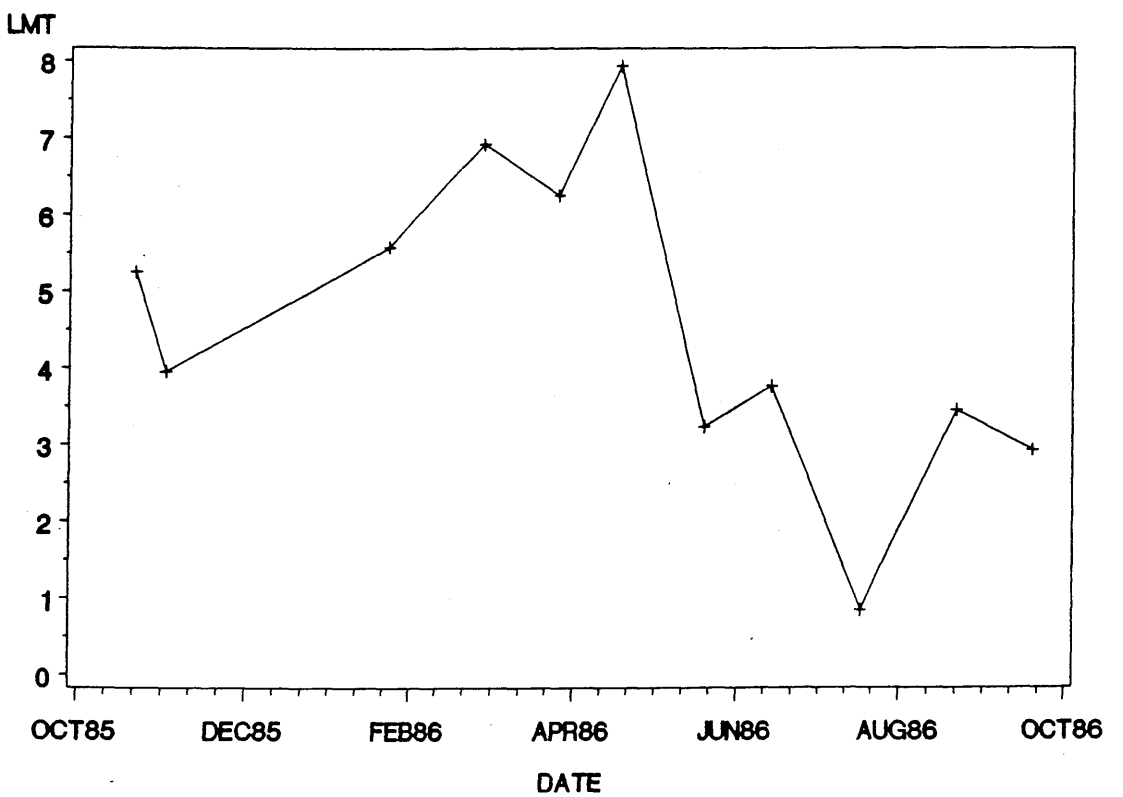


Fig. 5.3.1 Mean, Standard deviation and maximum and minimum values for the percentage GVF of Coelomic Space (% EMT).

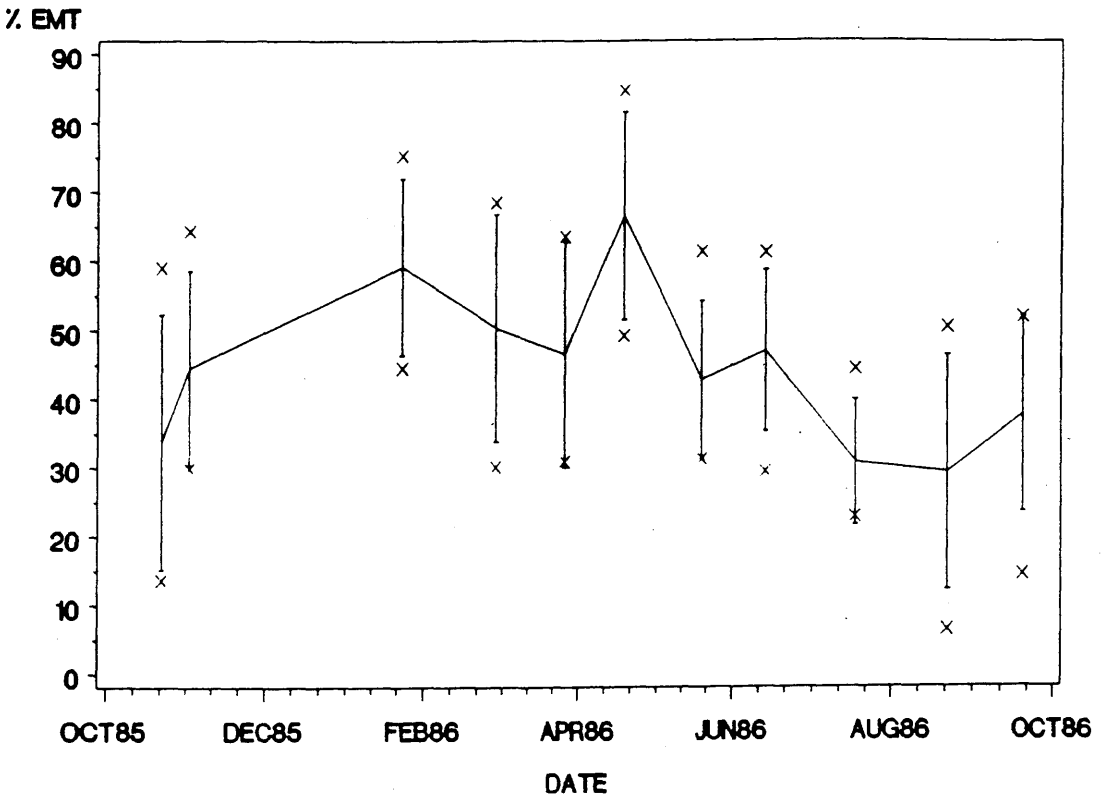
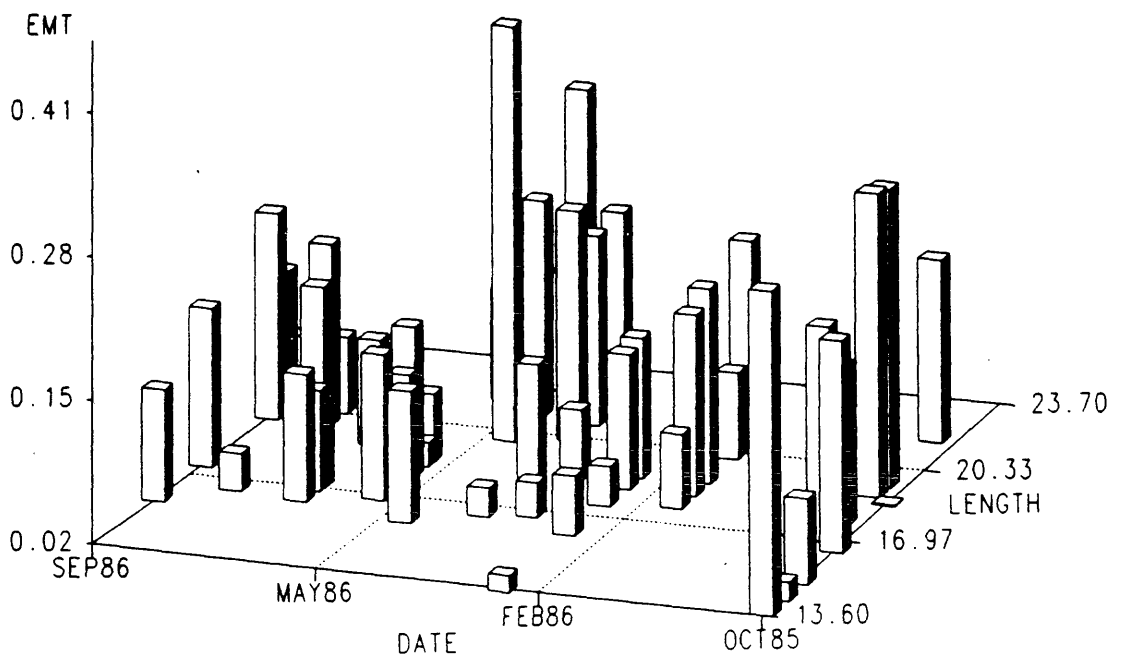


Fig. 5.3.2 Mass of Coelomic Space in individuals against length and sample date.



EMT: Mass of "empty space" in grams
LENGTH: Length in mm.

Fig. 5.3.3 Mean, Standard deviation and maximum and minimum values for the Standard Mass of Coelomic Space (EMT mg/mm^3).

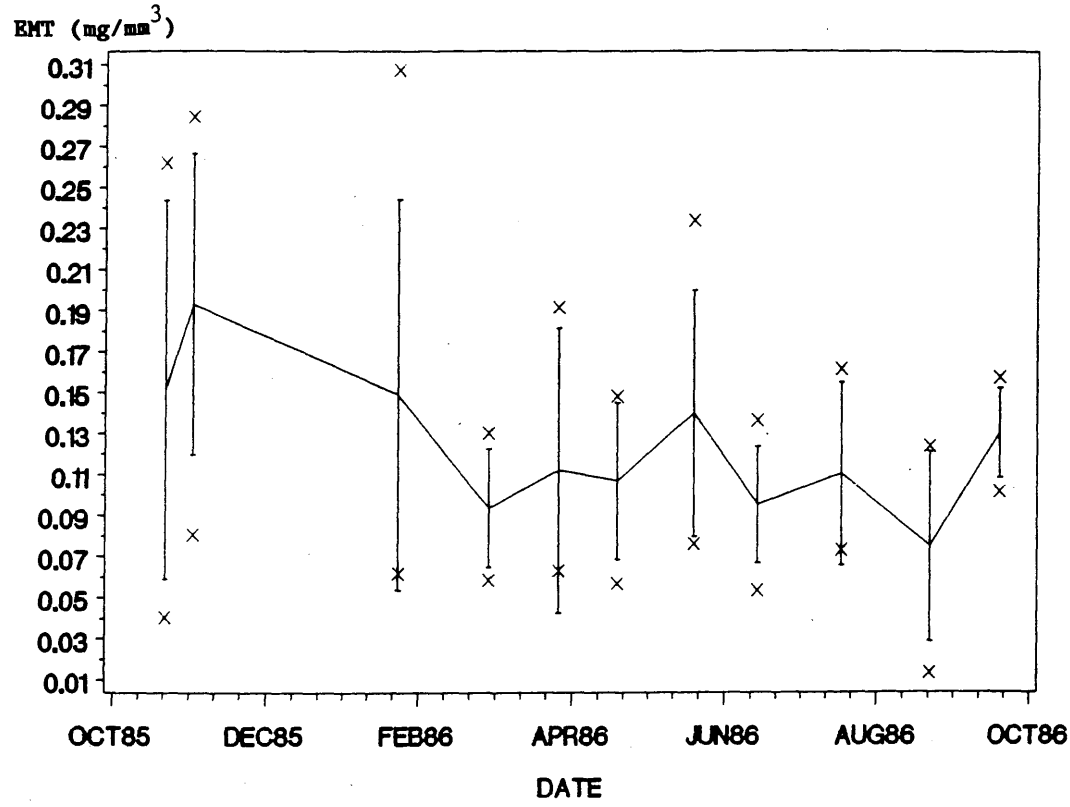


Fig. 5.3.4 Variance to Mean Ratio of the GVF of Coelomic Space (EMT).

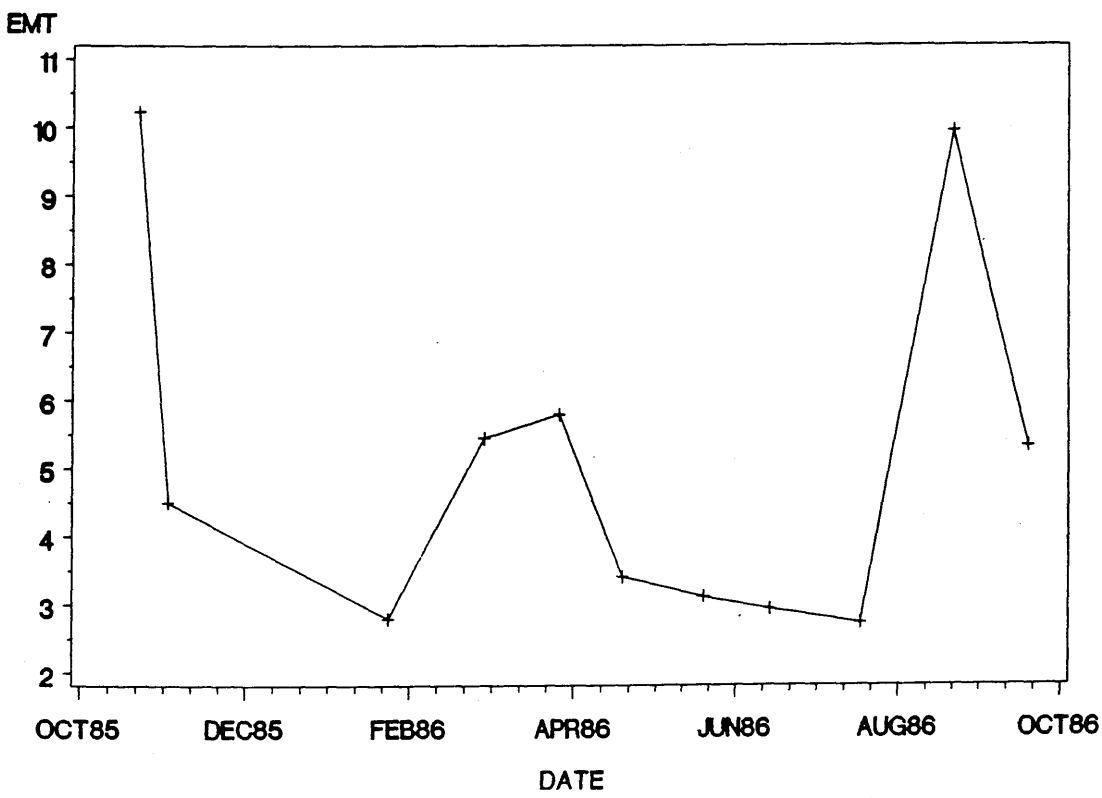


Fig. 5.4.1 Mean, Standard deviation and maximum and minimum values for the percentage GVF of Non Reproductive Tissue (% NRPD).

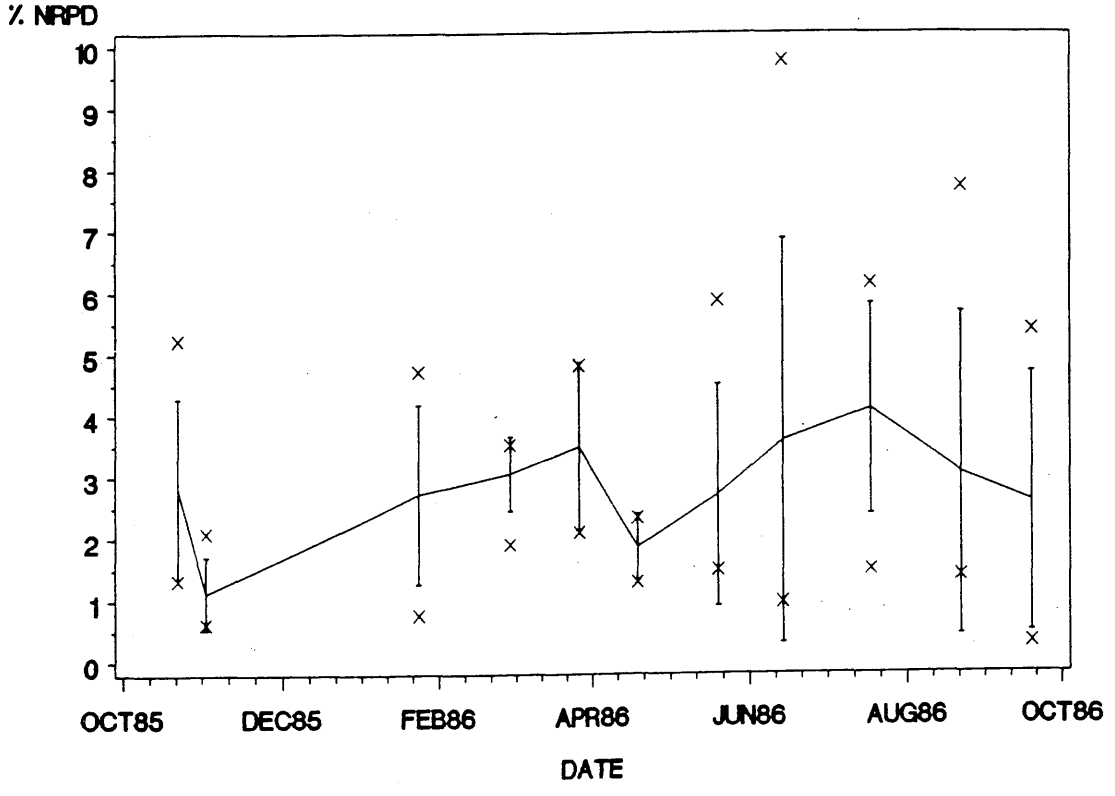
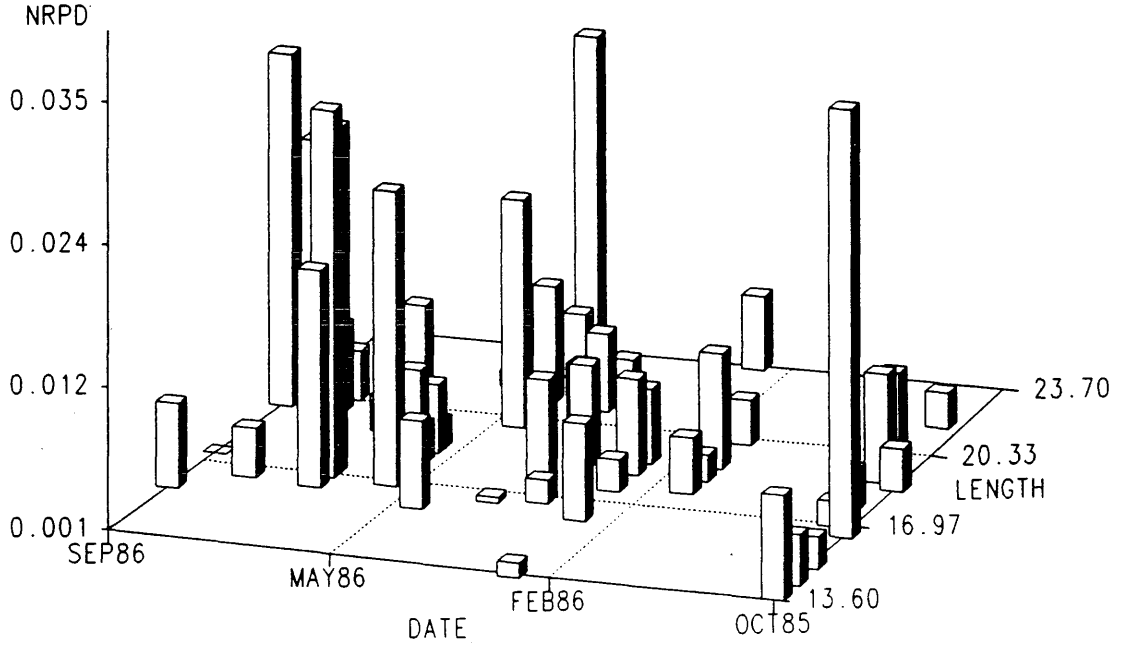


Fig. 5.4.2 Mass of Non Reproductive Tissue in individuals against length and sample date.



NRPD: Mass of nonreproductive tissue in grams
LENGTH: Length in mm.

Fig. 5.4.3 Mean, Standard deviation and maximum and minimum values for the Standard Mass of Non Reproductive Tissue (NRPD mg/mm^3).

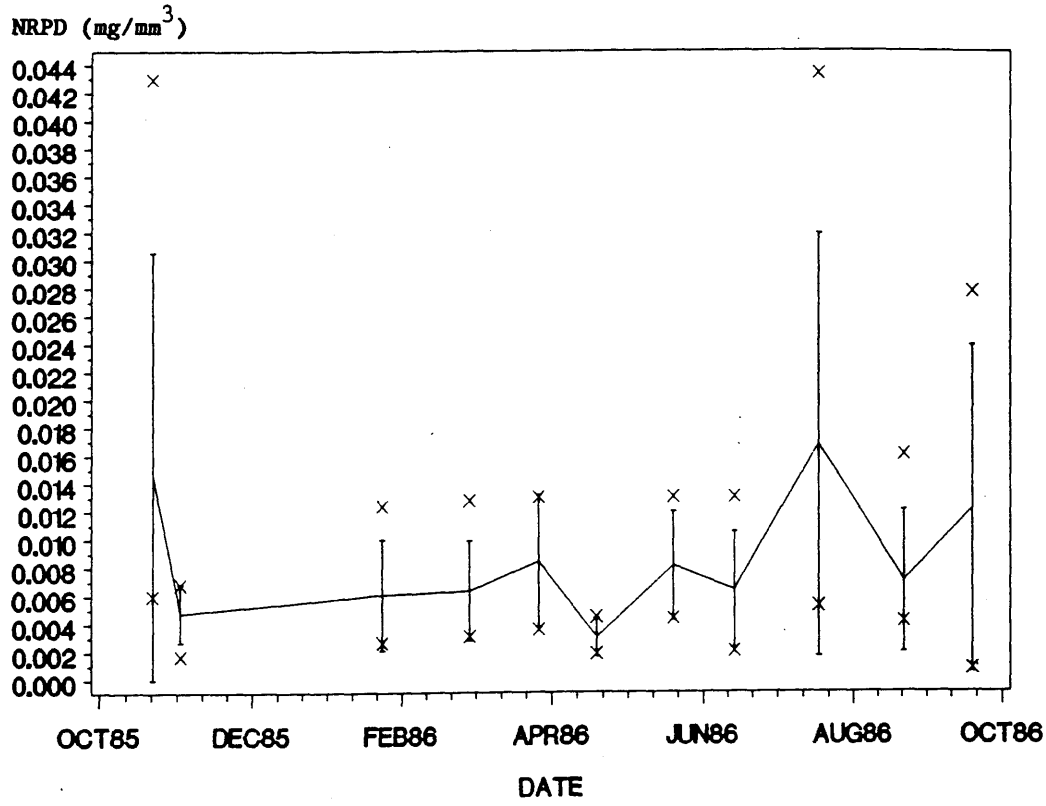
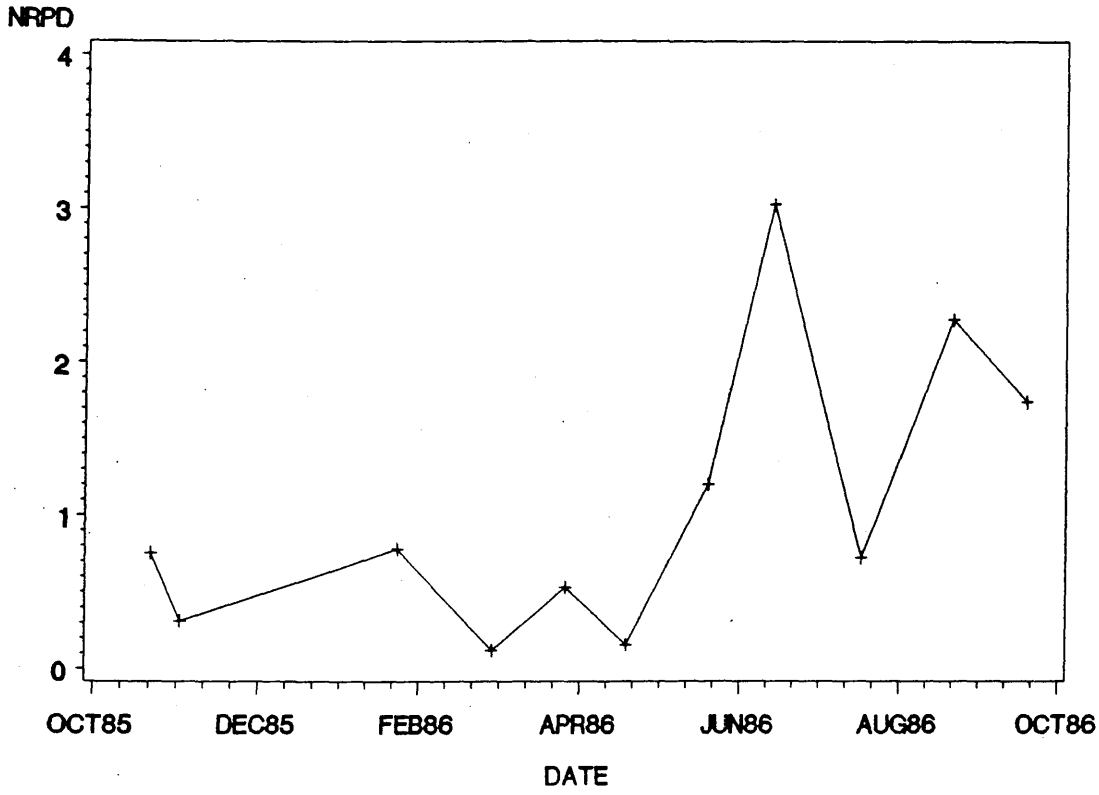


Fig. 5.4.4 Variance to Mean Ratio of the GVF of Non Reproductive Tissue (NRPD).



FEMALES: LOCH FYNE.

Fig. 5.5.1 Combined Plot of the Mean GVF of all the female gonad tissue phases (% VF).

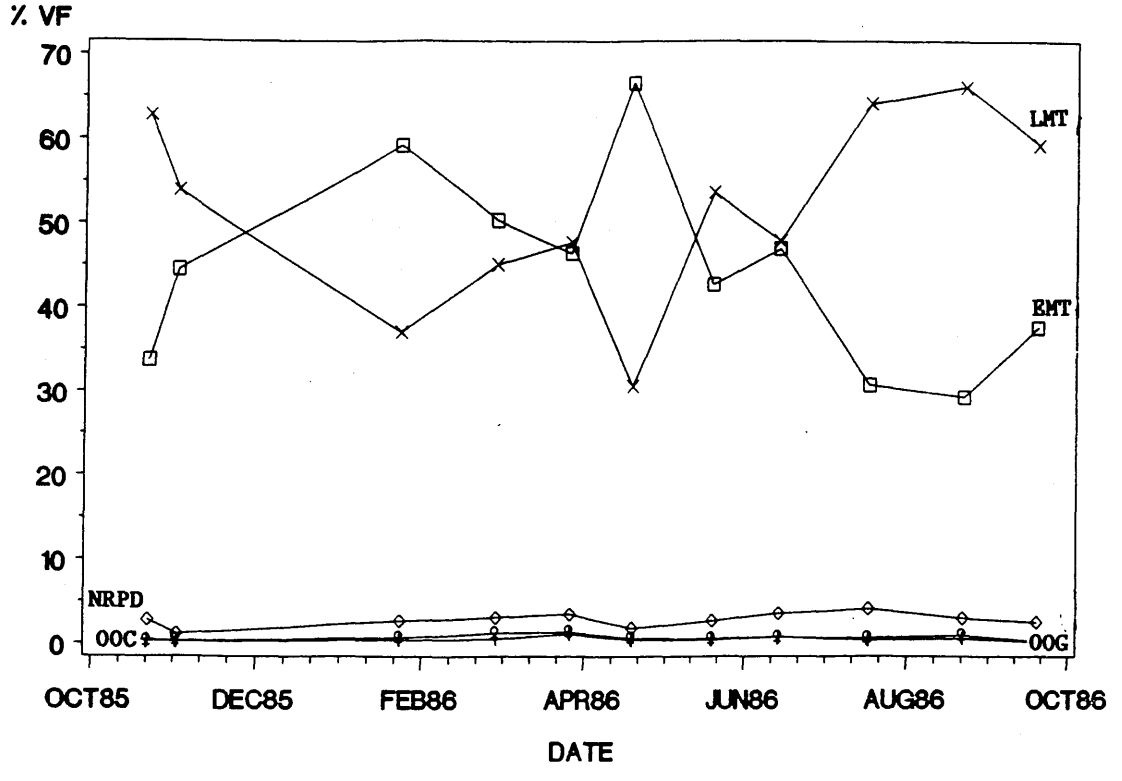
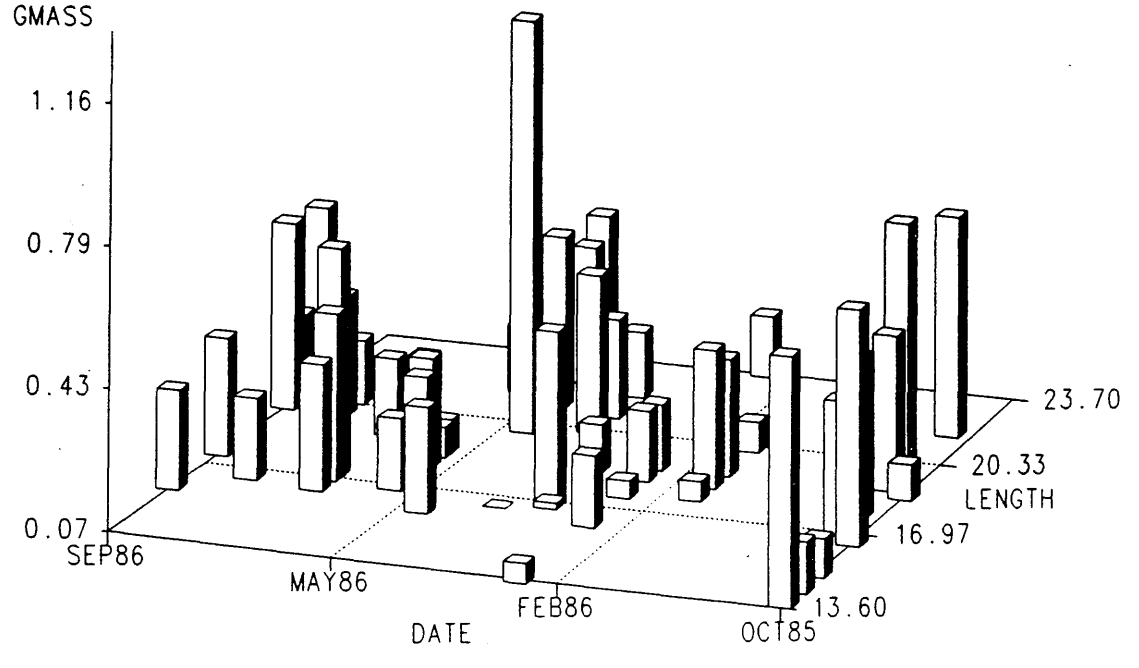


Fig. 5.5.2 Total mass of gonad in individuals against length and sample date.



GMASS: Mass of gonad in grams
LENGTH: Length in mm.

FEMALES: LOCH FYNE.

Fig. 5.5.3 Combined Plot of the Mean Standard Mass of all the female gonad tissue phases (ST. MASS mg/mm^3).

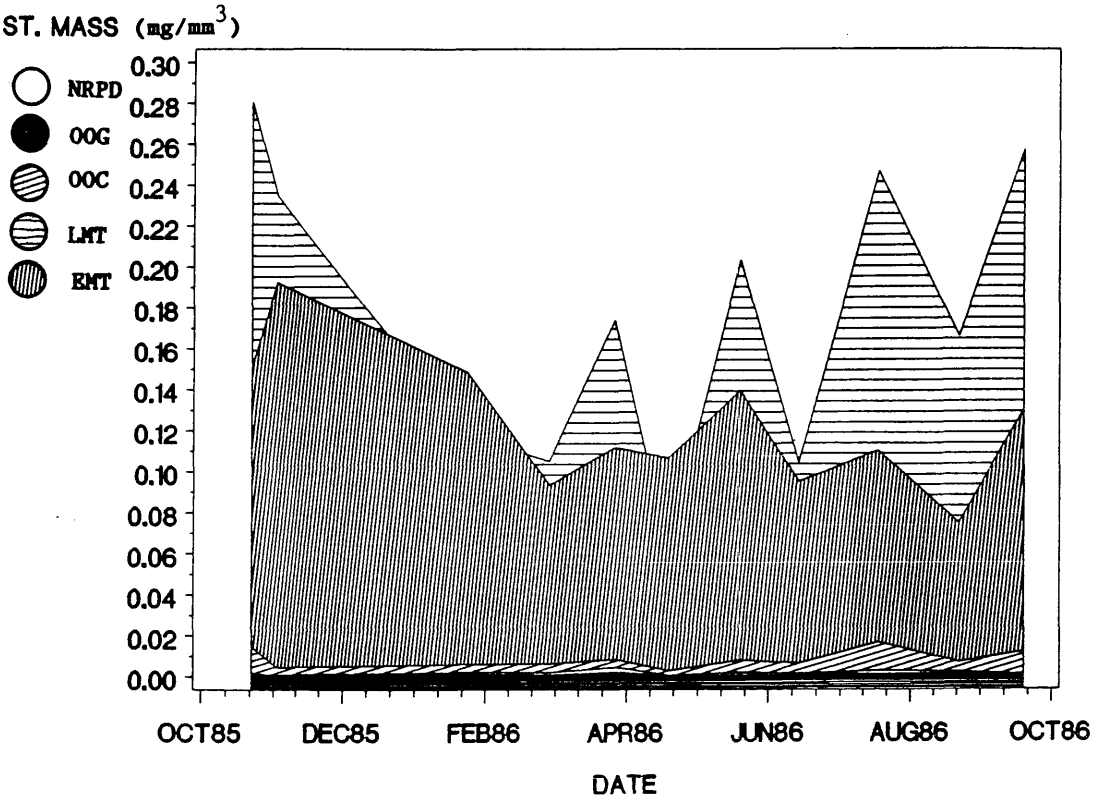
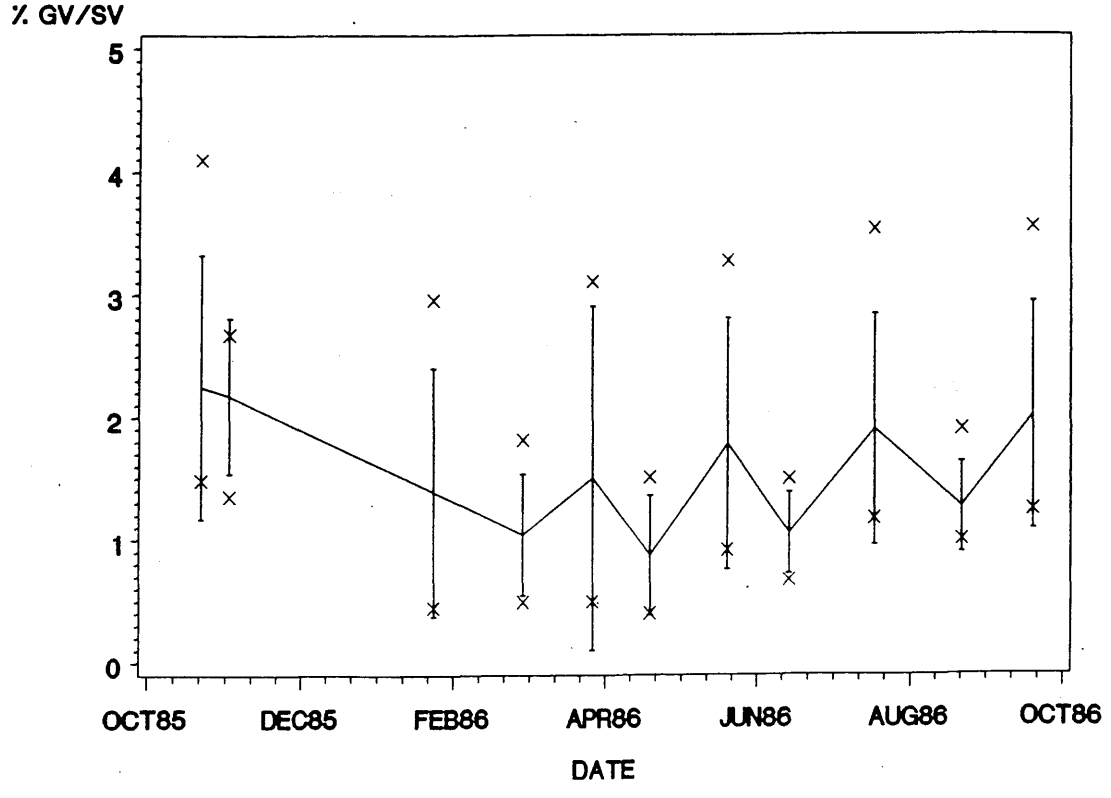


Fig. 5.5.4 Mean, Standard deviation and maximum and minimum values of percentage gonad volume per unit shell volume (% GV/SV).



5.4.4 Examination of Male Gonad Tissue from Loch Fyne.

Spermatogonia. Table 6. Figs. 6.0.1-4.

Spermatogonia achieve a peak in late February and again in October. The abundance of spermatogonia fluctuates at a lower level from May onwards. Isolated individuals appear capable of generating large numbers of spermatogonia which tends to reduce synchrony within the population. This is reflected in significant differences within and between samples.

Primary Spermatocytes. Table 6. Figs. 6.1.1-4.

Primary spermatocytes display a well defined cycle of production. This cell phase slowly accumulates from a winter minima to a peak in July of approximately 20% of the volume fraction, showing a gentle decline throughout the autumn and winter. The growth of the spermatocyte population is most rapid from April onwards. Variance to mean ratios are less than 2.0, but relative differences are significant both between and within samples.

Secondary Spermatocytes. Table 6. Figs. 6.2.1-4.

Secondary spermatocytes display a similar pattern of accumulation to the primary spermatocytes. Rising from a late January minimum to an August maximum. A slight decline in March, undoubtedly an anomaly of the small sample size for that month, and also in June, marks a departure from this trend. As with primary spermatocytes, these cells become less numerous during the autumn and winter. Secondary spermatocytes are the most asynchronous of all the cell fractions and are significantly different within and between samples.

Sperm and Spermatids. Table 6. Figs. 6.3.1-4.

Spermatid/sperm represent from 40% to 60% of the GVF. February and March offer the lowest mean values of standard mass for the

spermatid/sperm cell phase. Thereafter the masses increase to a plateau in April and May, followed by a gradual decline until July. Cells of this tissue phase then accumulate once more to a late autumn peak. The cycle is completed, with a marked fall in the number of cells between October and November, levelling off until late January and finally dropping to the annual low in February and March. The population displays greatest synchrony in May and during the months of September and October. Differences are again, significant.

Coelomic Space. Table 6. Figs. 6.4.1-4.

As before, the variation in GVF of coelomic or cell free space is reciprocal with the spermatid/sperm phase. When converted into the equivalent standard mass, the change in gonad volume is clear. The gonad must increase in volume as the amount of coelomic space is maximal during the autumn and falls to a minimum in February and March. The amount of coelomic space then increases rapidly to a peak in May is succeeded by a decline until late July, The process is then repeated with an early autumn trough, prior to a late autumn increase. This cell phase is highly synchronous within the population for much of the year but shows notable departures from this trend in October, February and August. Variation within and between samples is significant.

Nonreproductive Tissue. Table 6. Figs. 6.5.1-4.

This phase too, displays a marked peak in late February followed by a slight decline. Quantities of nonreproductive tissue remain relatively stable throughout the summer months, showing an increase in August before a late autumn decline which persists during the winter. Asynchrony within the population is notable in November and June. Differences between and within samples are significant.

Summary of Males from Loch Fyne. Figs. 6.6.1-4.

As with the males from the Firth of Lorn the spermatid/sperm and coelomic or cell free tissue phases are the most abundant. Both range

between 40% and 65% of the GVF. Primary spermatocytes form the second highest proportion and constitute between 5% and 15%. Spermatogonia, secondary spermatocytes and nonreproductive material represents less than 5%. Gonad volume clearly increases in response to the proliferation of cells and coelomic space.

TABLE 6.

Analysis fo Variance.
Males from Loch Fyne.

Cell Phase	Source	DF	F Value	PR > F
SPG	Within	9	10.07	0.0001
	Between	40	4.84	0.0001
SPC	Within	9	75.74	0.0000
	Between	40	5.77	0.0001
SSC	Within	9	26.35	0.0000
	Between	40	4.65	0.0001
SPT	Within	9	23.41	0.0001
	Between	40	23.21	0.0000
EMT	Within	9	4.17	0.0001
	Between	40	8.52	0.0000
NRPD	Within	9	173.40	0.0000
	Between	40	186.49	0.0000

Fig. 6.0.1 Mean, Standard deviation and maximum and minimum values for the percentage GVF of Spermatogonia (% SPG).

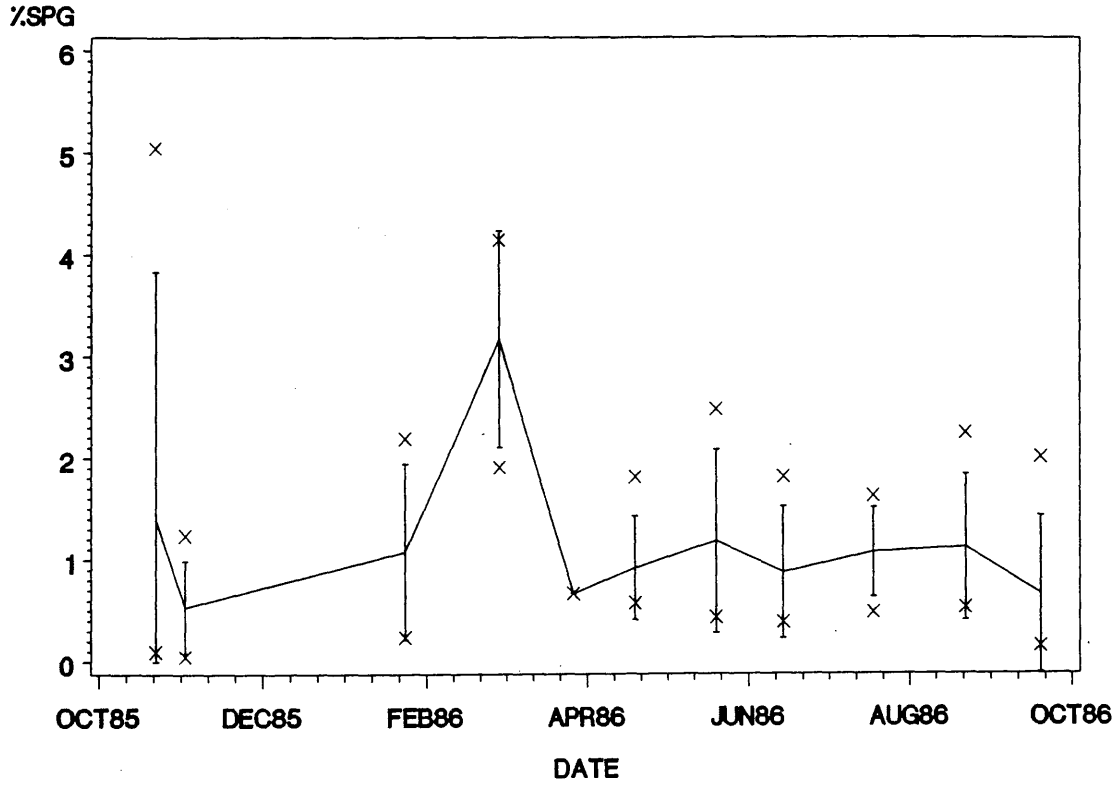
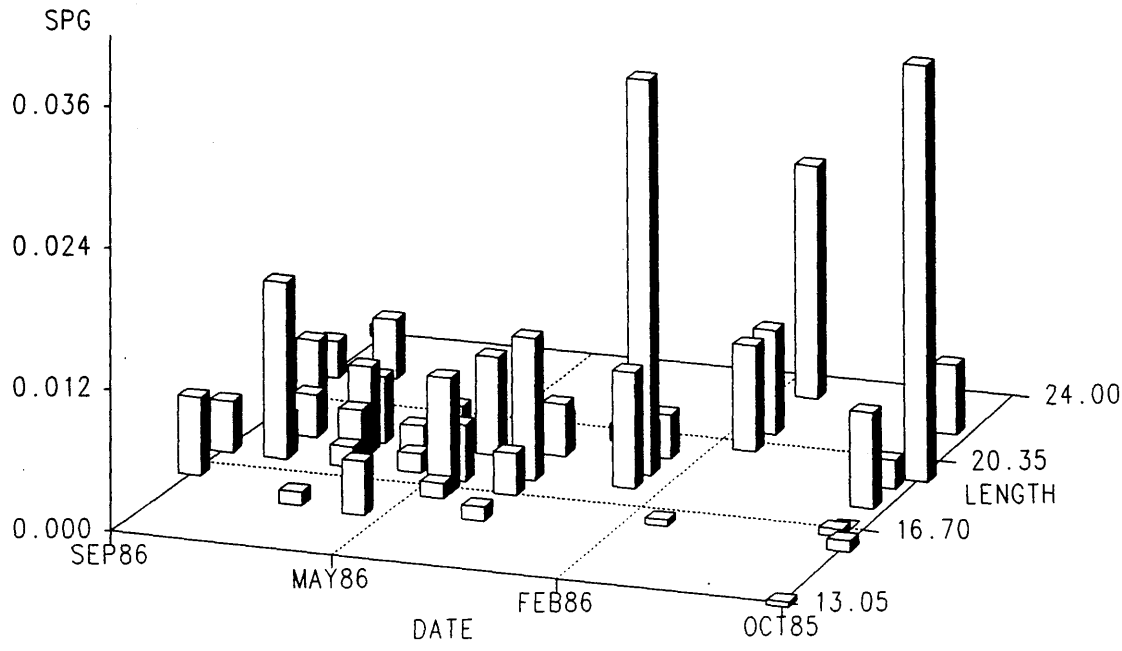


Fig. 6.0.2 Mass of Spermatogonia in individuals against length and sample date.



SPG: Mass of gonad in grams
LENGTH: Length in mm.

Fig. 6.0.3 Mean, Standard deviation and maximum and minimum values for the Standard Mass of Spermatogonia (SPG mg/mm^3).

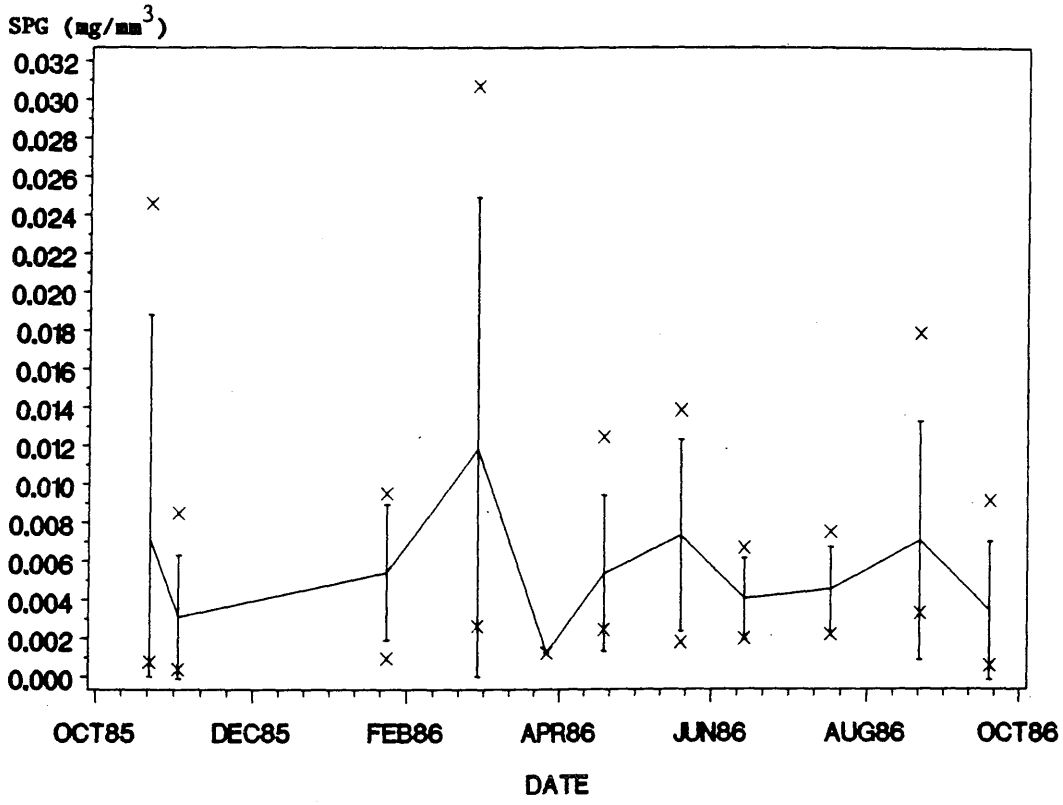


Fig. 6.0.4 Variance to Mean Ratio of the GVF of Spermatogonia (SPG).

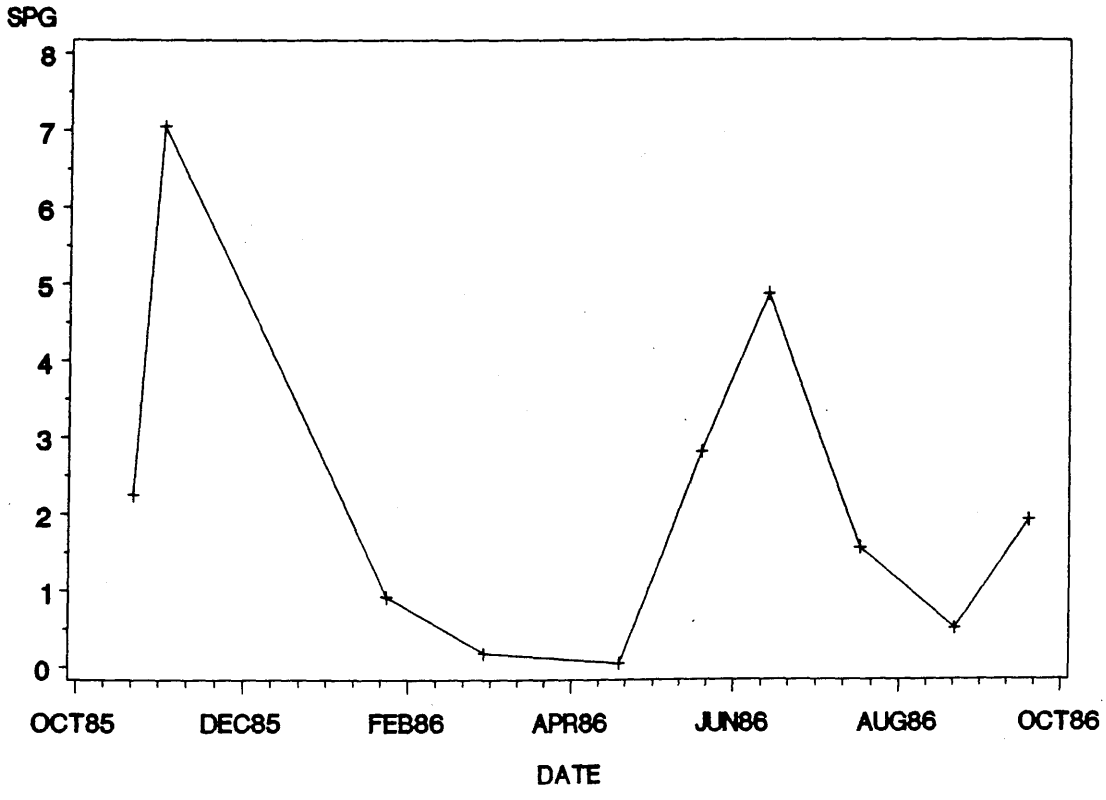


Fig. 6.1.1 Mean, Standard deviation and maximum and minimum values for the percentage GVF of Primary Spermatocytes (% SPC).

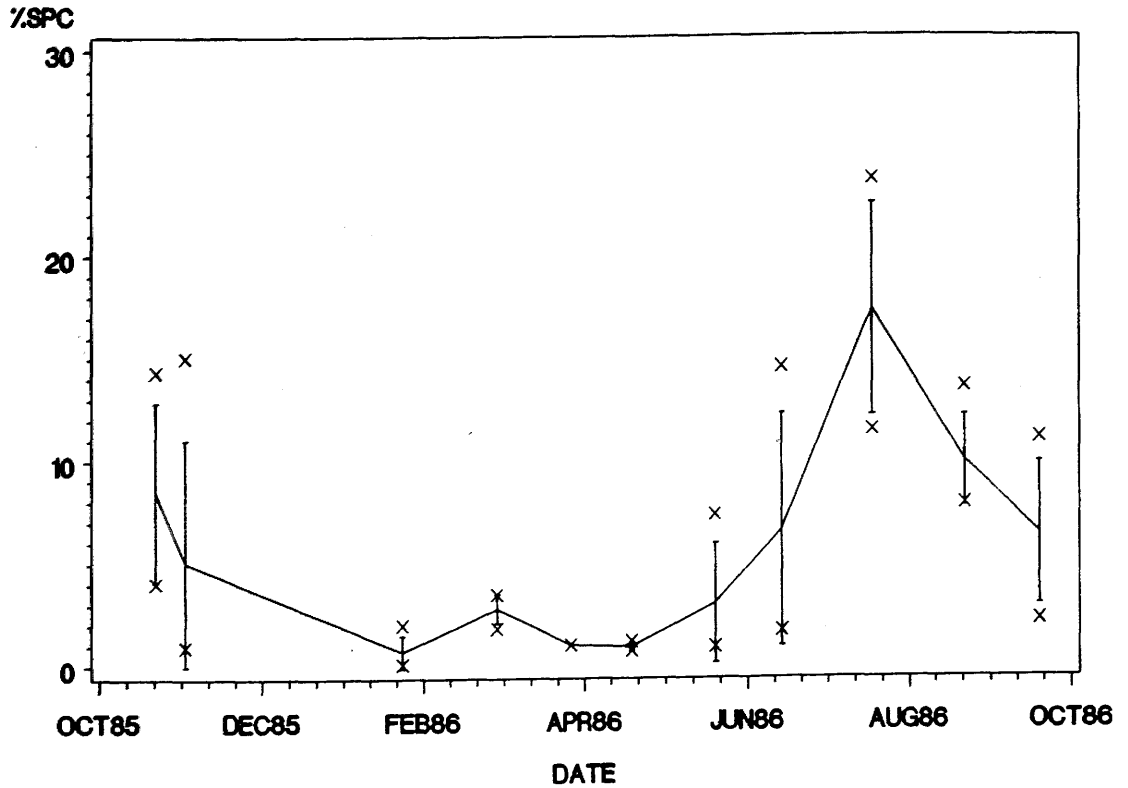
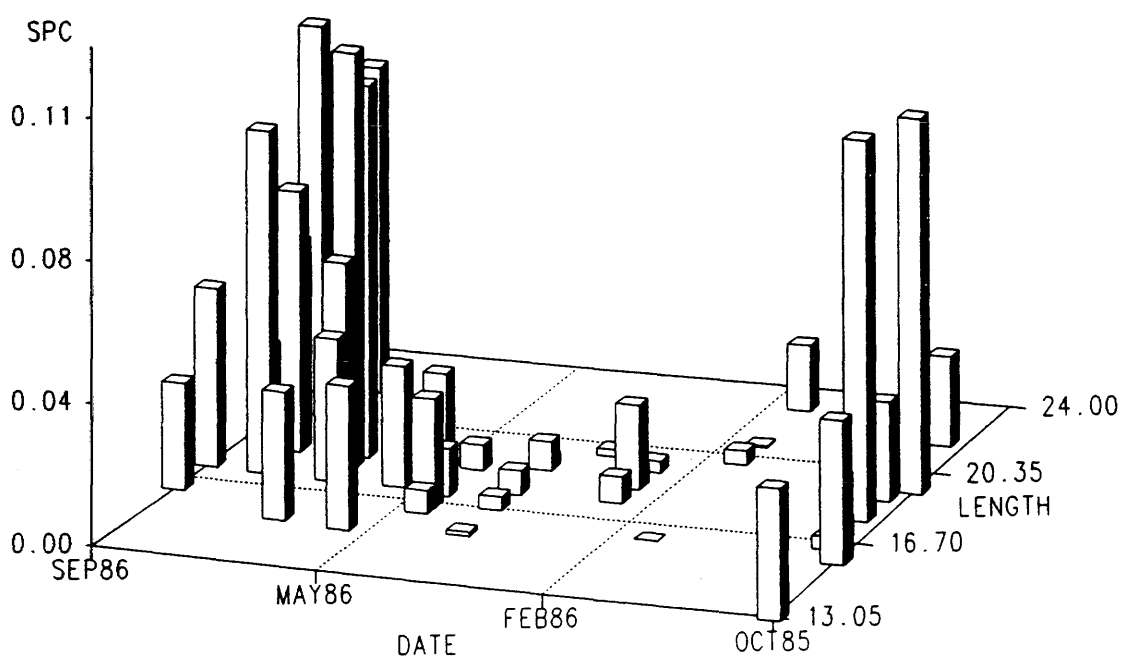


Fig. 6.1.2 Mass of Primary Spermatocytes in individuals against length and sample date.



SPC: Mass of gonad in grams
LENGTH: Length in mm.

Fig. 6.1.3 Mean, Standard deviation and maximum and minimum values for the Standard Mass of Primary Spermatocytes (SPC mg/mm^3).

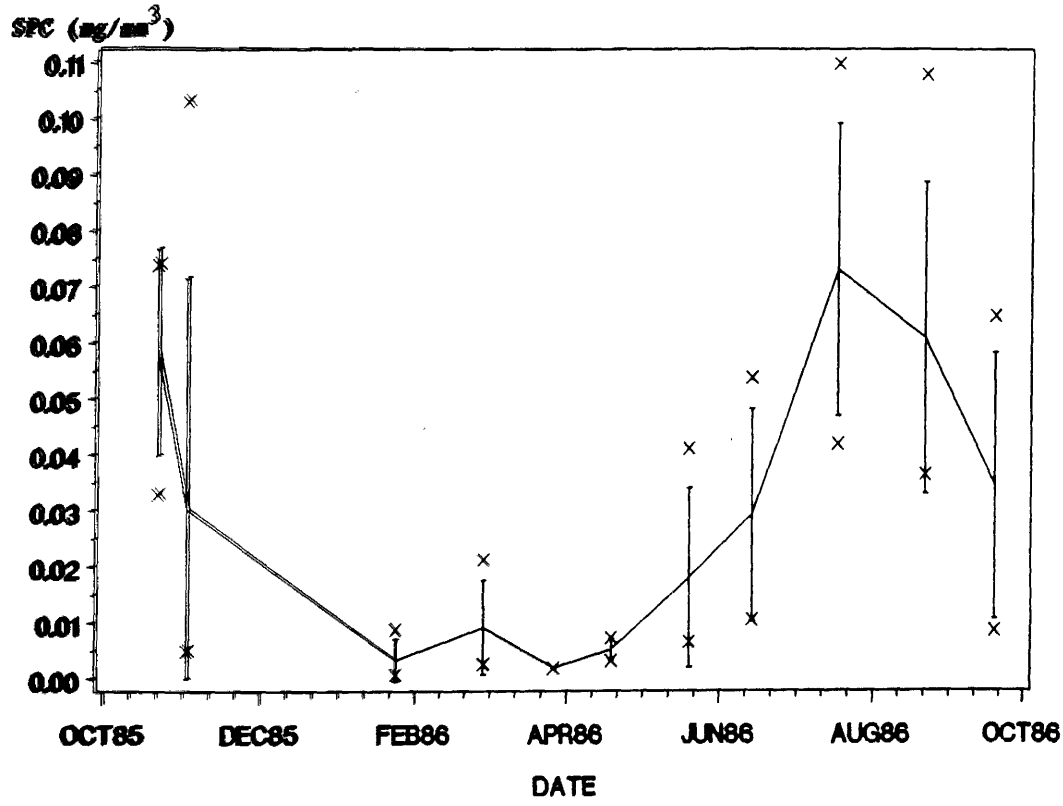


Fig. 6.1.4 Variance to Mean Ratio of the GVF of Primary Spermatocytes (SPC).

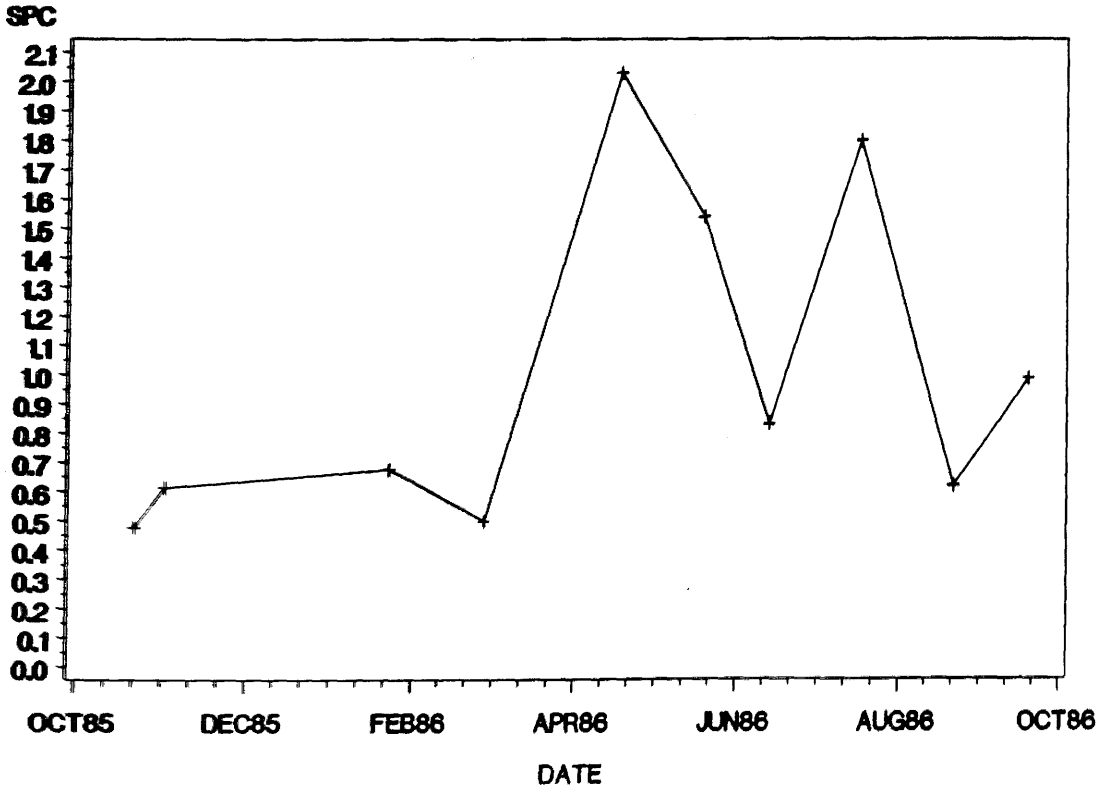


Fig. 6.2.1 Mean, Standard deviation and maximum and minimum values for the percentage GVF of Secondary Spermatocytes (% SSC).

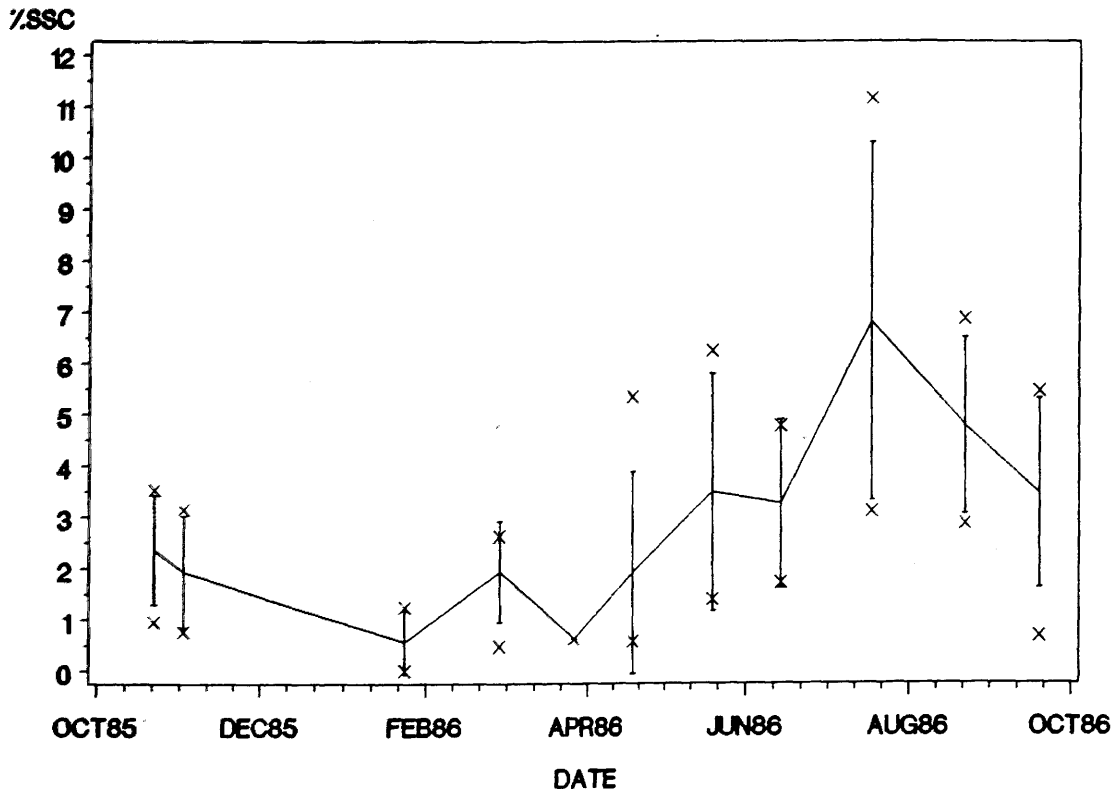
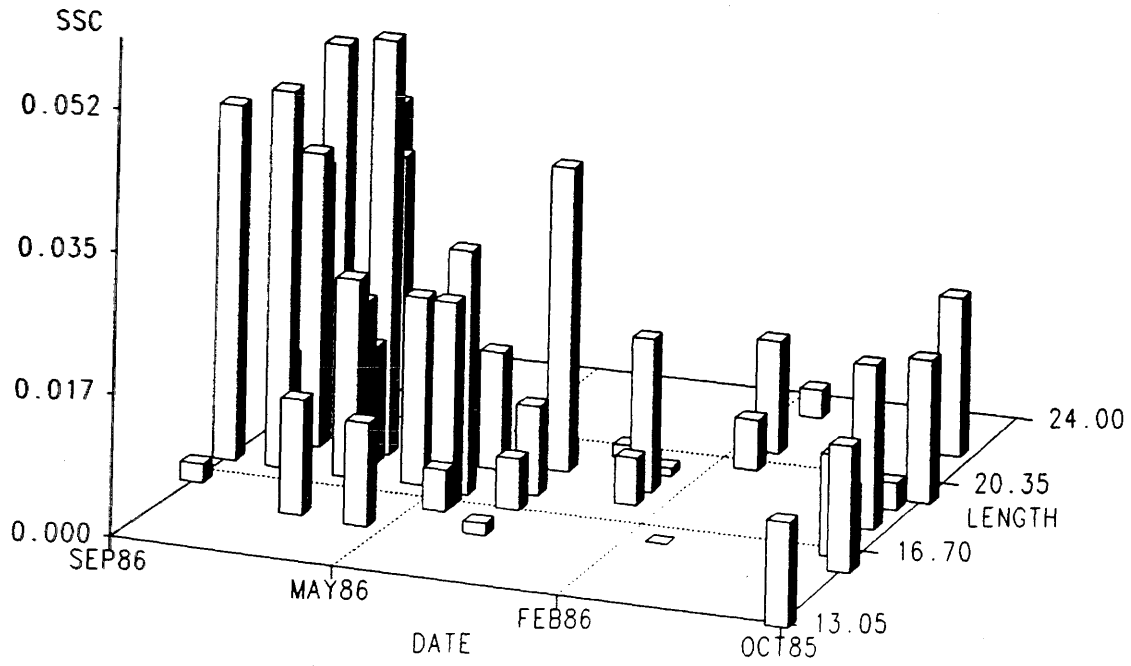


Fig. 6.2.2 Mass of Secondary Spermatocytes in individuals against length and sample date.



SSC: Mass of gonad in grams
LENGTH: Length in mm.

Fig. 6.2.3 Mean, Standard deviation and maximum and minimum values for the Standard Mass of Secondary Spermatocytes (SSC mg/mm^3).

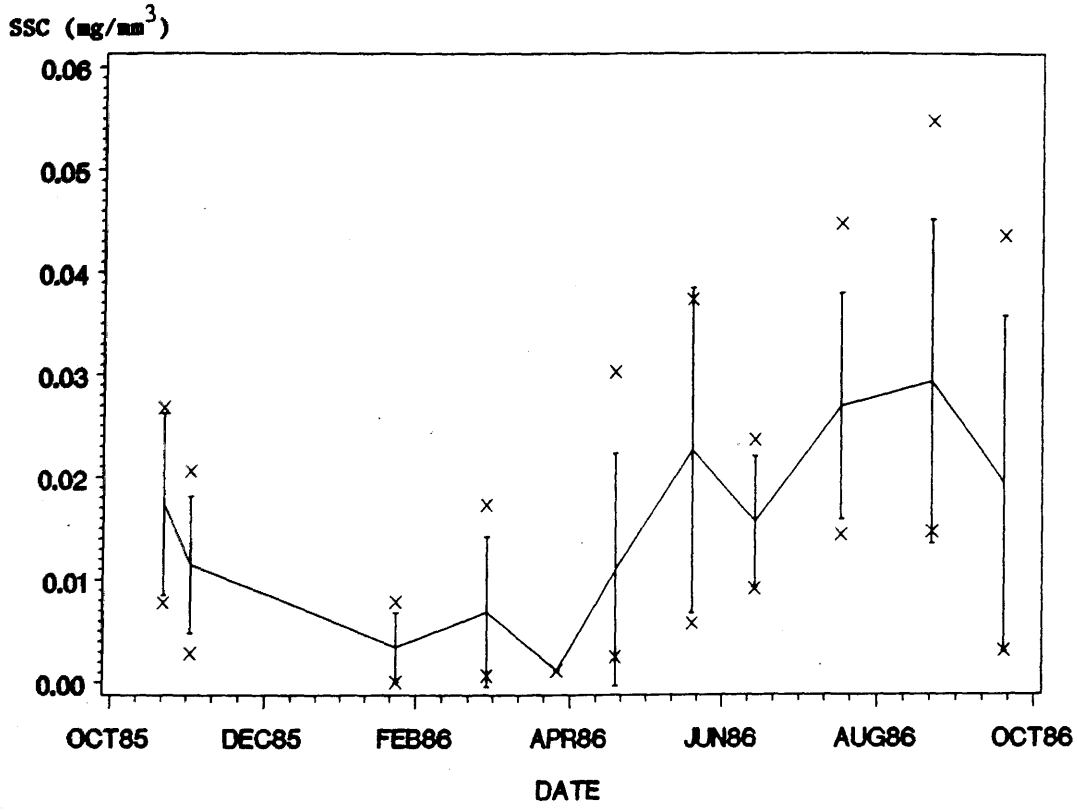


Fig. 6.2.4 Variance to Mean Ratio of the GVF of Secondary Spermatocytes (SSC).

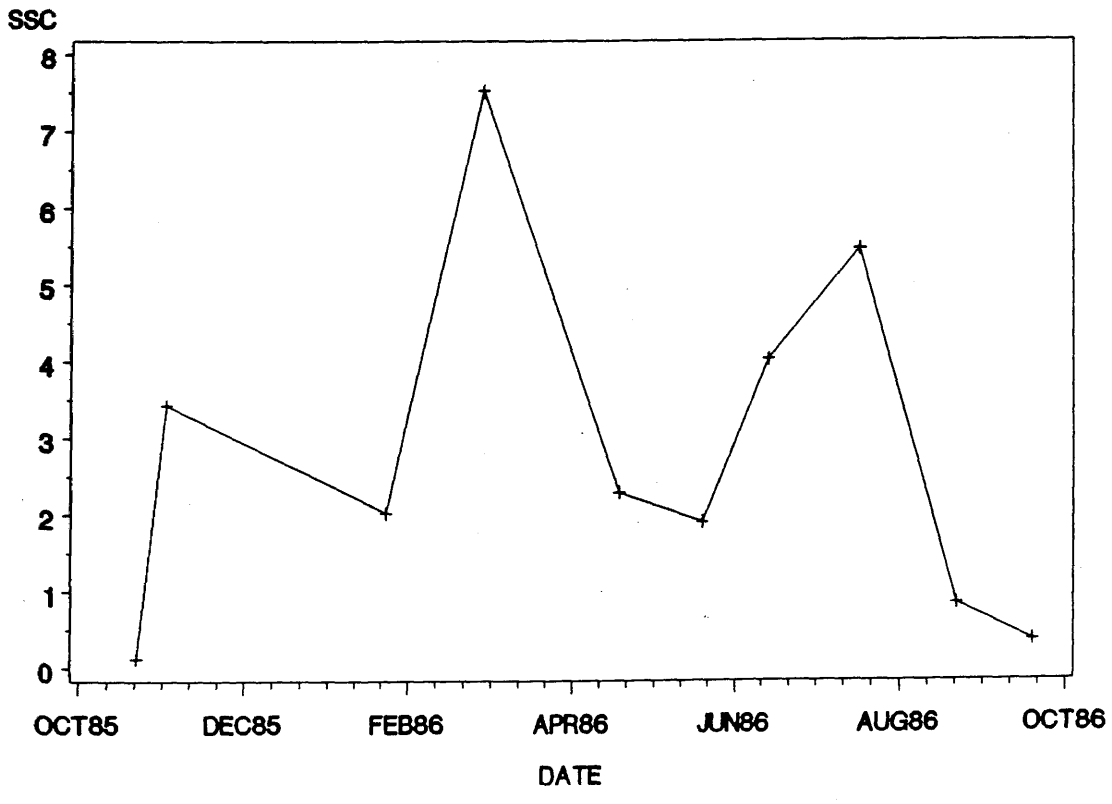


Fig. 6.3.1 Mean, Standard deviation and maximum and minimum values for the percentage GVF of Spermatids and Spermatozoa (% SPT).

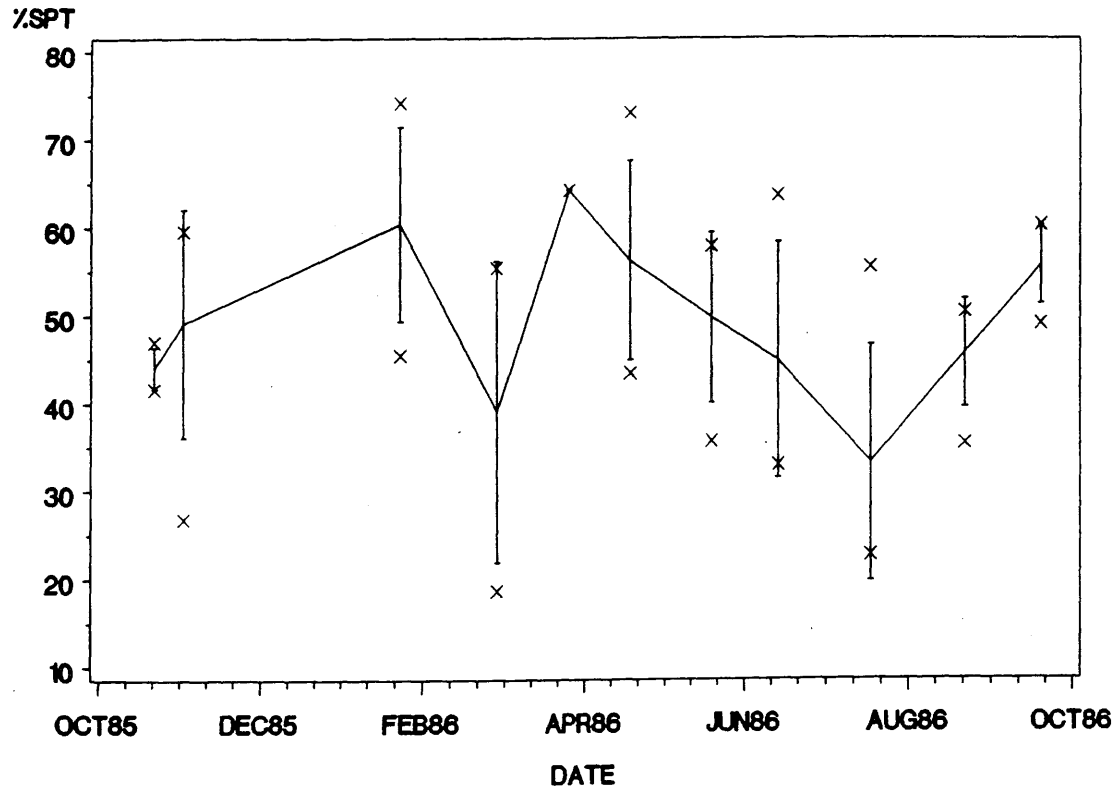
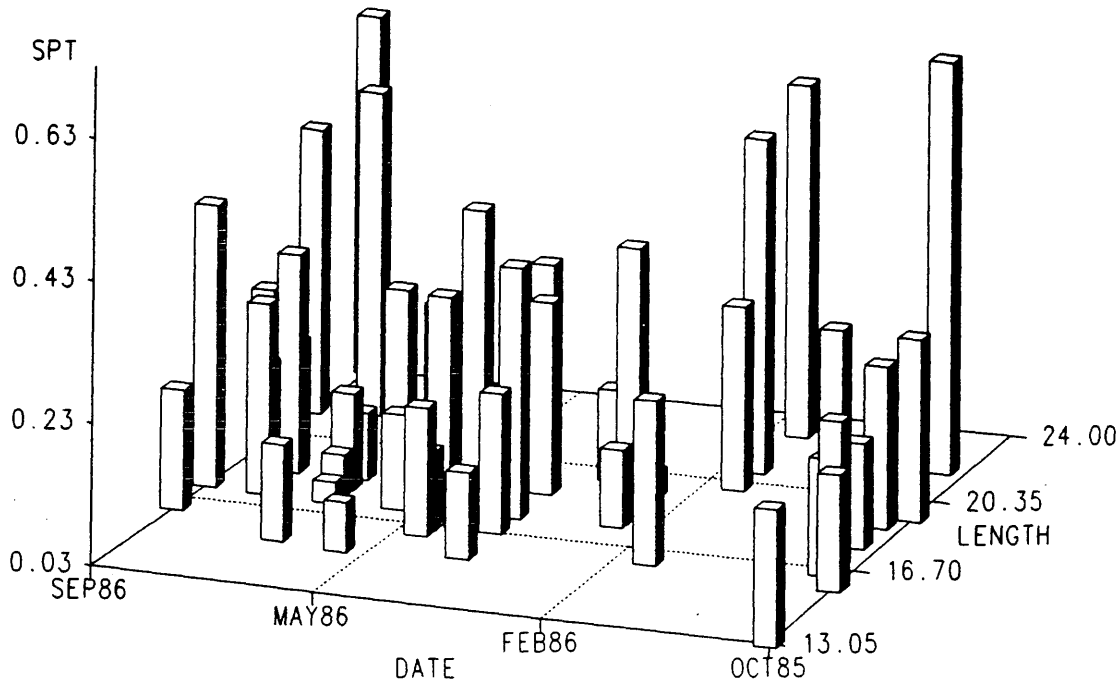


Fig. 6.3.2 Mass of Spermatids and Spermatozoa in individuals against length and sample date.



SPT: Mass of gonad in grams
LENGTH: Length in mm.

Fig. 6.3.3 Mean, Standard deviation and maximum and minimum values for Standard Mass of Spermatids and Spermatozoa (SPT mg/mm^3).

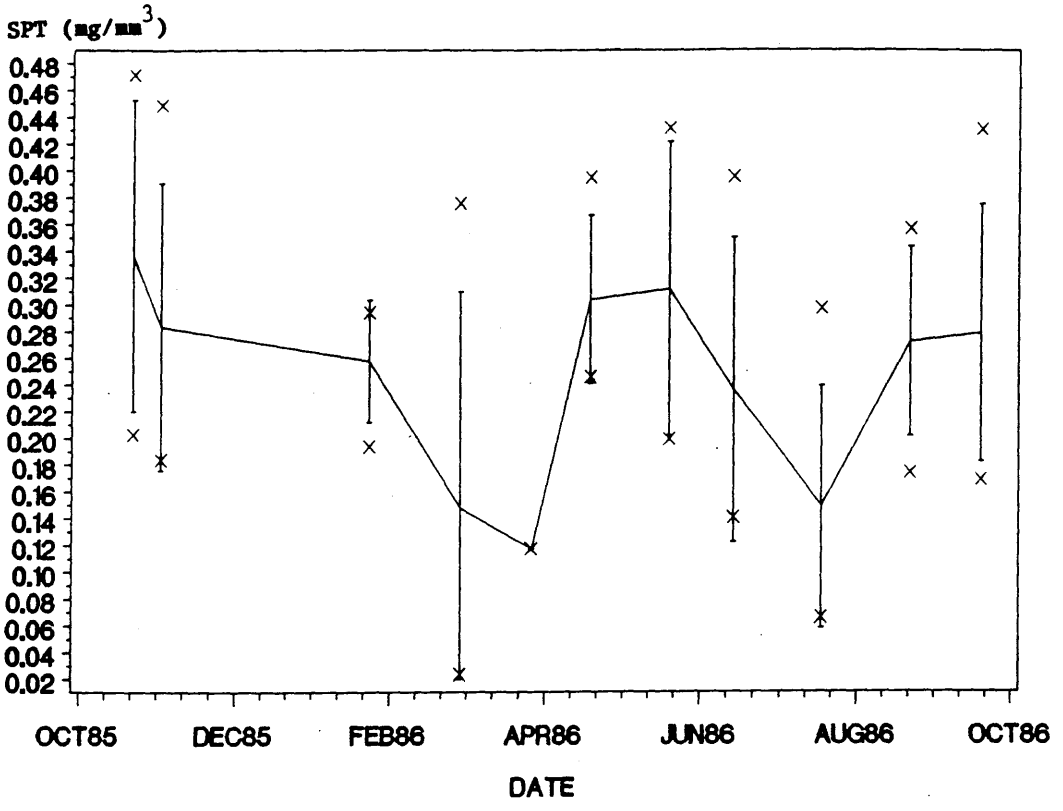


Fig. 6.3.4 Variance to Mean Ratio of the GVF of Spermatids and Spermatozoa (SPT).

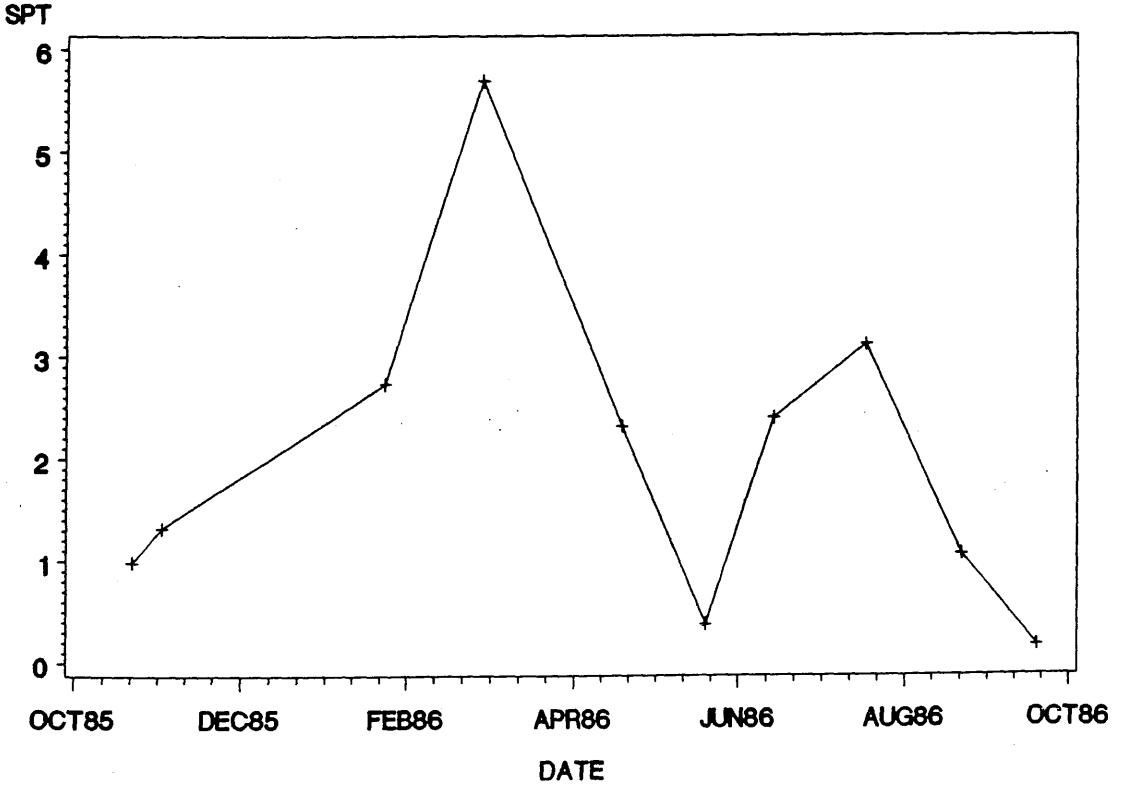


Fig. 6.4.1 Mean, Standard deviation and maximum and minimum values for the percentage GVF of Coelomic Space (% EMT).

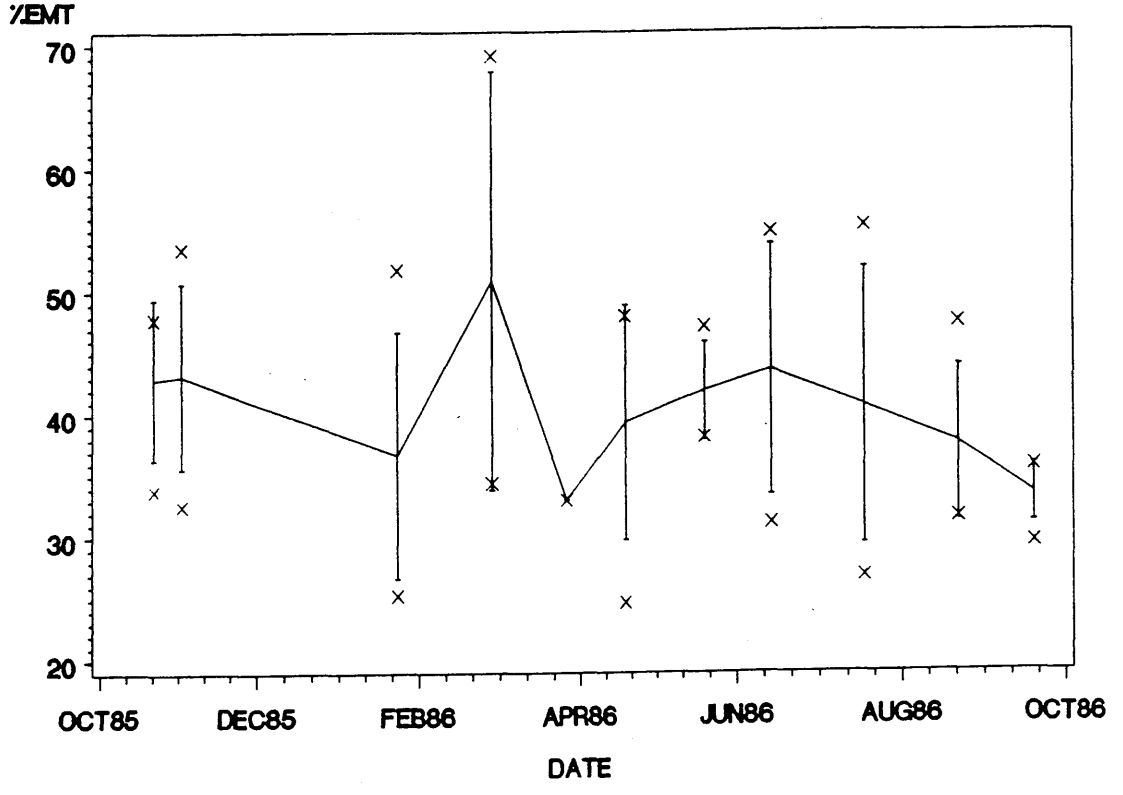
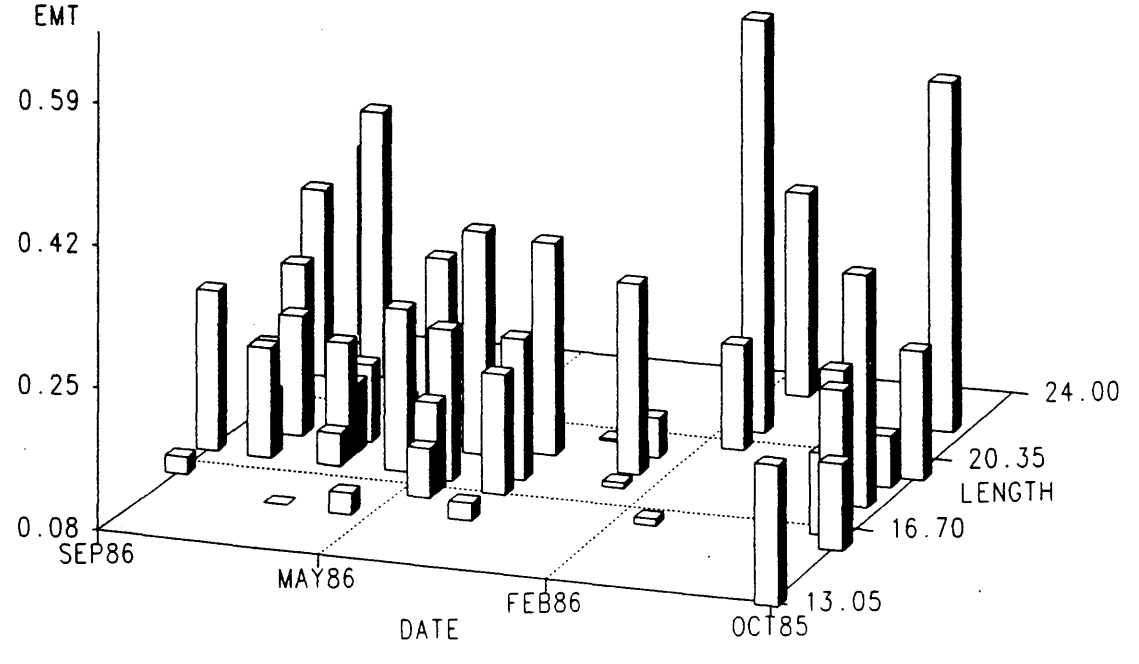


Fig. 6.4.2 Mass of Coelomic Space in individuals against length and sample date.



EMT: Mass of gonad in grams
LENGTH: Length in mm.

Fig. 6.4.3 Mean, Standard deviation and maximum and minimum values for the Standard Mass of Coelomic Space (EMT mg/mm^3).

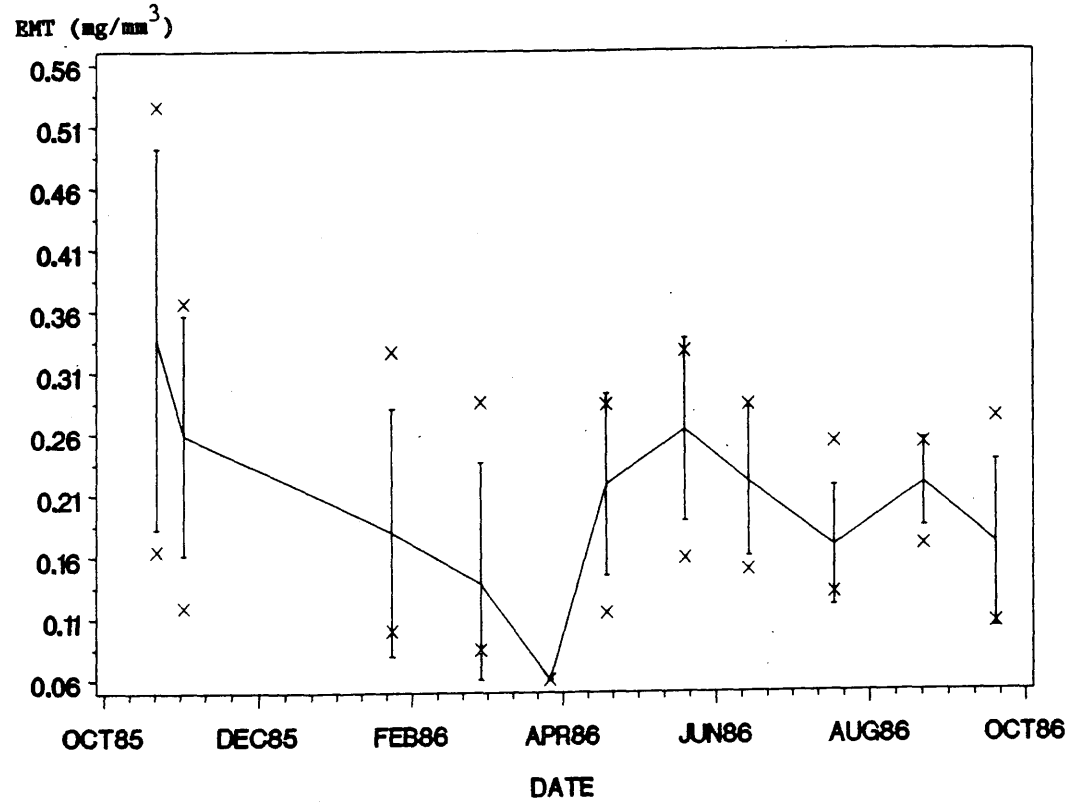


Fig. 6.4.4 Variance to Mean Ratio of the GVF of Coelomic Space (EMT).

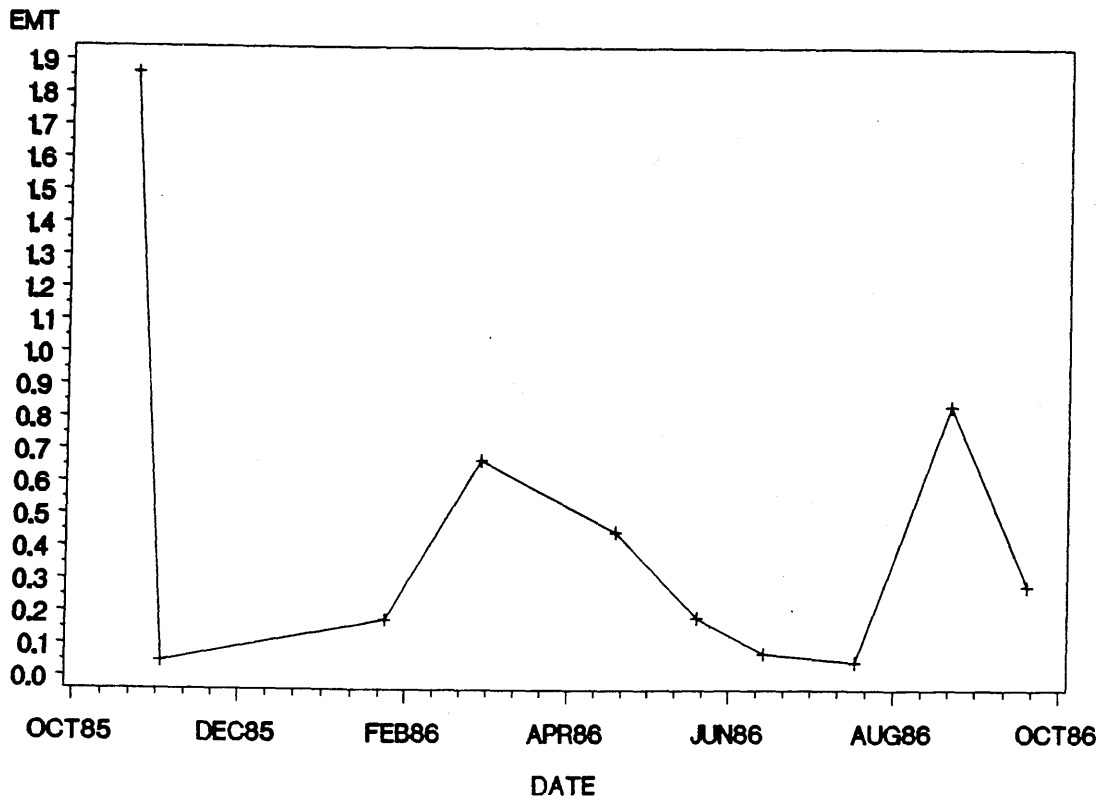


Fig. 6.5.1 Mean, Standard deviation and maximum and minimum values for the percentage GVF of Non Reproductive Tissue (% NRPD).

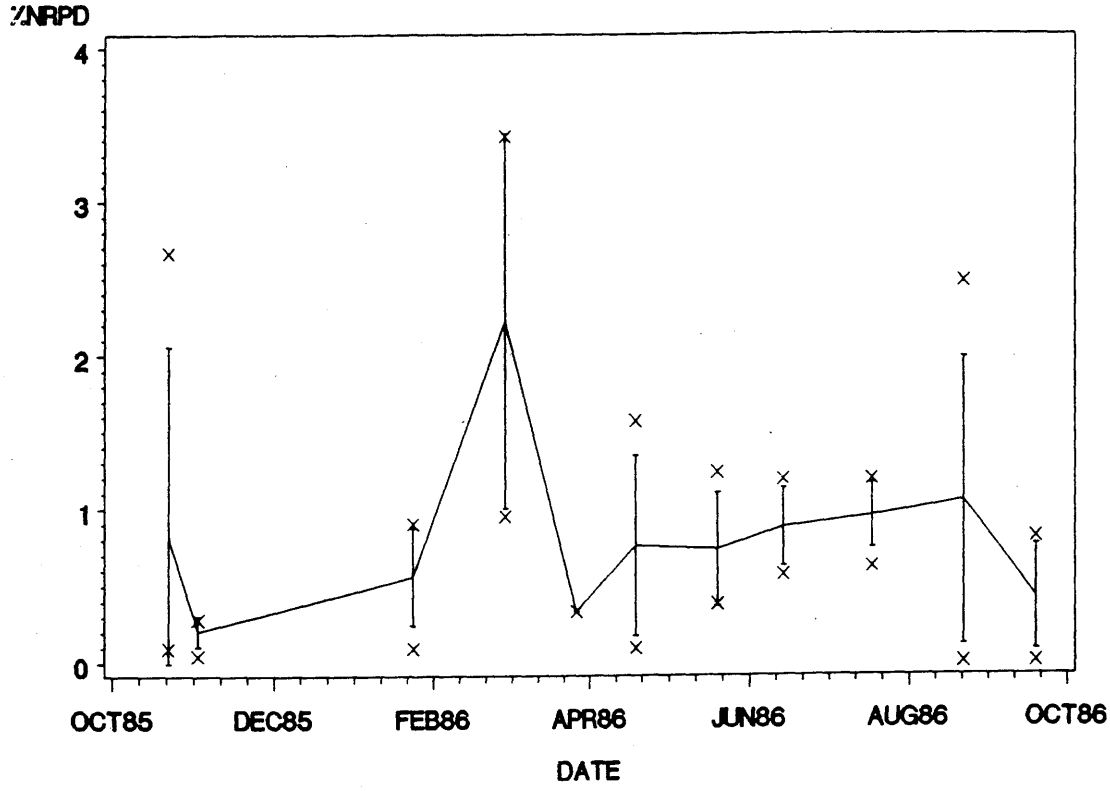
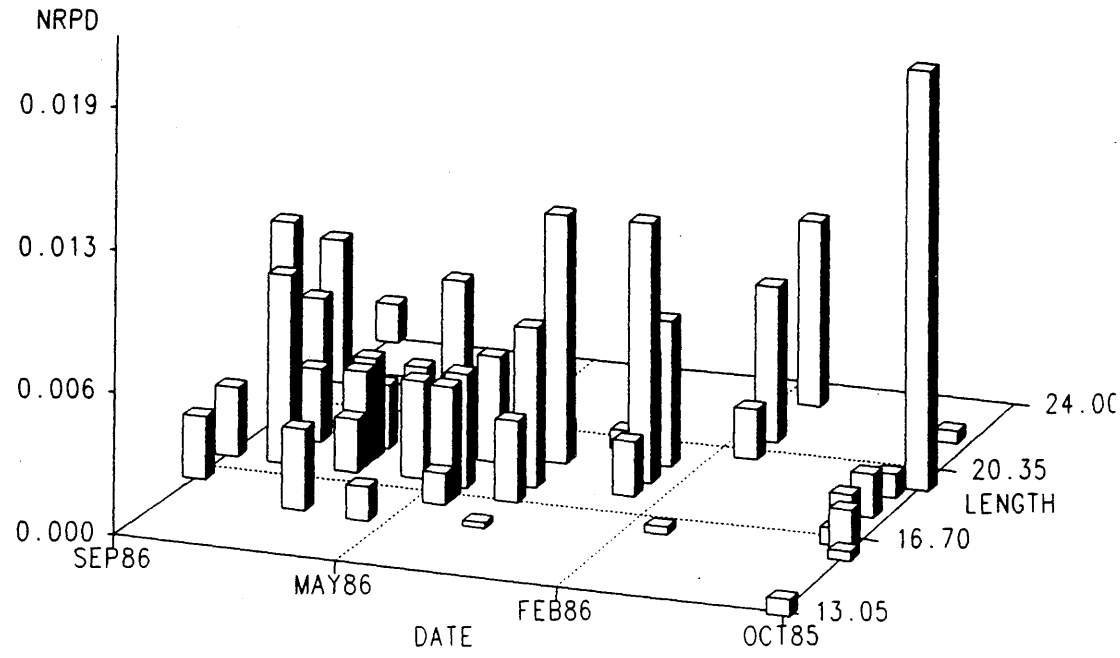


Fig. 6.5.2 Mass of Non Reproductive Tissue in individuals against length and sample date.



NRPD: Mass of gonad in grams
LENGTH: Length in mm.

MALES: LOCH FYNE.

Fig. 6.5.3 Mean, Standard deviation and maximum and minimum values for the Standard Mass of Non Reproductive Tissue (NRPD mg/mm^3).

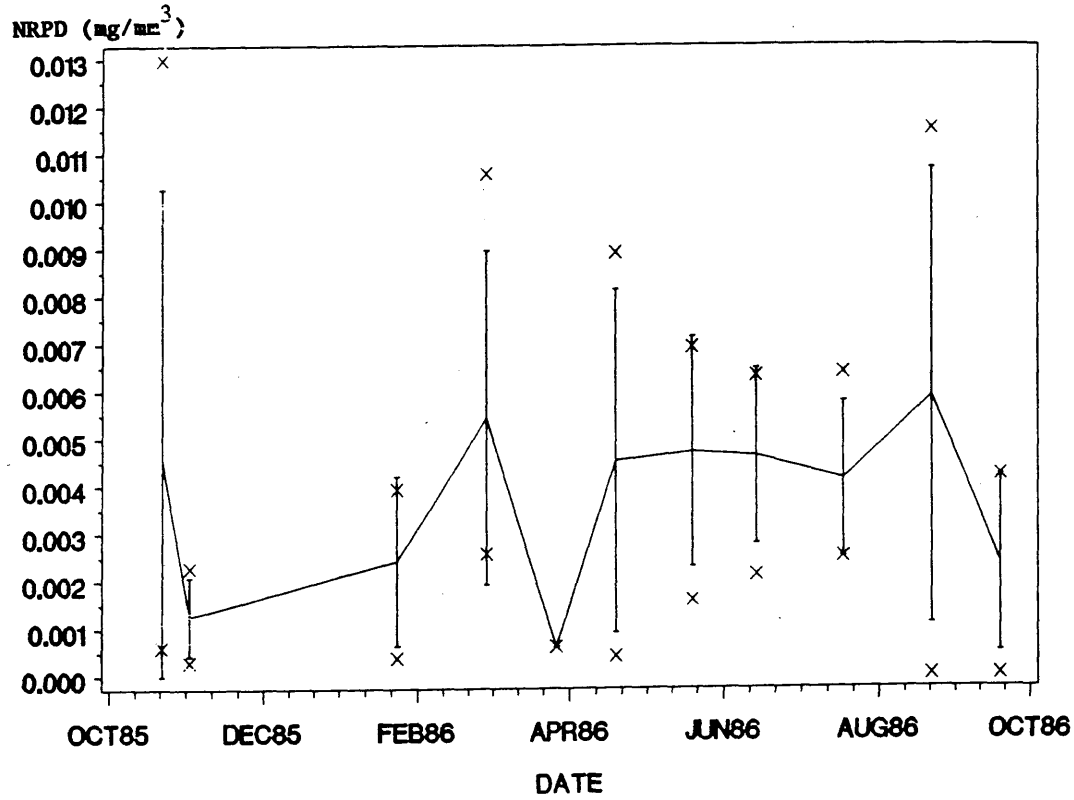
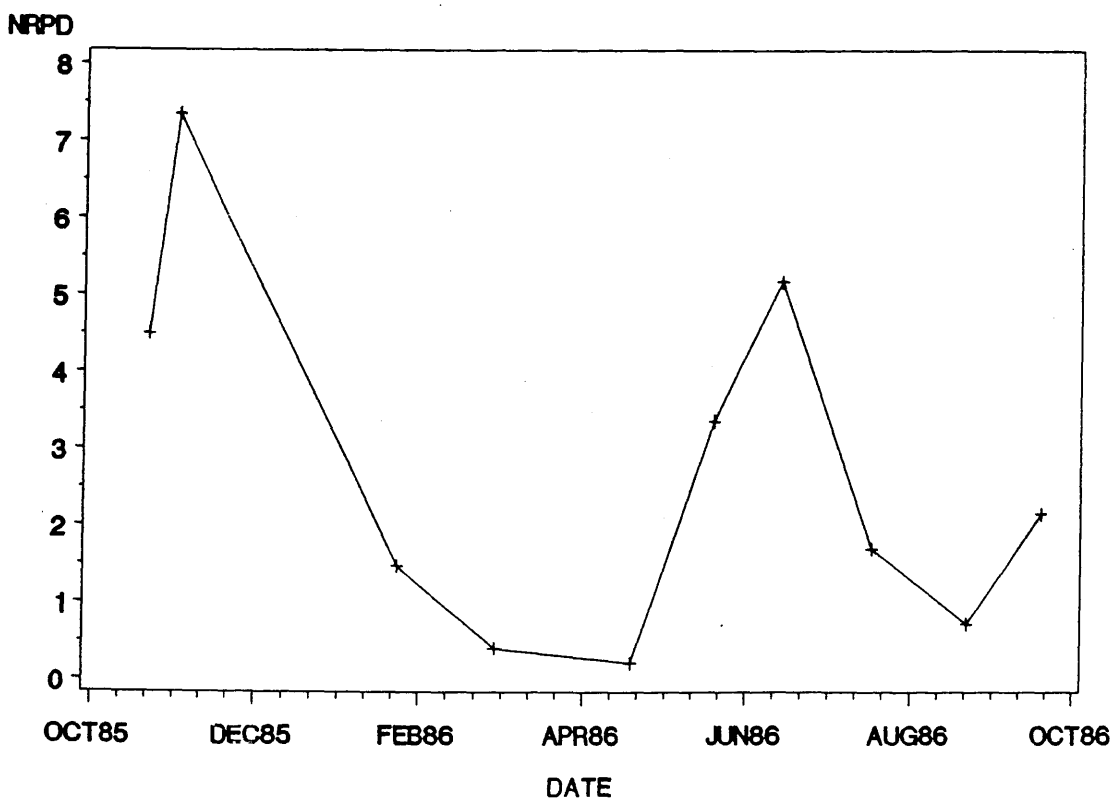


Fig. 6.5.4 Variance to Mean Ratio of the GVF of Non Reproductive Tissue (NRPD).



MALES: LOCH FYNE.

Fig. 6.6.1 Combined Plot of the Mean GVF of all the male gonad tissue phases (% VF).

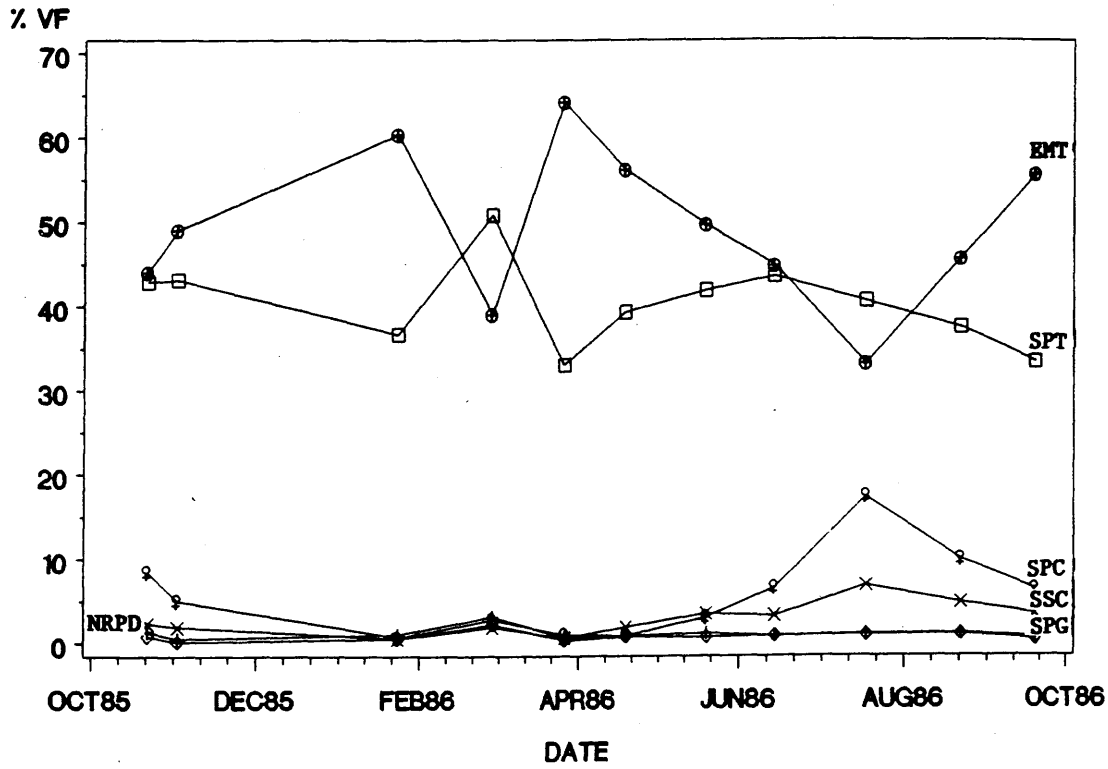
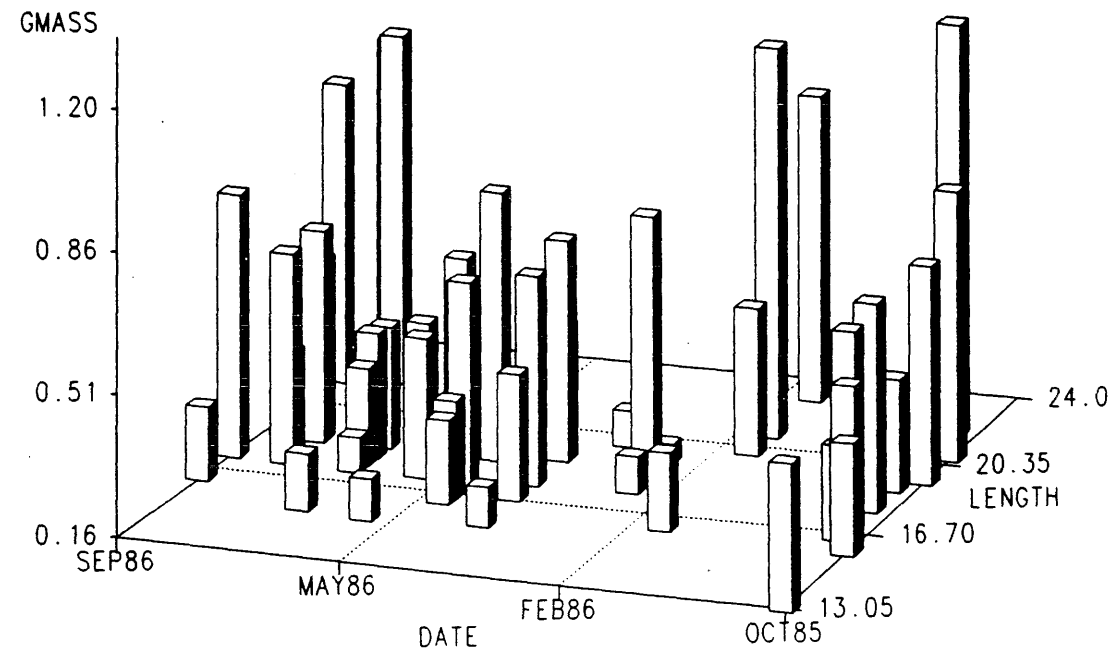


Fig. 6.6.2 Total mass of gonad in individuals against length and sample date.



GMASS: Mass of gonad in grams
LENGTH: Length in mm.

MALES: LOCH FYNE.

Fig. 6.6.3 Combined Plot of the Mean Standard Mass of all the male gonad tissue phases (ST. MASS mg/mm^3).

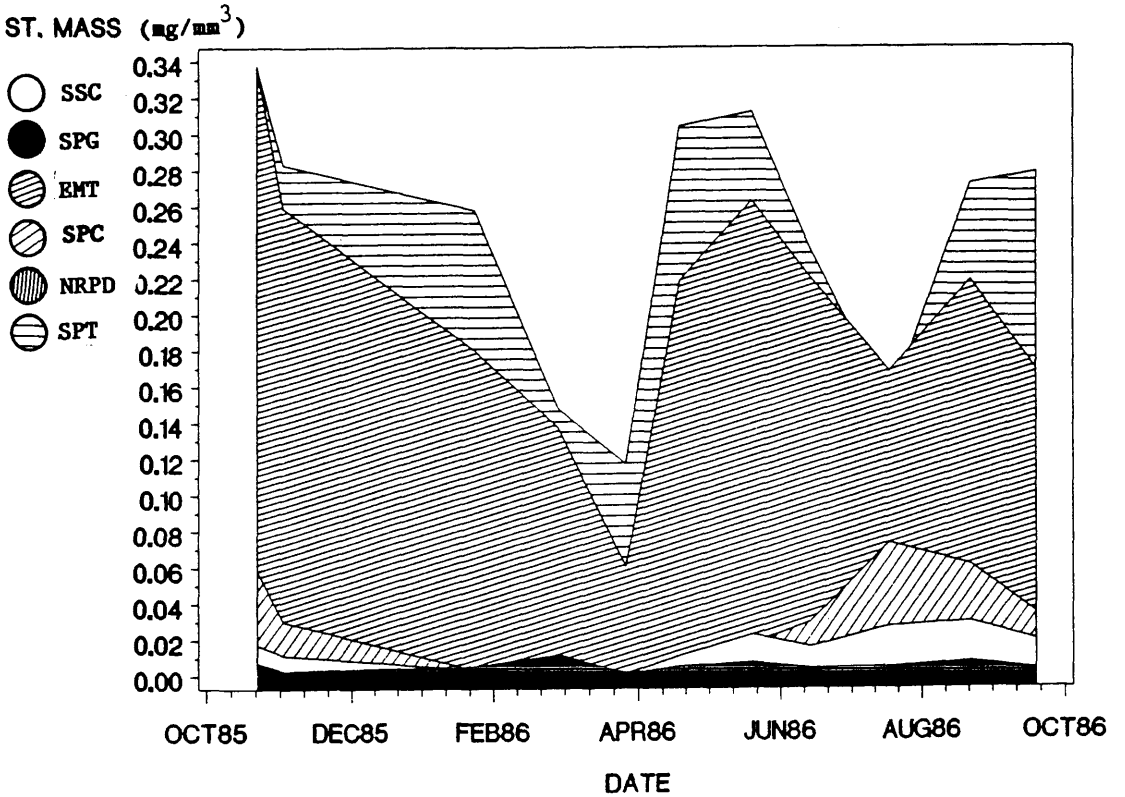
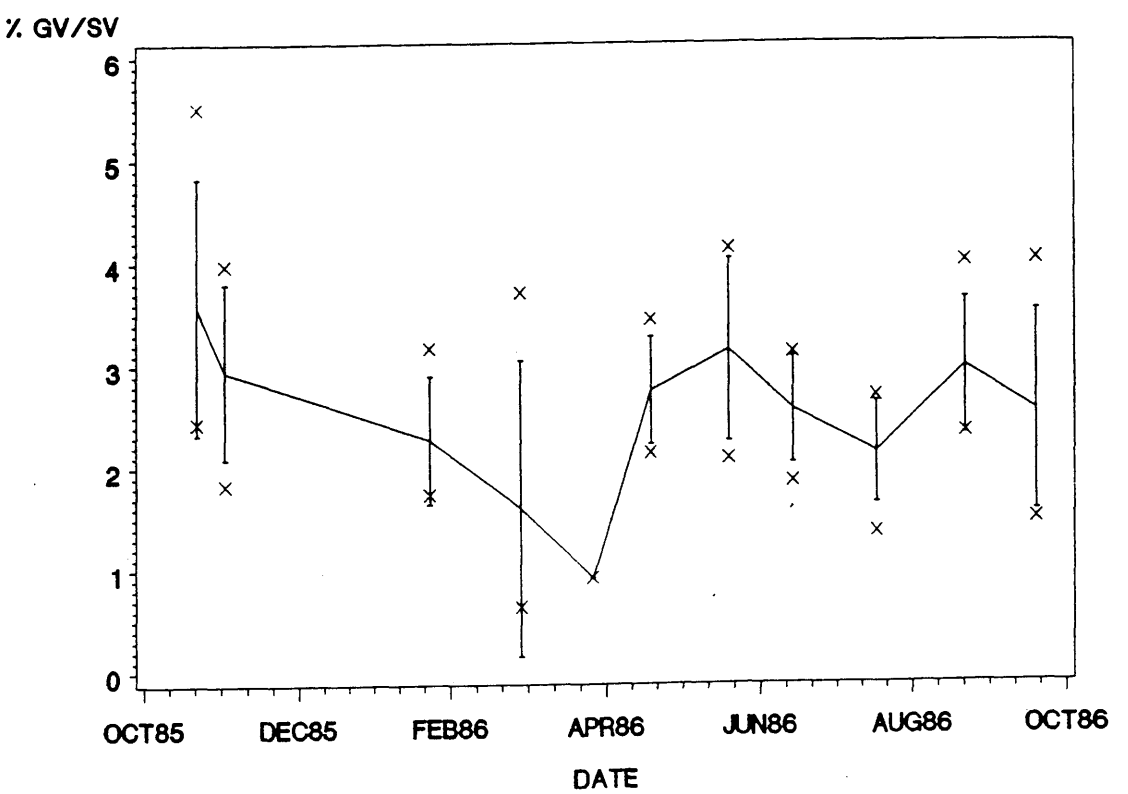


Fig. 6.6.4 Mean, Standard deviation and maximum and minimum values of percentage gonad volume per unit shell volume (% GV/SV).



5.4.5 Comparison of both Sexes and Sites.

Table 7. Figs. 7.0.1-2.

Comparison of the total gonad volume to shell volume ratio (the gonad volume ratio) confirms significant differences between male and female T. retusa from Loch Fyne and the Firth of Lorn. Examining the sites individually reveals the males to have consistently larger gonads than the females.

The shell volumes of all those animals sampled were not significantly different between the sexes at both sites, indicating no overt external sexual or geographic dimorphism.

Comparison of the standard masses of late mature oocytes and spermatids/sperm is more relevant to analysis of potential reproductive output. Analysis of variance indicates no significant differences in terms of volume or estimated mass of mature male and female gametes in the Firth of Lorn or Loch Fyne. There are, however, significant differences between sites.

Animals originating from the Loch Fyne population were found to be approximately twice as fecund as their counterparts from the Firth of Lorn during the largest of the spawning events. Loch Fyne specimens are also more productive, spawning possibly three times a year.

TABLE 7.

Analysis of Variance.
Comparison of Gonad Volume Ratio Between Site and Sex.

Source	DF	F Value	PR > F
Site	1	52.78	0.0001
Sex	1	47.24	0.0001
Site and Sex	1	2.43	0.1209

Analysis of Variance.

Comparison of Shell Volumes Between Site and Sex.

Source	DF	F Value	PR > F
Site	1	2.70	0.1019
Sex	1	0.80	0.3730
Site and Sex	1	0.94	0.3325

Analysis of Variance.

Comparison of Mass of Late Mature Oocytes with Spermatids/Spermatozoa.

Source	DF	F Value	PR > F
Site	1	15.35	0.0006
Sex	1	23.98	0.0001
Site and Sex	1	0.33	0.9566

Analysis of Variance.

Comparison of Gonad Volume Ratio.

Location	Source	DF	F Value	PR > F
Firth of Lorn	Sex	1 104	103.68	0.0095
Loch Fyne	Sex	1 98	170.75	0.0486

Analysis of Variance.

Comparison of Shell Volumes.

Location	Source	DF	F Value	PR > F
Firth of Lorn	Sex	1 106	0.00	0.9709
Loch Fyne	Sex	1 99	1.39	0.2408

Analysis of Variance.

Comparison of Mass of Late Mature Oocytes with Spermatids/Spermatozoa.

Location	Source	DF	F Value	PR > F
Firth of Lorn	Sex	1 104	0.86	0.3615
Loch Fyne	Sex	1 98	1.80	0.1113

COMPARISON OF REPRODUCTIVE DATA FROM LOCH FYNE
AND THE FIRTH OF LORN.

Fig. 7.0.1 Mean percentage gonad volume per unit shell volume (GV/SV %) of males (□) and females (◇).

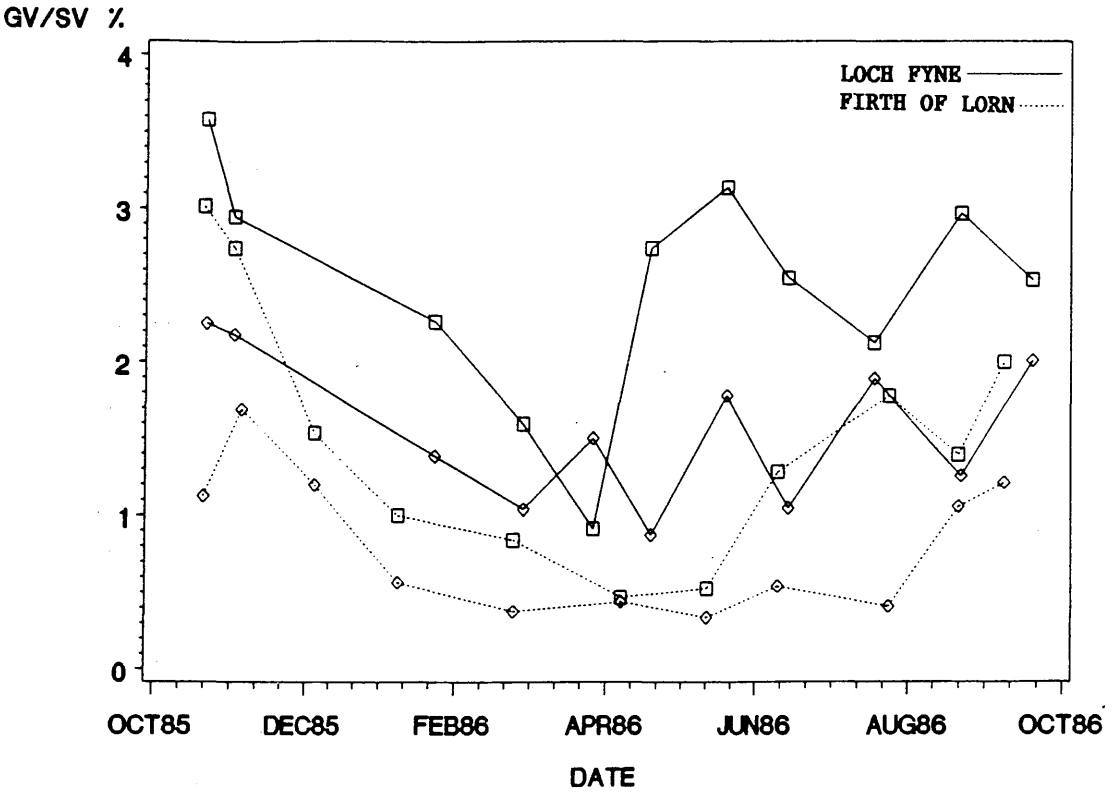
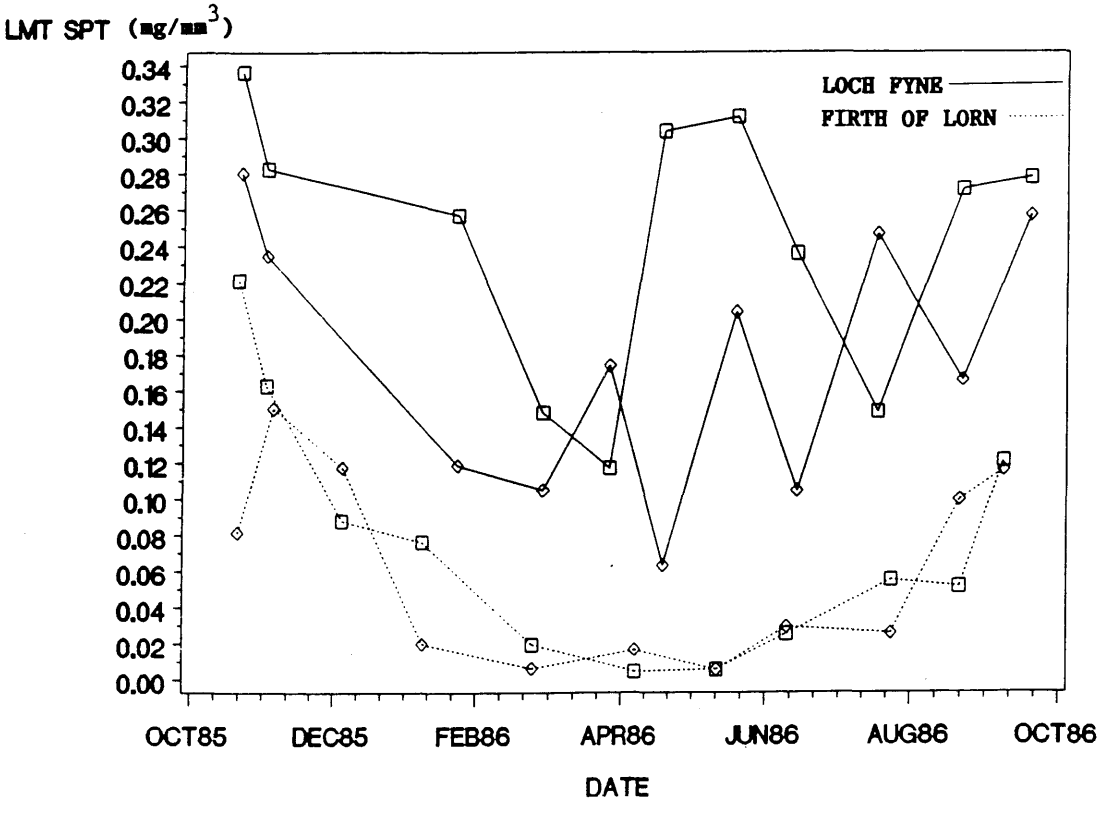


Fig.7.0.2 Comparison of the Standard Mass of the Late Mature Oocyte (◇) and Spermatid/Spermatozoa (□) cell phases (LMT SPT mg/mm^3).



5.5 DISCUSSION.

5.5.1 The Reproductive Cycle in the Firth of Lorn.

Terebratulina from the Firth of Lorn display a clearly seasonal reproductive cycle characterised by a single major spawning period, occurring between late November and January. December represents the month of greatest spawning activity.

5.5.2 The Reproductive Cycle of Females From the Firth of Lorn.

For mature females the stereological results suggest an overlap of gametogenic cycles between succesive generations of oocytes. Oogonia are present throughout the year and these provide a pool of generative cells. The pattern of proliferation is unclear and the precise time of the initiation of oogenesis is not easily defined.

A more marked cycle of events becomes apparent upon the initiation of vitellogenesis, as indicated by the accumulation of yolk in oocytes at an early stage of development. Vitellogenesis proceeds from around April onwards with the volume of oocytes increasing throughout the summer and autumn. The well defined cycle of the growth and spawning of late mature oocytes closely mirrors that of the subordinate oocyte cell phase and therefore, serves to indicate that at least two generations of cells are present within the gonad of females from the Firth of Lorn during any one season. The pattern of oogonial proliferation, though ill defined, also shows an autumn peak and this would represent a third generation of cells.

The hydrography of this region (see Chapter 1) implies rapid mixing of the water column and therefore little delay in an increase in the levels of surface productivity being reflected at depth. Moore (1931), who studied sedimentation of organic material in Loch Striven, found that the quantity of material sedimenting increased considerably at the time of the spring phytoplankton bloom, and even at a depth of 83m there was little delay in a detectable increase in sedimentation as a result of a diatom bloom in surface waters.

If it is assumed that the initiation of gametogenesis coincides with the time of maximum food availability, which in this locality is represented by the spring bloom occuring between March and April, and that vitellogenesis initially proceeds slowly, as a consequence of a low metabolic rate (Peck et al., 1988) then, on this time scale it is likely that a single generation of gametes will require in the order of 18 months to obtain sufficient nutrients (yolk) to support the brief lecithotrophic larval stage of T. retusa (Pers. obs.).

Observations of sectioned gonads reveal that few oocytes remain attached to the genital lamella in recently post spawned individuals, certainly less than the subsequent generation of late mature oocytes. The presence of early oocytes may represent a small body of vestigial cells, perhaps the product of late seasonal production, but which are not sufficiently developed enough to be spawned or to become atretic. These cells are retained and over-winter in the gonad, forming part of the following years reproductive output. The implication is that most of the reproductive effort is directed towards the production of a single generation of late mature oocytes each year, while conserving the small proportion of immature oocytes from the previous season's production.

The mechanism by which vitellogenic oocytes accumulate and assimilate nutrients has been discussed (see Chapter 4). Definitive conclusions can not be drawn from the information presented here, but it is certain that the aquisition and apportionment of nutrient reserves within the oocyte profoundly affect the observed pattern of development and maturation of late/mature oocytes. Subjective observations suggest that the majority of late/mature oocyte cells likely to be spawned during a given season are present within the gonad by August. The rapid increase in volume of the late/mature cell phase between August and November can only be accounted for by the increasing volume associated with the accumulation of yolk by individual cells.

A rapid increase in volume of this nature may represent some fundamental change in the ability of the vitellogenic oocyte to accumulate reserves, possibly the result of a number of factors. Perhaps the stage in the oocytes development has been achieved i.e. stage VI (see section 4.1.2), when the outer microvillar fringe of the

oocyte becomes fully functional. Alternatively nutrients, previously stored in the tissues of the outer mantle epithelium may become available. A combination of these possibilities is also reasonable given that when the oocytes' ability to accumulate nutrients is maximised, the capacity of the animal to supply both somatic and gametogenic requirements may be exceeded. Additional demands would be fuelled by the liberation of reserves stored during the spring and early summer.

Jones (pers. comm.) found no evidence to support the occurence of a minor phytoplankton bloom during the summer or autumn. The hydrography of this region is complex and poorly understood and therefore, a second burst of productivity cannot be ruled out. Greater abundance of food during August would serve to explain the rapid increases in oocyte volumes observed and account for similarly elevated activity in the male gonad.

The work of Olive (1983) demonstrates the effects of temperature upon the endocrine system of polychaetes. Should oocyte development be mediated by a similar system in brachiopods, it is possible, that above a critical temperature certain metabolic processes accelerate thereby speeding vitellogenesis.

Time does not permit the testing of such hypotheses, but the importance of these observations and the physiological consequences for the organism, demands future investigation.

Although gonadal density was derived from rather crude experimental estimates, the density data reveal that the relative densities of gonads from animals of differing states of maturity are approximately the same. Measurements of gonadal volume demonstrate that this fluctuates in concert with the increasing cell phase volumes. Cell free or coelomic space forms a large proportion of the gonad volume and is maintained to the extent that the density of the gonad remains relatively constant throughout the reproductive cycle. Presumably the physiological requirements of the gametes, particularly the oocytes demands a finite minimum of coelomic or cell free space from which nutrients can be drawn and into which metabolic waste can be voided. The potential significance of a minimum space requirement for

the gonads and the consequences in terms of reproductive strategy will be discussed below.

5.5.3 The Reproductive Cycle of Males From the Firth of Lorn.

Males from the Firth of Lorn also exhibit the same pattern of seasonality as the females, with evidence of a single major spawning period between October and February. Males appear to be able to produce viable gametes for a longer period which begins before the females are mature and ends after most eggs have been released.

Spermatogenesis is initiated between February and April as indicated by the proliferation of primary spermatocytes at this time, and proceeds throughout the summer. The growth of spermatogonia however, appears to precede that of the primary spermatocytes by approximately two months.

The increase in spermatocytes corresponds well with the expected time of the spring increase in productivity and therefore food availability. Investigations of the presumed storage layer (see section 3.2.2) imply that reserves contained within this tissue layer (predominantly protein) are utilised throughout the winter when productivity and hence food availabilty is minimal, a trend given support by the evidence of Peck and his coworkers (1986a, 1987) which suggests that protein is the main metabolic substrate of overwintering animals.

Spermatogonia were observed to start increasing during the winter. Should stored reserves be utilised to increase their number, this might imply that initiation of the earliest stages of spermatogenesis are to some extent independent of external food availabilty and influenced in part by the previous years stored reserves.

The description of spermatogenesis presented in section 4.3 suggested that the secondary spermatocyte cell phase is perhaps short-lived. Primary spermatocytes become increasingly numerous from May until August and constitute a significant proportion of the reproductive material within the gonad. Not until June do the primary

spermatocytes begin to undergo meiosis and thereby swell the volume of secondary spermatocytes. The peak in primary spermatocyte volume occurs at the same time as that of the secondary spermatocytes. Secondary spermatocytes rarely represent greater than 12% of the gonad volume fraction (GVF) while their parent cells may achieve 35% of the GVF. These facts combine to give weight to the assumption that the secondary spermatocyte cell stage is a brief transition between the production of a large body of primary spermatocytes which at the appropriate time undergo two meiotic divisions to produce spermatids, the time span between these divisions constituting the secondary spermatocyte cell stage.

The accumulation of spermatids and sperm commences in June proceeds slowly until August. Thereafter there is a rapid approximately three fold increase in this cell phase until spawning begins in November and December. The significance of such an increase in gamete production during August is intriguing as this echoes the rapid increase in the rate of vitellogenic activity noted within the female population at this time. Assuming that materials in excess of normal metabolic requirements are stored and that the reproductive effort expended by both sexes is the same (as will be discussed), then such a strategy has profound implications. Particularly with respect to the mechanisms involved in determining the rate at which gonial cell divisions are achieved and those factors which ensure that a large body of cells are at the same stage of development. Could the timely release of nutrients from some stored reserve serve as a possible synchronising agent ?

Environmental factors which influence the rate at which gametogenic processes proceed can have powerful synchronising (or desynchronising) effect on populations of organisms or cells within organisms (Olive, 1981a). Olive (1981b) cleverly demonstrated a link between a reproductive response and some endogenous timing factor which is described as a circa-annual rhythm in a phyllodocid polychaete. The organism is known to have a gonadotrophic hormone system perhaps allied to endogenous factors such as nutritional state and or an endogenous clock.

5.5.4 Comparison with Previous Studies.

Curry (1982a) attributed twin peaks derived from length frequency histograms to bi-annual spawning periods. Collins (1986) undertook a more detailed study, and censused sub-1mm animals which suggested that there was only one major period of settlement, corresponding to the autumn settlement (Curry 1982). Collins (1986) indicates that differences in the method of collection could account for some of the variation. Curry (1982) dredged, while Collins (1986) used a grab.

The clam dredge used for much of the sampling, conducted over the past 10 years is a particularly destructive method. Different population structures may be an artifact of intensive collection. The observed disparity could therefore be a reflection of differential recruitment.

It has been suggested by Comely, (1978) and in the present study that the area studied acts as a sink for a variety of allochthanous deposits, as a result of bathymetry and hydrographic conditions. Other populations in the Firth of Lorn may reproduce at different times and as a result of the prevailing currents be recruited into this locality.

The pattern of settlement detected by Collins (1986) confirms the evidence for a single major spawning period. Trophic conditions in the past may however, have been favourable for the overwintering of gametes, in sufficient number that a second minor spawning event could have occured, during the spring. It is not known if T. retusa has the physiological flexibility required to exploit temporal changes in food availabilty to produce additional generations of gametes, but evidence from Loch Fyne suggests that this may be possible.

5.5.5 The Reproductive Cycle in Loch Fyne.

Terebratulina from the Loch Fyne population offer a marked contrast in reproductive behaviour to those of the Firth of Lorn. The reproductive cycle is less seasonally defined, the animals more fecund and capable of spawning more than once a year.

5.5.6 The Reproductive Cycle of Females From Loch Fyne.

Females provide the clearest indication of the likely pattern of events which cause this difference in reproductive strategy. Oogenesis may begin as early as December, with similar connotations as have already been discussed, for the manner in which stored reserves are apportioned during the winter months.

A dramatic increase in oogonia occurs between March and April and is undoubtedly as a direct consequence of the substantial increase in the levels of productivity in the water column caused by the spring bloom. The quantity of oocytes also increases at this time, both these cell phases displaying an increase and subsequent decrease following an alternate monthly cycle.

The most startling feature of the reproductive cycle in this population is that spawning may occur between March and April, May and June, July and August and finally from October until the begining of the following spring. Each succesive spawning event is slightly larger than the previous one.

Late mature oocytes rarely constitute less than 20% of the GVF. Mature oocytes would therefore appear to be present within the gonads of most females at all times. The ability to spawn early in the season is perhaps a benefit of the successful overwintering of vitellogenic oocytes.

Sustained levels of high productivity, possibly the result of successive phytoplankton blooms thoughout the spring and summer permit individuals to successfully fuel the production of up to four generations of oocytes each year.

5.5.7 The Reproductive Cycle of Males From Loch Fyne.

The pattern of reproduction within male Terebratulina from this locality is less defined but supports the evidence for multiple spawnings within individuals.

As with all the germ cells described so far, spermatogonia are always present in mature animals, though the variabilty in their production reveals little of the reproductive cycle of the adult.

In contrast, primary spermatocytes display clear seasonality, increasing in number from April until August, and then declining. A simple annual cycle but bearing little resemblance to the multiple generations of sperm produced each year.

Secondary spermatocyte development is much the same as that of its precursor. Not until the spermatid/sperm cell phase is achieved is there an indication of the complete adult cycle.

Males from the Firth of Lorn were reported to contain sperm, presumed to be viable, over a greater time span than over which the females appeared capable of spawning. This is also reflected in the Loch Fyne population, where gonads harbour two major generations of spermatid/sperm spanning the times during which the females contain ripe gametes. Males seem capable of spawning between May and August, and between October and the following February. This would imply that sperm are overwintered and slowly released in a manner akin to continuous spawning. A more likely scenario is that both sexes retain their gametes over the latter part of the winter, perhaps resorbing a proportion of their number and thereby maintaining the adult sufficiently, to permit a successful spawning during the early spring.

Evidence of resorption of gametes is sparse and as will be discussed, the global definition of the nonreproductive cell phase would tend to mask increases in phagocytic activity.

Examination of the outer epithelial tissue layer, which is presumed to function as a storge tissue and might therefore be operative in maintaining the animal during the winter months, indicates that reserves are never depleted to the extent that occurs in Terebratulina from the Firth of Lorn. The implication being that the much higher sustained levels of productivity experienced in Loch Fyne (see section 1.3) are sufficient to permit greater fecundity and repetitive spawning events within individuals.

5.5.8 Comparison of Sexes.

In producing lecithotrophic larvae which do not posses a functional gut (Franzen, 1969) the burden of providing sufficient nutrients for the larval stage lies with the female, at least until metamorphosis. Similar volumes of gametes are produced by both sexes and this raises the question of potential differences in the effort invested in reproduction between sexes.

Vahl and Sundet (1985) questions the validity of the assumption that sperm production is 'cheap', siting the Islandic scallop (Chlamys islandica) as a possible example of sex linked differences in metabolic rate. Adult males having a higher metabolic rate than females or immature specimens of either sex. Vahl suggests that such differences exist because protein synthesis consumes more energy than any other biosynthetic process (Lehninger, 1975) and sperm contain relatively more proteinaceous substances than ova. Without experimental evidence (calorific values of reproductive output, for example) it is impossible to establish any conclusive evidence of differences in reproductive effort, and it would certainly be simplistic to attribute significance to observed differences in the pattern of gametogenesis, bases on such a premise.

Vahl and Sundet (1985) also suggests that there is an energetic cost inherent in the attainment of sexual maturity. Adult scallops having a higher metabolic rate than juveniles. Both Curry (1982a) and Collins (1986) report substantial juvenile mortality in demographic studies of T. retusa from the Firth of Lorn. Maturity of T. retusa appears to be achieved by the time a shell length of approximately 5.5 mm is attained (pers obs.). The additional stress incured by an increase in metabolic demand at the onset of maturity might be sufficient to contribute to juvenile mortality.

5.5.9 Comparison of the Reproductive Cycles.

The results clearly indicate that T. retusa from Loch Fyne are more than twice as fecund as those from the Firth of Lorn, in addition to being capable of multiple spawning events over the same time period.

The reproductive strategy of the individual and ultimately the population is determined by a host of factors, controlled intrinsically at the genomic level and extrinsically by the environment. In order to separate the influence of these characteristics, the effect of one or more of these variables must be removed.

Recently, a study conducted by Ansell et al. (1988) revealed that scallops with different reproductive cycles, translocated to a common ground, retained the critical features of the reproductive cycles of the parent populations. The cycle, even in specimens translocated at an early age, showed none of the differences characteristic of the species native to the chosen locality. These results strongly implied that genetic factors play a significant role in controlling the reproductive cycle, at least in some European scallop stocks.

Assuming that reproduction is under the control of some innate endogenous rhythm, and that larval dispersion may result in settlement of offspring on foreign grounds experiencing different environmental conditions to the parent population, then this prompts the question as to the stage of development at which this presumably genetically based clock is set.

An endogenous rhythm modulating a future reproductive cycle would presumably be initiated post-settlement. This would permit the progeny of populations settling on foreign grounds, as is likely with the planktrophic mode of larval development, to adopt a reproductive cycle tuned to local environmental cues.

Without the appropriate experimentation it is impossible to establish the extent to which genetic factors influence the differences between the reproductive cycles of Terebratulina in the Firth of Lorn and Loch Fyne.

It is interesting to speculate however, that certain features of the reproductive strategy might lead to a significant genetic link in the chronology of the reproductive cycle in T. retusa.

This species produces a lecithotrophic larva with a brief pelagic phase. At 10°C the larval phase lasts in the order of 72 hours. Some

larvae maintained in vitro were observed to be motile for 14 days, perhaps indicating a limited ability to delay settlement. The competence of the larvae to metamorphose after this time was not established.

Dispersion is therefore likely to be limited, particularly in the population from the Firth of Lorn. Although subject to currents in excess of a knot and a strong southerly residual flow (see section 1.2.1), there is evidence that this region is a sink for allochthanous deposits (Comely, 1978; Collins, 1986) and may therefore be part of a circulation or gyre within the deep depression in which the population are situated. This may serve to further restrict larval distribution and enhance recruitment to the parent population.

Similarly, current flows in Loch Fyne which are of very low speed compared with those of the Firth of Lorn (see section 1.2.2), would also reduce dispersion. The only major contribution to dispersion would be the result of excursions by the larvae, during the brief pelagic phase into the surface waters, which may experience more rapid current flow as a result of wind sheer for example.

Restricted dispersal is one of many potential constraints on gene flow. The larvae may for example be substrate specific or perhaps show some preference for settlement near to adults of the same species. Collins (1986) surveyed the density of settlement in the Firth of Lorn on a variety of substrates. This census revealed that initial colonisation appeared to be unspecific and the adult distribution was possibly function of selective post settlement mortality.

Preliminary substrate preference experiments in vitro, although not conclusive, did suggest some degree of substrate specificity, but no causative agent was identified (pers. obs.). N. anomala an inarticulate which is believed to have an extended planktonic phase (Rowell, 1960) appears to be highly selective (Collins, 1986), but the criteria governing the choice of substrate are unknown. Settlement on structurally complex substrates is common amongst brachiopods. Wisely (1969) Thayer (1975) and Doherty (1979) report rugophilic behaviour in brachiopod larvae. Recruitment onto or near to conspecifics is characteristic of a number of brachiopods (Mattox,

1955; Hartmann, 1966; McCammon, 1973; Foster, 1974; Thayer, 1975; Doherty, 1979; Richardson, 1981b; Stewart, 1981), and has promted comment regarding settlement stategy, but again nothing is known of the root cause of this behaviour.

Limited gene flow between breeding populations experiencing significantly different selective forces might therefore lead to similar differences being reflected within the genome.

The environmental data reveals both the populations under scrutiny to be subject to similar annual temperature variations with the maximum around October. Short term variations in Loch Fyne, presumably the result of meterological disturbances or incursions by different bodies of water (Jones, pers. comm), may cause fluctuations of a degree Celcius; more than 25% of the annual range. The Firth of Lorn diplays a similar range to Loch Fyne but the data available suggest greater stability.

The levels of productivity and therefore food availablity would seem to be the key factor in determining the differences in the sustainable levels of fecundity between the two populations. Loch Fyne experiences up to five times the productivity of the Firth of Lorn.

The initiation of gametogenesis appears to be closely linked with the annual cycles of productivity in both locations. Detectable proliferation of germ cells seems to occur from January, with the marked increases in primary spermatocytes and early vitellogenic oocytes corresponding to the spring bloom in March and April.

Without more detailed data regarding the utilisation of stored reserves and the amount of food available during the winter period it is not possible to isolate those factors which mediate the pre-spring bloom increase in germ cells.

Specimens from Loch Fyne are capable of an early spring spawning event which is undoubtedly a consequence of the overwintering of gametes whose final development is presumably fuelled by the spring bloom. The slow metabolic rate of these organisms (Peck et al., 1988, in press; Curry et. al., in press) may also affect the speed with which

nutrients can be acquired and the rate of gamete production.

Animals from Loch Fyne usually contain several generations of gametes, a feature particularly obvious in the females. More marginal trophic conditions in the deeper less productive waters of the Firth of Lorn are only sufficient for the production of a protracted but none the less synchronised, single spawning event.

5.5.10 Spawning Behaviour.

Having establibed the pattern of reproduction, the questions which now arise are: what are the cues likely to initiate spawning and more importantl y, why is the population releasing its gametes at this particular time during the year ?

These questions are particularly important in interpreting the reproductive strategy of the Firth of Lorn population. In producing a relatively small number of large eggs, which contain sufficient nutrient reserves to maintain the non-feeding larval stages through to metamorphosis, the females have adopted a strategy designed to maximise larval survival, but with minimal expenditure on the part of the adult (Sibley and Calow, 1986). Clearly an advantageous principle where larval or juvenile mortality may be high and food availability may be limiting.

The timing of the spawning of this population presents something of an enigma. Since the larval life history is short, settlement is likely to occur at a time when potential food availablity is minimal. Given a settlement peak in January and February (Collins, 1986) there would be an intervening period of approximately two to three months before the spring increase in productivity would make a significant contribution to food availablity.

In order to rationalise the differences between the time of spawning and that of settlement, requires detailed knowledge of the factors exercising control over these events.

The adoption of a given larval strategy presumably results from

selective pressures acting on a wide range of factors including the levels of energy available for reproduction, the advantages and disadvantages of larval dispersal, egg size and number and the individual probabilitites of larval survival.

Todd and Doyle (1981) presented a settlement timing hypothesis, which proposes that for a given species there is an optimum time to spawn, (when standing crop is at its peak) and an optimum time for the larvae to settle. They suggest that their intuitive model is based on the concept that in conditions of seasonally varying resources there is an optimum time for settlement.

Collins (1986) predicts a slow initial growth rate, resulting in a sigmoidal growth curve. This is perhaps an indication of the limited resources available to early post settled juveniles. Low metabolic rate (Peck et al., in press) may suggest that there is sufficient food within the environment, even during the latter part of the winter to sustain the larvae until the spring. It may be that winter storms are capable of causing the resuspension of organic material, although the cohesive nature of the sediments and their depth would make this an unlikey event.

Despite the presumed lack of food, there is remarkable settlement (Curry, 1982; Collins, 1986), begging the question as to the possible advantages of an "early" settlement ? Perhaps in becoming established prior to the spring bloom the animal is able to take full measure of the highly seasonal productivity. Collins (1986) reports a possible size refuge for juveniles of approximately 2 mm, below which they appear to be particulary vulnerable. A metabolically constrained growth rate could require an early settlement in order that the size refuge is achieved before the major sources of juvenile mortality become active.

Mileikovsky (1969), indicates the significance of interspecific relationships; predation being an appropriate example. The spawning of some clams in the North Sea apparently correspond to the time when predatory brittle stars are not feeding, (Thorsen, 1958). As a general observation the vast majority of the brachiopods from the Firth of Lorn were attached to the valves of the bivalve Modiolus, (Curry (1982a),

quotes a figure of 75% attachment to living mussels). Presumably these animals would be capable of filtering substantial quantities of suspended particulate material from the water column, including eggs and larvae. Should the winter be a time of relative quiescence in feeding activity, then it might be reasonable to suggest, that through adaptive synchrony, T. retusa may achieve an advantage in spawning during a period of relative impoverishment.

Peck et al. (in press) found that adult T. retusa utilises protein as its main metabolic substrate. The nutrient reserve contained within the oocyte and therefore the major metabolite of the larval stages is lipid. This transition would require a fundamental physiological change during the early life history. It is assumed that such changes would occur during metamorphosis and/or early in juvenile development. A physiological adjustment of this magnitude may incur a delay in the achievement of full feeding potential and therefore infer an advantage to early settlement.

Trophic conditions in Loch Fyne are far less stringent than those of the Firth of Lorn. Spawning appears to be more of a restriction imposed by the ability to generate gametes than the effects of a seasonally limiting food source. During the winter, recorded levels of productivity fall to values similar to those experienced in the Firth of Lorn. Winter is the only season in which the Loch Fyne population does not produce a significant, synchronised spawning event. This would suggest that the constraints imposed by a precise settlement 'target' or the larvae are inoperative, when food is not seasonally limiting. The implication being that food availabilty is an important factor in both the initiation of gametogenesis and ultimately the development of a spawning cycle which fa cilitates larval settlement at the appropriate time.

Selection pressures may not always operate at the level of larval development. Juveniles or adults could be the critical elements (Miliekowsky, 1969). Perhaps the juvenile has specific feeding requirements, or avoids predation by settling in the winter.

While persuing the idea of a metabolically constrained growth rate, it may also be possible that in order to survive the following

winter, juveniles must acrew sufficient reserves, a process which may require that the juvenile has the opportunity to exploit the entire growing season.

Space for settlement may be at a premium in an environment with a limited availability of hard substratum. T. retusa may be better equiped to compete by becomming established before a preponderance of larvae of other species commence settlement.

Spawning would reduce the energy demands incurred by the adult in maintaining gametes. Although this may not enhance the probabilty of adult survival during adverse conditions as prolonged physiological stress appears to cause the resorption of gametes (pers. obs.). Relieving the adult of this energy burden at this time may, however, ensure that sufficient reserves are available not only to maintain the adult but also to contribute to the observed proliferation of germ cells, during the latter part of the winter.

5.5.11 Possible Spawning Cues.

Finally it is necessary to examine those environmental cues responsible for stimulating spawning behaviour. As has already been discussed, reproductive cycles may have a strong genetically regulated component. Circannian endogenous reproductive rhythms have been demonstrated in invertebrates (Jegla and Poulson, 1970), but these cycles still required external physical cues for synchronisation.

T. retusa has been encouraged to spawn in vitro, by both agitation, temperature shock and by the introduction of viable gametes to the water (Curry, 1982, pers. obs.). Whilst many of the recognised chemical stimuli, salinity change and current flow failed to illicit a positive response (Pers. obs.).

Curry (1982), invokes temperature as the most plausible agent for the initiation of spawning in the Firth of Lorn. No conclusive evidence of a dominant environmental spawning cue was recorded during the present investigation. Although the development gonads does appear to approximate to the annual temperature cycle.

Thorsen (1946) has reported that gradual temperature changes are capable of inducing spawning, but according to Olive (1983), environmental temperature is a relatively noisey timing device. The stabilty of the short term temperature record from the Firth of Lorn and the very gradual change in the annual temperature cycle would seem to offer a rather imprecise mechanism for the initiation of a synchronised spawning event. Neither would temperature seem to function as as a suitable stimulus in Loch Fyne, where short term fluctuations may be considerable and certainly fail to correlate with periods of spawning activity.

The availailty of food, at least initially, would not seem to be paramount as a spawning stimulus, as indicated in the Firth of Lorn, where gametes are liberated at a time of impoverishment. The abundance of food appears unimportant with respect to spawning, but the substances or metabolites derived from detrital material may act as time cues. Temporal changes in the composition of the food could possibly be detected by the organisms and serve as exogenous regulators of reproduction (Giese and Pearse, 1974).

Pheromonally influenced gonad development has been known for some time. It is possible that pheromones act to mediate the initiation and synchronisation of gametogenesis (Giese and Pearse, 1974). The fact that the introduction of water containing viable gametes induces spawning in both sexes may serve to indicate that other hormonal stimuli are at work in bringing about epidemic spawning behaviour.

The ultimate cause of the synchronous annual spawning event in the Firth of Lorn and that of the repetitive spawning events recorded in Loch Fyne may not be the same. Presumably a suite of stimuli are required to initiate and synchronise spawning. The intergration of an endogenous rhythm founded within the genome and switched on at some time between settlement and the onset of maturtity could, through the endocrine system be linked to environmental cues. A reproductive cycle requiring genetic, hormonal, and environmental controls to be correctly phased would synchronise gametogenesis and prevent spawning at inappropriate times.

5.5.12 Stereological Method.

Stereological analysis is, in principle, an extremely informative methodology. In practice, however, the implementation of this proceedure is extremely time-consuming and if carried out manually can in no way be treated as routine. This technique is of limited use for the study of most reproductive studies, unless combined with an image analysis system.

The results of the present study reveal a number of these flaws. Primarily, subjectivity is still an inherent problem when the designation of a given cell phase, often a false division of a dynamic process, is decided by eye. In addition, the manner in which the various cell phases within the gonad are divided may result in the masking of important cytological events. A typical example of this is, the cell phase designated as nonreproductive (NRPD). Hindsight would dictate that this phase should be subdivided to reveal the proliferation, growth and decline of accessory cells and phagocytic material.

Resolving spermatid from sperm is time consuming and difficult to achieve with any degree of precision. Unifying these cells into a single phase (SPT) will necessarily increase the period of time which the male specimens are capable of spawning, and possibly reducing the observed level of reproductive synchrony within the population.

Stereological analysis emphasises the change in volume of a given cell phase, and reveals little of the actual numbers of cells involved; a particularly important factor when dealing with cells such as vitellogenic oocytes which by definition increase in volume.

Combining the stereological methodology adopted in this study, with measurement of oocyte diameters as suggested by Grant and Tyler (1983a,b) or alternatively the stereological determination of oocyte volumes as demonstrated by Morven and Ansell (1988) would greatly enhance the ability of the investigator to interpret the reproductive cycle, the cytological processes involved and their intergration with physiology.

CHAPTER 6.

GENERAL DISCUSSION AND CONCLUSIONS.

CHAPTER 6.

6.0 GENERAL DISCUSSION.

6.1 THE REPRODUCTIVE STRATEGY.

To fully appreciate the significance of the reproductive strategy of T. retusa, it is necessary to view this work in a wider context. What selective forces, for example, could have operated to result in the adoption of the observed mode of reproduction , How does this compare with the known strategies of extant brachiopods , and ultimately, what are the likely palaeobiological implications ?

The first of these questions has to some extent already been broached in the discussion of the reproductive cycle. There are a number of interesting points which should be developed. These can be roughly divided into three categories of 'constraint'; physiological, morphological and environmental. Although it is difficult and often misleading to apportion precedence, exploring a limited number of factors helps to clarify these complex phenomena.

6.1.1 Physiological Constraints.

Perhaps the most thoroughly documented aspect of brachiopod physiology is metabolic rate. (Hammen et al., 1962; Shumway, 1982; Peck et al., 1986, 1987, 1988). Experimental evidence suggests that brachiopods have a relatively low metabolic rate.

A low metabolic rate will necessarily place constraints upon the acquisition and apportionment of reserves, and trade-offs between metabolism growth and reproduction will result. Intrinsically, a low metabolic rate will imply a slow rate of growth and suggests that the processes of accumulation of stored reserves will be equally retarded.

The storage materials detected in the outer mantle epithelium and perhaps the caecae (see section 3.2.2) are likely to be used to supplement food in gamete production. The time required to accumulate sufficient reserves and there allocation during reproduction will have

a profound bearing on the reproductive strategy. This requirement is reflected in life histories of a number of brachiopods which are iteroparitous and produce non-feeding lecithotrophic larvae, some of which have a brief pelagic phase. In other brachiopods the young experience some degree of brood protection by the parent until near metamorphosis. Development of this type is likely to be favoured where larval survival may be poor, perhaps as result of intensive predation, specific habitat requirements or seasonal food availability.

A low metabolic rate may therefore be a fundamental factor in dictating that nonplanktotrophic development is the sole reproductive mode, exhibited by articulate brachiopods.

The larvae of articulate brachiopods are not known to feed, and present knowledge of their embryology would suggest that they have no functional gut (Long, 1964, Franzen, 1969). The burden of providing the larval stage with sufficient nutrients to achieve metamorphosis lies with the parent. Parental investment in individual propagules is necessarily high but relatively few are produced.

It is difficult, however, to generalise about the total energetic cost of such a strategy in comparison to that of planktotrophy (Jablonski and Lutz, 1983) although some authors invoke the conservatism of the nonplanktotrophic strategy as being the more efficient (Chia, 1974). Olive (1983) suggests that this mode of reproduction is not cost effective and is only adopted when morphological and environmental constraints preclude gamete accumulation and mass discharge.

Planktotrophic larvae are subject to the effects of long term exposure to predation, widespread dispersal, perhaps to areas unsuitable for settlement, and the vagaries of planktonic production. This mode of development would incur high larval mortality and therefore select for the production of large numbers of gametes.

6.1.2 Morphological constraints.

A recent paper by Peck and Holmes (in press) adds weight to a long

standing argument about the functional effeciency of the lophophore. Their results suggest that the size of brachiopods is limited by the amount of space available to house the lophophore between the valves of the shell. As the animal increases in size it requires relatively more space to acomodate the lophophore, and this may be achieved by a consumate reduction in the relative volumes of other internal organs. Assumptions are also made from the work of La Barbera (1986), suggesting that as brachiopods get larger they have proportionally less surface area of lophophore with which to obtain resources. Both these factors would affect the reproductive strategy.

The volume of the gonad is likely to be restricted, a design constraint which would appear to be compounded as the size of the individual increases. This would have a direct influence on fecundity, as egg size tends to be highly conservative within species (Chia 1974). Despite the relative reduction in gonad volume, it is also possible that larger specimens will have a relatively reduced ability to obtain food associated with less space to store materials in excess of immediate requirements; reserves which may be important in the reproductive cycle.

As a consequence of limited space and the potential restictions in the acquisition and storage of resources it is possible that brachiopods suffer the same constraints experienced by other organisms restricted not by anatomical inefficiencies but by small body size.

Underlying the distribution of many reproductive strategies is the relationship between body size and the mode of development. Because of the massive mortality incurred by planktotrophy, species following this developmental mode must release immense numbers of gametes. As body size becomes small, the absolute energy available for reproduction is also reduced (Giesel, 1976). Thus, below a certain size an animal may not be able to produce enough planktotrophic larvae to ensure recruitment to and maintenance of the population. Here the most efficient strategy is to invest more energy per offspring in a few eggs that have a relatively good chance of surviving to complete their development.

Chia (1974) speculates that most small animals are producers of

small numbers of large eggs, and may be viviparous or even brood protecting. The reason given for this is that the energy available to animals of small body size is relatively less and consequently less will be available for gamete production.

Chia's suggestions, hinge on the assumption that the major factor in determining the reproductive strategy is the amount of energy available for gamete production. Restrictive allocation of energy may result from a low metabolic rate, morphological constrictions, as well as environmental factors such as limited availability and/or quality of food.

6.1.3 Environmental constraints.

Brachiopods are significant members of a number of present day marine communities. Work in the fjordic waters of both New Zealand and British Columbia (Grange et al., 1981; Richardson, 1981; Tunnicliffe & Wilson, 1988), in the Mediterranean (Emig, 1987) and in the Caribbean (Jackson et al, 1971) are illustrations of there dominance in certain areas.

Brachiopods appear to have survived long periods of geologic time, not by adapting in situ to any change in environmental conditions, but by remaining in specialised refuge habitats , insulated from traumatic environmental fluctuations (Curry et al. in press.).

According to Crisp (1974) the nonplanktotrophic strategy is appropriate to organisms occupying time stable habitats, especially those where energy supplies are low and pelagic losses are likely to be high. Curry et al. (in press) predict that brachiopods would be favoured and most abundant in areas where the rate of renewal of particulate food resources was low, where the available particulate food, if abundant, were of low quality or where other conditions such as low oxygen levels or cold temperatures act to limit the effectiveness of competitors with higher metabolic rates.

Many of the well documented habitats of extant brachiopods (for examples Tunnicliffe and Wilson, 1988; Emig, 1981; Curry, 1982;

Jackson et al 1971) would certainly to conform to the above description. The Firth of Lorn displays many of these characteristics, with highly seasonal and relatively low levels of productivity, possibly reflecting sub-optimal conditions (Comely, 1978).

In contrast, Loch Fyne offers greater abundance of food, but is perhaps refuge-like to the extent that the brachiopod population is limited to below a depth of approximately 20m. The cause of this restriction was not established. All the larger brachipods censussed occured on the almost vertical barren rock of the cliff face or where possible adopted a cryptic habit. There is little or no algal overgrowth at this depth, and most of the rock face is clear of sediment. Collins (1986) proposes that grazing pressure could impose an impact on the distribution, and certainly no great proponderance of gastropod or echinoid grazers were observed over the range of the population at Kenmore Point.

It is generally believed that brachiopods are poor spacial competitors (e.g. Doherty, 1979) and it is argued that competion for space decreases with depth and like predation (Stanley, 1974; Whitman and Cooper, 1983) is reduced in cryptic environments. The distribution of brachiopods may therefore reflect a withdrawal from intense spacial competion and predation (e.g. Jackson et al., 1971; Logan, 1977, 1979; Whitman and Cooper, 1983). Competition-induced small body size may therefore result in nonplanktotrophic reproduction.

Reference to the reduced of availablity of internal space is particularly appropriate with respect to T. retusa. Reproductive output in animals from Loch Fyne where food rarely appears to be a limiting factor, can only be increased by increasing brood frequency which may indicate that there is a maximum feasible gonad size.

Certainly, the Thecidine brachiopods of the Carribean, which rarely exceed 5 mm in length would support this contention. Both Lacazella and Thecidellina brood small numbers of larvae (pers. obs.). Their cryptic habit, small size and hence reproductive strategy are possibly as a consequence of intensive spacial competion within the reef environment (Jackson et al. 1971). Collins (1986) argues that paedomorphic brachiopods are the expression of an adaptive requirement

for early reproduction, constrained by slow initial growth rate. While large brachiopods appear to be better spacial competitors (Doherty, 1979), in tropical reef environments, all the articulates are paedomorphic.

Since Thorsens classic review of invertebrate reproductive strategy (Thorsen, 1946) a number of workers have refined Thorsens observations (Vance, 1973a,b; Strathmann, 1978; Valantine and Jablonski, 1983). Although there is no unifying model, it is clear that strategic patterns exist.

The Antarctic species Lyothyrella uva is one of the largest living brachiopods, and displays brooding behaviour (Peck et al., 1987). The primary constraint at higher latitudes is that of highly seasonal productivity confined to the brief polar spring and summer. Planktotrophy would necessarily require a pelagic phase extended not only by low temperatures but also that of low metabolic rate. Most sessile marine invetebrates at these latitudes have therefore evolved nonplanktotrophy often with a high degree of parental care.

Similarly, the incidence of planktotrophy in many marine invertebrates tends to decrease with depth. Many taxa from the deep sea exhibit nonplanktotrophic strategies (Thorsen, 1946). The patchy distributions and poor trophic conditions characteristic of some deep sea environments would tend to promote the economies which appear to be inherent in this strategy. The reproductive biology of deep sea brachiopods is unknown but it is to be expected that they would conform to this mode of development.

6.1.4 Pale obiological Implications.

Study of the reproductive strategy of extant faunas will permit more informed interpretation of fossil assemblages and assist in our understanding of the mechanisms involved in the rates of both speciation and extinction.

The decline of the Brachipoda has elicited many explanations, from adverse environmental conditions (Rudwick, 1970), increased predation

(Stanley, 1974), competitive displacement (Thayer, 1979), to lack of flexibility in reproductive development, (Valentine and Jablonski, 1983). Much of the speculation centres upon a supposed or inferred inferiority to bivalve molluscs which expanded in both diversity and number as the Brachiopoda diminished (Tunnicliffe and Wilson, 1988).

In the light of an improved understanding of the functional morphology of extant species it will perhaps be possible to make more accurate predictions of the reproductive traits of the fossilised taxa.

Some evidence of brooding exists for a number of extinct species. Rudwick (1964) reported possible brood pouches in the Devonian Uncites, though Jux and Strauch offer an alternative interpretation. Similarly, Pentagonia (Dutro, 1971), Pennsylvanian Cardinaria (Cooper, 1956), Permian Megousia (Fergusso, 1969) and Amphipella (Grant, 1981) may have brooded.

It is unknown whether all Palaeozoic articulate brachiopods possessed nonplanktotrophic larvae, but as pointed out by Valentine and Jablonski (1983), in view of the highly cosmopolitan distributions obtained by some taxa (e.g. Boucot, 1975) that some did not. Because the group exhibits latitudinal gradients resembling the distribution of those modern planktotrophs (with a distinct low latitude maximum) rather than those of modern nonplanktotrophs including living articulates (with a much reduced or absent equatorial maximum) it has been suggested that some Palaeozoic brachiopods may have adopted a planktotrophic strategy (Rudwick, 1970; Jablonski and Lutz, 1983).

Demographic studies in support of this hypothesis, use the detection of juvenile settlement peaks (Noble and Logan, 1981). The fact that so little is known about the population structures of extant faunas, where the is a much greater chance of collecting a representative census of the various size spectra and without the problems associated with selective preservation, means that such results are equivocal.

In an evolutionary context, an animal has two choices in the mode of gamete production, that of numerous small eggs or a small number of large eggs. Chia (1974) argues that planktotrophic development

resulting from small eggs has certain clear advantages and may be considered as the "primitive type of development". The condition of this hypothesis is that when the the energy for gamete production is at a minimum, there will be a shift in reproductive strategy to a more efficient mechanism of reproduction in terms of per unit energy cost. But this incurring the penalties of limited dispersal and mutation.

The limited reproductive strategy reflected in this Phylum would not only affect the ability to successfully colonise diverse environments, but also strongly influence the patterns observed in past assemblages.

Inarticulates are primarily planktotrophic while the articulates are apparently exclusively nonplanktotrophic. Jagersten (1972) argues that the planktotrophic inarticulate brachiopod larvae retain some of the characters of the ancestral lophophorates and there is fossil evidence for planktotrophy in Ordovician articulate brachiopods (Chuang, 1971). Thus planktotrophy may represent a primitive state in the Brachipoda; it is not clear if the similarities between articulate larvae are as a consequence of convergence and repetitive derivation from ancestors with similar planktotrophic larvae, or if planktotrophy was lost only once and the inarticulates have been nonplanktotrophic since their separation from the inarticulates (Strathmann, 1978a).

Steele-Petrovic (1979) has suggested that one factor leading to the dominance of benthic assemblages by bivalve molluscs rather than brachiopods in the post Palaeozoic is the low larval dispersal capabilty of articulate brachiopods. Valantine and Jablonski (1983) have suggested that the articu late brachiopods declined because the articulates were locked into a nonplanktotrophic larval type and thus have only persisted in habitats and latitudes in which this mode of development is not disadvantageous. Articulate brachiopods for which reliable information is available, seem to share a characteristic suite of reproductive specialisations including hermaphroditism, low fecundity and direct development.

Logically, the limited information regarding the biology of extant species would suggest that a number of factors play important

roles and the relative influence of each varies between both environment and taxa.

The longevity of many brachiopod lineages implies that they are genetically conservative. Should such conservatism be linked with some ancestral selection for a reduced metabolic rate, the two would form a powerful mechanism of adaptive and evolutionary restraint. Curry et al. (in press) explore the physiological consequences of low metabolic rate not least of which is the influence on the reproductive strategy, which in turn affects extinction and speciation.

Planktotrophic species have a relatively long pelagic phase which will have the ability to disperse over a wide geographic area in a single generation. Local catastrophies are unlikey to eliminate a species over its entire geographic range, and the larvae from other persistent populations are likely to replenish populations reduced by local extinctions. These effects will combine to produce geologically long lived species, and lineages characterised by low extinction rates. In contrast, species having nonplanktotrophic development will tend to have smaller more continuous geographic ranges. As a result of the more restricted geographic and often ecological range of such species, local catastrophies and random population fluctuations are more likely to result in extinction. Consequently, nonplanktotrophic species will tend to be short lived, and these lineages will be characterised by high extinction rates (Jablonski and Lutz, 1983).

Dispersal capabilty is also likely to affect the rates of speciation. Planktotrophs with wide ranging dispersal maintaining geneflow while nonplanktotrophs with little genetic communication between disjunct populations having a higher chance of speciation. A low disperal capabilty is also often reflected by geographic differentiation into morphological variants or subspecies. Consistent with these predictions are the recent immunolgical findings of Collins et al.(1988). Using immunological distances as taxonomic indicators they deduced that there were biochemical differences between six populations of T. retusa, including those from Loch Fyne and the Firth of Lorn.

6.2 GENERAL CONCLUSIONS.

The Recent articulate brachiopod Terebratulina retusa is dioecious. The onset of maturity occurs at shell length of approximately 5.5 mm. While there is no external sexual dimorphism apparent, the testes are white/cream in colour and ovaries are yellow/orange.

Two gonads occur as four palmate lobes, a pair in each valve and consist of a reticulate connective tissue band, the genital lamella.

Gametes are borne on the margins of the genital lamellae which are inserted into an extensive mantle sinus, the vascula genitalia. The vascula genitalia is an anterior extension of the coelom which opens posteriorly to the visceral cavity and to the exterior via metanephridia, the latter acting as gonoducts. Genital lamellae are fused along one margin to the inner mantle epithelium.

Both the inner and outer mantle epithelia are united by connective tissue pillars. The outer mantle epithelium was identified as a storage tissue. Temporal changes in the stored reserve, predominantly protein with variable amounts of glycogen and glycoprotein, were subjectively observed to reflect the pattern of the reproductive cycle.

Germ cells proliferate at the base of the genital lamella. As no transition stages between peritoneal cells and germ cells were observed, it is likely that gametes originate from a separate gonial cell line.

Oogenesis is devisible into six stages defined by key ultrastructural developments :-

Stage I. Oocytes are generally less than 3 μm in maximum diameter, contain a finely granular nucleus, and no granular cytoplasm. These cells are attached to the distal regions of the genital lamella by enveloping peritoneal cells, which form a follicular capsule.

Stage II. Oocytes display signs of vitellogenesis. The ooplasm includes lipid granules and free ribosomes. Nuclear chromatin is condensed. The follicular capsule is attached to the genital lamella.

Stage III. Ooctyes produce an accessory cell and the oolemma is thrown into simple digitate microvilli. Organelles proliferate. The follicular capsule is attached to the genital lamella.

Stage IV. Oocytes typically exceed 15 μm diameter. Accessory cells proliferate and form a variety of junctional complexes with other accessory cells and the vitellogenic oocyte. The oolemma elaborates. Both lipid and proteinaceous yolk granules become more abundant. The follicular capsule is attached to increasingly distal regions of the genital lamella.

Stage V. Oocytes exhibit distinctive elaboration of the oolemma and microvilli. The pseudolemma and perivitelline space are formed allowing the oolemma to produce endocytotic vesicles. Lipid and proteinaceous yolk granules continue to accumulate. Some proteinaceous granules migrate to the cortex of the vitellogenic cell. The follicular capsule remains attached to distal regions of the genital lamella.

Stage VI. Oocytes possess few accessory cells, are no longer attached to the genital lamella and exhibit an intensely plicate microvillar surface layer. A clearly defined band of cortical granules forms at the periphery of the oocyte. Yolk accumulation continues, with the mature oocyte measuring approximately 130 μm in diameter.

The vascula genitalia is eventually occluded by proliferating gametes. Immediately prior to spawning the follicular envelope is lost. Oocytes which are not released during spawning become atretic.

Post-spawned individuals contain two forms of amoebocyte. These

cells are attributed with a phagocytic role as they were observed to contain fragments of necrotic oocyte. In fresh specimens the phagocytic amoebocytes occur as red/orange pigmented droplets along the distal margin of the genital lamella.

Spermeo- and spermatogenesis follow a developmental sequence common to many animals. Spermatogonia proliferate to produce primary spermatocytes which undergo two meiotic divisions, resulting in four spermatids. Spermatids elaborate, producing a doughnut shaped mitochondrion, a spherical nucleus, an axially displaced acrosomal vesilcle and a tail anchoring fibre complex. Excess cytoplasm is lost and presumbly resorbed. Mature sperm are of the 'primitive type', the head measuring approximately 2·μm and the tail 50 μm.

Cells containing lipid granules intersperse the mass of developing cells within the testes and these cells may serve a nutritive role.

Stereological analysis clearly reveals the different reproductive cycles exhibited by two geographically separate populations of T. retusa. The Firth of Lorn population reproduces annually, spawning between late November and the end of January. At Kenmore point the population spawn repeatedly throughout the spring and summer, each succesive spawning event is of greater magnitude than its predecessor, culminating in the greatest spawning activity in late autumn.

By categorising the constituent cell fractions of the ovary and testes the pattern of gonad development can be mapped. In the Firth of Lorn a single generation of oocytes may require 18 months to aquire sufficient reserves to fuel the brief non-feeding, lecithotrophic larval stage.

The initiation of gametogenesis appears to be mediated by the mobilisation of reserves stored in the outer mantle epithelium during the winter. Primary productivity increases dramatically in the spring and both vitellogenesis and spermatogenesis show a corresponding increase. T. retusa from Loch Fyne successfully overwinter gametes and as a consequence are capable of spawning during the spring.

In females from the Firth of Lorn vitellogenesis proceeds from April onwards, corresponding to the time of the spring phytoplankton increase. Specimens from the Firth of Lorn exhibit a marked increase in gametogenic activity from August until spawning begins in late autumn.

Comparison of the total gonad volume to shell volume ratio (the gonad volume ratio) confirms significant differences between male and female T. retusa from Loch Fyne and the Firth of Lorn. Examining the sites individually reveals the males to have consistently larger gonads than the females.

The shell volumes of all those animals sampled were not significantly different between the sexes at both sites, or between sites, indicating no obvious external sexual or geographic dimorphism.

Comparison of the standard masses of late mature oocytes and spermatids/sperm is more relevant to analysis of potential reproductive output. Analysis of Variance indicates no significant differences in terms of volume or estimated mass of mature male and female gametes in the Firth of Lorn or Loch Fyne. There are, however, significant differences between sites.

Animals originating from the Loch Fyne population were found to be approximately twice as fecund as their counterparts from the Firth of Lorn during the largest of the spawning events. Loch Fyne specimens are also more productive, possibly spawning three times a year.

Productivity in Loch Fyne may be five times higher than in the Firth of Lorn. Trophic conditions are likely, at least in part, to be responsible for the disparity in reproductive output and reproductive cycle between the populations studied.

Temperature is considered to be a particularly 'noisy' environmental cue for the spawning behaviour of both brachiopod populations. There is, however, a clear annual change in water temperature which approximates to the cycle of development of the gonads.

Spawning behaviour can be stimulated in vitro in ripe specimens by

the introduction of stripped gametes into the water column, temperature shock and agitation. Unripe specimens can rarely be induced to spawn.

The evolution of the observed reproductive strategy i.e. a relatively high degree of investment in a small number of propagules, is considered to be a response to the constraints imposed by a low metabolic rate. Morphological and environmental constraints which result in non-feeding lecithotrophic or direct developement may be secondary adaptations in the Brachiopoda.

If low metabolic rate is the ultimate cause of the restricted modes of reproduction within the Articulata, the two would conspire to be a powerful evolutionary restriction, limiting the capability to disperse and mutate.

KEY TO ABREVIATIONS ON FIGURES AND PLATES.

A	Anchoring fibre complex
Ac	Accessory cell
Agr	Agranular endoplasmic reticulum
An	Anulus
Ao	Atretic oocyte
Az	Acrosome
Bm	Basement membrane
Ca	Caecum
Cg	Cortical granule
Ci	Cilium
Cr	Chromatin
Ct	Connective tissue
Ctp	Connective tissue pillar
Dj	Desmosome-like gap junction
Er	Endoplasmic reticulum
Ev	Endocytotic vesicle
Fc	Follicular cell
Fe	Follicular envelope
Gc	Golgi complex
Gg	Glycogen granule
Gl	Genital lamella
Gv	Golgi vesicle
Ger	Granular endoplasmic reticulum
Ie/Ime	Inner mantle epithelium
Lg	Lipid granule
Ly	Lysosome
Mc	Mucus cell
Mi	Microsome
Mtc	Mitochondrion
Mv	Microvillus
N	Nucleus
Ne	Nuclear envelope
Nep	Nephridiopore
No	Nucleolus
Np	Nucleopore
Ns	Nephrostome
Oe/Ome	Outer mantle epithelium
Ol	Oolemma
Ooc	Oocyte
Oog	Oogonia
Pd	Proteinaceous droplet
Pe	Peritoneal cell
Ph	Phagocytic cell
Pg	Proteinaceous granule
Pl	Pseudolemma
Ps	Primary spermatocyte
Pvs	Perivitelline space
S	Spicule
Sf	Shell fibres
Sg	Spermatogonia
Ss	Secondary spermatocyte
Spt	Spermatid
Sz	Spermatozoa
T	Tail
V/Ve	Vacuoles
Vg	Vascula genitalia
Vo	Vitellogenic oocyte

APPENDICES.

APPENDIX I

AUTOMATIC TISSUE PROCESSING PROGRAMME

Dehydration	50% Ethanol	2 hours
	80% Ethanol	1 hour
	8% Phenol Meths	1 hour
	8% Phenol Meths	2 hours
	8% Phenol Meths	1 hour
	100% Ethanol I	1 hour
	100% Ethanol II	1 hour
Clearing	Chloroform I	1 hour
	Chloroform II	1 hour
Impregnation	Wax Bath I	2 hours
	Wax Bath II	2 hours

POLYWAX with a melting point of 57^{o}C was used for impregnation and a Shandon Elliot machine was used to automatically process the tissue.

APPENDIX II

STAINING PROCEEDURE

De-wax	Hiclear I	10 minutes
	Hiclear II	2 minutes
Hydration	Absolute alcohol	30 seconds
	95% Ethanol	30 seconds
	70% Ethanol	30 seconds
	Distilled water	30 seconds
Stain	Harris Haematoxylin	5 minutes
Wash	Distilled water	
Blue	2% Pottasium acetate	2 seconds (as required)
Wash	Distilled water	
Dehydrate	70% Ethanol	30 seconds
	95% Ethanol	30 seconds
Counter Stain	Papanicolaou's stain OG6	3 minutes
	95% Ethanol	Wash
	Papanicolaou's stain EA50	3 minutes
Dehydration	95% Ethanol	30 seconds
	Absolute alcohol	30 seconds
	Xylene	2 minutes
Mount	DPX Resin	

Papanicolaou 1941.

APPENDIX III

GUTARALDEHYDE-OSMIUM FIXATION FOR ELECTRON MICROSCOPY

Primary fixation — 1 or 6 hours

2.5% Glutaraldehyde
0.2M Sodium cacodylate buffer (pH 7.25)
1.9% Sodium chloride

Washing — 2 x 15 minutes

0.2M Sodium cacodylate buffer (pH 7.25)
2.5% Sodium chloride

All the preceeding steps were carried out at 4°C. Specimens are stable at this stage in the fixation and can, if required be stored at 4°C for some months.

Post fixation — 1 hour

1% Osmium tetroxide
0.2M Sodium cacodylate buffer (pH 7.25)
2.5% Sodium chloride

Washing — Several changes of distilled water

Dorange (Pers.comm.).

APPENDIX IV

ENBLOC PREPARATION FOR TRANSMISSION ELECTRON MICROSCOPY FOR GLUTARALDEHYDE-OSMIUM FIXED MATERIAL

Wash		Distilled water	5 minutes
		Distilled water	5 minutes
Stain	0.5%	Uranyl acetate	30 minutes (in dark)
Dehydration	30%	Ethanol	10 minutes
	50%	Ethanol	10 minutes
	70%	Ethanol	5 minutes
Stain	1%	P-Phenylene diamine in	
	70%	Ethanol	30 minutes
Dehydration	70%	Ethanol	5 minutes
		Absolute alcohol	10 minutes
		Absolute alcohol	10 minutes
		Epoxy propane	5 minutes
		Epoxy propane	5 minutes
		Epoxy propane	5 minutes
Impregnation	1:1	Araldite resin/Epoxy propane mix in a rotator and in open vials to allow evaporation.	5 hours (or overnight)
Embedding		Embed in fresh araldite	
Polymerisation		In oven at $60^{o}C$	48 hours

ARALDITE RESIN

Hardner	DDSA	10ml
Resin	CY212	10ml
Accelorator	BDMA	0.4ml

Araldite resin was prepared and stored in syringes in a freezer. Syringes were packed in silica gel to avoid moisture contamination of the resin. Araldite was prepared up to 4 weeks prior to use.

APPENDIX V

STAINING PROCEEDURE FOR ARALDITE RESIN EMBEDDED MATERIAL

Stain	2%	Uranyl acetate in alcohol (exclude light)	10 minutes
Rinse	30%	Alcohol	
Wash		Immerse in three changes filtered distilled water	30 seconds
Rinse	0.02N	NaOH	
Stain		Lead citrate (Venadile 1965)	10 minutes
Rinse	0.02N	NaOH	
Wash		Immerse in three changes filtered distilled water	30 seconds
Drying		In air	

Sections were viewed with a CORINTH 500 TEM.

Photographic images were recorded on AGFA-GEVAERT 70 mm roll film.

APPENDIX VI

REPARATION OF OSMIFIED MATERIAL FOR SCANNING ELECTRON MICROSCOPY

Dehydration	30% Acetone	5 minutes
	50% Acetone	5 minutes
	70% Acetone	5 minutes
	90% Acetone	5 minutes
	100% Acetone	5 minutes
	100% Acetone (anhydrous)	5 minutes
	100% Acetone (anhydrous)	5 minutes

Critical point drying

Liquid Carbon dioxide 4 x 15 minutes

Coating

Coating was carried out in a Polaron coating unit

Coating material	Gold
Atmosphere	Argon
Voltage	750 V
Current	26 mA
Time	6 minutes

Mount Silver colloidal paint or double sided sticky tape. Mounted on aluminium stubs.

Specimens were viewed with a PHILIPS S.E.M. 500

Photograhic images were recorded on Ilford FP4 120 roll film.

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