146

STUDIES ON SOME INTRACELLULAR PARASITES OF THE MARINE BIVALVE, TELLINA TENUIS (DA COSTA)

Studies on a coccidian parasite of the ovary and a mycoplasma-like organism with an associated virus in the digestive gland of the marine bivalve, Tellina tenuis (da Costa)

J.S.Buchanan B.Sc. (Wales) M.I.Biol.

Unit of Aquatic Pathobiology

University of Stirling

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A B S T R A C T

STUDIES ON SOME INTRACELLULAR PARASITES OF THE MARINE BIVALVE *TELLINA TENUIS* (DA COSTA)

This study is divided into four sections beginning with a consideration of the effects of an hitherto undescribed coccidian parasite of the ovary of this bivalve on the dynamics of a particular population from Kames Bay, Millport, Isle of Cumbrae, Scotland. The parasite was found to bring about complete or partial castration of female *Tellina tenuis* but had no effect on males. The general biology of *Tellina tenuis* is reviewed and discussed in relation to observations that were carried out over one year on the age structure, growth, density, reproduction and degree of parasitization of this bivalve. The population parameters were found to have changed little over the last fifty years. There was not found to be any significant change in the condition index that could be related to the destruction of the gonad by the parasite. This is the first description of a coccidian parasite of the ovaries of any marine mollusc.

The second section describes the life cycle and developmental stages of the coccidian parasite. The parasite is believed to be monoxenous with sporogony and anisogamy occurring in the ovary of the host. Endogenous stages were observed in the primary germ cells of the gonadal follicles. A diagnosis is presented based on the number of sprozoites per sporocyst and sporocysts per oocyst. The name *Merocystis tellinovum* (*sp. nov.*) is proposed for this coccidian. The genus *Merocystis* belongs to the family Aggregatidae within the sub-order

Eimeriorina of the order Eucoccidiorida. An ultrastructural study of both sexual and asexual stages is presented in support of this diagnosis.

The third section of this study is an investigation of the observation that a large proportion of the Tellina tenuis examined histologically contained inclusion bodies within the secretory cells of the digestive gland. These inclusions were comprised of dense masses of pleomorphic mycoplasma-like organisms. The first part of this section describes the morphology and ultrastructure of these organisms and the histopathological effects on the host digestive gland. A description of the normal digestive gland is given including observations of the cyclic changes in appearance that take place in response to the influence of tidal rhythms. This is believed to be the first description of a mycoplasma-like organism from a marine invertebrate.

The fourth section is concerned with a series of experiments to determine the nature of a virus seen in association with the mycoplasma-like organism. The virus was isolated from the host cells by density gradient centrifugation and its morphology was compared with a second virus isolated by Hill (1975) through the medium of a fish cell culture. It was found that these two viruses were quite distinct from each other. Attempts were made to propagate both viruses in an established cell line from the Atlantic salmon and the results are described and discussed.

TABLE OF CONTENTS

SECTION TWO: Studies on a coccidian parasite of the ovary of Tellina tenuis for which the name Merocystis tellinovum (species nova) is proposed.

SECTION THREE: Light and electron microscopical observations of a mycoplasma-like organism infecting the secretory cells of the digestive gland of Tellina tenuis.

SECTION FOUR: Studies on two viruses isolated from *Tellina tenuis*.

SEE VOLUME TWO FOR LIST OF PLATES

ERRATA AND ADDENDA

Vol 1

1) Page 25 delete Haskin, 1954 and insert Hancock, 1965

2) Page 69 delete Small and Marszalek, 1969. Line 4

3) Page 100 delete Howard and Gourlay, 1974

4) Page 101 delete Hill, 1971

5) Page 143 delete Hill

6) Page 144 delete Howard and Gourlay, 1974

7) Page 147 delete McEwen C R, 1967 and substitute:

Lysaght A.M., 1941. The biology and trematode parasites of the gastropod Littorina neritoides L. J. mar. biol. Ass. U.K. 25: 481-488

8) Page 155 Add Sindermann C.J., 1975. Marine disease problems in the United States. Proc 3rd. U.S.-Japan meeting on Aquaculture. Publn. of Japan Sea Regional Fisheries Research Laboratory. pp 35-38

9) Page 172 Trager W., 1974. Some aspects of Intracellular parasitism. Science 183 pp 269-273

Vol. 2.

The captions to plates 39 and 40 should face plates 41 and 42 and vice versa.

GENERAL INTRODUCTION

Tellina tenuis (da Costa) is a common eulamellibranch bivalve mollusc found in huge numbers between the tide levels on most sheltered sandy beaches of the British Isles. Inter-tidal sandy beach communities dominated by Tellina species are common throughout the world and are characterized by low productivity and the long life, slow growth and late maturity of the constituent species.

Many studies have been carried out in Scotland during the last fifty years on the distribution, seasonal growth and population dynamics of Tellina tenuis as well as on the structure and adaptations of the animal to its habitat. These have shown the far reaching effects that the animals have on the physical, chemical and biological environment as well as suggesting general principles applicable to other marine bivalves and to benthic marine invertebrate populations as a whole. Their fundamental importance in the marine food chain as an important constituent of the diet of demersal fish has been well demonstrated.

Tellina tenuis, in common with all inter-tidal bivalves, carries a heavy load of bacterial, protozoon and metazoan symbionts. This aspect of the biology of Tellina has previously been neglected, so that it was decided to study this subject and to try to determine the importance of parasitism and disease as a factor controlling population numbers. The availability of baseline data that has been collected over a long period of time revealed the basic stability of the populations in the long term despite wide fluctuations in numbers and recruitment that take place from year to year.

SECTION ONE

SECTION ONE: Observations on the distribution, growth and reproduction of a population of Tellina tenuis at Kames Bay, Millport, with particular reference to the effects of a coccidian parasite of the ovaries.

1.1 Introduction

Tellina tenuis is the overwhelmingly dominant member of the sand-dwelling community of animals in the inter-tidal zone at Kames Bay, Millport. Many studies have been carried out on the distribution, growth, age structure and reproduction of this bivalve over the last fifty years and these have been facilitated by the accessibility and abundance of the population. Dramatic fluctuations in recruitment have been seen to take place from year to year and many ingenious theories have been erected to explain these oscillations including the possible effects of parasitism and disease. Following on the discovery of a coccidian parasite (to be described in detail in Section Two of the present study) of the ovary of Tellina tenuis it was decided to examine one population in detail in order to attempt an assessment of this parasite on the reproductive potential of the total population.

1.2 Review of the Literature

The bivalve mollusc Tellina tenuis (da Costa) belongs to the super-family of Tellinacea which belong to the order Heterodonta of the Lamellibranchia. The terminology of Morton and Yonge (1964) is adopted throughout the present study.

The super-family Tellinacea includes the families Semelidae, Donacidae, Asaphidae and Tellinidae. There are 15 genera in

the family of the Tellinidae and 7 of these belong to the genus Tellina (Yonge, 1949).

Tellina tenuis is the most common of the 7 species and it is the only inter-tidal representative. The remainder inhabit different grades of sandy substrate in the sub-littoral.

The Lamellibranchia can be found in all soft substrates of the seas and have been living there, virtually unchanged, since Paleozoic times. The shallow-burrowing 'eulamellibranch' forms have flourished since the Mesozoic era. Simpson (1953) noted that the mean age of living bivalve genera is a remarkable 78 million years and that the proportion of bivalve genera still extant is estimated at 139 out of the total of 423 known from the fossil record. This stability is reflected in the numbers of symbionts, commensals and parasites usually found in association with bivalve molluscs.

b) Structure and adaptations of Tellina tenuis

i) Feeding

The structure and adaptations of the Tellinidae are discussed in detail by Yonge (1949). He described them as deposit feeding Eulamellibranchia. This view has been challenged by subsequent authors such as Holmes (1950), Trevallion,(1967) and Pohlo (1968), who have shown that the 'eulamellibranchia' are suspension feeders as well as bottom feeders. Morton (1967) describes the Tellinacea as the most adept of burrowing bivalves which have become specialised for feeding on surface deposits. This he contrasts with the filter or suspension feeding habit of most shallow-burrowing lamellibranchs. Trevallion (1971) believes that Tellina tenuis is probably a suspension feeder as well as a deposit feeder as it is specialised for life in firm sand washed by the tide. This

habitat harbours little organic debris for deposit feeders to browse on. Observations of the gut contents during the course of the present study showed that it was packed with pennate and centric diatoms of the kind found in the plankton thus confirming the suspension feeding habit of Tellina

ii) Anatomy

The basic pattern of the anatomy of all bivalves is chracterized by the enclosure of the body within a two-piece shell with the consequent loss of the head together with the radula, buccal mass and associated glands (Morton and Yonge, 1964) The role of food catching has passed to the highly developed gills or ctenidia which entrap food particles in mucus rather in the manner of a sticky sieve (Tammes and Dral, 1955). The ctenidia are paired, each consisting of two plate-like demi-branchs hanging in the mantle cavity either side of the foot The demibranchs are composed of 2 flat lamellae covered in rows of cilia. The powerful beating action of the cilia coupled with phasic movements of the adductor muscles, pull a strong current of water through the inhalant siphon. The main sensory organs are to be found at the edge of the mantle. The mantle secretes the shell and two symmetrical mantle flaps enclose the whole body. The mantle cavity is relatively large in Tellina tenuis so that the siphons and foot can be accomodated when the valves are closed. Powerful posterior and anterior adductor muscles hold the valves tightly together Text figure 1 presents a generalized diagram of the anatomy of Tellina tenuis.

iii) External features

The valves of Tellina are ovoid and very flattened. An

average specimen is 15 mm long and 10 mm wide but only 3 mm thick. They are thin and smooth with a glossy external surface and are variously coloured white, pink, rose red and creamy yellow. A feature of the genus is the relatively large external ligament which is sufficiently strong that paired empty valves are often encountered extended flat on the surface of the sand. A study of the structure and method of deposition of the shell was made by Trueman (1942). The chief external features of Tellina to be seen when the animal is feeding are the very long mobile siphons and the narrow blade-like foot adapted to burrowing in compacted sand.

iv) Behaviour

The position of the animal at low tide has been observed to be horizontal with the right valve uppermost. When covered by the tide it is believed that the animal assumes an upright position (Yonge, 1949; Holme, 1950), and makes vertical migrations, coming nearer the surface at high tide. Holme describes the inhalant siphon as ranging over the surface of the sand, drawing in material lying on or just above the surface of the sand. This behaviour has not been observed in the present study except in the abnormal conditions of aquaria where oxygen concentrations fall to low levels in the bottom strata. Trevallion (1971) and Yonge (1949) have reported seeing siphons extending 5 cm above the sand surface. Trevallion has drawn attention to the predatory activities of young plaice which feed on the tips of the siphons of Tellina.

c) The Tellina community concept

Communities are societies of organisms with many interactions between the individuals (Tait, 1968). The concept of community in relation to tellinids has been discussed Jones

(1951) and Thorson (1955). Jones' (1951) description of north-east Atlantic area benthic communities defined the boreal shallow sand association as occurring on relatively exposed coasts in north-west Europe. The important species include Arenicola maritima, Tellina tenuis, Donax vittatus, Nephthys caeca and Bathyporeia pelagica. Jones described Petersen's (1918) Macoma community as a boreal, shallow mud association, common on the more sheltered coasts of north-west Europe, in estuaries and in the Baltic. The constituent species include Arenicola maritima, Macoma balthica, Mya arenaria, Cardium edule and Corophium volutator. Thorson (1957) identifies a number of 'iso-communities' inhabiting 'level-bottom' marine habitats, each of which has a characterizing species. He identifies 4 types of 'Tellina habitats'. These were defined as shallow water communities inhabiting exposed beaches from the tidal zone to a depth of 5-10 metres. The characterizing genera are Tellina, Donax and Ancilla. The substrate is pure, often hard, sand and the characterizing species seem to have a long life, slow growth and a rather late maturity; the standing crop thus comprises several generations. The Kames Bay Tellina community, defined by Stephen (1938), fits Thorson's description of a Boreal Lusitanian (i.e. North-Eastern Atlantic and North Sea) Tellina community. The distribution of this type is as follows:

Danish North Sea Coast

German North Sea Coast off the mouth of the river Elbe and off the E. Friesian Islands (Hagmeier, 1930; Caspers, 1952)

Scottish West Coast and North Sea Coast

Rio de Faro sand flats, Portugal (includes Tellina incarnata and Aequipecten bispinosus)

Other Tellina communities are (1) the T. distorta/T. donacina community of the Mediterranean (Vatova, 1934, 35, 36, 49, cited in Thorson (1957); (2) the T. tenera community of the New England coast and the inshore part of the Texas coast (Hedgpeth, 1954); and (3) the T. lilacina community from New Zealand (Oliver, 1923). Other Tellina communities exist in other parts of the world, for example in Senegal, but no details are available. The global distribution of Tellina species underlines the relevance of selecting this genus for study since it seems likely that principles learned from the intensive study of one population may be applicable to similar far flung communities.

Thorson (1950) places T. tenuis with Cardium edule and Cummingia tellinoides and the sea urchin Echinus esculentus as having a four year life cycle and quotes Stephen (1932) as showing that even for the four year species such as T. tenuis there is considered to be heavy mortality after breeding. Subsequent work and observations made during the present study have shown that there are in fact at least eight year groups in most T. tenuis populations. Stephen's observations on heavy mortalities post spawning are discussed in 1.4.b of this study.

d) The population ecology of Tellina, age structure, growth and density

The population ecology of tellinids has been the subject of considerable investigation over the last fifty years. Studies by Ansell and Trevallion (1968, 1972) on the seasonal growth and biochemical cycle of Tellina tenuis and Donax vittatus defined certain characteristics of the seasonal activities of bivalves from shallow water. In winter the animals are

inactive and gametogenesis proceeds slowly at the expense of food reserves until the first spring blooms of phytoplankton. At this time growth recommences, food reserves are renewed and the gonadal tubules rapidly proliferate until the temperature rises above a certain minimum so initiating spawning. Somatic growth, spawning and gonad renewal continue throughout the summer in response to available food, tide and weather conditions. Ansell and Trevallion (1967) recorded a marked drop in carbohydrate levels post-spawning in Donax but not in Tellina. The relationship between glycogen levels and gametogenesis has been discussed by Gabbott (1975).

In population studies of Tellina tenuis at Kames Bay, Millport, spanning nearly twenty years, Stephen (1928, 1929a and b, 1930, 1931, 1932, 1933, 1938 and 1953) estimated the rate of growth and the number of year groups by measurement of shell length rather than by counting annual rings. He observed the seasonal state of the reproductive organs and made a detailed study of the density and size distribution at fixed points extending well into the sub-littoral. Barnett (1969 and 1971) analysed the length frequency data of the populations at Kames Bay and Hunterston Sands before and after the nuclear powered electricity generating station at Hunterston became operational. He divided the population up into year groups using the probability paper method of Harding (1949). The mean lengths of the year groups thus obtained were used in the construction of a Ford-Walford plot (Hancock, 1965) to show up the differences between the two populations. The Hunterston population in the years following the onset of the influence of thermal effluent from the power station showed higher growth rates than pre-effluent years especially in the younger animals. Barnett showed that shell height increased in proportion to the cube root of shell weight. Hunterston shells were found

to be significantly lighter than those from Millport for a given flesh weight. Thus warm water definitely affected the growth rate of Tellina. Trevallion and Ansell (1967) quoting A D McIntyre state that shell length frequency modes on a Ford-Walford plot for Tellina at Firemore, Loch Ewe, were in agreement with the figures of Barnett for Kames Bay.

Stephen (1938) recorded a density of 7,388 m^2 at Kames Bay at one point. Densities of 4,000 m^2 were regularly recorded by Clark and Milne (1955) in a survey of inter-tidal fauna of several Scottish sea lochs. McIntyre and Elefetheriou (1968) recorded densities of 624 m^2 at Firemore, Loch Ewe, and 3,750 m^2 at Kames Bay.

e) Parasitic castration in molluscs

The presence of certain helminth parasites has been known to eliminate or reduce the production of eggs by molluscs. A review of this aspect of parasite induced pathology is given in Malek and Cheng (1973). Most of the work that has been done in this field relates to the effects of larval trematodes on snails. If the larval trematodes are rediae then the host's gonadal tissues are totally or partially destroyed as a result of direct ingestion. Studies by Hurst (1927), Rees (1936), Malek (1952), Coelho (1954), Pan (1965), Etges and Gresso (1965) and Sturrock (1967) showed that the number of eggs produced by the hermaphrodite snail hosts of human infesting schistosomes and other trematodes is reduced. The presence of dendritic sporocysts of Bucephalus spp. in the gonads of the American oyster, Crassostrea virginica results in a decrease in the number of eggs produced (Galtstoff, 1964, Cheng and Burton, 1965). Malek and Cheng (1973) define parasitic castration as the total or partial elimination of gamete formation in hosts by parasites. This can be the result of two processes, one

mechanical and the other physiological. Parasitic castration has been suggested as a method to control certain species of undesirable molluscs (Cooley, 1962, and Cheng, 1963).

Wesenburg-Lund (1934) was the first to have reported that molluscs parasitized by larval trematodes become abnormally large. A review of this phenomenon, sometimes called gigantism, is given by Cheng (1967). He also throws some doubt as to whether enhanced growth involving soft tissues actually does occur (Cheng, 1971).

Sex reversal is another phenomenon associated with parasitic castration. Malek and Cheng (1973) reviewed the literature relating to sex reversal and found the evidence inconclusive. They believed that the only conclusion that can be drawn is that the development of secondary sex characteristics in molluscs is governed by gonadal hormones and if the gonads are destroyed there may be a reduction in the size of the external genitalia.

The enhanced growth of parasitized snails described by Pan (1965) has been questioned by Cheng (1971) who found that greater amounts of calcium were deposited in the shells of parasitized snails and that there was no increase in soft tissue weight.

The effect on shell morphology has been studied by Sturrock and Sturrock (1970, 1971). No comments relating to shell distortion in parasitized pelecypod molluscs could be found.

Almost all the information relating to the effect on longevity relate to species harbouring schistosomes. In all cases the longevity was reduced (Pan, 1965).

1.3 Materials and Methods

a) Description of the habitat.

Kames Bay (Grid reference 549171) is a small sandy bay facing south and sheltered from the east and west by rocky prominences. The area of the sand at low tide is approximately 11 acres (Watkin, 1942). The slope of the beach is remarkably constant and the surface is normally flat apart from some build up of sand towards High Water Springs. The nature of the sand is also constant except around boulders where accumulations of finer, more muddy sediment and organic debris collect. These large boulders provide useful marks for position fixing on the otherwise featureless expanse of sand. The surface of the beach below the level of High Water Neap tides is usually wet and brackish (Smith, 1955), at low tide owing to the upwelling of ground water at the head of the beach. This appears to have a significant effect on the distribution of T. tenuis.

b) Method of sampling

Four stations were chosen corresponding with Mean High Water Neap tide level (MHWN), Mean Tide level (MTL), Mean Low Water Neap tide level (MLWN) and Mean Low Water Springs tide level (MLWS). A transect line of sight taking the south end of Little Cumbrae Island with Holy Island off Arran and Butcher's Rock (a prominent boulder corresponding with MLWN) (Plate 4) was adopted. Samples were taken 10 metres either side of this line. This transect corresponds with that adopted by Elmhirst (1931), Stephen (1928) and Watkin (1942). At each of the stations a galvanised iron square of sides 25 mm was pushed into the sand and the resulting block excavated and sieved through a 2 mm sieve. The bivalves were transferred to labelled Kilner jars containing sea water and transported back to the laboratory.

Considerable difficulty was experienced in taking an accurate sample at the early stages of the survey as the amount of surface water seeping into the hole caused by the excavation would entrain Tellina from the surrounding area thus giving artificially high counts. The method used by Watkin (1942) of digging drainage channels and pools around the sample area to carry off surface water was adopted during this survey. Under ideal circumstances samples should have been taken from a minimum 1/10th cubic metre with transportation of the cube to the laboratory for careful sieving through a 0.5 mm sieve. The unit of 1/16th metre was adopted for expediency since the survey was carried out single-handed but it was found that the large numbers of Tellina captured coupled with the uniform conditions encountered justified using the smaller unit. Watkin (1942) and Stephen (1928) employed a unit of 1 square foot which they then multiplied by a factor of 10.7638 to give them numbers per square·metre.

c) Sub-sampling

The high densities of 2,000+ Tellina per square metre encountered below MTL necessitated sub-sampling. The method adopted was to number each individual from each sample by laying the entire catch for each month on a glass sheet overlying squared paper using one numbered square per individual. A table of random numbers (Fisher and Yates, 1963) was consulted so that a sub-sample of 30 individuals could be selected from each group.

d) Processing of samples

In the laboratory individuals were first measured using a micrometer caliper gauge and then weighed. The first large sample taken was measured for length, width and thickness of shell and these measurements correlated with whole weight, wet

flesh weight, dry flesh weight and shell weight to find the best correlation coefficient. In measuring wet flesh weight, the soft tissues were removed, blotted once each side on filter paper and weighed on aluminium foil. The tissues were then dried in an oven at 85°C for three weeks and re-weighed. Shell weight was measured by carefully removing all soft tissues and air drying before weighing. At the time of removal of tissues from the shell the animals were examined under a dissecting microscope for sex and the presence of coccidia in the ovary and scaled as follows:

1. no parasites
2. lightly parasitized (25% parasites; 75% eggs)
3. medium parasitization (50% parasites; 50% eggs)
4. heavy parasitization (75% parasites; 25% eggs)
5. totally parasitized (no eggs).

The sex and degree of maturity of the individual and an estimate of the number of digenean metacercaria in the mantle cavity were also recorded.

e) Methods of determining condition

The collected data were recorded (cf. appendix, pages 163-182) and transferred to punched cards for computer processing. After transforming to natural base logarithms the regression of wet flesh weight on length was calculated for each sampling position on each date for the months of April, July, October, December 1974 and January 1975. Pooling of all the data gave a common regression equation of:

$$\log W = 3.278 \log L + 1.543$$

where W = wet flesh weight in mg and L = length in mm.

In order to determine the condition of both parasitized and non-parasitized individuals a length of 15 mm was taken as

representing a standard animal and the regression constant for each group applied (Table 2).

f) Method of determining age structure

The age composition of the population was determined by the method described by Harding (1949) and modified by Cassie (1971) for separating a polymodal length frequency histogram into a series of constituent normal distributions by the use of probability paper. It was not possible to distinguish clearly growth rings in Tellina.

g) Method of determining growth rate

Growth rates were estimated by following monthly changes in the position of the modes of the length frequency histograms constructed from samples taken at the beginning, middle and end of the growing season (Figures 2-5).

1.4 Observations and Results

a) Age and growth rate estimates at different levels on the beach

Haskin (1954) has reviewed methods of measuring growth rates in bivalves. There are three basic methods:

1. measuring the distance between successive winter rings;
2. marking measured animals and re-measuring at frequent intervals;
3. following the shift in the size frequency peaks corresponding to different age groups per unit time.

The last method has been employed in the present study owing to the impossibility of measuring growth rings and the difficulties involved in mark and recapture experiments.

Percentage length frequency histograms are presented on pages 31-34, (Figures, 2,3,4 & 5). These have been plotted for the

months of April, July, October 1974 and January 1975 for the sampling stations corresponding to the levels of MHWN, MTL, MLWN and MLWS. A smoothed length frequency histogram is given for December 1974 at the Mean Tide level only (Figure 3). These histograms illustrate general trends in the growth rate of Tellina at different levels on the beach.

At MLWS in April (the start of the growing season) modes at 10, 12 and 14 mm are marked. No Tellina were taken at this station below 7 mm in length. By July the 10 mm peak had moved to 12 mm and the 12 mm to 14 mm representing a net increase in length of 2 mm for both these age groups in the first half of the summer. The October sample at MLWS shows the emergence of a mode at 5 mm and the January histogram shows modes at 6, 9, 11, 13 and 17 mm. These are believed to correspond to 2+, 3+, 4+, 5+ and 7+ year groups and thus match the major peaks of 5, 9, 12 and 14 mm measured at Firemore Bay, Loch Ewe (Trevallion and Ansell, 1967) in April. It appears that a 6+ year group is missing from the population at Kames Bay.

At the next higher sampling site, corresponding with MLWN, a greater growth rate is found. Marked peaks at 11.5, 15.5 and 18 in April become peaks at 12, 14, 16 and 18 by July. It is believed that the modes have shifted from 9 to 12 mm and from 11.5 to 14 representing a net increase in length of 3 mm and 2.5 mm for the 3+ and 4+ year groups respectively.

The October histograms for this site (MLWN) mirror those of MLWS by the appearance of a mode representing the first appearance of the previous year's spat in the samples.

Growth rates at MTL can be estimated from well marked modes at 8, 10, 12, 15 and 18 in April. By the end of the 6 month

growing season these modes had become 7, 11, 13, 15 and 17 indicating that growth of the year groups was as follows:

Mode April (mm)	Mode October (mm)	Year of origin	Year group	Increment
3	7	1972	1+	4 mm
8	11	1971	2+	3 mm
10	13	1970	3+	3 mm
12	15	1969	4+	3 mm
15	17	1968	5+	2 mm
-	-	1967	6+	-
18	-	1966	7+	-

Figure 6 represents a length frequency diagram for a sample plotted on probability paper using the method of Harding (1949) modified by Cassie (1950). This is a useful method for the analysis of polymodal frequency distributions. The large sample collected in December 1974 was used in the construction of this plot.

The following modes were found:

Mode	Standard deviation	Year of origin	Year Group
5	-	1973	1+
8	1.1	1972	2+
11.3	1.0	1971	3+
12.7	1.0	1970	4+
-	-	1969	5+
16.0	1.0	1968	6+
17.7	1.0	1967	7+

The 1969 year group appears to be missing from the sample. The method of sampling failed to capture the spatfall from the summer of 1974. These would be expected to appear as a mode of 1-2 mm in December. Subsequent sampling in 1975 and 1976 showed that spatfall had indeed taken place in the summer of 1974 and appeared to be average.

From the modes of the histograms (Figures 2 to 5) and an analysis of the Harding Plot for December the greatest difference in growth rate appeared to be between Mean Tide level and Mean Low Water Springs. The modes in October 1974 were as follows:

Year of origin:	1+	2+	3+	4+	5+	6+	7+	
MWLS	-	6	9	11	13	and	17	mm (October)
MTL	-	7	11	13	15	and	17	mm (October)
MTL	5	8	11.3	12.7	16		17.7	(December, Harding Plot)

At first sight this appeared surprising because the population at MLWS has more time available for feeding as they are covered by the tide for longer periods. Against this, the mean density (Table 1) was found to be 928 individuals per square metre at MTL as against 1,854 per square metre at MLWS so that inter-specific competition for available food would be halved.

b) The effects of parasitic castration by the coccidian on the condition index of parasitized and non-parasitized females relative to males

In view of the fact that parasitic castration has been shown to cause gigantism in snails parasitized by trematodes it was decided to assess whether or not the presence of the parasite at levels of 75% parasites to 25% eggs up to 100% parasitization (i.e. heavily parasitized) were significantly different in weight of shell, weight of wet flesh and weight of dried flesh than females with under 25% parasites and males.

The method of Ansell and Trevallion and Ansell and Lander (1967) was adopted and the regression of the logarithm of total weight on the logarithm of length was calculated for each sample and the weight corresponding to an animal of 15 mm in length calculated from the appropriate equation. Values for the

constants of each regression for the months of April, July, October, December and January are given in Table 2 . These months were chosen as they correspond with the beginning of the growing season, the peak spawning period, the end of the growing season and the over-wintering period.

The figures of wet weight and whole weight are approximately 25% higher than Ansell and Trevallion's (1967) figures for Tellina from Kames Bay. The seasonal fluctuations follow the same pattern. The total weight was calculated from combined shell weights and wet flesh weights. Wet flesh weight reached a maximum in July and fell by 37% to a minimum in October post-spawning. No significant difference could be detected between parasitized and non-parasitized females and males. Ansell and Trevallion calculated the wet weight of soft tissues for males and females and found females to be slightly heavier but no statistical significance was attached to this. This was not reflected in dry flesh weight.

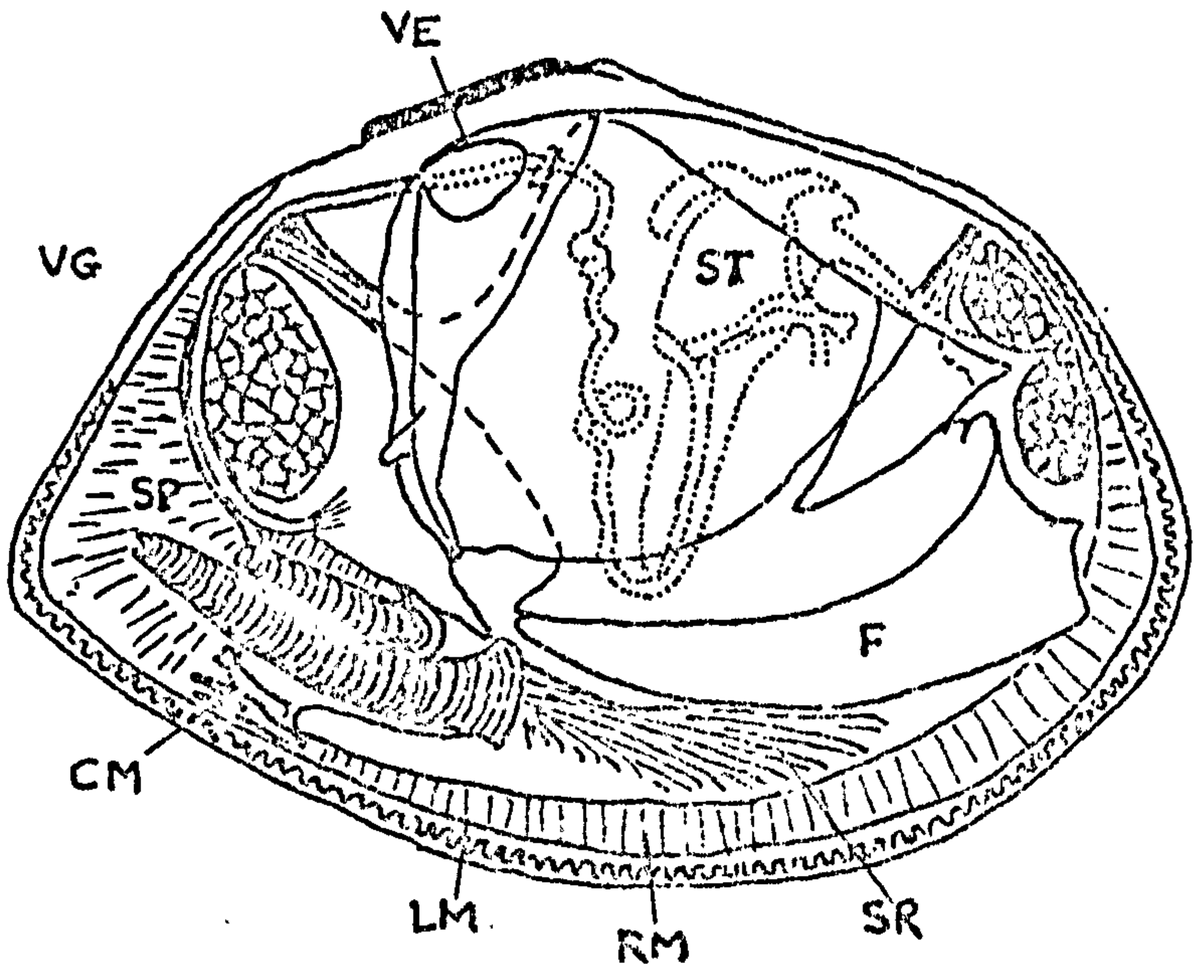
It must be concluded that the presence of the coccidian does not significantly affect growth or body weight in Tellina.

c) Reproduction in Tellina tenuis

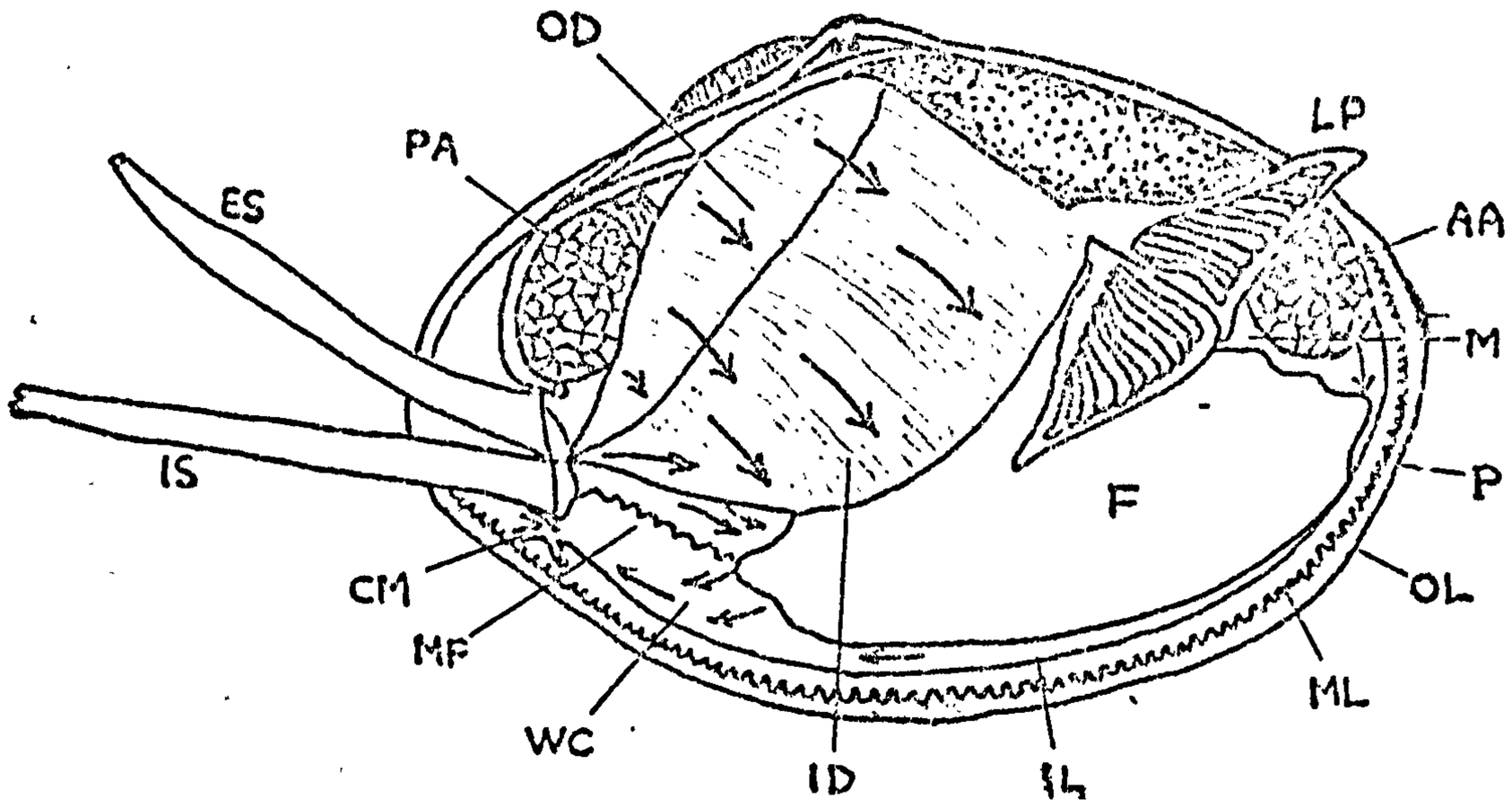
i) Anatomy

The reproductive system of Tellina tenuis observed by monthly histological examination consisted of a mass of tubules (Plate 6) covering the digestive gland which, according to their state of development, ramified outwards and downwards from the region of the heart. The sexes could be distinguished by the colour of the gonad in fertile specimens. The testis appeared as a compact yellow-white multilobed structure on either side of the visceral mass, almost covering the dark brown digestive gland.

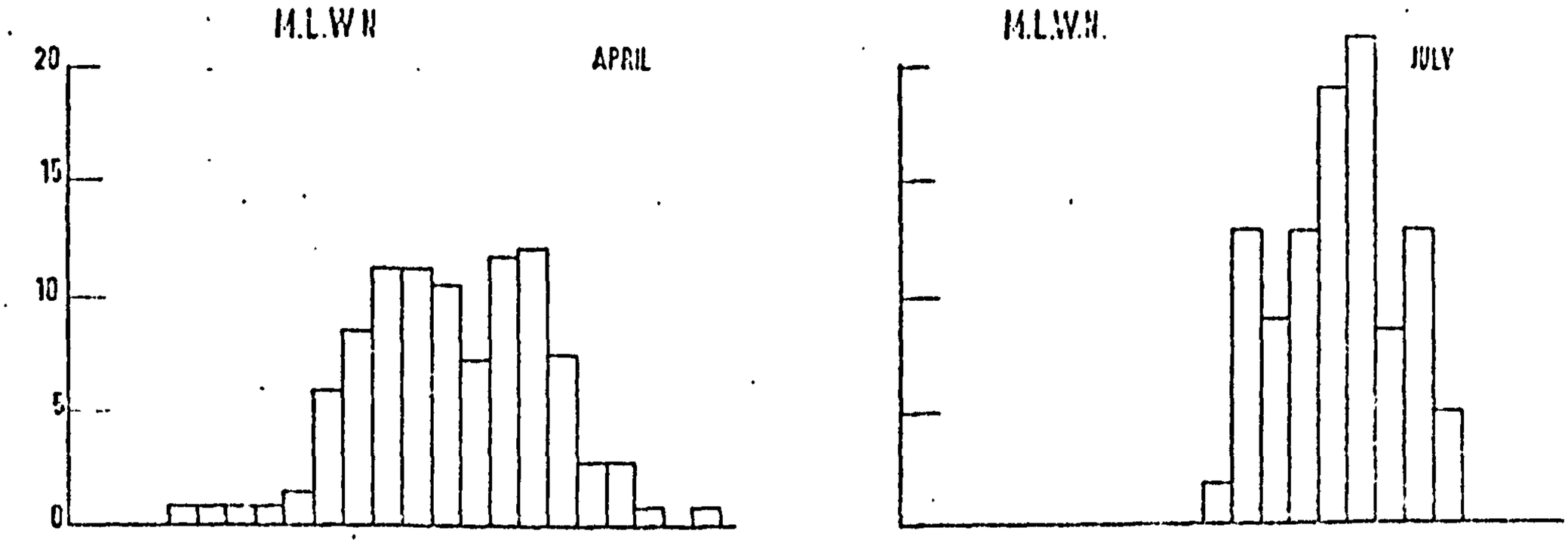
TEXT FIGURE 1



Tellina tenuis, drawing of a cleared and stained specimen (x 5) (after Yonge, 1949). LM, RM, longitudinal and radial muscles of the mantle edge; SR, siphonal retractor; ST, stomach; VE, ventricle VG; visceral ganglion. Alimentary canal indicated by dotted lines.

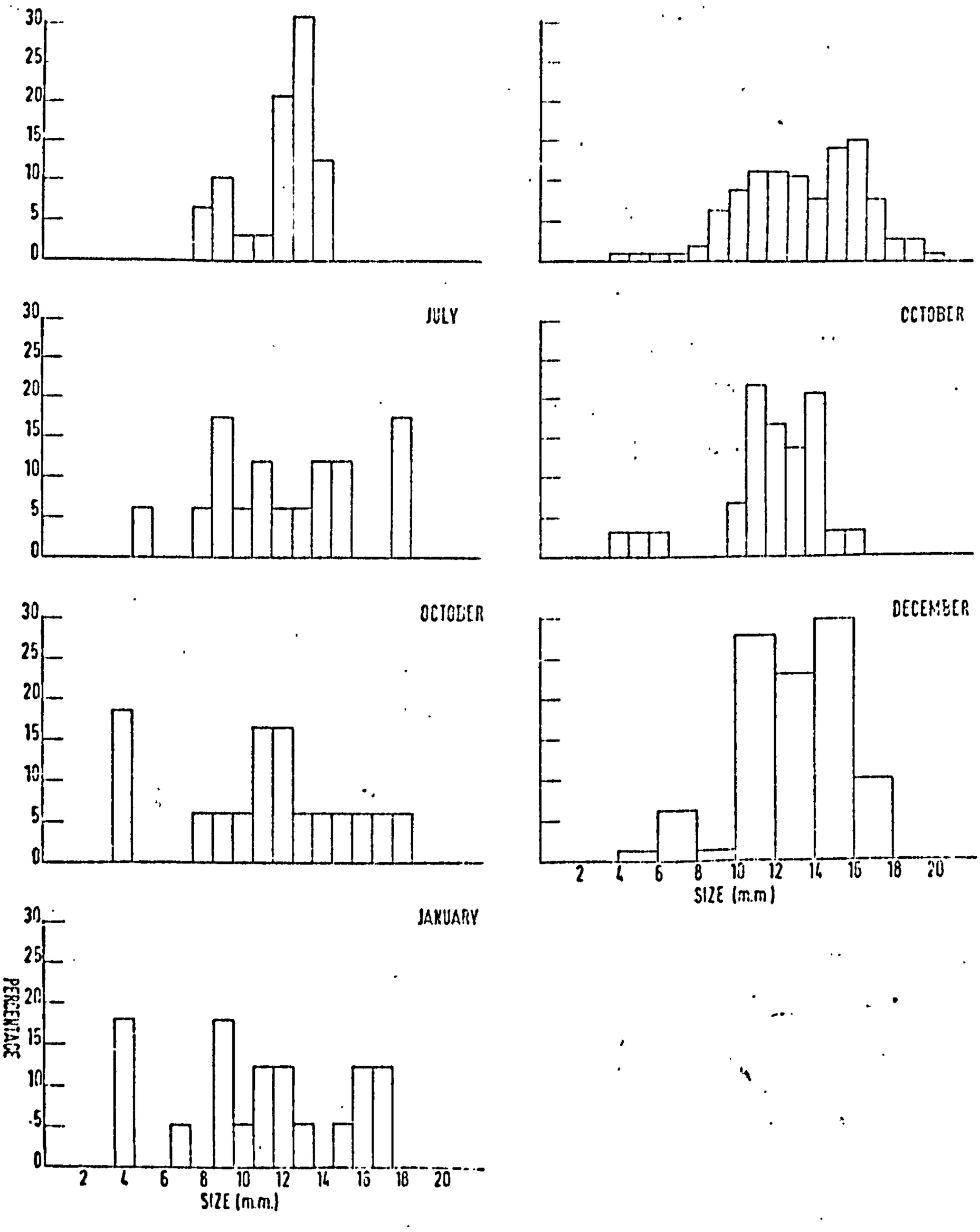


Right shell valve and mantle lobe removed. AA, anterior adductor; CM, cruciform muscle; ES, exhalant siphon; F, foot; ID, inner demibranch; IL, inner lobe of mantle edge; IS, inhalant siphon; LP, labial palp; M, mouth; MF, mantle fold; ML, middle lobe of mantle edge, OD; outer demibranch; OL, outer lobe; P, periostracum; PA, posterior adductor; WC, waste canal. Arrows indicate the direction of ciliary currents.



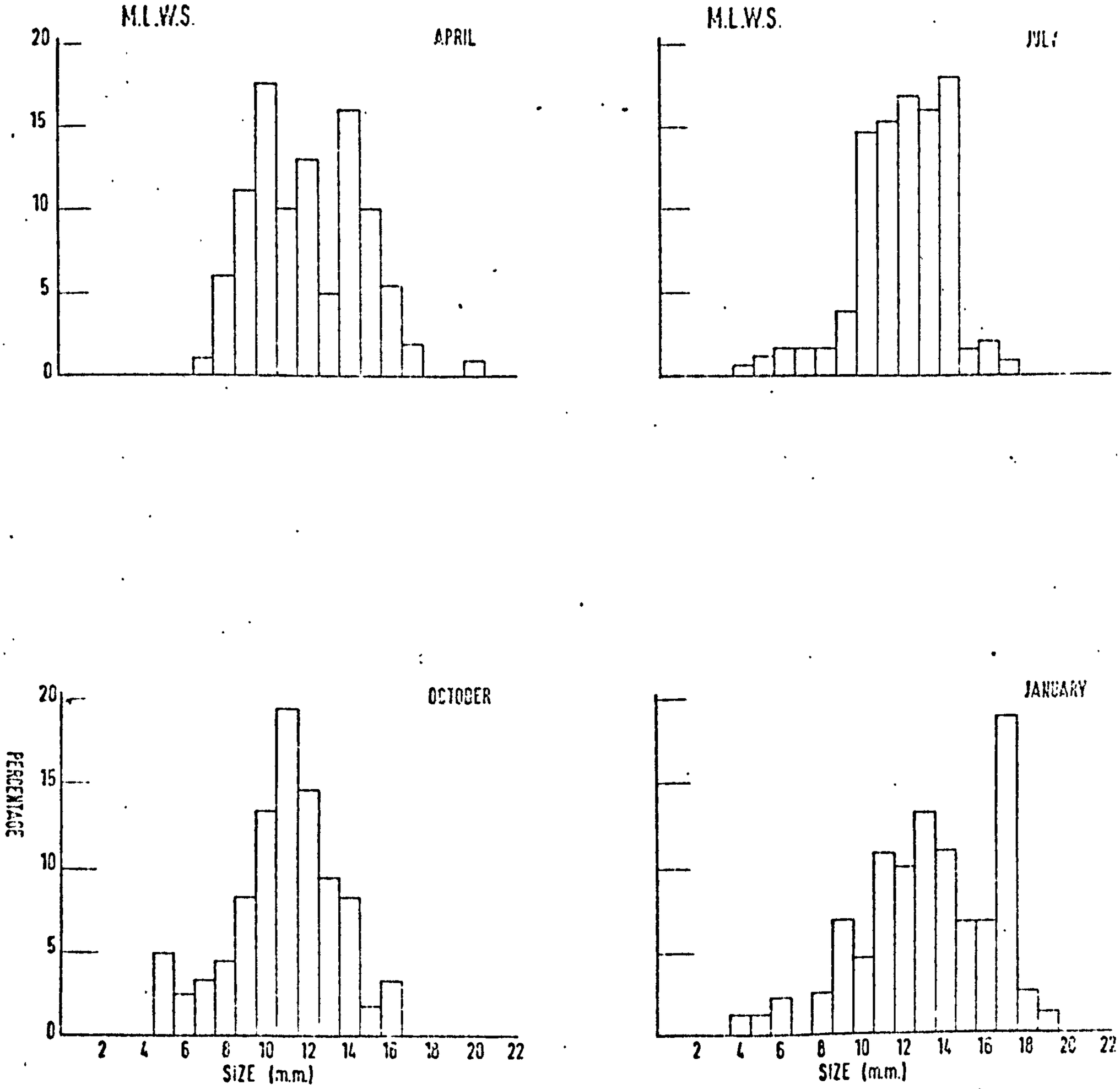
PERCENTAGE SIZE FREQUENCY DISTRIBUTION AT M.L.W.N. OF TELLINA TENUIS AT KAMES BAY, MILLPORT IN 1974.

FIGURE TWO



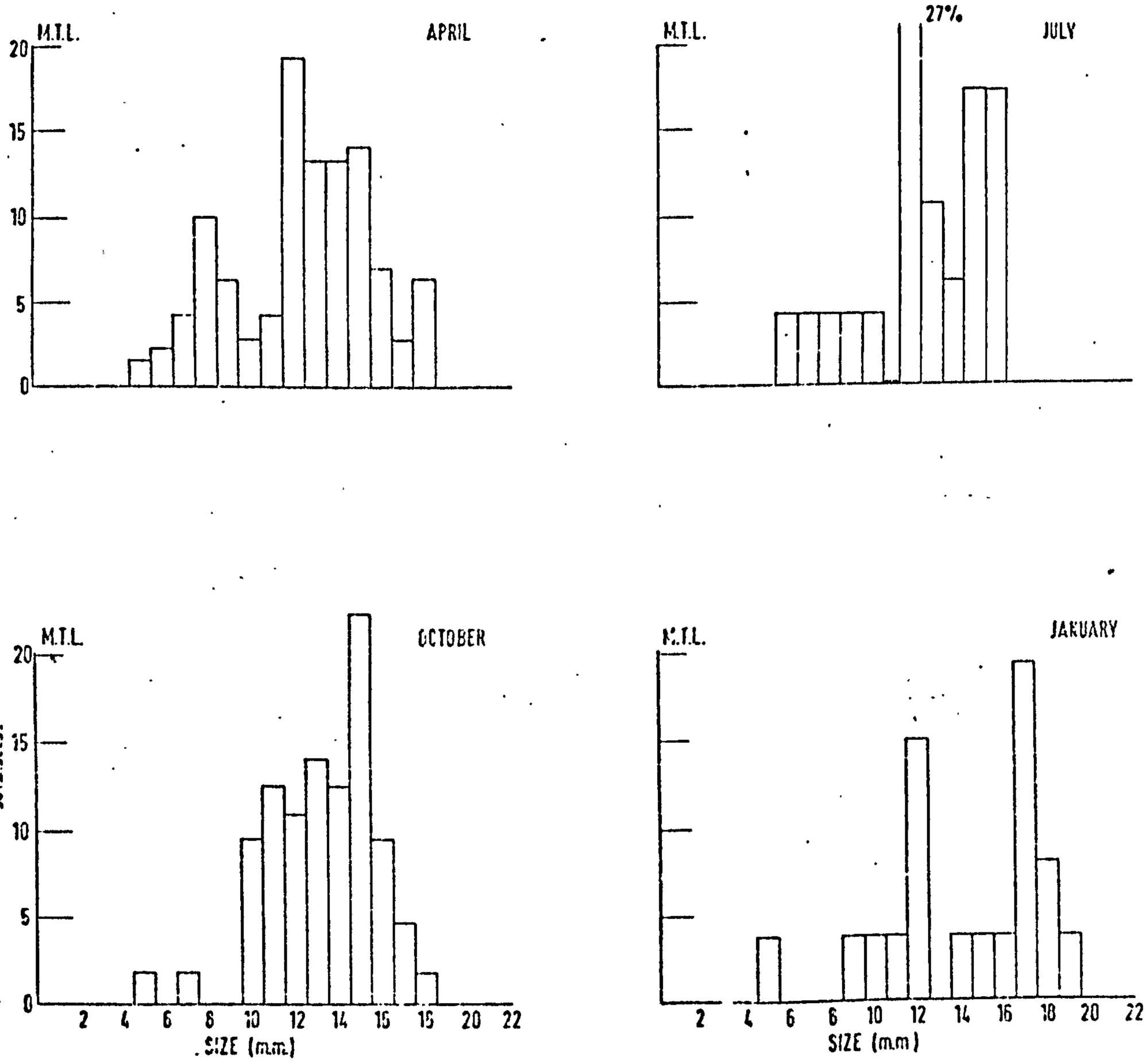
PERCENTAGE SIZE FREQUENCY DISTRIBUTION AT M H.W N AND M L W N OF TELLINA TENUIS AT KAMES BAY, MILLPORT IN 1974

FIGURE THREE



PERCENTAGE SIZE FREQUENCY DISTIBUTION AT M.L.W.S. OF TELLINA TENUIS AT KAMES BAY, MILLPORT IN 1974.

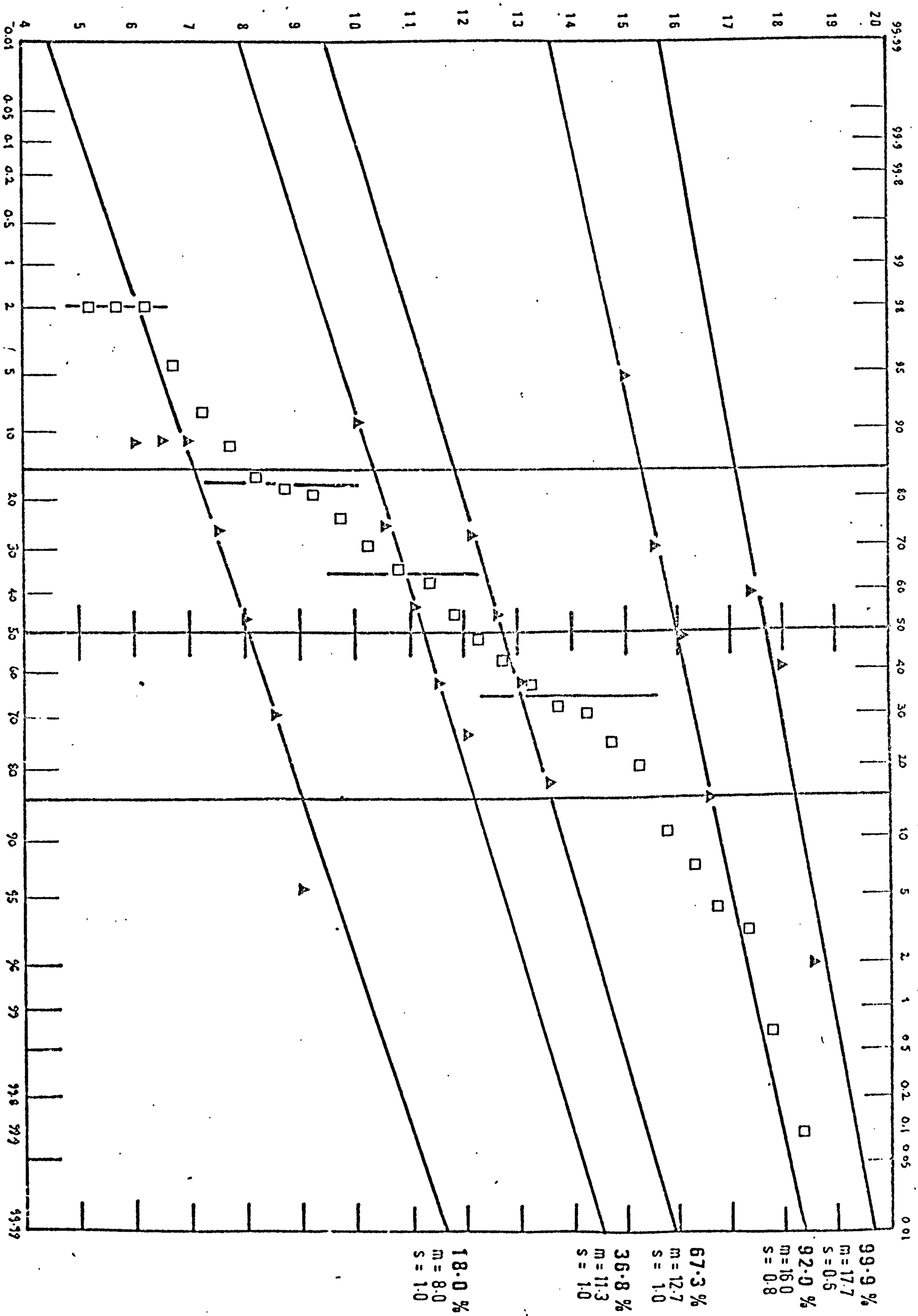
FIGURE FOUR



PERCENTAGE SIZE FREQUENCY DISTRIBUTION AT M.T.L. OF TELLINA TENUIS AT KAMES BAY, MILLPORT IN 1974.

FIGURE FIVE

Cumulative Length-frequency Plot (after Harding, 1949)



Text Fig. 6. Tellina tenuis survey, December, 1974. Kames Bay.

Kames Bay Tellina tenuis survey 1974-1975

Table 1: Densities per square metre (Stephen's equivalent in brackets for 1926-27) based on samples 1/16th M^2 (against Stephen's 1 sq. foot samples).

	MHWN	MTL	MLWN	MLWS
April	496(398)	2,352(807)	2,464(1,506)	2,096(2,679)
May	416(-)	2,080(-)	2,080(-)	1,696(-)
June	104(452)	912(861)	1,584(1,980)	2,592(1,979)
July	272(-)	384(-)	2,048(-)	880(-)
August	80(538)	464(1,097)	1,952(2,313)	1,088(2,238)
September	336(355)	1,185(548)	2,288(1,269)	1,056(2,206)
October	368(258)	1,072(828)	624(1,392)	2,992(2,055)
November	160(-)	560(-)	1,392(-)	2,304(-)
December	- (-)	708(-)	- (-)	- (-)
January	352(495)	400(592)	928(1,700)	1,920(-)
February	400(-)	80(-)	992(-)	1,680(-)
March	No sample	944(-)	2,800(-)	2,096(-)
Means	298 416	928 788	1,741 1,695	1,854 2,231
Standard Deviation	±140 ±101	±683 ±199	±693 ±394	±663 ±272

TABLE ONE

Table 2:

Seasonal changes in the weight of Tellina tenuis from Kames Bay, Millport. The total weight of the standard animal was calculated from the regression of the logarithm of total weight on the logarithm of length; values for the constants M and C in the regression equation log weight (mg) = M log length (mm) + C are given, W = weight in mg.

Date and No. in sample	Length/Whole weight	Length/Wet weight
8 April Parasitized (16)	Y = 3.462X + 1.846 W = 427.4	Y = 3.422X + 1.463 W = 174.9 mg
8 April Non-parasitized (25)	Y = 3.228X + 1.879 W = 429.9	Y = 3.039X + 1.541 W = 185.8 mg
9 July Parasitized (4)	Y = 3.849X + 1.768 W = 397.0	Y = 3.540X + 1.543 W = 217.5 mg
9 July Non-parasitized (25)	Y = 3.292X + 1.857 W = 416.8	Y = 3.632X + 1.532 W = 217.6 mg
16 October Parasitized (7)	Y = 3.030X + 1.841 W = 369.7	Y = 3.044X + 1.406 W = 136.4 mg
16 October Non-parasitized (14)	Y = 3.113X + 1.853 W = 390.6	Y = 3.216X + 1.428 W = 151.6 mg
23 December Parasitized (28)	Y = 3.038X + 1.874 W = 400.0	Y = 3.193X + 1.445 W = 156.6 mg
23 December Non-parasitized (27)	Y = 3.095X + 1.737 W = 433.7	Y = 2.812X + 1.576 W = 186.4 mg
24 January Parasitized (17)	Y = 3.032X + 1.901 W = 424.8	Y = 3.194X + 1.477 W = 168.5 mg
24 January Non-parasitized (10)	Y = 3.477X + 1.824 W = 427.3	Y = 3.503X + 1.418 W = 161.4 mg

Kames Bay Tellina tenuis survey 1974-1975

Table 3: Percentage of each sex measured at five times in the year including the percentage of females parasitized with coccidia assessed as either light, medium or heavy.

	April	July	October	December	January
Total number sampled	85	85	90	177	85
% Light par.	30.0	7	63.3	37.30	22.5
% Medium par.	42.2	18	9.1	35.82	35.0
% Heavy par.	35.4	75	27.27	26.86	42.5
% Total Females	41.88	46.5	38.8	44.3	33.75
% Total Males	46.74	44.5	15.3	20.53	30.50
% Total Neuter	11.38	9.0	45.9	35.17	35.75
% Females (corr.)	51.62	47.57	61.75*	61.88*	51.62
% Males (corr.)	48.37	52.43	38.25	31.12	48.37

Note:

The corrected ratio of males to females is based on the assumption that half of the neuters will differentiate to become male and half female.

* The high proportion of females relative to males in October and December was due to an artificially high percentage of males being classified as neuter owing to the cryptic nature of the male gonad after spawning.

TABLE THREE

Table 4: Seasonal changes in weight of standard length (140-160 mm) Tellina both parasitized and non-parasitized (mean values)

	Length (mm)	Total wt. (mg)	Shell wt. (mg)	Wet wt. (mg)	Dry wt. (mg)	% Total wt. shell	wet	dry
8 April 1974								
parasitized	155.0	407	195	162	2.05	47.91	39.80	5.04
non-parasitized	147.0	322	154	121	1.70	47.82	37.58	5.27
9 July 1974								
parasitized	150	356	155	175	3.70	43.54	49.16	10.39
non-parasitized	150	309	129	156	3.40	41.74	50.48	11.00
10 October 1974								
parasitized	149	326	175	104	-	53.68	31.90	-
non-parasitized	152	315	171	124	-	54.28	39.36	-
23 December 1974								
parasitized	148.5	298	129.6	80.72	1.50	43.48	27.08	5.03
non-parasitized	148.9	307	147.64	91.58	1.118	48.09	29.83	3.64
January 1975								
parasitized	150	310	150	127	-	48.39	40.90	-
non-parasitized	150	301	146	102	-	48.50	33.88	-

Table 5:

Means and standard deviation from the mean of the lengths, whole weight, wet flesh weights and dry flesh weights of Tellina tenuis from Kames Bay, Millport.

	$K = \frac{W}{L^3}$	Length	Whole wt.	Wet flesh weight	Dry flesh weight	No. of sample
April, Par.	88	148.0 ± 14.9	153.75 ± 69.9	116.9 ± 43.0	1.46 ± 4.9	16
April, Non-par.	90	135.0 ± 25.97	115.7 ± 66.9	99.27 ± 50.4	1.375 ± 5.8	25
January, July, October, Par.	*	140.7 ± 26.49	142.1 ± 88.0	102.14 ± 63.7	1.346 ± 0.727	28
January, July October, Non-par.	*	154.56 ± 23.9	113.27 ± 95.9	99.29 ± 75.82	1.537 ± 0.819	28
October, Par.	80.77	134.85 ± 28.9	97.71 ± 82.61	70.85 ± 38.39	1.086 ± 0.908	7
October, Non-par.	85.66	133.79 ± 25.87	124.0 ± 69.34	72.47 ± 41.78	1.144 ± 0.657	14
December, Par.	74.0	142.6 ± 26.05	144.8 ± 83.9	96.8 ± 64.6	1.309 ± 0.540	28
December, Non-par	75.1	136.15 ± 18.7	110.12 ± 49.9	66.8 ± 21.09	1.000 ± 0.262	32

* January, Par. K = 90
January, Non-par. K = 100

* July, Par. K = 93
July, Non-par. K = 86

The ovary was more translucent and grey. Individual ova could be seen under a 10 X hand lens, each enclosed within a gelatinous mucopolysaccharide capsule (Plates 23,29). Histological sections showed that in mature specimens the gonads extended ventrally into the foot as far as the pedal ganglion, surrounded the alimentary canal and penetrated between the lobes of the digestive gland. The tube shaped gonadal follicles opened via a pair of short gonoducts into the inner dorsal pallial cavities.

Histological observations of approximately 1,200 Tellina tenuis from Kames Bay, Loch Ewe and West Sands, St. Andrews throughout one year showed that this is a gonochoristic species, i.e. individuals are either all males or all female. No hermaphrodites were found although on a number of occasions the presence of oocysts of the coccidian parasite of the ovaries in the gonadal follicles of immature male Tellina during December and January strongly suggested that previously female animals were redifferentiating as males. Further experimental work is necessary to back up this circumstantial evidence in order to establish whether sex reversal does take place at the end of each breeding season.

ii) Maturation of the gametes

Primary germ cells lining the gonadal follicles developed into oogonia which grew towards the lumen of the follicles. The oogonia developed into oocytes recognizable by their large size, darkly staining granular cytoplasm and an enlarging vesicular nucleus. The oocytes were attached to the walls over a large area in the early stages of their development. As they developed they extended towards the centre of the follicle becoming oblong, piriform and connected to the follicle wall by

a slender stalk. New oogonia were continuously developed between the ripening oocytes from the primary germ cells and could be recognized by their less densely staining cytoplasm and a round nucleus with a distinctive staining nucleolus (Plate 12).

The coccidian endogenous stages were restricted to the primary germ cells. It appeared that cells that escaped penetration long enough to become oogonia were thereafter refractory to the parasite trophozoites. Germ cells containing the parasite were often observed detached from the follicle walls and floating free in the lumen of the gonadal tubules. A detailed description of the endogenous and exogenous stages of the coccidian are given in Section 2.4(a) of the present study.

The sperm, in common with amphineurans, archaeogastropods, other bivalves and most cephalopods (Fretter and Graham, 1963) having external fertilization, possessed spherical masses of mitochondria clustered around the posterior end of the head. There was a thin covering of cytoplasm around the tail and the head was distinctly corkscrew shaped. The sperms of T. fabula were observed to have a much heavier 'pitch' to the corkscrew than those of T. tenuis.

iii) The ratio of the sexes

From each sample collected at four different heights on the beach at Kames Bay in April, July, October, December and January sub-samples were taken by the method outlined in 1.3(c). The sex of each individual and the degree of maturity of the gonads were established. At the same time the degree of parasitization by the coccidian M. tellinovum was measured. Table 3 sets out the results.

It was found that a ratio of approximately 1:1 existed between the sexes. In October 45.9% of the bivalves were undifferentiated sexually. It is believed that spawning is completed by September (Stephen, 1938, Watkin, 1942, Ansell and Trevallion, 1967).

The preponderance of females over males in the winter months is attributed to sampling error. It is always easier to determine females because of the presence of the coccidian oocysts in the empty follicles. These show up clearly through the membranes of the ovarian follicles indicating that the host was female whereas spent males, unparasitized females and sexually undifferentiated individuals were hard to sex and tended to be lumped together as neuters. This resulted in an artificially high number of individuals being classified as neuter and for this reason the winter figures show a ratio of females to males of 3:2. A corrected ratio based on the assumption that half the neuters will become male and half female is given at the foot of table 3.

A far higher ratio of young (2+ age group) Tellina were male than female suggesting that the species is protandrous.

iv) Spawning

Females were found to contain mature ova from May to September. It appeared most likely that the annual reproductive cycle was set off by temperature fluctuations in spring or early summer.

The spawning trigger is believed to be a combination of thermal, mechanical and hormonal stimuli acting at different times at different levels on the beach. The chief stimulus is believed to be lunar periodicity (Fretter and Graham, 1963).

Histological observations on the gonads of more than 1,200 individuals suggested that each individual spawns for short periods over the whole breeding season.

d) Density of the population

Table 1 gives the number of Tellina tenuis per square metre based on samplings of 1/16th square metre for 12 months from April 1974 to March 1975. Stephen's (1926-7) equivalents are given in brackets.

The mean values show that the population has changed little since the work of Stephen. The density of individuals per square metre falls progressively from a maximum at MLWN to zero above MHWN tide mark. It is believed that numbers fall off in a like manner below the MLWN level to a depth of about 5 metres below MLWS (Clark and Milne, 1955).

e) The seasonal pattern of parasitization by the coccidian

The degree of parasitization of the ovaries was based on an assessment of the ratio of coccidian oocysts to mature ova in the gonadal follicles. The coccidian oocysts stood out under the low power of the dissecting microscope by virtue of the refractile nature of their contents (Plates 6-9).

Table 3 sets out the ratios of light, medium and heavily parasitized Tellina throughout the year. It was found that the percentage of heavily parasitized individuals rose to a peak in July when 75% of the females were categorized as heavily parasitized as against 7% lightly so. By the end of the spawning season in October it was found that 63% of the females were lightly parasitized and only 27% heavily so. This suggested that a large proportion of the coccidian oocysts were

shed at the peak spawning period in late July and August.

The ratios of the percentage of light, medium and heavy categories were approximately even throughout the winter and spring. During the winter approximately 300 Tellina tenuis were examined in detail for signs of parasitization. It was found that approximately one third of the population were female and contained the parasite, one third were male and one third were undifferentiated. Thus the coccidian appeared to survive overwintering in females not undergoing sex reversal. In the spring the number of coccidia mirrored the increase in maturing ova reaching a peak in July.

1.5 Discussion

a) Age structure and growth rate

Barnett (1971) followed the age composition of Tellina at Kames Bay and at the neighbouring beach of Hunterston. He plotted length frequency diagrams for each month and followed the monthly changes in the position of the modes. He found that considerable overlap takes place in the length frequencies of different year groups but by separating the polymodal length frequency histograms into a series of constituent normal distributions using the probability paper method the mean sizes of each of the younger groups could be estimated. Barnett noted that it was almost impossible to use the method with the older year groups since these were not well separated.

He calculated the growth rate using the estimated mean lengths for year groups in the early winter of each year after growth had stopped. These he called the mean specific growth rates, being the increase in length (mm) per unit length (1 mm) per unit time (1 year). He found that Tellina reached a mean

length of 3 to 4 mm by their second winter. Shell length increased in proportion to the cube root of shell weight.

An analysis of a Ford-Walford plot (Hancock, 1965) for a population of Tellina tenuis at Firemore, Loch Ewe (Trevallion and Ansell, 1967) showed that shell length frequency modes were: 3, 6.2, 9.3, 12.2, 13.9 and 15.3. These were believed to represent respectively 1+, 2+, 3+, 4+, 5+ and 6+ year groups. The intercept of the 45 degree slope was 18 mm. This data agrees with Barnett's assessment of size lengths at the end of the growing season, however, the theoretical maximum of 18 mm length does not correspond with the observed lengths of many Tellina at Kames Bay which were over 20 mm in length.

Stephen's (1932) assessment of the rate of growth differed from Barnett (1972) and Ansell and Trevallion (1970) in one fundamental respect. Stephen noted the presence of a 3 mm mode in April 1927 which constituted 80% of the population at MLWS. This mode moved to 5 mm by June and at the end of the growing season had doubled in length to 6 mm. He states that "at L.W.M. in Kames Bay in October, 1927 there were four year groups represented in the population, namely the spat of 1927 (0+ group), mostly 3 to 4 mm; the 1926 spat (1+ group), ranged around 7 mm; the missing 1925 spat (2+ group), which would have ranged around 9 mm; and lastly the 1924 spat (3+ group) ranged around 11 mm. There may, of course, be a few representatives of older groups, but, if present, they form a very insignificant part of the population".

The modern interpretation of the age structure of the population of Tellina at Kames Bay using the probability paper method of polymodal frequency analysis - a method that was not available to Stephen - shows that he differed by a year in his assessment

of the age groups.. According to post-war workers the 3-4 mm group in April is actually a 1+ group and not 0+.

Watkin (1942), working on the same population 9 years later, makes the same assessment of growth rate as Stephen. He sampled in March and April only and ascribed the 3 mm mode he observed in April as belonging to the previous year spat.

It seems that Stephen's and Watkin's interpretation of the data was based on the observation made by Stephen that no specimens of Tellina tenuis passing the 2 mm sieve were retained by the 1 mm sieve during the winter months. We are left with the puzzling observation that large numbers of 1 to 2 mm Tellina spat were found in August and October but none at all during the winter months. Stephen sampled with a 1 mm sieve in August and found that by October this spat had spread in large numbers from below the low water mark up to the mean tide level, noticeably diminishing in numbers in the higher levels. These were individuals still between 1 to 2 mm in length in October when growth normally ceases.

It is hard to understand why both Stephen and Watkin believed that the spat of 1 mm length grew to 3 mm over the winter. There is no doubt that they both observed large numbers of recently settled spat having a mode at 1 mm in October. From this it must be assumed that they accepted that spat of 1 mm in October would grow to 3 mm by April. For example, Watkin found three well defined modes in April at 3, 10 and 13 mm with an ill-defined mode at 6 mm indicating to him the presence of four year groups which he described as 0+, 1+, 2+ and 3+. He found that the mode at 3 mm (representing, he believed, the previous year's spatfall) "is well marked at the low water stations, much more so than the upper stations". The mode changed to 4 mm in the

upper stations "indicating a more rapid growth in the high water stations where the population is considerably less".

As a mode at 1-2 mm was absent in April it must be concluded that either the spat had grown to 3 mm (or even 4 mm) over the winter or they had been killed off in an epizootic in November. Both suggestions seem unlikely because somatic growth ceases in the winter and the large number of spat recorded would surely be evident in the winter sample as dead shells.

This anomaly has not been commented on by subsequent workers in the field despite the fact that Stephen's and Watkin's work is invariably referred to in any ecological study of Tellina tenuis.

The present study confirms the observations of Stephen and Watkin that at higher levels on the shore growth rate is faster and older broods dominate in the composition of the population, as evidenced by the pattern of modal distribution throughout the year at the 4 stations sampled. The population is less dense so that it may be imagined that the reason for greater growth relates to less intraspecific competition for available food. This theory was first advanced by Stephen. The effects of siphonal predation by Pleuronectes platessa will also be more marked at lower levels on the beach and this would be bound to be reflected in growth rate (Trevallion, Steele and Edwards, (1970), Trevallion and Ansell (1971), Trevallion et al (1973).

b) Reproduction and spawning

The long spawning season of Tellina results in the appearance of spat throughout the breeding season from early June to October.

The failure of recruitment in any one year has usually been

attributed to the weather conditions prevailing at the time of spatfall. An offshore wind at the critical time of settlement would tend to blow the spat out to sea. For complete failure of recruitment in any one year it would be necessary to postulate gales from the north for a large part of the summer. However, the prevailing currents and winds at Millport are both from the south west. From these facts it seems more likely that factors other than weather may be more important in determining recruitment in any one year. It is not impossible to imagine, however difficult to prove, that the observed destruction of the reproductive potential of a large number of females by the coccidian parasite coupled with unusually high mortalities from disease, predation and adverse climatic conditions over the winter could bring about failure of recruitment and the loss of a complete year class.

c) Density of population

Stephen (1930), by sampling 1/4 square metre areas within a few yards of each other at St. Andrews, showed that considerable variation exists in populations at any one place. Holme (1949) thought it possible that there may have been differences in drainage or other conditions between Stephen's quadrats which might account for the observed aggregations.

The coefficient of dispersion (Salt and Hollick, 1964) was not measured in this present study but repeated sampling at Kames Bay and St. Andrews suggested that a significant degree of aggregation was the case at least at high densities. The extreme patchiness of the populations is a feature already commented upon.

d) Parasitic castration

Parasitic castration is often accompanied by prolonged life,

gigantism or both. This parasite induced syndrome has been reviewed by Cheng (1971) and Kuris (1974). The effect of the coccidian was not truly castratory in that the gonad was not permanently destroyed. Nevertheless, the use of the term may be justified in that the reproductive potential of a high proportion of females, each having the potentiality to produce approximately 1×10^7 eggs in one spawning season, was largely destroyed by the effects of the parasite.

Parasitic castration in other species of mollusc has been caused not only by the ovophagous habit of some parasites but also by pressure induced atrophy and hormonal effects amounting to sex reversal in some dioecious species. Rothschild (1936, 1938 a and b, 1941 a and b) and Lysaght (1941) observed that a high percentage of giant forms of Peringia were infected with larval trematodes which may bring about partial or complete destruction of the gonads. It was suggested that the unusual growth rate was either due to gonad destruction or compounds from the parasite. Boettger (1952, cited in Pearre, 1975) suggested a hormonal deficiency might be a possibility as the time of gonad ripening in some gastropods produced a decreased growth rate. Some host gastropods showed real gains in body weight and in others initial gains were followed by stunting (Pan, 1965), while some gained only in shell weight (Cheng, 1967).

It is considered that the effects of the coccidian might not be wholly deleterious to the population for the following reasons:

1) Heavily parasitized individuals were not significantly lighter than their non-parasitized sisters and thus were not measurably affected by the presence of the coccidian.

2) The heavy mortalities thought to take place post spawning

amongst first-time spawners owing to the metabolic demands of gonad production might be avoided in castrated females.

3) Older, larger individuals have a higher reproductive potential and are less susceptible to predation than younger, thinner shelled Tellina.

A large proportion of the available energy budget of mature Tellina has to go into gonad production. The attack by the coccidian trophozoites on the primary germ cells was observed to arrest development of a large proportion of the host oocytes before energetically expensive yolk production began. This would leave a surplus of energy funds which, if not dissipated on humoral defence mechanisms, could be reinvested in glycogen storage and shell construction thus increasing the survival chances of over-wintering Tellina. Thus the presence of the coccidian could indirectly help first-time spawners to over-winter by allowing a build-up of reserves and removing the spawning stress known to cause heavy mortalities in most lamellibranch populations. The condition index of males, lightly parasitized females and heavily parasitized females was not significantly different throughout the year. All categories showed the seasonal changes in weight noted by Ansell and Trevallion (1967) but no differences could be detected in weight between these three categories. (Table 4).

Kuris (1974) believes that parasitic castration may be an important component of population regulation systems for many marine organisms. He listed coccidians, digenean trematodes, metacercariae, dinoflagellates, nematomorphs, ellobiopsids, fecampid turbellarians, fungi and cestodes as sometime parasitic castrators and cited numerous examples from both vertebrates and invertebrates.

e) Fluctuations in numbers and factors affecting spat settlement

Coe (1956) analysed fluctuations in numbers of populations of marine invertebrates including the Pismo clam (Tivela stultorum) and the bean clam (Donax gouldii). Donax gouldii was recorded at densities of 20,000 per square metre in the inter-tidal zone at La Jolla, California. Approximately half the population died after spawning one year after these densities were recorded. In the following year mortalities increased as soon as spawning began until by the end of the year no individuals remained alive. Miles of sandy beaches thickly strewn with empty shells "bore vivid testimony as to what had occurred". It was presumed that mortalities had been caused by a protozoan parasite. This cycle had been reported on several times in the past and is an extreme example of the common phenomenon of resurgence in littoral populations. The explanation of this phenomenon is believed to be due to chance combinations of conditions of wind, temperature and tides. A favourable current could bring swarms of larvae to a beach at the exact time of transformation and settlement. Adverse currents have evidently been the cause of the complete failure of many sedentary marine invertebrates to re-establish themselves in formerly populous localities. The reproductive strategy of most marine invertebrates including Tellina is such that relatively few individuals can produce a maximum population in a single generation given favourable conditions. Over-population must be followed by rapid decline in numbers owing to intraspecific competition for the available resources of space and food.

Ansell (1972) commenting on Stephen's (1938) observations on the fluctuations in brood strength of Tellina noted that successful settlements are never so widely separated that the species is

absent. This is not the case for the mactrid Spisula subtruncata which has only been observed to occur in Kames Bay for the life time of a single successful settlement and at very infrequent intervals. Ansell's observations on the distribution, growth and seasonal changes in Donax vittatus from MLWS and below in Kames Bay led him to the conclusion that the failure of Donax settlement was not due to any failure by the adults to produce gametes but to hydrographic conditions prevailing leading to the presence or absence of larvae at critical periods within the bay, or to biotic factors affecting the settlement of such larvae and their post settlement survival.

Trevallion (1971) found that heavy mortalities occurred in populations of Tellina tenuis from Loch Ewe after a summer of high rainfall and low sunshine. She showed that the condition of animals based on measurements of ash-free flesh dry weight were closely linked to changes in temperature which had a direct bearing on the food supply.

Stephen (1928) described a very heavy mortality that effectively removed an entire year group from the population at Kames Bay. The empty shells were found in large numbers at the end of the summer so that predation can be ruled out. Stephen had been following this population with particular interest as the year class involved had been one of superabundance - roughly 2½ times that of subsequent years. He believed that spawning stress was the most likely cause of this mass mortality particularly as these were first-time spawners.

Stephen (1938) found a correlation between abnormally high temperatures in the Atlantic in the early 1930's and good broods of Tellina and other invertebrates.

Trevallion, Steele and Edwards (1970) have suggested that grazing by young flatfish on the tips of the siphons of feeding Tellina can divert growth of the prey into siphon regeneration. This is supposed to take place at the expense of gonad development. They advance the theory that when Tellina density drops below a critical level this predation would cease and thus permit recovery of the predated Tellina. Thus Tellina populations could be controlled through inhibition of spawning by a functional response of predators to prey density and this mechanism could account for the sparse recruitment on Loch Ewe beaches in 1964 and 1967.

McIntyre (1970) noted that the same sequence of good and bad years of recruitment of Tellina is not found either on every beach of an open firth or on beaches inside several adjacent sea lochs, thus he believed factors other than large scale environmental effects must be sought. He classified these factors as follows:

1) Predation at the spawning stage
2) Dispersal at the planktonic stage
3) Wave action at the settlement stage.

He believed that these are the controlling factors that lead to local differences in population structure and persistent failures in recruitment. He attributed the more regular recruitment on beaches of the Clyde sea area to the richer environment which allows Tellina to withstand cropping and achieve reproduction. McIntyre makes no mention of the possible effects of parasitism or disease on Tellina population dynamics.

1.6 Summary and Conclusions

1. This section deals with the results of preliminary investigations into the distribution, growth and reproduction

of Tellina tenuis as affected by the presence of a hitherto undescribed coccidian parasite of the ovaries.

2. Collections were made at four levels on the beach at Kames Bay, Millport over one year (1974) and the density and lengths measured at each month. The months of April, July, October, December 1974 and January 1975 were selected for the measurement of shell length and weight, wet and dry flesh weights, sex and the degree of parasitization.

3. The population was found to follow the same range and, within the limits of sampling error, to have the same density as that recorded by previous workers in the last fifty years.

4. The density maximum of approximately 4,000 per square metre is to be found at MLWN and falls progressively to zero above MHWN. The population merges with Tellina fabula at depths of about 1 metre below MLWS.

5. The size frequency distribution shows a regular gradation from MLWS to MHWN. Smaller individuals predominate at and below MLWN and become proportionately fewer above MLWN.

6. Growth is more rapid at higher levels than at lower levels, and older broods dominate in the composition of the population.

7. Tellina tenuis appears to be a protandrous gonochoristic species with a sex ratio of approximately 1:1.

8. Maturation of the gametes took place in April and spawning was believed to occur every neap tide from June to October.

9. There were indications that sex reversal took place in a proportion of the population during the winter although this has yet to be proved.

10. A coccidian parasite was detected in at least 90% of female Tellina. The trophozoites were found in the primary germ cells of the oocytes in the gonadal follicles. No traces were found of the coccidian in any male Tellina in Kames Bay or in samples from the North, West and East of Scotland.

11. The percentage of heavily parasitized females reached a peak in July at 75%. In October this fell to 27% with 63% categorized as lightly parasitized.

12. The parasite does not appear to cause gigantism amongst females and no significant changes could be found in weights or lengths of "standard" animals whether male, or lightly parasitized or heavily parasitized female. The parasite only causes the temporary death of the reproductive capacity of females.

13. It is concluded that the parasite does not have any marked deleterious effect on the population which appears to have remained remarkably stable in all its parameters over the last fifty years. It is believed that this stability probably reflects a long association between the parasite and its host.

SECTION TWO

SECTION TWO: Studies on a coccidian parasite of the ovary of Tellina tenuis for which the name Merocystis tellinovum (species nova) is proposed.

2.1 Introduction

The presence of masses of parasitic oocysts in the gonad of the female was first noticed by Stirling (pers. comm.) from a population of Tellina tenuis at Aultbea, Loch Ewe. A survey of populations from the north, east and west coasts of Scotland showed that a very high proportion of fertile female Tellina harboured this parasite and in very many cases the parasite destroyed the reproductive potential of the female host.

The present study constitutes an investigation into the biology of the parasite. It was found to possess all the features of a typical Eimeriorine coccidian with gametogony and merogony taking place within the primary germ cells of gonadal follicles. The oocyst was found to contain 64 sporocysts each containing 2 sporozoïtes. The parasite is believed to belong to the genus Merocystis of the family Aggregatidae Labbé, 1899, belonging to the sub-order Eimeriorina Léger, 1911, of the order Eucoccidiorida Léger and Duboscq, 1910. The terminology of Levine (1970) is adopted throughout. A diagnosis is presented and the name Merocystis tellinovum (species nova) is proposed.

The fine structure of the infectious stages and micro- and macrogametogenesis is described together with the morphology of the biflagellate gamete.

The host/parasite relationship is examined in relation to the inflammatory responses of the host such as phagocytosis, leuco-

cytosis and encapsulation.

2.2 Review of the Literature

The coccidia have recently been the subject of a comprehensive review in a volume edited by Hammond and Long (1973). A chapter by Levine reviews the history and taxonomy of the group and the terminology proposed by Levine is adopted in the present study.

Levine takes a conciliatory approach to the inherent problems of the taxonomy of the coccidia by rejecting both the narrow view that they are all merely members of the genera Eimeria and Isospora of the family Eimeriorina (plus a few others) and the broad view that they are all members of the sub-class Coccidiasina as opposed to the sub-class Gregarinisina.

Of the 8 families of the Eimeriorina, 4 occur in invertebrates and 4 in vertebrates. Very much more is known about the latter group because of the severe economic effects of the disease of coccidosis in domestic animals. In contrast little is known about the marine species as they have largely been neglected since the original descriptions of the late 19th century.

The history of the taxonomy of the coccidia is tangled and involved in the extreme (Levine. 1973). Suffice it to say that the Committee on Taxonomy and Taxonomic Problems of the Society of Protozoologists (Honigberg et al, 1964) erected very detailed taxa, however since 1964 a completely new classification has had to be attempted owing to the wealth of new information gained from electron microscopical studies.

The traditional basis for classification has been the morphology of the oocyst. Detailed descriptions of endogenous stages and

the location of development within the host tissues are rarely included and the vast majority of descriptions are confined solely to the morphology of the oocyst at sporulation (Marquardt, 1973). According to Levine and Ivens (1970) the exact location of the parasite within the host's digestive tract is only known in 17 of the 95 named species of Eimeria from ruminants. Further, only portions of the life cycle are described for 15 out of the 95 species and complete life cycles have been described for only 2 of the entire list.

Levine (1962) estimated that with the use of quantitative and qualitative characteristics there can be at least 2,654,736 different species in the genus Eimeria alone, based on possible permutations of numbers of sporocysts per oocyst, and the number of sporozoites per sporocyst. Obviously such slight morphological differences do not provide adequate justification for erecting new species. Conversely, similarities in oocyst morphology are not necessarily justification for lumping new species together. Marquardt (in Hammond, 1973) recommends the following taxonomic determinants be taken into account in any description of a new species: (i) size; (ii) variability of the oocyst wall; (iii) shape and shape index; (iv) colour and (v) immune responses. With the exception of the latter these have been described in the present study.

The fine structure of protozoan parasites has now become the basis for determining systematic relationships even to the extent of adopting ultrastructural features as the chief criteria for distinguishing between sporozoa. (Honigberg et al, 1964; Garnham, 1969 a and b; Levine, 1969 a and b, 1971).

The electron microscope has allowed detailed investigation of

development, structure and function in the coccidia. Scholtyseck (in Hammond, 1973) presents a comprehensive review of the fine structural characteristics of the group.

The coccidia are distinguished from all other protozoan cells by the ultrastructure of their motile cells. The sporozoite and the merozoite are both bounded by a characteristic multi-stratose pellicle and contain an apical complex comprising a polar ring, conoid, rhoptries and micronemes. Also typical of the group is the micropore which functions as an ultracytostome (Scholtyseck, in Hammond, 1973)

There appears to be no record of a coccidian from the eggs of any pelecypod mollusc. Chytridiopsis ovicola Léger and Hollande 1917 was originally thought to have a possible relationship to the coccidians and Dolfus (1922) cited in Sprague (1971) treated it as an agent of coccidiosis in oysters. Sindermann (1970) was equally positive that it was an Haplosporidan. Sprague (1970 and 1971) attempted to clarify its taxonomic position and argued that it belonged to the Microsporida based on the ultrastructural studies of Manier and Ormières (1968) who showed that Chytridiopsis socius possessed a polar filament. Assuming the generic position is constant it then follows that C. ovicola is a microsporidan. Strangely, C. ovicola was only found in a single sample of Ostrea edulis from Marennes, France. Becker and Pauley (1968) described a similar parasite to Chytridiopsis in the ova of the Pacific oyster, Crassostrea gigas.

The only coccidian in the eggs of a marine invertebrate seems to be Ovivora thalassemae(Lankester). The host is the echiuroid worm Thalassemae neptuni and the life cycle has been described by Mackinnon and Ray (1937) cited in Kudo (1966).

2.3 Materials and Methods

a) Collection of specimens

Tellina tenuis were collected from between the tide levels at the following beaches during 1974 and 1975:

Kames Bay, Millport, Isle of Cumbrae	549171
Inverasdale, Loch Ewe	865823
Firemore, Loch Ewe	884818
Aultbea, Loch Ewe	890867
West Sands, St. Andrews	174505
Stevenston beach, Firth of Clyde	268403
Barassie beach, Firth of Clyde	327322
Hunterston beach, Firth of Clyde	522185
Troon beach, Firth of Clyde	
Seamill beach, Firth of Clyde	473197
Lunderston Bay, Firth of Clyde	

With the exception of Firemore beach the usual densities of Tellina were of the order of 1,000 or more per square metre. The animals were transported to the laboratory in Kilner jars packed in ice and transferred to aquaria containing sea water at 10 degrees C. The sea water was circulated through charcoal and fibreglass wool by means of an "Eheim" filter. A minimum 24 hours were needed to allow clearance of sand grains from the gut.

b) Preparation of material for light microscopy

Fresh smears from the gonad were examined microscopically after the addition of methylene blue (Sprague, 1965). Specimens found positive were fixed in alcoholic Bouin's solution or 10% buffered formalin, embedded in "Paraplast" and sectioned in a Leitz ultramicrotome at 5 um thick. Some sections were stained with Heidenhain's iron haematoxylin and eosin and others were treated by the periodic acid-Schiff method of McManus. Some of

the PAS preparations were counterstained with Weigert's iron haematoxylin. Formalin fixed sections were subjected to the Fuelgen nucleal reaction. Measurements were made by means of a calibrated ocular micrometer.

c) Preparation of material for electron microscopy

1 mm^3 blocks of tissue were fixed in a 2.5% solution of glutaraldehyde in 0.05 M sym-collidine buffer of pH 7.4 containing 0.03 M sucrose. After a minimum period of 2 hours in glutaraldehyde at room temperature the tissues were rinsed in 6 changes of buffer solution and subsequently post fixed in 1% osmium tetroxide in buffer at 4°C. Dehydration was carried out in a graded alcohol series followed by gradual infiltration over 72 hours from 100% propylene oxide through to 100% Araldite/Epon resin mix. This was found necessary in order that the thick walled oocysts of the parasite might be adequately impregnated with resin.

Sections were cut first at 2 um thick from resin embedded blocks using glass knives in a Huxley hand microtome. These were examined unstained in a Leitz Laborlux light microscope fitted with phase contrast optics. This method allowed rapid location of the desired stages in the coccidian life cycle. Ultra-thin sections were then taken by the use of glass knives in a Reichert Ultramicrotome and stained either in lead citrate and uranyl acetate or in a combined 1% potassium permanganate/1% uranyl acetate stain in doubly distilled water. This 10 minute stain was found to give excellent contrast although somewhat 'grainy' at magnifications above 25,000. Copper grids were coated in Formvar and reinforced with vacuum deposited carbon film. Sections were viewed in AEI Corinth and JEOL 100 electron microscopes.

d) Preparation of material for scanning electron microscopy

Suspensions of oocysts in acetone were placed on coated stubs and shadowed with gold/palladium before examination in a Cambridge Scanning electron microscope.

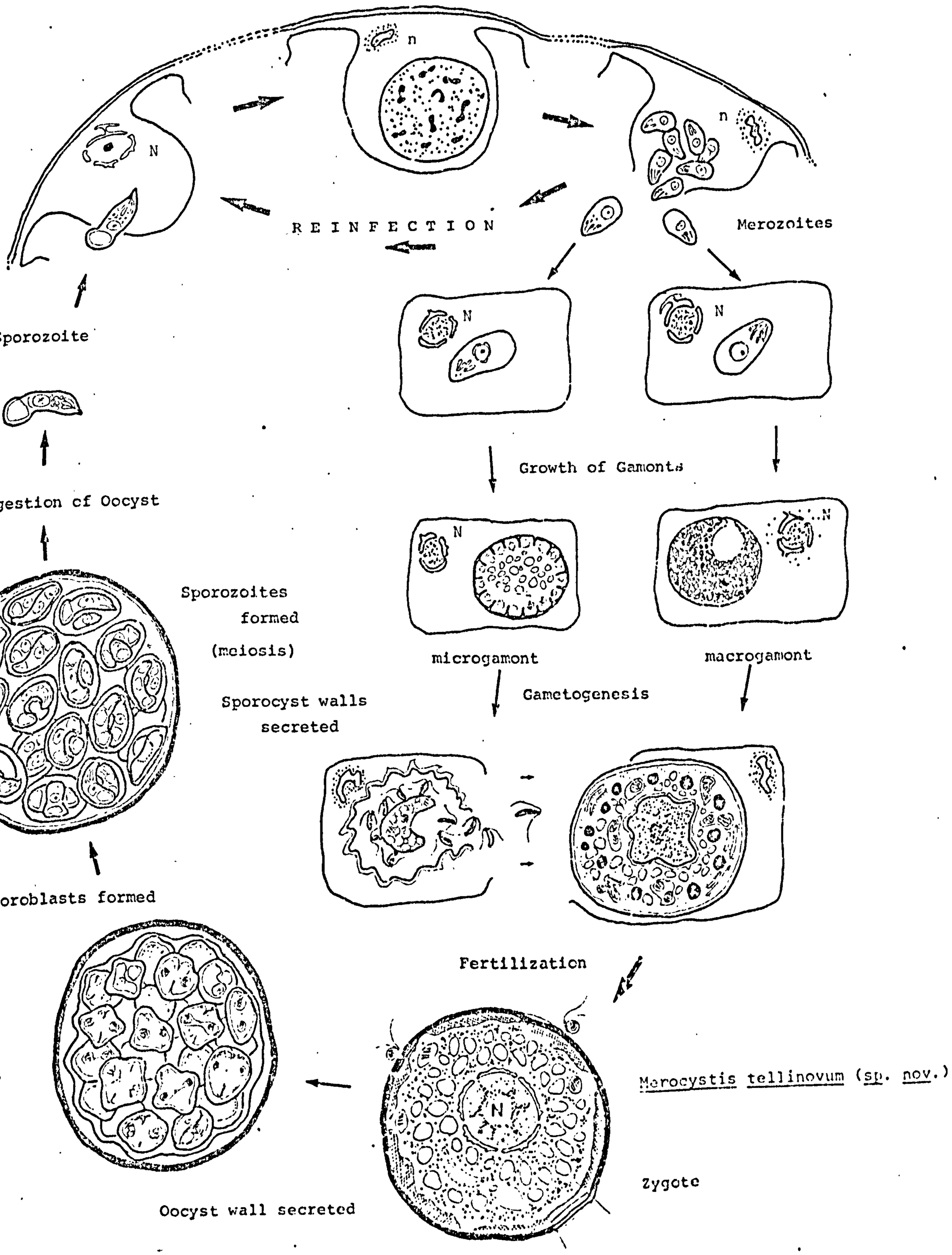
2.4 Observations

a) The life cycle

The three phases of the typical coccidian life cycle - sporogony, merogony and gametogony - were observed. The terminology of Hammond (1964) is used throughout. All the stages in the life cycle were believed to be haploid except for the zygote. Meiosis was believed to be associated with sporogony (Scholtyseck, 1973).

A diagrammatic representation of the life cycle of the coccidian is presented in text Figure 7. New infections were believed to be initiated by the ingestion of sporulated oocysts in the course of filter feeding by neighbouring Tellina tenuis.

Sporozoites were thought to excyst from oocysts in the gut of the host having been subjected to the action of digestive enzymes. They were thought to penetrate through the gut wall of the host and to invade the primary germ cells lining the ovarian follicles (Plates 24-29). A sporozoite free in the intralobular haemocoele of the digestive diverticuli is illustrated in Plate 57. The elongated sporozoites transformed into spherical trophozoites whereupon the first nuclear division occurred (Plate 59). The immature schizont (meront) reproduces asexually by multiple fission resulting in the formation of between 8 and 16 merozoites (Plates 29 & 30). Breakdown of the host primary germ cell released these merozoites to invade new cells. Several cycles of merogony were thought to take



Text Figure 7

place before the onset of gametogony. Light micrographs of the first stages of gametogony are presented in Plates 24 & 30.

Gametogony involves the formation of two sexually differentiated cells from the haploid merozoite, the macrogamont and the microgamont. A detailed description of gametogony is given on pages 76 to 79.

Fertilization of the macrogamete by the motile microgamete resulted in a zygote which transforms into an oocyst shortly after fertilization. Sporogony of the oocyst took place in the lumen of the gonadal follicles and is illustrated in Plates 10-18. These exogenous stages are described in detail below.

b) Exogenous stages (Plates 10 to 20, 25 to 28 and 30).

<u>The oocyst</u> This was spherical to sub-spherical with a range in diameter of 23.44 to 29.30 µm (mean 26.37 µm). The oocyst wall appeared smooth, thin, colourless and double layered under the light microscope. Micropyle, oocyst residuum and polar granules were absent. The sporocysts occupied the whole space of the oocyst sac. Sporocysts averaged 64 in number and were smooth and oval. Their size range was 3.22 µm long by 2.93 µm wide. Each sporocyst contained two sporozoites each 3.30 µm long and 0.85 µm wide. The sporozoites were crescentic or comma shaped, the 'head' of the comma being represented by a refringent globule. Squash preparations of oocysts usually showed sporozoites lying head to tail although electron microscopy revealed that the pairs lie at right angles to one another. All stages of sporogony through to maturation occurred in the lumina of the gonadal sac (Plates 25 to 28 and 30).

c) Endogenous stages

The endogenous stages developed in the primary germ cells of

the mature female Tellina tenuis. A description of oogenesis in uninfected Tellina is given in 1.4(c). It appeared that primary germ cells that escaped penetration by trophozoites long enough to become oogonia were thereafter refractory to the parasites as all endogenous stages of the coccidia seemed to be limited to primary germ cells. Germ cells containing endogenous trophozoites were often observed detached from the follicle walls and floating free in the lumen of the gonadal tubules. (Plates 21, 22, 25 and 28)

The following stages were observed:

1. Numerous small trophozoites 3-4 µm in length could be seen inside primary germ cells. These had single or double chromatic structures (Plates 29 and 24)

2. Schizonts containing up to 64 irregularly arranged comma shaped merozoites 3 x 1.5 µm in size each with a posterior refractile globule (Plates 11, 19 and 20).

3. Mature macrogametocytes 10-15 µm in diameter with chromophilic granules around the periphery and a conspicuous nucleus. These were common (Plates 20 to 25).

4. Thin walled microgametocytes ca 12 µm in diameter containing few to numerous microgametes conspicuous as discrete dense chromophilic granules evenly arranged around the periphery. of the microgametocyte. These were much less common than macrogametocytes (Plates 25, 27, 28 and 30).

5. Zygotes with a chromophilic periphery at various stages of cleavage to form sporocysts (Plates 28, 31 and 32).

d) Diagnosis

Merocystis tellinovum sp. nov.

The name Merocystis tellinovum sp. nov. is proposed for this

coccidian.

HOST: Tellina tenuis (da Costa) (Bivalvia, Eulamellibranchia)

HABITAT: Primary germ cells and oogonia

LOCALITY: Scotland

MORPHOLOGY: Oocyst with 64 sporocysts, each with two sporozoites, spherical to sub-spherical with a mean diameter of 26.3 µm and with a thin, smooth, double layered wall. Micropyle, oocyst residuum and polar granules are absent. Sporocysts oval, thin walled with a mean size of 4.2-3 µm each containing two comma shaped sporozoites each slightly longer than the sporocyst and lying at right angles to each other. Sporulated oocysts are slightly larger than unsporulated oocysts. Monoxenous with merogony, gametogony and sporogony in the same host.

e) Distinctive features of Merocystis tellinovum

i) Relative size

Measurements of sporulated oocysts of M. tellinovum both from fixed preparations and living material indicate that the size range of the oocyst falls between 23.44 and 29.30 um in diameter.

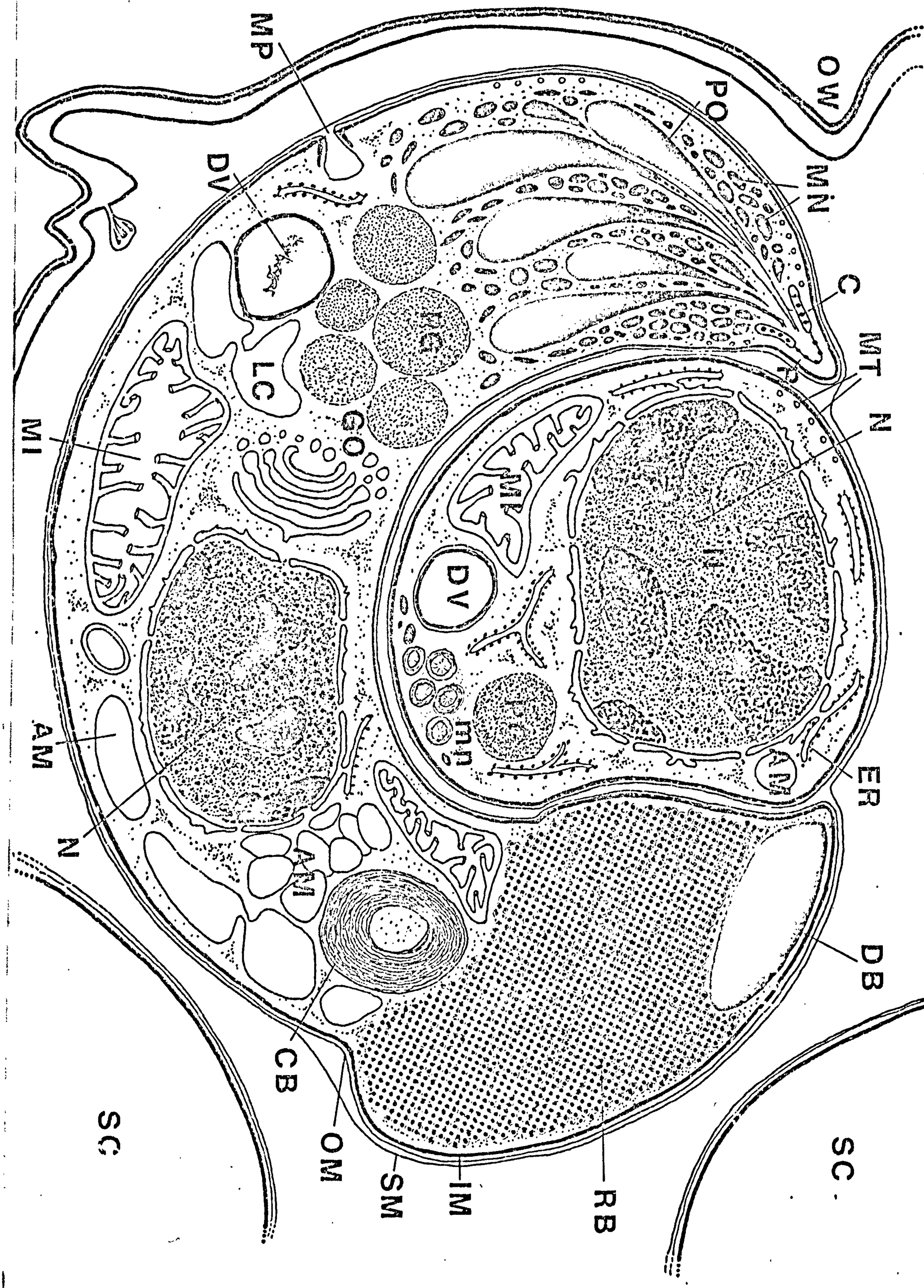
COMMENT

The oocyst falls within the size range of almost any species of Eimeriorine coccidia. For example, Eimeria bovis oocysts are 23-34 um by 17-23 um in size. Some oocysts are ellipsoidal, for example Eimeria ellipsoidalis which measures from 12-32 um by 10-29 um.

ii) Variability of the oocyst wall

It was noticed that a small percentage of oocysts had a crenulate margin wall. It was first considered that this might

Text Figure 8



be an artefact caused by shrinkage during post-fixation. To test this a large number of oocysts from heavily infected individuals were prepared for scanning electron microscopy by the critical point drying method (Small and Marszaleck, 1969). This revealed that the surface of the outer wall varied from smooth through veined to highly crenulate (Plates 61, 62 and 63). Transmission electron microscopy showed that the wall became greatly thickened and could only be successfully embedded by means of vacuum impregnation techniques. (Plates 36, 43-46)

COMMENT

It is possible that the survival value to the species conferred by the thickening of the oocyst wall is one of resistance to adverse conditions especially since the proportion of thick walled oocysts was much higher in winter. Christensen and Porter (1939) showed that there were striking differences in the oocyst structure of individual specimens of the same species of E. auburnensis.

iii) Shape and shape index

Oocysts from M. tellinovum are constant in shape being spherical to spheroidal (Plates 16 to 20).

COMMENT

Levine (1971) reported that the shape index (i.e. length divided by width) of species of coccidia is remarkably constant. Davis et al (1957) working on E. alabamensis (of the nuclei of epithelial cells in cattle) noted that the oocysts may be pyriform, elliptical or spherical. It is probable that the pyriform shape results from the pressure of oocyst upon each other in crowded conditions.

iv) Colour

Dense masses of oocysts seen through the tunica propria covering

the visceral hump appeared white (Plates 5-9). This feature made it easy to pick out infected individuals as the oogonia and oocytes of Tellina appeared colourless and the coccidian oocysts showed clearly white amongst them. Frequently the oocysts completely filled the ovarian follicles (Plates 8,9 & 21).

The use of an apochromatic lens to determine the colour of the oocyst wall showed that it was transparent. The white appearance of the oocyst in normal microscopy was therefore considered to be due to the refractile nature of the sporozoites.

v) Cross infection experiments and host susceptibility

Indirect cross infection experiments involving Ostrea edulis were attempted but proved negative. Observations on the closely related species, Tellina fabula, failed to reveal the presence of coccidia. Bivalves such as Donax and Cochlodesma found in close association with T. tenuis were similarly uninfected. A sample of 100 Tellina fabula from below the Low Water Springs tide level at Kames Bay were examined histologically as were a large sample of Donax Vittatus from St. Andrews West Sands. Both species appeared free of coccidia in the ovary. Tellina tenuis is found in association with Cochlodesma sp. in Aultbea, Loch Ewe. This species, too, proved negative. In general it would appear that these protozoa are highly host specific.

f) Transmission and scanning electron microscopy of the sporozoite and the merozoite (infective stages)

Sporozoites and merozoites are motile infective stages able to penetrate host cells. . In the life cycle the mobile comma shaped sporozoite is the initial stage whereas the merozoite is derived asexually by schizogony.

The mature sporozoites have a mean length of 3.3 um and a width

of 0.85 μm. They are comma shaped and have the characteristic structure of the coccidian sporozoite (Plates 36,38,39,43 & 44). The following ultrastructural features are commonly found in coccidia. A general review is given by Scholtyseck in Hammond (1973), however brief comments regarding the significance and function of each of these features have been added to each of the observations in order that the reader may relate this catalogue of structures to those of other known genera of the coccidia.

i) The pellicle (Plates 40, 43, 44, 47 and 48).

In both sporozoites and merozoites the pellicle was made up of an outer unit membrane and two closely adpressed inner membranes. This feature is diagnostic of all coccidian sporozoites. The space between these outer and inner membranes varies between 15 and 20 nm. The mean width of the pellicle is 40 nm.

COMMENT

Scholtyseck, Mehlhorn and Friedhoff (1970) in studies of the Eimeriorina found that the outer membrane is continuous and encloses the whole cell in contrast to the inner membrane which is perforated at the anterior end at the polar ring. No perforations of the inner membrane were seen in this study of M. tellinovum. A diagrammatic representation of the pellicle is given in text Figure 8 and the various layers are illustrated by the electron micrographs in Plates 47 and 48.

ii) The polar ring

This structure was not seen in free sporozoites although its development could be made out in sections of mature oocysts.

COMMENT

The polar ring is formed by a thickening of the inner layer of

the double membrane of the pellicle. Roberts and Hammond (1970) suggested that it was the site of attachment of the microtubules but further work is required to elucidate its fine structure.

iii) The sub-pellicular microtubules

These were seen at the anterior end of the merozoite and sporozoite (Plates 40, 48 & 60).There appeared to be up to 24 regularly spaced microtubules running tangential to the long axis. It was thought that the pellicle inner layers and the microtubules connected and that the two structures were a single functional unit. Slow, twisting movements of the sporozoites were seen in smear preparations of living material and it may be that the function of the microtubules relates to cell motility.

Microtubules in the developing sporozoites can be seen in Plate 44. Merozoite subpellicular microtubules are illustrated in Plates 37 and 38.

iv) The conoid

This structure was not clearly seen although its presence could be deduced from studies of the developmental stages (Plates 37,38).

COMMENT

The conoid was first described by Gustafson, Agar and Cramer (1954) in the trophozoite of Toxoplasma gondii as a hollow, cone-like structure situated at the apical end. It is believed to be formed from a spinal band of microtubules and may be compressible and extensible. The presence of a conoid has been shown in all sporozoites and merozoites of the coccidia but it

is absent from haemosporidians and piroplasms. Further studies are required on M. tellinovum motile stages to determine the size and shape of this organelle. The function of the conoid is thought to be to assist in penetration of the host cell.

v) Rhoptries (Paired Organelles)

Rhoptries were seen in both sporozoite and merozoites as well as in the late stages of sporocyst development within the oocyst. They were clavate electron dense structures with the narrow end extended towards the anterior portion of cell. Plates 43 & 48 show ultrastructural features of these rhoptries.

COMMENT

Many more than two of these paired organelles were commonly seen and in this respect they resembled the sporozoites of Eimeria. Scholtyseck (1973) comments that more than two paired organelles can be seen in the merozoites of Babesia ovis from the tick Rhipicephalus bursa as well as in Toxoplasma, Besnoitia and Sarcocystis genera.

The function of the rhoptries is not known although there are some indications that the paired organelles have a gland-like appearance and that they may secrete a proteolytic enzyme to assist in the mechanical function of the conoid. The narrow neck-like portions may serve as secretory ductules. This suggestion is supported by the results of Schrevel (1968) who found acid phosphatase activity in the rhoptries of the gregarine Selenidium.

vi) The micronemes

The size and shape of the micronemes is shown in Plates 38 & 43. In longitudinal section they appeared oval and in transverse

section circular or spherical. Frequently they appeared dense and structureless but some sections showed them to be surrounded by a membrane and to have a lacuna with characteristic fibrillar processes of the polar filament of the myxosporida.

COMMENT

In most sporozoa, micronemes are small osmiophilic convoluted cord-like structures with a diameter of 60-90 nm. They were first described by Gustafson et al (1954) in Toxoplasma. They called them toxonemes. Subsequently appellations such as 'cytoplasma-strange, sarconemes, lankesterellonomes, convoluted tubules, radial clubs, rod-shaped granules' have all been suggested but the primacy of the term micronemes is now generally accepted (Jacobs, 1967).

It has been suggested that micronemes have a secretory function and that they may be linked with rhoptries as a common functional system aiding the penetration of the host cell.

vii) Micropores

These structures were not clearly visualised in developing sporozoites but are believed to be represented in Plate 44. A micropore-like structure was frequently seen in the oocyst wall and is believed to be involved in enzyme secretion resulting in the formation of the parasitophagous vacuole surrounding the oocyst. These structures are illustrated in Plates 45 and 46.

COMMENT

Micropores are organelles believed to be formed by the fusion and invagination of the pellicle layers. They have been variously styled micropyles and ultracytostomes and are implicated in the uptake of nutrients by the sporozoite.

Senand (1967) reported two to three side canals branching off from the invagination of the outer membrane in Sarcocystis.

viii) Crystalloid inclusions

The posterior end of the mature sporozoite was observed to be filled with a dense aggregate of granules having a diameter of 40 nm and an interparticle spacing of 6-9.5 nm. The particles were not membrane bound nor were membranes associated with the margins of these inclusions. A sequence of crystalloid formation was inferred from Plates 37-42.

COMMENT

The term crystalloid inclusions for these paracrystalline arrays by Garnham et al (1962, 1969), who observed that the refractile cytoplasmic vacuoles of Leucocytozoon sp corresponded, at the fine structural level, to aggregations of closely packed crystalline material resembling Echo virus in structure.

Dasgupta (1968, 1971) in studies of the ookinetes and early oocysts of Plasmodium cynmolgi bastienelli believed that the crystalloid aggregations were viral in nature and corresponded with clumps of Feulgen positive material in degenerating oocysts. Terzakis (1968, 1969 and 1971) noted similar inclusions in Plasmodium gallinaceum and described them as resembling nothing else but a virus. Terzakis measured the particles at 35-55 nm and described them as having a trilaminar membrane and packing in a hexagonal pattern. Davies and Howells (cited in Trefiak and Desser, 1973) postulated that the crystalloid is a virus infection in Plasmodium berghei. Galluci (1971) has reported crystalline inclusions from late zygotes and ookinetes of Haemoproteus columbae.

Trefiak and Desser (1973) listed two types of crystalloid

inclusion, Type I they characterized as lipo-protein and Type II as probably viral. The crystalloid inclusions described above corresponded with Type I and were believed to be formed from the lipid and protein components present in the macrogamete.

g) Sexual stages of Merocystis tellinovum

The sexual phase of the life cycle of Merocystis tellinovum resembles that of other Protozoa with differentiated gametes. The male gamete is flagellated and a detailed description of the development and structure of this important stage of the life cycle is presented.

i) The Microgamont

The term microgamont is used for the parent cell of the microgamete. The ultrastructure of the microgamont of M. tellinovum is shown in Plate 51. Light microscopy showed (Plate 30) that the number of developing microgametes per microgamont was not large and it was estimated that 30 to 50 microgametes were produced. Microgamonts were much less commonly seen than macrogamonts.

ii) Microgametogenesis and the microgamete

It was possible to recognize four stages in microgametogenesis. These were as follows:

a) The nuclei of the microgamonts became arranged around the periphery of the cytoplasm (Plates 52 & 53). Each nucleus is associated with a mitochondrion having characteristically tubular christae.

b) The surface of the microgamont cytoplasm became elevated above each nucleus (Plates 51 & 52). The basal bodies from which the flagella arise could be seen on opposite sides of

the developing nucleus of the microgamete. The chromatin content of the nucleus became progressively condensed.

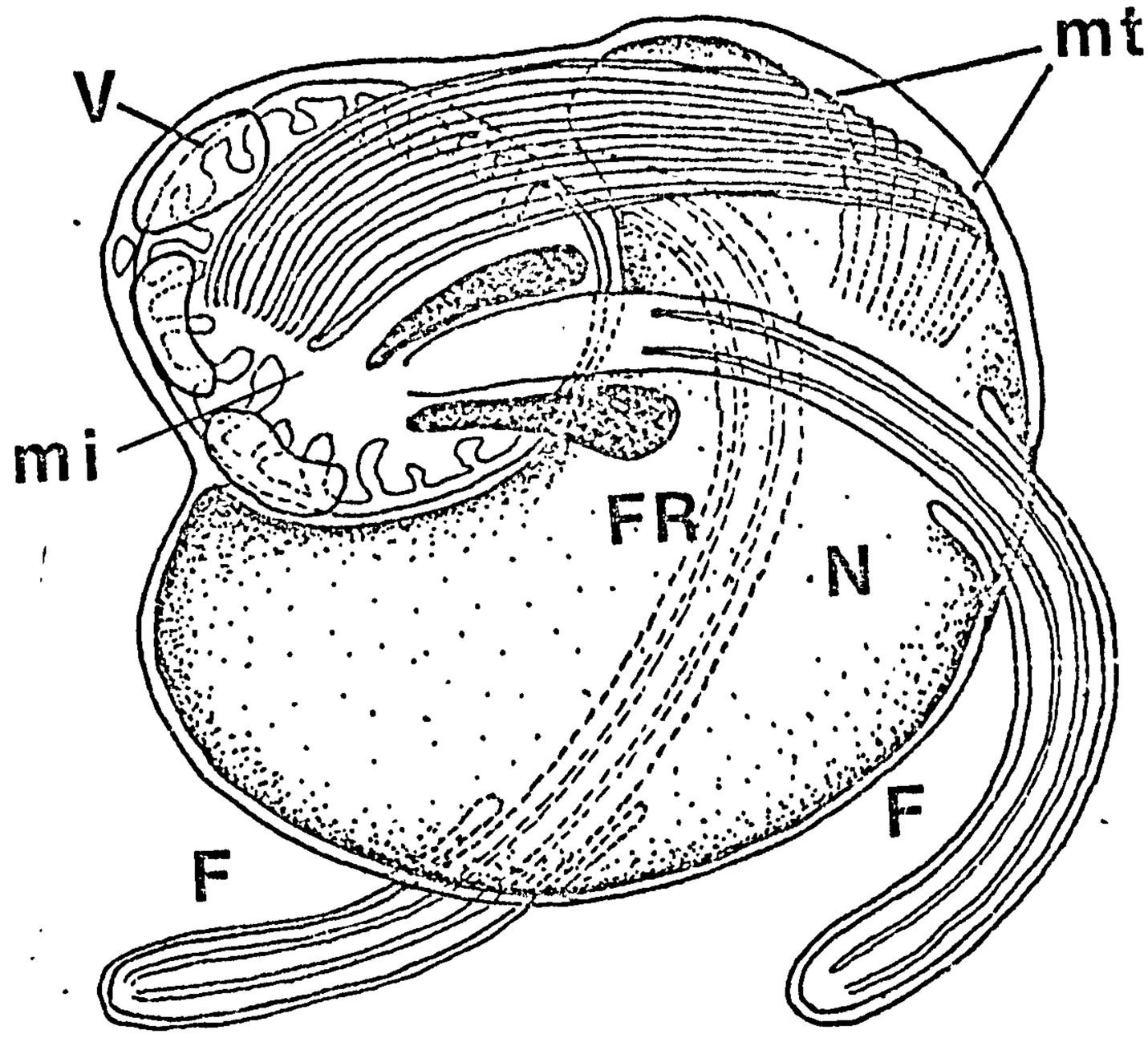
c) The nucleus became contained within a cylindrical outgrowth. A residual nucleus was not seen nor was fusion and elongation of mitochondria associated with the nucleus. These features are common in Eimeriorine microgametogenesis.

d) The immature spheroidal microgamete became separated from the microgamont. The residual cytoplasmic masses of microgamonts, sometimes contained within the ruptured bilayered pellicle, and sometimes free in the lumen of the gonadal follicles were frequently encountered in electron microscopical observations. These are illustrated in Plates 55 and 56.

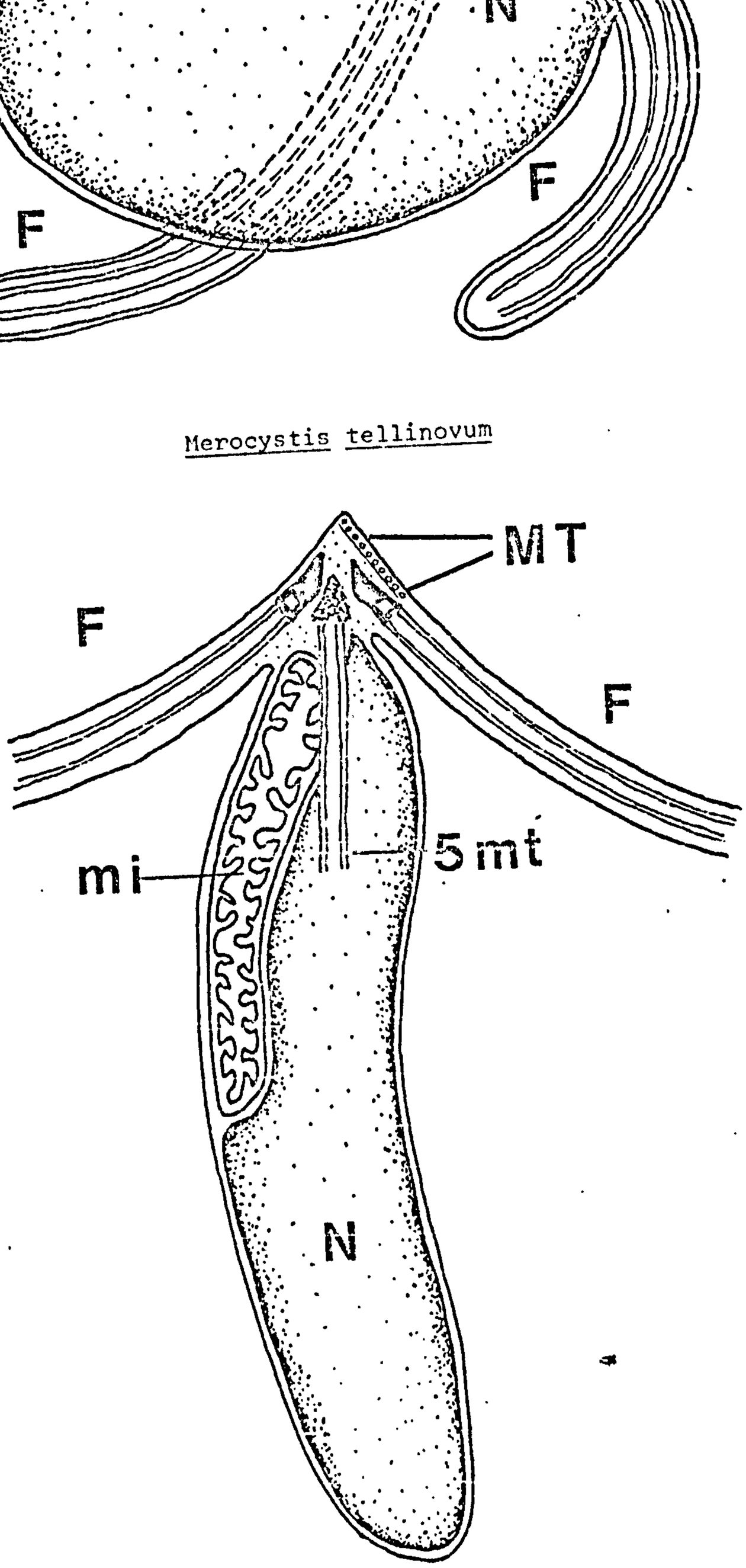
The microgamete at maturity was believed to be spheroidal, approximately 0.1 um in diameter and biflagellate. A diagrammatic construction is given in text Figure 9. The short flagella were deeply inserted within the bounding membrane. A spherical mitochondrion lay within a cup-shaped nucleus consisting of condensed chromatin and a number of vesicles, believed to be involved with penetration of the macrogamete, covered the anterior surface. A band of 12 microtubules was observed to run antero-posteriorly under the bounding membrane of the gamete between the insertions of the flagella (Plate 54).

COMMENT

Great significance has been attached both to the morphology of the coccidian microgamete and the number and arrangement of the flagella by taxonomists concerned with the phylogeny of the Sporozoa (Scholtyseck, in Hammond, 1973). The occurrence and arrangement of the flagella is taken as an indication of the



Merocystis tellinovum



Toxoplasma gondii (after Scholtyseuk)

Text.Fig. 9

phylogenetic relationship of the coccidia with certain groups of the Mastigophora.

Three different types of microgamete have been described. These are:

a) lens or cup-shaped protococcidian types;

b) elongate, slender and slightly curved microgametes of the Eimeriorina, and

c) filiform microgametes of the Haemosporina which supposedly have no mitochondria in contrast to the first two types.

The microgamete of Merocystis tellinovum most closely resembles the protococcidian pattern. These more or less spheroidal microgametes lack the polarity of the Eimeriorina. The microgamete of Coelotropha durchoni (Porchet Hennere, 1970) has two free flagella and numerous sub-pellicular microtubules. The mitochondrion is positioned in close association with the basal bodies of the flagella. The close resemblance of the M. tellinovum microgamete to this pattern indicates a taxonomic affinity with the protococcidia, however, that order is not believed to be merogonous nor are the members obligatorily intracellular. All the protococcidia so far described have been discovered in marine invertebrates.

The macrogamont This sexual form derives from the merozoite. The literature relating to macrogametes is comprehensively reviewed in Hammond. The macrogamont of Merocystis tellinovum was recognizable by the following features.

1) The large central nucleus with a compact electron-dense nucleolus (Plate 53).

2) The pellicle represented by one unit membrane with underlying osmiophilic material. Microgametes are seen adhering to the outer layer in Plates 53 and 54.

3) The presence of large granules which are probably analogous to the wall-forming bodies characteristic of all the Eimeria species so far described (Plates 53 and 54).

4) Lipid and amylopectin-containing cytoplasmic inclusions probably forming food reserves for the development of the oocyst (Plates 53 and 54).

COMMENT

Hammond reports that a canal-like outgrowth of the nucleus approaches the surface and a microgamete enters only at this place (Plate 53). The microgamete penetrates directly into the nucleus of the macrogamete which probably undergoes an immediate change in appearance. Light and electron micrographs of the ensuing stages post fertilization are given in Plates 10 to 20.

2.5 Discussion and Conclusions

Each of the fine structural features described above has been followed by a brief comment for the reasons already mentioned. The discussion is therefore brief.

i) Phylogenetical considerations and suggestions for future work.

The functional micromorphology of Merocystis tellinovum as revealed by the techniques of electron microscopy show that most of the typical fine structural features associated with the motile stages that distinguish the coccidia from all other protozoan cells were present. However, detailed analysis of the developmental processes such as endopolygeny, schizogony, asexual reproduction and sporogony have not been attempted in the present study. Further observations of these developmental stages would probably establish the phylogenetic position of this parasite. Preliminary observations suggest that the genus may be intermediate between the Protococcidiorida and the

Eucoccidiorida.

The physiology of Merocystis tellinovum has not been touched upon. The processes of locomotion, excystation, cell penetration, intake and digestion of nutrients, cytochemistry and studies on enzyme activity of organelles should now follow these preliminary electron microscope observations. These should be studied in conjunction with parasite-host relationships such as the formation of the parasitophorous vacuole, degenerative changes and host encapsulation. The pathogeneity of this particular coccidian appears to be a function of the size of the endogenous stages. The development of the oocyst to 30 um in diameter seemed to result in mechanical disruption of the primary germ cell wall.

Levine (1963) calculated that the genus Eimeria has been described from perhaps 1 or 2% of chordates and 5.7% of mammals. He estimated that we might expect to find 34,000 species of Eimeria.

There are approximately 100,000 genera in the phylum Mollusca (Morton, 1970) and it does not seem unreasonable to expect that there exist many more than the 12 genera (Kudo, 1966) that have so far been described. It seems quite probable that where bivalves of one species can be found at densities approaching those of Tellina at Kames Bay then they will harbour coccidia at some stage of their life-cycle.

Tellina tenuis, for example, is host to two species, one of which has been described in detail in the present study. The other species is Hyaloklossia pelseneerii, Leger and Duboscq (1915). This coccidian was frequently seen in the kidneys of Tellina from Kames Bay and West Sands, St. Andrews. No studies have

been made on Hyalokiossia since the original description of Leger and Duboscq. (Plates 68, 69 and 70).

Levine (1973) has pointed out that "there is work ahead for several generations of Protozoologists in the field of Taxonomy". It seems certain that there are very many coccidia in marine invertebrates awaiting description.

2.6 Summary and Conclusions

1) An hitherto undescribed coccidian parasite of the ovary of Tellina tenuis was examined both by light and electron microscopy.

2) A diagnosis is presented and the name Merocystis tellinovum (sp. nova) is proposed.

3) This is the first coccidian parasite of the ovaries of any bivalve to be described. The effect on the reproductive potential of the host has been assessed in the first section of this study.

4) The life cycle is described including the stages of sporogony, merogony and gametogony.

5) The ultrastructure of the sporozoite is described in relation to other coccidia to which they bear morphological similarities.

6) The ultrastructure of the microgamete, microgametogenesis, fertilization and the macrogamont immediately post fertilization are described.

7) The host-parasite relationship is examined and discussed.

8) Suggestions for future studies on this parasite are proposed.

SECTION THREE

SECTION THREE: Light and electron microscopical observations on a mycoplasma-like organism infecting the secretory cells of the digestive gland of Tellina tenuis.

3.1 Introduction

During the course of routine histological examinations of T. tenuis from various beaches in the North, West and East of Scotland, some specimens were found to contain cytoplasmic inclusion bodies in the secretory cells of the digestive gland. These inclusion bodies contained densely packed spherules closely resembling mycoplasmas (vide infra) and will be referred to as mycoplasma-like organisms or MLO.

In order that an appreciation of the cytopathic changes brought about by the MLO on the digestive gland could be understood a detailed study of the histology and ultrastructure of the normal digestive gland was made. The cyclical changes of absorption, digestion and breakdown in the gland and the fine structural features of the secretory and absorptive cells are described in relation to other members of the Lamellibranchia.

The first section of this review deals with the literature concerning the lamellibranch digestive gland. The appearance of this highly labile organ is very changeable and it was necessary to form clear ideas of normal versus diseased tissue before a description of histopathological changes could be attempted.

3.2 Review of the Literature

a) The lamellibranchiate digestive gland

Studies on the structure and function of the digestive gland of the Lamellibranchia have revealed that there exist a

remarkable uniformity throughout the group despite the diversity of form and habitat exploited by this the largest grouping of the bivalvia. A voluminous literature exists on the subject but, to date, no studies of the fine structure of Tellina tenuis digestive gland have been published.

The digestive diverticula are organs of absorption and intracellular digestion (List, 1902, Vonk, 1924, both cited in Owen, 1955; Yonge, 1926b; Owen, 1955; Dinamani, 1957; Saleuddin, 1965, cited in Sumner, 1966 a and b; B.S.Morton, 1969a, 1970a and d and 1973; J.E.Morton, 1956). They are also considered to be organs of secretion (Mansour, 1946; Mansour and Zaki, 1946). Owen (1955) suggested that the function of secretion could be derived from the fragmentation of spherules budding from the distal ends of the absorptive cells into the lumen of the digestive tubules. These may carry the waste products of intracellular digestion to the stomach and there disrupt to release enzymes of digestion.

b) Basophil cells

The pyramidal basophil cells occupying crypts in the digestive acini have been shown by Sumner (1966 a and b) and McQuiston (1969) to be secretory in function. Sumner refers to these cells as basiphil cells. In the present study they will be refered to secretory cells or basophil cells. These cells are traditionally ascribed the function of nests of young cells (Yonge, 1926b) from which 'spent' digestive cells and new tubules arose. Owen(1970, 1972a and b, and 1974) ascribes both functions to these crypts of young cells and recognizes two distinct types of basophil cell (1) secretory and (2) young, undifferentiated cells which can either become secretory or absorptive.

Langton (1975) based a quantitative study on synchrony of the

digestive diverticula of Mytilus edulis on the appearance of the absorptive cells. He classified these (after Platt, 1971) as:

i) Normal (holding) phase
ii) Absorptive
iii) Disintegrating
iv) Reconstituting

He found that 90% of the tubules were of Types (i) and (ii) and that the relative percentage of these two varied according to the degree of exposure related to the tidal rhythm. The mechanism Langton proposed for excretion was that residual bodies were released individually.

c) Classification and general features of mycoplasmas

The basis of nomenclature and classification of this group of organisms was laid in 1956 by Edward and Freundt and has now been universally adopted. The name Mollicutes for this class of 'soft-walled' organisms was proposed in 1967. The Mollicutes comprise only one order, Mycoplasmatales, which contains three families, the Mycoplasmataceae, Acholeplasmataceae and Spiroplasmataceae (Freundt, 1976). The mycoplasmatales are similar to the L-phase of true bacteria (Kleinberger-Nobel, 1962). It was once generally held to be the case that mycoplasmas were derived from unknown bacterial parents that had lost the ability to revert although there is no conclusive evidence for this derivation.

The chief biological characteristics separating these prokaryotic organisms from other classes of the lower protista is the lack of a cell wall and the inability to synthesize mucopeptide polymers and their precursors. The form of the organism is variable and includes rings, globules, filaments and small elementary bodies which can pass through filters which would hold back bacteria. Particles as small as 125 nm have been

separated by differential filtration and are capable of growing in tissue culture or artificial media. The growth requirements for parasitic species can be provided by a protein rich medium (usually supplied as serum protein) containing unsaturated fatty acids and cholesterol. Mycoplasmas are resistent to penicillin and other antibiotics that inhibit polymerization of cell wall precursors. The genome size is either 1 x 10^9 daltons or 4.5 x 10^8 daltons, i.e. half as great as bacteria or, at most, on the same level as the smallest bacteria. Virus genomes are in the range 10^6-10^7 daltons (Dales, 1963).

Much of the work on the morphological and ultrastructural characterization of the order Mycoplasmatales has been reviewed by Boatman (1973, 1974); Smith (1971); Maniloff and Morowitz (1972) and Zavadova (1972). The plant associated mycoplasmas are reviewed by Davis and Whitcomb (1971). Abstracts of the proceedings of the first conference of the International Organisation for Mycoplasmology (I.O.M.), September, 1976, include review papers by Freundt on species differentiation, Razin on ultrastructure and morphology, Whittlestone on isolation and detection, Bove on plant and insect mycoplasmas and Cole on mycoplasma viruses.

The bulk of the literature on the ultrastructure of the mycoplasmas is concerned with Acholeplasma laidlawii of the family Acholeplasmataceae (Edward and Freundt, 1969, 1970). Only a very few of the 37 species of the genus Mycoplasma have been adequately characterized from the point of view of morphology and ultrastructure. Those that have include M. hominis (Bredt, 1974), M. gallisepticum (Maniloff et al, 1967), and M. pneumoniae (Boatman and Kenny, 1971). Boatman (1973) points out that, ideally, the growth and development of myco-

plasmas should be followed by light microscopy despite their minuteness owing to fixation artefacts that occur when preparing specimens for electron microscopy.

Bredt (1970) working on M. hominis, demonstrated the extraordinary heterogeneity of these organisms. In ten strains he recognised three basic forms.- These were coccoidal (0.3 to 0.8 µm in diameter), diploforms and filamentous forms (0.3 to 0.4 µm in diameter). Some doubt has been cast on the relevance of the morphology of cultured forms of mycoplasmas by Hirumi and Maramorosch (1973) who maintain that fixation of MLO in host tissues minimizes the alterations sometimes observed in mycoplasma preparations made from pure cultures. It has been recognized by these authors that high speed centrifugation affects both morphology and ultrastructure. Nevertheless, this method is frequently used to separate mycoplasmas prior to fixing the cells by various chemicals (Maniloff et al, 1965 and Maniloff, 1971). These problems have been discussed by Rodwell (1965) and Lemcke (1972). Bernstein-Ziv (1969 and 1971) describes the effects of centrifugation and fixation on the morphology of M. gallisepticum. Cells harvested at various periods during growth by centrifugation at 39,000 g for 10 minutes were examined by Bredt by negative staining. The negatively stained cells were apparently unfixed and, surprisingly, remained intact despite vacuum drying and exposure to the electron beam. Terminal 'blebs' were seen on some cells and short filaments. Subsequent work by Bernstein-Ziv (1971) on the effects of hypotonic solutions on cells of M. gallisepticum showed that filaments could be induced within one minute. In a few cases cells exposed to hypotonic solutions did not form filaments and this was also true for cells exposed to an isotonic

solution of 0.4 m sucrose even though subsequently transferred to a hypertonic fixative. These experiments emphasized the pliability of mycoplasmas. Morphological variations are probably only a reflection of the physical conditions under which organisms are grown or examined.

Black et al (1971) working on the morphology and ultrastructure of human T-mycoplasmas used a lengthy schedule involving first glutaraldehyde fixation, centrifugation, embedding in agar and secondary fixation using veronal acetate buffered osmium tetroxide. Most of the cells they observed were spherical or ovoid although rod shaped filamentous and partly branched forms were also observed. Work on M. bovirhinis by Boatman (1973) showed marked similarity to cultures of M. hominis. The cells are largely filamentous and despite the fact that a wide range of osmolalities were employed, no noticeable differences were reported in the final preparations. Kammer et al (1970) employed a scanning electron microscope as did Boatman and Kenny (1970) working on M. felis but found that the resolution capability of 20 nm was not sufficient to show surface detail. In general the morphology revealed by the scanning electron microscope was predominantly ovoid with lobulate, star-shaped and filamentous variations.

Observations of thin sections of mycoplasmas show that the cytoplasmic components are less subject to variation than the shape of the cells. With a few exceptions they consist exclusively of three components: a bounding 'unit' type membrane enclosing a cytoplasm containing randomly distributed ribosomes and a nuclear area devoid of ribosomes containing fibrillar material. Some species have additional features such as 'blebs' in M. gallisepticum (Morowitz and Maniloff, 1966 and Maniloff and

Morowitz, 1967), and striated structures (Biberfield and Biberfield, 1970, and Boatman and Kenny, 1971). These striated fibrils have been thought to resemble actin-like contractile proteins and are believed to be involved in cellular movements and cell division by virtue of their contractile nature (Razin, 1976).

3.3 Materials and Methods

The collection of specimens, sampling sites and preparation of material for light and electron microscopy has been described in Section Two(3).

Histochemical observations

Cryostat sections of alcohol fixed material were stained by a modified phloxine/tartrazine method in which zinc chloride was used (see Appendix 3). Acridine orange staining of cryostat sections were examined in a Leitz Orthoplan microscope equipped with fluorescence optics. The various steps are covered in Appendix 4.

3.4 Observations and Results

a) The appearance of normal digestive gland tissue

i) General features

Light and electron microscopical examination of the digestive gland cells of Tellina tenuis showed that they have two types of cells, absorptive and secretory, forming the lining of the tubules. The digestive diverticuli followed the pattern of the majority of bivalves in that they were divisible into three main regions. These are:

1) A dendritic system of main ducts arising from the caecum lined with non-ciliated epithelium.

2) Short unbranched secondary ducts lined with ciliated epithelium.

3) Irregularly branching blind-ending acini lined with secretory and digestive cells.

ii) The secretory cells

These were found in clusters of four to twelve cells in crypts round the lumen of the acini. Under the light microscope they were darkly staining basophilic pyramidal cells (Plates 72 & 87). Mitotic figures were often seen with the light microscope and pairs of cells with incomplete septa representing later stages in cytokinesis were frequently seen under the electron microscope (Plates 76 and 77).

Mature secretory cells appeared very similar to vertebrate pancreatic exocrine cells (Lima-de-Faria 1969) and they had the fine structure of protein-secreting cells. The basal nucleus was large with a conspicuous nucleolus (Plates 76 & 77). The endoplasmic reticulum was well developed and granular and a well defined Golgi body was situated in the centre of the cytoplasm above the nucleus and proximal to the lumen of the tubule. The Golgi cisterni gave rise to electron dense secretory vesicles which accumulated in the cytoplasm at the apex of the cell prior to release into the lumen (Plates 78 and 79).

The cells were fully differentiated with the typical permanent and semi-permanent features such as flagella and desmosomes Lysosomes were evident in the cytoplasm by bodies containing tubular crystalline arrays, ie., Owen's peroxisomes, were not observed.

iii) The absorptive cells

These cells were highly labile and it was found that their appearance varied greatly depending on the metabolic state of the animal at the time of fixation. These stages have been defined by J E Morton (1956) and McQuiston (1969) for Lasea rubra, a small beach clam, and have been defined as:

1) Formation
2) Absorption and phagocytosis
3) Digestion
4) Breakdown
5) Development and formation.

The most commonly observed phase during the present study was that of breakdown owing to the fact that animals were usually kept for two to three days after capture as indicated previously. Observations of Tellina fixed at various stages of the feeding cycle directly from the beach showed that the stages of the feeding cycle recognized in absorptive cells of other Lamelli-branchs such as Dreissena, Cardium, Anodonta, Macoma and Ostrea described by Morton (1969b, 1970 a, b and c, 1971) were present.

Plates 72 & 74 show absorptive cells during breakdown. Fragmentation spherules formed by large 'blebs' of cytoplasm budding off from the distal ends of these cells can be seen in the lumina of the tubules (Plates 72 & 80). These spherules contained numerous profiles of degenerate mitochondria. There was a marked absence of lysosomes or residual bodies such as described by Owen (1972). These cells had a well developed 'brush border' of microvilli (Plates 72, 80 &81). The cytoplasm contained numerous food vacuoles which appear to be at different stages of absorption by the cytoplasm. These electron dense

vacuoles were osmiophilic suggesting a high lipid content. In contrast to the secretory cells, there was a marked absence of Golgi profiles and endoplasmic reticulum. Mitochondria were numerous.

b) Host-parasite interactions

The MLO seems to have an affinity for one specific cell of one component of the digestive tract; inclusions have only been found in the secretory cells of the terminal acini of the digestive diverticula.

i) Light microscopy of the cytopathic effect caused by the MLO:

The first sign of infection appeared to be a small eosinophilic inclusion body lying above the nucleus and proximal to the lumen of the tubule. It was thought that the this small inclusion then enlarged until the normally pyramidal secretory cell was distended to three or four times normal size and became spherical in outline. Cells were then believed to burst leaving their skeletal outline as evidence of their former presence. These ghost cells were a diagnostic feature of heavily infected Tellina. (Plates 80 to 91 and 101 to 104).

Under the oil immersion objective of the microscope the contents of these inclusions appeared to be uniformly granular although occasionally distinct spherules staining with different intensity could be distinguished (Plates 93, 94 and 101-103).

The cell contents were avidly phloxinophilic (Plate 92) and fluoresced bright yellow to orange when stained by acridine orange (Plates 95-100).

The first observable histological change in the nucleus was the development of an "owl's eye" appearance (Girardi et al. 1966) The nuclei had a pale nucleoplasm and prominent nucleoli with pronounced margination, or rimming, of the chromatin (Plate 93).

ii) Electron microscopy of infected secretory cells

Electron micrographs showing secretory cells at various stages of degeneration are given in Plates 110-112 and 118.

The features of nuclear degeneration observed by light microscopy and described above are amplified by Plates 114, 115, 118 and 119.

Following infection by MLO the nuclear envelope appeared to become swollen and the nucleus to shrink which produced a characteristic lobed and irregular outline (Plate 119). Karyorhexis and finally karyolysis are represented in Plate 115

The cytoplasm of the secretory cells at an early stage of infection was granular and electron-dense. The cisterni of the rough endoplasmic reticulum became greatly swollen (Plate 111, 113 and 118) and profiles of endoplasmic reticulum became arranged in concentric whorls around the cytoplasmic inclusion body containing MLO. The expansion of this cytoplasmic inclusion body resulted in the mechanical compression of the cytoplasmic organelles against the cell membrane. The electron-dense nature of the necrotic remnants of the cytoplasm suggested coagulation of the protein elements (Sparks, 1972). Ultimately the cells were thought to rupture releasing MLO into the lumina of the tubules (Plates 101, 102 and 129). The persistent outline of these dead cells was a diagnostic feature of infected Tellina. The electron microscope showed that the margins of the dead cells were thickened with amorphous electron-opaque material Plate 73 shows the margin of one of these cells with the necrotic remnants of the nucleus adhering to the cell margin.

c) The morphology and ultrastructural features of the MLO

In the present study MLO were observed by negative staining following ultracentrifugation (Plates 147/8) and by phase

contrast light microscopy (Plates 102 & 103) as well as by transmission electron microscopy.

i) The cell boundary

The cell boundary appeared to be a trilaminar membrane typical of unit membrane structure and was composed of a light area about 5 nm thick bounded on either side by electron dense regions to give a membrane with an overall thickness of 11 nm. The outer layer appeared to be more dense than the inner layer and this is believed to represent the presence of a capsular substance (Domermuth et al, 1964a).

ii) The capsular matrix

A capsular matrix was seen in some preparations where phosphotungstic acid had been used to negatively stain MLO isolated from Tellina digestive gland homogenates (Plates 158 & 159). These closely resembled the globular sub-units described by Hummeler et al (1965). These may play a role in the attachment of mycoplasmas to cell surfaces prior to penetration.

iii) The cell contents

Numerous ribosomes having a mean diameter of 14 nm were loosely arranged within the plasma membrane. Ribosomes were largely absent from the region believed to contain the nuclear material. This region consisted of unbounded fibrillar and granular material. Membrane bound intracytoplasmic lacunae were often seen (Plate 122).

iv) Morphology

The basic form is a coccoid sphere 0.3 to 0.8 um in diameter. Diploforms and filamentous forms were occasionally seen

Plates 109, 123) as were small, densely staining 'blebs' attached by a narrow neck to the parent cytoplasm. The MLO could be typed according to their size and the electron opacity of the cytoplasmic matrix. These were as follows:

1) Small, compact spherules with highly opaque cytoplasmic matrices (elementary bodies)
2) Small, intermediate spherules containing moderately opaque cytoplasmic matrices (initial bodies)
3) Intermediate spherules with moderately opaque cytoplasm (intermediate bodies)
4) Large, intermediate spherules with low opacity cytoplasm (large bodies)
5) Large, fully developed spherules with low opacity cytoplasm - usually containing virus particles (giant bodies)
6) Filamentous forms, usually containing virus particles.

v) Replication

Replication of the MLO appeared to be by budding, fragmentation and binary fission. Cells thought to be undergoing binary fission are illustrated in Plates 108, 109, 113, 116 and 123.

Cytoplasmic fragmentation is illustrated in Plates 132 and 133 It is not certain that this is a form of reproduction or results from the presence of the virus.

Occasionally large filamentous forms (Plates 109 and 123) appeared to be undergoing fragmentation. It was thought that cytoplasmic division might lag behind genome replication resulting in multinucleate filaments. These later fragment to give simple cells. This method of replication is common amongst the Mycoplasmatales and is thought to be responsible for the characteristic 'chain of beads' appearance of filamentous forms.

a) Cyclical changes

The normal secretory and absorptive cells closely resembled those of other Lamellibranchiate species. The cyclical activity observed appears to conform most closely with that described by Langton (1975) for Mytilus edulis. In general there was a lack of synchrony. Tubules in some areas of the gland appeared coordinated with each other whereas in other areas they were not. This might be explained by food reaching various parts of the multibranched system at different times. For example, a small group of tubules served by one duct might receive food from the stomach before another group of acini served by another duct. Thus individual tubules might pass through the digestive cycle at different rates because of uneven distribution of food throughout the tubules (Plate 85).

b) Regeneration of the secretory cells

A study by Thomson et al (1974) on starvation of the mussel Mytilus edulis and its effects on the structure and function of the digestive gland showed that the secretory or basophil cells degenerate first. The cells became densely staining and the endoplasmic reticulum appeared disorganized. They distinguished between two types of basophil cell and concluded that the stress induced degenerative changes in the digestive tubules themselves do not affect the capacity of the tissue for recovery.

Mix and Sparks (1971) working on oysters found that the repair of the epithelial lining of the digestive tubules following irradiation was effected by division of the basiphil cells. Purchon (1971) considered that the means by which degenerate basophil cells were replaced is obscure.

Yonge (1926) considered that the basophil cells were immature

absorptive cells because of their high mitotic rate. Owen and Pal (1970 and 1971) found no evidence that basophil cells could give rise to absorptive cells. Thomson et al (1974) suggest that their flagellate basophil cell (Type II) may differentiate into a mature digestive cell under suitable conditions. Williams (1969) measured the biochemical composition of mussels in an attempt to determine the effect of the parasite Mytilicola intestinalis and found no difference between parasitized and non-parasitized animals. Williams concluded that analysis of whole animals or while tissues is least useful in assessing physiological condition not because analyses are insensitive but because the parameters he measured such as the carbohydrate/ protein ratio tend to be regulated independently of the degree of stress.

The infection of the basophil or secretory cells and the consequent destruction and atrophy of portions of the digestive gland did not appear to cause starvation and loss of condition of the host. It must be concluded that the rapid regenerative powers that are a function of the cyclical mode of feeding and digestion probably prevent the parasite from harming the host. It was generally found that older individuals of 7+ and 8+ year groups had an accumulation of inclusion bodies and a build up of brown cells in the haemocoele of the digestive gland. This suggested that the mycoplasma-like parasites probably accumulate with age in the host.

c) Artefacts and mycoplasma cell morphology

Any consideration of the morphology of mycoplasmas demands an awareness of the problems of alteration and distortion that may be caused by preparative techniques. Unfortunately, past

papers dealing with the morphology of these delicate organisms have not always given details of the composition of the fixing fluid, the osmolality of the fixative buffer combination and pH, temperature and duration of the fixation schedule. However, the fixation of the MLO in the host tissues probably did much to minimize alterations to the morphology of the cells. This approach to the morphology was recommended by Hirumi and Maramorosch (1973).

The effect of phage-virus (MVT) on morphology

The basic coccoid form having a diameter of 0.3 to 0.8 µm appeared to be invariate whether the organism was viewed by phase contrast electron microscopy, thin section electron microscopy or negative staining following density gradient centrifugation. The only exceptions to the basic morphology appeared to be found amongst those cells containing virus particles. These cells were distended and appeared to be two to three times the diameter of MLO in which virus particles could not be seen. Neither Cole (1976) nor Gourlay (1973) report similar swelling of mycoplasmas infected with phage (Plates 108 & 123). A description of the virus, its morphology, replication and buoyant density will be found in Section IV.

d) The cell wall of the MLO

The interpretation of structures resembling the early formation of a cell wall in those cells harbouring phage-virus particles suggested that the organism might be Rickettsial or Chlamydial rather than mycoplasma-like. However, close observation of the plasma membrane of those cells that did not contain virus particles or were at an early stage of infection failed to reveal any hint of a cell wall. From these observations it was concluded that either the additional layer of membrane is a host

response to the presence of virus or that the cells might be L-phase variants of bacteria that have become adapted to a parasitic intracytoplasmic mode of existence and are being stimulated to revert to walled forms under the influence of the phage. A third possibility exists that these virus-containing cells might be a different organism. However, this is not considered likely.

e) The capsular matrix

A capsular matrix has been found to surround many different species of mycoplasmas (Green and Hanson, 1973; Howard and Gourlay, 1974; Wilson and Collier, 1976). The matrix stains with ruthenium red, a dye that reacts strongly with acidic high molecular weight substances. The presence of carbohydrate on the cell surface of mycoplasmas is indicated by the lectin binding ability of many species (Kahane and Tully, 1975, cited in Razin, 1976). These carbohydrate groups may be part of a capsular carbohydrate polymer or membrane glycolipids, glycoproteins or lipopolysaccharides (Kahane and Marchesi, 1974; Smith et al, 1974). They are believed to be important antigenic determinants as well as playing some role in cell surface attachment. The capsular matrix of the MLO is thought to be that illustrated in Plates 151 and 152.

The membrane of M. pulmonis gives the appearance of being composed of globular sub-units (Hummeler et al, 1965) which bear a close resemblance to those observed in Tellina MLO.

f) The range of habitats

The range of habitats known to contain Mollicutes is very broad. Mycoplasma-like organism have been isolated fror sewage and soil. Acholeplasma laidlawii (Tully and Razin, 1968) has been

isolated from a wide variety of hosts as well as from soil and a thremophilic, acidophilic prokaryote has been discovered in self-heated coal refuse piles (Belly and Brock, 1973). The agent of yellows disease in plants has been identified as a mycoplasma-like organism (Maramorosch et al, 1970; Davis and Whitcomb, 1971 and Hill, 1971).

The plant MLO's have proved extremely difficult to cultivate. Razin (1973) attributes this to their intracellular location within the plant and the insect vector. Mycoplasmas in animals are rarely intracellular which Razin believes may indicate a stricter adaptation of the parasite to conditions which may be difficult to stimulate in a cell-free medium.

g) Intracellular parasites of undetermined taxonomic status from the aquatic environment and their possible relation with MLO from Tellina tenuis

There appear to be features of the MLO in Tellina tenuis that closely resemble the organisms causing a condition described as epitheliocystis reported by Hoffman et al. (1969) from the blue gill (Lepomis macrochirus) and Wolke et al.(1970) from the striped bass (Morone saxatilus) and white perch (Morone americanus). Recently, Paperna (pers. comm., 1976) has observed epitheliocystis organisms in Sparus aurata and Liza ramada which differ from the above in details of their fine structure although they cause similar hypertrophy of epithelial cells. In all the above cases the organisms apparently occupy a giant vacuole within living cells. The cytoplasm is pushed aside and forms a cortex. The final cyst formed by the organism's reproductive processes is contained within a single unruptured and greatly hypertrophied cell. Wolke et al. described an electron-dense structure bearing little resemblance to aunit membrane lying between the organisms within the cyst and the cyst wall or basement lamina.

They described this structure as being composed of connective tissue, fibres, nuclei and cellular organelles bound on their outermost surface by epithelial cells which appeared to be undergoing pressure necrosis. Wolke suggested that possibly the coalescence of several epithelial cells had resulted in what seems to be a single large cell surrounded by a corona of nuclei. Paperna noted that in S. aurata the final large cyst is similarly formed by the coalescence of several epithelial cells but that the corona of nuclei was separated from the plasmatic wall of the epitheliocystis inclusion by a distinct membrane. Paperna contrasted this with the form of the cyst wall in Liza ramada where the plasmatic layer itself was divided by desmosomes into cellular units while no distinct separation could be seen between the plasmatic layer and the outer layer containing the nuclei. These differences in the relationship between host and parasite probably reflect the biological and taxonomic diversity of the causative organisms of epitheliocystis.

h) An analysis of the various prokaryotic organisms that have been found to form cytoplasmic inclusions within a membrane bound vacuole

Anderson et al (1965) compared the ultrastructure of a mycoplasma, several rickettsiae and ornithosis "virus" (also known as organisms of the Psittacosis lymphogranuloma-trachoma group (PLT) or Chlamydozoaceae of the genus Bedsonia or Miyagawanella). All were capable of forming intracytoplasmic inclusions but the only organisms that were found contained within a membrane lined vacuole were Rickettsia sennetsu and the PLT organism. R. sennetsu differs from other rickettsias in this respect as well as in being pleomorphic, tightly packed or wrapped around each other in a tight ball. All other rickettsiae are free in the cytoplasm of the host and are usually cylindrical in shape.

All have a cell wall and a plasma membrane and contain ribosomes and DNA strands. The cell wall is frequently visualized as a rippled trilayered structure. Anderson et al found that the plasma membrane was frequently not well demonstrated in R. sennetsu and that this lead to the mistaken interpretation that the host membrane lining the vacuole was the cell wall and the cell wall was mistaken for the plasma membrane. The overall appearance of R. sennetsu is very similar to the initial bodies of the PLT organism (Mitsui, 1964). The intravacuolar position of both R. sennetsu and PLT organisms makes it clear that both are enclosed within a cell wall and a plasma membrane. In the PLT group the plasma membrane is never visualized in the elementary body stage although Anderson et al say that it seems probable that the trilayered structure seen in the elementary body is the cell wall and that the inner plasma membrane is retracted along with internal components and thus is not readily seen.

Wolke (1970) studies of the ultrastructure of the epitheliocystis associated organism refers to:

1) the intracytoplasmic location with a membrane lined inclusion;
2) the presence of both a cell wall and a plasma membrane;
3) the presence of four forms, i.e. giant bodies, initial bodies, intermediate and elementary bodies.

The presence of morphologic evidence that the agent of epitheliocystis is not Rickettsial but similar to the ornithosis agent is based on the reproductive forms observed by electron microscopy. Wolke acknowledges that this is a risky procedure but comes to the same conclusion as Hoffman (1964) that the epitheliocystis agent is probably a member of the Bedsonia group.

Paperna recognized several morphological units distinctly different from the Bedsonial or Rickettsial organisms. The individual units he saw that were comparable to elementary bodies of PLT organisms are formed as inclusion bodies of the mother unit, whereas in PLT organisms elementary bodies are formed through a succession of binary fissions or budding. He noted that the rounded units formed by binary fission in epitheliocystis from S. aurata remain interconnected by cytoplasmic bridges. Higashi (1955) reported plasmatic bridges interconnecting large units of PLT organisms after completion of binary fission.

In conclusion, it must be said that there are many features of the mycoplasma-like organism described in the present study that require further elucidation before the taxonomic position becomes clear. In referring to the organism as an MLO the following considerations have been taken into account:

1. The method of replication appears to follow that described for mycoplasmas

2. No viruses have yet been described from chlamydia or rickettsia whereas mycoplasmas are usually found in association with viruses.

3. The organism does not appear to have a cell wall when the virus is absent. The presence of virus seems to either induce the formation of a cell wall or to make the fact that a cell wall exists more visible. It may be that those cells that do not appear to have a cell wall might possess an inner plasma membrane that is not visible because it is closely adpressed to the outer wall.

4. Conventional tests using Giemsa or Ziehl-Neilson stains were negative for chlamydia and rickettsia.

The vexing question of the taxonomy can only be resolved by culturing the organism.

SECTION FOUR

SECTION FOUR: Studies on two viruses isolated from *Tellina tenuis*.

4.1 Introduction

At the start of these studies of intracellular parasites of *Tellina tenuis*, the cytoplasmic inclusion bodies observed in the digestive gland were originally thought to be caused by the presence of masses of virus-like particles observed by electron microscopy and described by Buchanan (1973). It was only later realized that these virus-like particles were, in all probability, phages and that the mycoplasma-like organism was the cause of the inclusions within the secretory cells. An explanation of this misconception may be sought in Plate 106 where it can be seen that the presence of paracrystalline arrays of virus-like particles quite obscures the cells of the host MLO. It was for this reason that Hill (1975) undertook a series of experiments with the object of isolating this virus in fish cell cultures. In due course Hill isolated a virus in an established cell line of bluefin fibroblasts. He called this isolate TV1. This was the first isolate of a virus from a marine invertebrate by cell culture. The question then arose as to whether TV1 was the same virus as that seen in the secretory cells of the digestive gland. Hill had proven the pathogeneity of this virus to the Pacific oyster, *Crassostrea gigas* and had reported the presence of masses of crystalline arrays of virus particles of the same size and shape as those seen in *Tellina*. The experiments described in this section were carried out in order (1) to establish the morphology of TV1 (Weymouth isolate) and (2) to compare this with isolates prepared from fresh material by density gradient centrifugation and to test the effects of TV1 and extracts of filtered *Tellina* digestive gland on an established cell line of Atlantic salmon fibroblasts.

4.2 Review of the literature on mycoplasma viruses

In view of the fact that mycoplasmas and MLO's are known to harbour viruses it seemed likely that the particles in the MLO from Tellina were phages. They will be referred to in the present study as MVT standing for mycoplasma virus of Tellina.

Cole (1976) reviewed the subject of mycoplasma viruses and described 9 morphologically distinct particles from 6 groups although infectivity has only been established for half of these, all from Acholeplasma laidlawii (Gourlay, 1971 and 1973). The lack of a suitable host culture system has prevented the demonstration of infectivity for the remainder. The following table summarises Cole's classification:

Virus Group	Host Species	Number of carrier strains
MVL 1	A. laidlawii, A. granularum etc.	50+
MVL 2	" "	3
MVL 3	" "	1
SVC 1	Spiroplasma citrii, CSO, SRO	14
SVC 2	" "	12
SVC 3	" " + SMCA	15
VLP's	M. hominis and various MLO's	6

Gourlay (1970) described the first virus to be isolated from a mycoplasma although their existence had long been suspected. Since then Gourlay (1973) has isolated a total of three morphologically distinct viruses all from strains of Acholeplasma laidlawii (MVL 1 - 3).

The first virus was designated MVL 1 and research with a similar L 1 agent (Liss and Maniloff, 1971) has shown that it is a detergent and ether resistant rod-shaped particle (16 x 90 nm) containing single-stranded DNA. It originates from and propagates in A. laidlawii (Gourlay, 1974; Maniloff and Liss, 1974).

MVL 2 was also isolated from A. laidlawii by Gourlay (1972).

The L 2 phage-virus is spherical, 80 nm in diameter, enveloped and ether sensitive and has double stranded DNA. Little more is known about the properties of MVL 2.

Gourlay (1973) isolated a third virus from a spontaneous plaque in a culture of A. laidlawii. MVL 3, as this has been designated, is a short tailed (25 nm) polyhedral (57 x 61 nm) ether resistant, double stranded DNA virus.

The numerous isolates described by Maniloff and Liss (1974), all of which resemble MVL, are considered by Gourlay to be strains of the 3 different Mycoplasmatales viruses that have so far been isolated. Maniloff and Liss dismiss the question of whether or not the various isolates are the same or different as secondary when compared to the poor state of our knowledge of any one of the fifty or more isolates of Mycoplasmatales viruses. Indeed, mycoplasma cell isolates are very uncertainly classified. They believe that most, if not all, mycoplasmas have a virus associated with them. Our knowledge is deficient to the extent that no serological studies have been performed on any isolate.

Cole et al (1974) first demonstrated the presence of 3 morphologically distinct virus-like particles in Spiroplasma citrii which they designated SVC 1, 2 and 3. S. citrii is the prototype genus and species of a new group of motile, helical, sterol requiring mycoplasmas which is the actiological agent of some diseases in plants and arthropods. The infectivity of all three virus-like particles of spiroplasmas has not been established, owing to the lack of an indicator system.

Cole (1976) pointed out that the confirmed presence of viruses, especially tailed phage-like particles, presents an opportunity to study virus-host interactions in cultivable membrane bound prokaryotes that are unencumbered by cell walls.

SVC 1 is a rod shaped particle (14-15 nm x 230-280 nm) and SVC 2 is a long tailed polyhedron (75-83 nm x 52-58 nm) indistinguishable morphologically from group B bacteriophages. SVC 3 is a short tailed polyhedron resembling MVL 3 although smaller (13-18 nm x 37-44 nm). Like A. laidlawii, S. citrii have been seen to carry all three strains simultaneously.

The biological significance of mycoplasmatales viruses was reviewed by Clyde (1973) who worked with virulent and avirulent strains of three species of mycoplasmas in an effort to characterize virus isolates in terms of direct or indirect influence on donor mycoplasma virulence. Clyde reached the conclusion that until a virus free indicator cell line is available it is dangerous to assume that the appearance of plaques in tests proves the presence of virus in samples. He cautioned that workers surveying different species of mycoplasmas for virus must be prepared to characterize presumed new viruses by classical methods as well as demonstrating some biological link between isolates and donor organisms. The donor organism that Clyde was referring to is Acholeplasma laidlawii on which all phage assays have been conducted.

b) Phage and L-forms of bacteria, Rickettsiae and Chlamydiae

The inability of phage to penetrate L-forms has been interpreted by the idea that in the absence of a bacterial cell wall phage receptor sites must be absent. Taubeneck (1961) observed that the transformation of Proteus mirabilis to its L-phase resulted in the loss of the ability to adsorb bacteriophage. However, phage is capable of maturation in the L-form if the bacterium is lysogenic prior to L-transformation (Taubeneck, 1963). There have been no records of phage-viruses in the Rickettsiae or Chlamydiae.

c) The viruses of marine invertebrates

A report by Hill (1975) on the properties of a virus isolated from Tellina tenuis was the first report of any virus being isolated in cell cultures from any marine invertebrate. There have been a number of reports of the presence of viruses or virus-like particles in marine invertebrates. Bang (1971) reported on a transmissible disease from the shore crab, Carcinus maenas. A further publication by Bang (1974) described the pathogenesis and auto-interference of a virus disease in crabs. Bonami et al. (1972) ascribed an epizootic disease of Ostrea edulis to an unidentified virus. Papers by Bonami and Vago (1971), Bonami Vago and Duthoit (1971) and Bonami et al. (1975) describe a disease of a decapod crustacean, Macropipus depurator, thought to be caused by a virus. Buchanan (1973) described virus-like particles in the digestive gland of Tellina tenuis. These are now thought to be phage particles infecting a mycoplasma-like organism that causes a disease of the secretory cells of the digestive diverticuli (this volume pp 84-105). Couch (1974) described the presence of free and occluded virus, similar to Baculovirus, in the hepatopancreas of the pink shrimp, and Farley et al. (1972) drew attention to the presence of a herpes-type virus in the nuclei of Crassostrea virginica. The morphological features of this virus closely resemble those of the Lucké virus associated with kidney tumours in the frog, (Fawcett, 1956, and Zabernard, Vatter and Mckinell, 1966). Their evidence suggested to them that the herpes-type infection in oysters is enzootic under ambient temperature conditions and that elevated temperatures favour the spread of the infection or activation of the infection from an occult to an overt phase - or both. Experimental studies of oysters as carriers of virus were carried

out by Hedstrøm and Lycke (1963) following on the outbreak of two epidemics of infectious hepatitis associated with the consumption of raw oysters. They found that oysters are able to separate and concentrate the virus during filter feeding. Moreover, the stability of the virus used in their experiments (poliovirus type 3) was considerably greater than the same virus free in seawater. Oysters did not cleanse themselves of virus particles when transferred to fresh uninfected water o

Rungger et al. (1971) described virus-like particles in association with lesions in the muscles of Octopus vulgaris. Oedematous tumours were found scattered throughout the musculature of the tentacles. Electron microscopy of the tumour tissue revealed hexagonal virus-like particles 120-140 nm long and about 100 nm wide although they must have been elongate rather than hexagonal to have those dimensions. The particles were aggregated and sometimes surrounded by a multi-layered membranous envelope

4.3 Materials and methods

The method of collection, transportation and holding of specimens was the same as that described in 2.3.1.

The following methods were employed for virus isolation by zonal density centrifugation:

a) Preparation of samples

The digestive glands were excised and suspended in 5 volumes of tris saline on ice. Homogenization was carried out either in a Ten Broek grinder by hand or mechanically by a Silversen's small-head blender. Initial clarification was carried out by centrifugation in an MSE bench centrifuge at 2,000 r.p.m. for 15 minutes. Portions of the supernatants containing virus in suspension were treated in the following ways:

i) stored at -20^{o}C until required;

ii) fixed by adding equal volumes of 10% buffered formalin and stored at room temperature;

iii) concentration of the virus by initial centrifugation in 20% sucrose for 1 hour at 35,000 r.p.m, collecting the pellet and resuspending in tris saline;

iv) shaken at room temperature with a detergent, 0.4 sodium deoxycholate, and spun at 2,000 r.p.m. again for 15 minutes.

v) shaken for 1 minute with trifluorochloroethane (Arklone, I.C.I.) followed by centrifugation at 3,000 rpm for 10 minutes. The upper aqueous layer thought to contain the majority of virus particles was separated from the lower fluorocarbon layer and layered onto the gradient.

b) Preparation of gradients

20%, 30%, 40%, 50% and 60% (w/v) sucrose solutions were prepared in tris saline (0.1 M NaCl, 0.01 M Tris and 0.001 M EDTA, pH 7.5).

The concentrations were adjusted accurately by means of an optical refractometer, Equal volumes of each solution were layered in the centrifuge tubes by successively adding lighter solutions over the 60% sucrose. The 6 ml MSE cellulose nitrate tubes were left overnight to allow diffusion into a linear gradient. Before centrifugation the tubes were overlaid with 1 ml of the extract and 'capped' with .2 ml of liquid sterile paraffin to prevent evaporation.

Another method used for the separation of virus was to layer 1 ml of the extract onto 4 mls of 20% sucrose overlying 1 ml 50% caesium chloride which acted as a "pad" at the base of the tube.

Caesium chloride gradients were prepared by dissolving 1.7 gm of CsCl in 3.5 ml of distilled water. 5 mls of the CsCl solution were placed in the 6 ml capacity centrifuge tubes, followed by 1 ml of the extract and again 'capped' with sterile liquid paraffin.

c) Centrifugation of the gradients

Sucrose gradients were run in an MSE 65 mk 2 super-speed ultracentrifuge using a titanium swing-out rotor holding 3 x 6 ml tubes (No 587389). The rotor head and centrifuge bowl were precooled to $4^{o}C$ and the samples spun down at 35,000 rpm (100,000g) for 1 hour in the case of sucrose gradients and 36 hours in the case of CsCl gradients.

d) Unloading the gradients

This was done by puncturing the base of each tube with a needle and collecting 0.3 ml fractions in vials. The refractive inded of each fraction was estimated and graphed and the vials containing visible opalescent bands (Plates 154, 155) were prepared for electron microscopy by the methods described below.

e) Preparation of grids for electron microscopy

The difficulty of wetting grids with diluent before staining was overcome ny adding 0.01% Bacitracin (Sigma) to the diluent. Coated grids were used throughout (Polaron 483 copper).

f) Methods of negatively staining preparations

For purified preparations containing troublesome amounts of sucrose the method adopted was as follows:

i) One drop of 0.01% Bacitracin was placed on the coated grid and left for 15 seconds. This was drained and replaced with one drop of virus solution and the grid allowed to dry

ii) The sucrose washing system was prepared by placing 3-4 layers of filter paper in the bottom of a small petri dish. An inverted plastic stopper for use as a well was placed in the petri dish and filled with a 0.1 M solution of ammonium acetate. Distilled water can also be used. A bridge of filter paper to form a wick between the well and the base is made and the whole moistened with the ammonium acetate or distilled water cleaning solution. The grid was placed film side uppermost on the wick and left for 30 minutes. When using distilled water alone, 15 minutes was usually found adequate for the removal of all the sucrose. Ammonium acetate seemed to work well in cleaning up dirty preparations.. This technique is a modification of a method devised by Mr. I.M.Roberts of the Plant Diseases Research Establishment , Dundee (pers. comm.)

iii) Following sucrose removal grids were placed film side down on a large drop of stain on paraffin film or a waxed slide and left for 3-5 minutes.

g) Types of stain used

i) Phosphotungstic acid (PTA) - a 1-2% aqueous solution (w/v) pH adjusted to 7.2 with NaOH. This was the stain

most commonly used as it is stable over a long period, is unaffected by buffer solutions and is a good all-round stain for most types of viruses. However PTA stains very poorly when sucrose is present and does not have high penetration powers.

ii) Ammonium molybdate - used as a 2% aqueous solution, pH was adjusted to 6.5 with IN NaOH. This stain did not have any advantages over PTA.

iii) Uranyl acetate - a 2% aqueous solution. This gave higher resolution than (i) and (ii) and excellent contrast. However, the stain precipitates if mixed with buffers above pH 4.5 (except ammonium acetate) and is unstable in strong tungsten light.

iv) Sodium silicotungstic acid, pH 9. This stain produced results of high resolution and contrast although grainy in comparison with PTA.

h) Preparation of Atlantic salmon monolayer cultures of cells for virus propagation

A monolayer culture of fibroblast-like Atlantic salmon (AS) cells (Nicholson and Byrne, 1973) was obtained from Flow Laboratories Limited. For transport purposes the cells were shipped as a 100% monolayer in a flask devoid of medium.

On receipt of the cells Eagle's minimal essential medium (EMEM) supplemented with 15% foetal bovine serum was added and the cells incubated at 22°C for 24 hours. (The protocols for the preparation of growth media, cell dispersants and buffers is given in the appendix.)

After 24 hours the growth media was decanted from the monolayer

and the cells rinsed in 1:5000 ethylene diamine tetrazolium (EDTA) in a phosphate buffered saline (PBSA) to chelate the remaining divalent ions and remove any remaining foetal bovine serum. After this washing the cells were dispersed with 0.25% T/E/P (Trypsin/EDTA/PBSA) solution (for details, see appendix). The action of trypsinization was followed under the low power of an inverted Leitz microscope as it was found that, if allowed to proceed too far, the action of the dispersant would free large areas of the cell sheet from the glass. Once individual members of the cell sheet had rounded up the trypsin/EDTA was removed and the action of the dispersant checked by the addition of a small quantity of fresh media. The cells were then dispersed by vigorous pipetting and scraping using a pasteur pipette with the tip bent over at right angles. Dilutions of the original cell suspension were made in growth media to give suspensions of approximately 10^4-10^5 cells per ml (Wood, 1972). From this first flask replicate 25 cm^2 plastic flasks were seeded with AS cells and incubated at 12°C. The recommended temperature for maximum cell growth is 25°C (Nicholson and Byrne, 1973) but cell cultures derived from poikilothermic vertebrates will survive and multiply over unusually wide temperatures (Wolfe and Quimby, 1969). (The temperature of 12°C was recommended by Dr Hill for the growth of TV1 in Blue fin and RTG-2 cells.) AS cells will grow and multiply throughout the range of 2-28°C although little growth occurs at 4°C (Nicholson and Byrne, 1973). Flasks of cells were stored at 4°C using only EMEM until required.

It was found that actively dividing cells forming sheets that were 80-90% confluent could be obtained in four days. After seven days the cells were passaged from 1 to 3 flasks. By

experience it was found that cells grown in 25 cm^2 disposable plastic flasks could be split 1:4 after one week at 12°C. Leaving confluent cell sheets for longer than fourteen days made passaging more difficult because of the tendency of the cell sheet to lift off and contract into ropey strands of tissue.

Nicholson and Byrne (1973) found that AS cells will multiply through a wide range of pH but that the optimum pH was 7.4. Earles BSS was chosen as a buffer as it has a higher bicarbonate content and higher buffering capacity than Hanks or Geys. It is particularly useful for cultures that produce much acid or have a large cell population.

i) Maintenance of AS cells

Long term maintenance can be achieved by incubating lightly seeded cultures at 4°C. Cultures of AS cells have been maintained in this manner for as long as 6 months without a medium change or loss of viability (Nicholson and Byrne, 1973). Active-growth can be restored by simply incubating the cultures at 20°C for several days. It has been reported that the preservation of cells by freezing at -70°C in glycerol is moderately successful (Wood, 1972).

j) Sources of inocula

Two sources of inocula were used in this experiment. The first was TV1 harvested and kindly supplied by Dr B J Hill from tissue culture at Weymouth. This had been stored at 4°C as the supernatant from the infected tissue culture and was the same specimen used for morphological studies by negative staining described in Section IV 4.4 Ex3. Three different inocula were prepared from the Weymouth sample:

1) Undiluted, unfiltered Weymouth isolate

2) Undiluted but filtered (Leitz - 2 µm filter) Weymouth TV1 isolate

3) Diluted x 100 in MEM, filtered (Leitz - 2 µm filter) Weymouth TV1 isolate.

The second source of Tellina virus was prepared from a natural population of T. tenuis from West Sands, St. Andrews, Fife. The excised digestive glands were homogenized in a Ten Broek grinder held in a beaker of ice for 15 minutes until the majority of cells were broken open. Smears examined under phase contrast (using a Leitz Laborlux microscope) showed only cell debris. After grinding the tissue one sample was frozen overnight and thawed the next day and centrifuged at 5,000 rpm on a bench centrifuge for 20 minutes. A second sample was centrifuged without freeze thawing. In both cases the supernatant was collected and diluted 1:10 with MEM and passed through a 0.45 um millipore filter to eliminate bacterial contamination.

k) Inoculation procedure

0.5 ml of the six different inocula was introduced onto the tissue culture monolayer and incubated for 15 minutes at room temperature before being discarded and replaced with growth medium. Four replicates were set up from each inoculum plus four controls.

l) Incubation

The inoculated flasks were incubated at 12°C and examined daily under a Leitz inverted microscope fitted with phase contrast optics for evidence of cytopathic effects (CPE).

m) Preparation for electron microscopy

After a marked CPE had developed the cell sheet was dispersed

by trypsinization. The cells were spun down at 1,200 rpm in a bench centrifuge and the supernatant removed with a pasteur pipette. The cells were fixed by resuspending them in 2.5% glutaraldehyde in sym-collidine buffer, pH 7.2 for 1 hour at room temperature. The cells were recentrifuged at 2,000 rpm for 10 minutes and post-fixed in 1% osmium tetroxide in buffer at 4°C. A drop of molten agar was then added to each centrifuge tube and the solidified pellet removed, rapidly dehydrated in a graded alcohol series and embedded in Araldite-Epon mix resin over two days. Sections were cut on an LKB Mk III ultramicrotome and viewed in an AEI Corinth microscope.

4.4 Observations and Results

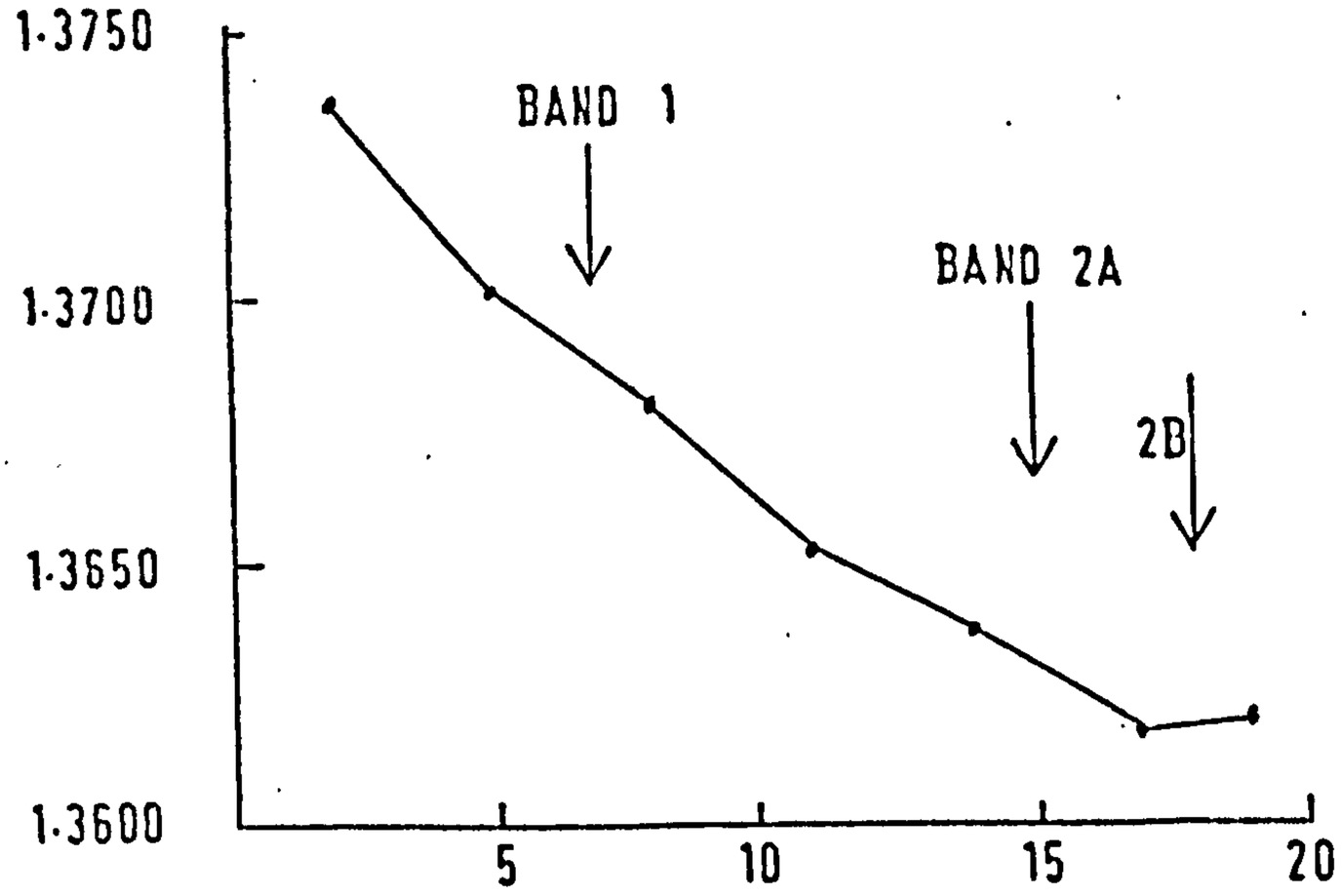
Experiment 1

The isolation of a virus from digestive gland homogenates of Tellina tenuis by density gradient equilibrium (isopyknic) centrifugation.

The methods have been described in 4.3.a-d.

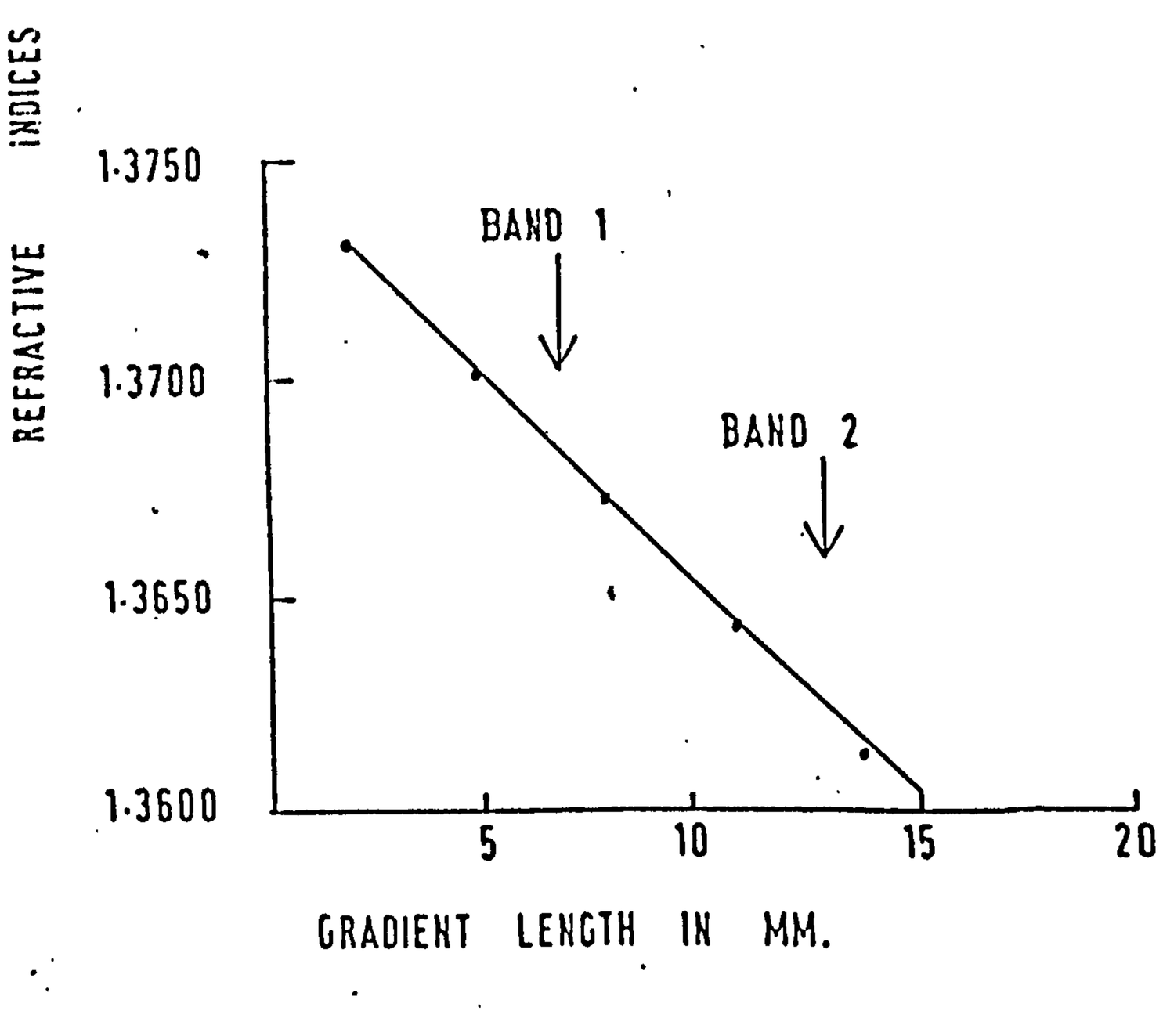
The effect of centrifuging 2 stock solutions, one washed in 0.4 sodium deoxycholate and the other unwashed in the detergent was to produce bands of the type shown in Plate 154. The buoyant density of the lower band in tube A was 1.38 and the top band was 1.32. In tube B the lower band was also 1.38 and the top band was 1.30. Graphs of the refractive indices of the fractions examined against height in the tube are given in text Figure 10

Electron microscopy of negatively stained preparations showed that the top band consisted exclusively of uniform rod-like particles that resembled the striated rods seen in thin sections of the mycoplasma-like organism (Plates 156 - 8). The



REFRACTIVE INDEX ρ

BAND 1	-1·3685	1·38
BAND 2A	-1·3630	1·32
BAND 2B	-1·3619	—



REFRACTIVE INDEX ρ

BAND 1	-1·3687	1·38
BAND 2	-1·3625	1·30

BOUYANT DENSITIES OF BANDS CENTRIFUGED THROUGH CAESIUM CHLORIDE TO EQUILIBRIUM FROM EXTRACTS OF TELLINA DIGESTIVE GLAND.

TEXT FIG. 10

dimensions of these rods were 150 nm to 200 nm in length and 20-50 nm in width. The striations of the rods occasionally appeared to be caused by the intersections of twin helically coiled fibres forming the structure of the rods.

The lower band was made up of densely staining particles of the type shown in Plates 152 & 157. These had a buoyant density of 1.38 and this is consistent with virus particles having DNA as their genome. The CsCl solution seemed to have a disruptive effect on these particles when compared with the results obtained from similar experiments using sucrose gradients.

Experiment 2

The isolation of a virus from sucrose gradients

Repeated attempts to form reproducable bands in the sucrose gradients were unsuccessful. Nevertheless it was usually possible to find virus-like particles by resuspending the pellet that collected at the base of the tube after centrifuging for 1 hour at 100,000 g. This experiment was repeated using stock solutions prepared in each of the methods outlined in 4.3.j. Methods (i), (ii), (iv) and (v) were discarded as either not giving reproducable results or not affecting the outcome of the experiments. A simple method was evolved that enabled repeated separation of a virus from the digestive gland homogenate.

Instead of preparing gradients as outlined in 4.3.b, 20% sucrose (w/v) was layered over 50% caesium chloride solution. The stock sample after initial clarification was spun through the sucrose at 100,000 g. An opalescent band was observed at the interface of the sucrose and caesium chloride solutions. This was removed by puncturing the side of the tube with a hypodermic needle and drawing off the opalescent band (Plate 160. The sample

was stained with phosphotungstic acid, pH 7.2 and the results are illustrated by the electron micrographs (Plates 152 & 153).

The particles have an estimated diameter of 55-60 nm and appeared to be spherical rather than hexagonal in outline.

Experiment 3

Observations on the morphology of a virus isolated from tissue culture by Hill (1975)

The virus used in this experiment was kindly supplied by Dr B J Hill of the M.A.F.F. Fish Diseases Laboratory at Weymouth.

Hill's virus was isolated in Blue fin fish tissue culture filtered from digestive gland homogenates prepared during the present study and known to contain virus particles of the type illustrated in Plates 136-141. These were believed at the time of these experiments to be the aetiological agent causing the lesions and necrotic foci in the digestive gland. It was subsequently realised that the lesions were produced by myco-plasma-like organisms which themselves contained virus particles (Plates 121 to 127).

Hill's virus (TV1) was stained by the following acid, neutral and alkali stains and examined on the Philips 301 electron microscope at the Regional Virus Laboratory, Ruchill Hospital, Glasgow, under the supervision of Dr C R Madeley.

i) Uranyl acetate (3% pH 4.5)
ii) Potassium phosphotungstate (3% pH 7.0)
iii) Sodium silicotungstate (3% pH 9.0)

The results are illustrated in Plates 136 to 141.

These viruses were hexagonal in outline and appeared to have 4 morphological units at the edge of each facet. The diameter

was measured at 59-65 nm.

Experiment 4

The effects of two virus isolates on an Atlantic salmon tissue culture

a) Cytopathic effects

Of all six virus suspensions incubated in AS cells only the Weymouth isolate of TTV gave a recognizable CPE. A summary of the CPE observed for each replicate is given below.

	1st pass	2nd pass	3rd pass	4th pass
1) Undiluted, unfiltered Weymouth TTV	+	+	+	-
2) Filtered Weymouth TTV	-	+	+	-
3) Diluted x 10, filtered Weymouth TTV	-	+	+	-
4) As above but freeze-thawed, 12 flasks	All negative, cell sheet healthy			
5) Undiluted, filtered St. Andrews	Contamination, cell necrosis			
6) Diluted x 10, filtered St. Andrews	No CPE, cell sheet healthy			
7) Diluted x 100, filtered St. Andrews	No CPE, cell sheet healthy			

In the absence of a suitable assay technique such as plaque formation or a susceptible overlay the only method available to determine the presence or absence of virus was electron microscopy. Pellets from tissue culture showing CPE were examined by thin section electron microscopy and the presence of numerous polyhedral hexagonal particles of approximately 60 nanometres was revealed (Plates 148 to 151). The particles were not found in cell nuclei, nor as paracrystalline arrays but

scattered throughout the cell cytoplasm. The results were closely similar to those of Moss and Gravell (1969) who investigated the ultrastructure and sequential development of IPN virus.

The appearance of the CPE began with focal contraction of the cells which was followed by progressive cytopathology. Two days after the onset of a CPE little remained of the cell sheet (Plates 142 to 147).

Freeze-thawing appeared to destroy the infectivity of the virus. The Weymouth isolate that had been subjected to freezing at -70°C followed by thawing did not produce any visible effect on the monolayers of AS cells.

b) Electron microscopy

Plates 148 & 149 showed that the ridges of the crumpled convoluted nucleus were packed with condensed chromatin. The cell centre was occupied by numerous smooth vesicles and elements of smooth endoplasmic reticulum together with vacuoles which appear to be coalescing (VA). Many appear to enclose 'plugs' of cytoplasm.

The Golgi apparatus had disappeared as a recognizable entity and the cisternae of the endoplasmic reticulum became flattened although certain segments were dilated. Dense bodies were considered to be acid-hydrolase-containing lysosomes or residual bodies.

Ribosomes were dispersed as sub-units and polyribosome clusters were absent.

Thecal formations of smooth membranes appeared to envelop plugs and peninsulas of cytoplasm. These ultimately became islands

surrounded by membrane in autophagic bodies.

Multiple concentric membranes, resembling myelin (Plates 150, 151), may have resulted from fusions of engulfed or invaginated membranous material during incorporation of virus into the cytoplasm of these AS cells. A similar appearance was described by Godman (1963).

4.5 Discussion

Experiment 1

The virus particles isolated by isopyknic centrifugation were of uniform size and shape. It was expected that it might be possible to isolate two viruses having (a) the morphology of Hill's TV1 isolate, and (b) the same size and shape as those seen in thin sections of the MLO (Plates 114-127). This was not possible. Only one type of particle could be found apart from those forming a distinctive band of buoyant density 1.32 and having a uniform rod like structure (Plates 156 and 159). It does not seem likely that these particles correspond with the striated rods seen in thin section (Plates 124 to 127) or that they are phage tails although it is conceivable that they might be. There is no evidence that the virus seen in thin sections of the digestive gland tissue is a tailed phage. Cole (1976) described spherical viruses from mycoplasmas. He calls these phage-viruses.

Experiment 2

Substantially the same results were obtained where sucrose gradients were used. It was usually possible to obtain reproducable results by these methods. Again the particles were of only one size and shape (Plates 152 and 153). No tailed particles were seen. No particles of the size and shape as those described as having been isolated from Tellina by Hill were seen whether using sucrose or caesium chloride gradients.

Experiment 3

Observations on the morphology of Hill's TV1 isolate suggest that there are two morphologically distinct viruses involved. The first can be isolated from digestive gland homogenates and corresponds with the morphology of the virus seen in thin sections in the MLO. This virus is presumed to be a phage. The second virus is hexagonal in outline and has a diameter of 60-65 nm. There is a distinct impression of four morphological units at each facet edge. It is not clear whether these are 'holes' or capsomeric units. The appearance of these viruses is intermediate between adenoviruses with their clear cut icosahedral form having 252 capsomeres and size of 65 nm and reoviruses having 92 morphological units and a diameter of 75 nm. Morphologically they most closely resemble the infectious pancreatic necrosis (IPN) viruses described by Moss and Gravell (1969). These viruses are of uncertain taxonomic affinities as there is some argument concerning the composition of the genome. Moss and Gravell (1969) believe it to be double stranded RNA whereas Hill (1975) favours single strandedness based on metachromatic staining properties (red/orange) when stained with acridine orange.

The properties and partial characterization of TV1 virus are described by Hill (1975). Hill (1976 in press) described 4 Tellina virus isolates which he has designated TV1-4; 3 viruses from oysters and 2 isolates from winkles and limpets (WV1 and LV1). A further isolate from the common shore crab Carcinus maenas is also mentioned. These appear to have very similar properties to TV1. Hill concludes that a group of viruses exist in the marine environment that have very similar properties and morphology as the IPN group. All can be

neutralized by polyvalent IPN antiserum. The site of replication within the viruses respective hosts is not yet known although Hill has shown that Crassostrea gigas (the Pacific oyster) that had been exposed to a single inoculum of tissue culture grown virus (TV1) showed a progressive increase in areas of specific fluorescence in the digestive gland post inoculation and that ultrathin sections of inoculated individuals revealed paracrystalline arrays of virus particles of the size and morphology of the virus associated with the MLO in Tellina although he did not specify whether these were secretory or absorptive cells.

Experiment 4

The virus induced abeyance of nuclear function (RNA and subsequently, DNA synthesis) is reflected in the very condensed chromatin and loss of turgor of the nucleus of the AS cells infected with TV1. The nucleus appeared to collapse and crumple. The disaggregation of polyribosomes was thought to be a visible reflection of the profound depression of cell protein synthesis by some product formed under viral direction.

The cytopathogenicity of TV1 closely resembled that described by Godman (1973) for Echovirus type 9 (Cocksackievirus A 25) in the cytoplasm of monkey kidney cells.

These preliminary observations suggest that AS cells are susceptible to TV1 but not to inoculi of filtered digestive gland homogenates from populations of Tellina tenuis known to carry heavy infections of the MLO containing virus MVT. Mycoplasma virus would not be expected to grow in tissue culture and so the absence of positive results is only to be expected if MVT is a phage.

4.6 Summary and conclusions

The following table sets out the properties of the two viruses from Tellina tenuis.

	Hill's virus (TV 1)	Mycoplasma virus (MVT)
1)	Can be propagated in AS fibroblasts, BF cells.	Does not affect AS fibroblasts
2)	Has not been found in any digestive gland homogenates by E.M.	Can be visualized in digestive gland homogenates regularly and repeatably
3)	Particles have a diameter of 60-65 nm.	Particles have a diameter of 55-60 nm.
4)	Particles are hexagonal in outline	Particles are spherical in outline
5)	Structural units appear as holes rather than capsomeres	Definite capsomeric substructure
6)	Buoyant density unknown. Thought to have single stranded RNA genome.	Buoyant density of 1.38 consistant with double stranded DNA containing viruses
7)	Site of replication unknown	Replicates within MLO

It is concluded that two viruses exist in Tellina tenuis One that is always found in association with a mycoplasma-like organism, designated MVT, with the properties listed in the table above; and a second virus (TV1) with properties that suggest to Hill that it is probably a member of the IPN group but for which the site of replication is unknown.

In view of the proven pathogeneity of the second virus (TV1) for the commercially important epicuristic bivalve, Crassostrea gigas, it is obviously a matter of urgency to investigate the aetiology of this new virus.

5. General Discussion

During recent years a great deal of attention has been focussed on shallow, sandy bottom animal communities. This has been motivated by three principle factors, firstly, the potential of these accessible areas of the sea bed for the artificial rearing of flatfish; secondly, the growing threat of marine pollution and lastly, the need for more basic data on the structure of communities in unpolluted coastal waters so that baselines can be set as a reference against which the effects of pollution may be assessed. Much of the work in Scotland in this field has been coordinated by the Department of Agriculture and Fisheries for Scotland at their Marine Laboratory in Aberdeen. This has lead to an upsurge of interest in the biology of Tellina tenuis as this bivalve is the overwhelmingly dominant species on most clean, sheltered sandy beaches around our coasts.

Most of the published accounts of this biology deal with the structure and distribution of populations, seasonal cycles in the growth and biochemical composition of individuals and the effects of predation by flatfish on growth and reproduction. There have been no studies on the effects of disease and parasitization on the specie and it was for this reason that the present study was undertaken. Extensive use of existing data on the Tellina population at Kames Bay, Millport, has been made in this study and against this baseline it was found that there had been little change in the last fifty years. Nevertheless, underlying this basic stability the age composition of the population fluctuates widely. Whole year classes can disappear and recruitment has been known to fail three years in a row. It now seems possible that the factors of disease and parasitization, previously discounted as unimportant alongside environmental influences, contribute significantly to these fluctuations.

Sindermann's (1970) review of recent widespread epizootics and mortalities in marine fish and shellfish classifies pathogens as primary or facultative. There is no evidence that either the coccidia or the MLO described in the present study are as virulent as primary pathogens such as Aerococcus viridans which causes "gaffkaemia' in lobsters, or the haplosporidan protozoan Minchinia nelsonii responsible for the widespread mortalities amongst oysters on the U.S. Atlantic coast (Farley, 1968). It seems more likely that the MLO, and perhaps Hill's TV 1 virus, can be classified with the facultative pathogens such as vibrios pseudomonads, aeromonads, pasteurellas and viruses (Couch, 1974; Snieszko, 1974; Farley et al., 1972).

These facultative pathogens have attracted much attention because of the rapid upsurge of interest in mariculture. They are believed to attack weakened or stressed individuals and have been observed to cause severe mortalities in conditions of overcrowding, poor water quality and temperature abnormalities. Most of the commercially exploited species are pelecypod molluscs such as oysters, clams and mussels. It seems likely, from observations made in this study, that Tellina, in the natural environment, can experience all the above conditions given a summer of high rainfall and low sunshine. If such a summer followed 2 or 3 years of optimal conditions during which the population density had gradually built up, a situation could arise of overcrowding and consequent starvation. Add to this brackish water conditions and temperature fluctuations so that the animals are under severe physiological stress, then conditions are probably ripe for the rapid spread of a facultative pathogen such as the MLO is believed to be. The destruction of the secretory cells of the digestive gland faster than they can be replaced by the host will inevitably lead to starvation and death.

There have been many reports of oyster mortalities where it has not been possible to ascribe an aetiological agent. Examples of these are "Opening Disease" of Australian oysters which has caused losses of upt to 80% of the stock, "Matsushima Disease" in Japan and mortalities of Long Island oyster spat. These are only three of the several examples cited by Korringa (1975) in a recent review of the problems of oyster farming. He mentions that most of these diseases have been attributed to viral agents. Until Hill's (1975) report of the isolation of TV 1, there had been no reports of virus isolation from any marine invertebrate but now we have grounds for optimism that mollusc viruses are amenable to culture in fish cell lines. We may now hold the key to at least some of the mysterious mortalities of unknown aetiology. Unfortunately, evidence of actual disease has not yet been linked to Hill's isolation of a virus from Tellina tenuis.

Apart from the observations of Hoffman et al.(1964) and Wolke et al (1970) and Paperna (1976, pers comm.) there appear to be no records of intracellular bacteria from fish or shellfish. A report by Glude (1975) of intracellular inclusion bodies associated with mortalities of Pacific oysters (Humboldt Bay disease) is believed to be a stage in the life cycle of the amoeba Vahlkampfia. It seems likely that intracellular bacteria have been overlooked in the search for pathogens owing to their cryptic and refractory nature. It would be unreasonable to suppose that this form of parasitization is unique to Tellina, indeed, the indications from recent observations are that this type of cytoplasmic inclusion body can be found in Tellina fabula Donax vittatus and Mytilus edulis. These observations have not yet been confirmed by electron microscopy. Further progress in this field depends upon finding a method of culturing the

causative organisms. Unfortunately, intracellular bacteria are only amenable to culture in embryonated eggs or in cell monolayers. To date no molluscan cell lines are available and the temperature required for egg incubation appears to present an unsurmountable obstacle.

Host-parasite relations

Intracellular parasitism represents the ultimate in ecological specialization for nothing less than the intact living host cell satisfies the nutritional and environmental needs of the parasite. Perhaps the greatest problem posed to parasitologists is the nature of the relationship between the host and the parasite. Trager (1974) has suggested that the most exciting and challenging field in parasitology seems to be to discover how intracellular parasites induce one host to provide shelter but not a closely related species. We do not know how the invader escapes the host cell's digestive processes or yet how they gain entry to the host cells. It is felt that the most important result of the present study has been the discovery of Tellina tenuis as an animal approaching the ideal for future experimental studies in this field. Here we have an animal that exists in huge numbers between the tide levels and harbours a variety of intracellular parasites. Its small size enables it to be readily transported and maintained in aquaria. The animals survive well without feeding for 3-4 weeks in aerated tanks of seawater provided the temperature does not exceed 10°C. The fact that Tellina tenuis is not a commercially or epicuristically valuable specie is unimportant as the answers to these questions on the nature of the host-parasite relations will be applicable to all living systems both verterbrate and invertebrate, plant and animal owing to the fundamental unity of living systems at the molecular level.

The presence of large numbers of coccidian oocysts and trophozoites both in the kidney and the ovary of Tellina presents an opportunity to study the inflammatory response to these parasites. No definitive studies appear to have been conducted into how amoebocytes combat infection in natural diseases. Most of the work on this topic has dealt with the ingestion of all sorts of particulate matter injected into oysters mainly. Malek and Cheng (1974) have emphasized the need for more research in this area because of its fundamental importance in the field of applied malacology.

Both Sprague (1971) and Sindermann (1975) have made urgent pleas for more basic research into diseases of marine organisms and emphasize that the agents of infectious disease, as well as physical and chemical pollutants, are important factors in any ecosystem. Biological factors have largely been neglected because of preoccupation with the problems of pollution ecology. They stress the need for gathering information about the life history and ecology of disease agents so that vital information can be accumulated regarding the vulnerable stages and restrictive environmental requirements such as salinity and temperature. Sprague stated that "... the basic problems (of disease) are likely to be solved only by sustained and concentrated effort on the part of many specially trained people over a long period of time".

Many and various were the parasites and symbionts encountered between the shells of Tellina during the course of these studies; such as the metacercarian Cercaria strigata; the encysted cercaria of the echinostomatid Himasthla; a Dicyemid mesozoan of the ovary; numerous ciliated protozoa - mostly hymenostomes and hypotrichs; parasitic copepods and a species of Nematopsis. Each represented a fascinating thesis problem on its own and it was hard to resist the temptation to turn aside into these rich fields.

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REFERENCES

REFERENCES

Anderson, D.R., H.E.Hopps, M.F.Barile and B.C.Bernheim, 1965. Comparison of the ultrastructure of several Rickettsiae, Ornithosis virus and Mycoplasma in tissue culture. J. Bacteriol. 90 (5): 1387-1403

Ansell A.D., 1972. Distribution, growth and seasonal changes in biochemical composition for the bivalve Donax vittatus (da Costa) from Kames Bay, Millport. J. exp. mar. Biol. Ecol. 10: 137-150

Ansell A.D. and Lander K.F., 1967. Studies on the hard-shell clam Venus mercenaria in British waters, III. Further observations on the seasonal biochemical cycle and on spawning. J. Appl. Ecol. 4: 425-435

Ansell A.D. and Trevallion A., 1967. Studies on Tellina tenuis (da Costa) I. Seasonal growth and biochemical cycle. J. exp. mar. Biol. Ecol. 1: 220-235

Bang F.B., 1971. Transmissible disease, probably viral in origin, affecting the amebocytes of the European Shore Crab Carcinus maenas. Infect. Immun. 3: 617-623

Bang F.B., 1974. Pathogenesis and auto-interference in a virus disease of crabs. Infect. Immun. 9: 1057-1061

Barnett P.R.O., 1969. Affects of temperature on the benthos near Hunterston Power Station. Chesapeake Science, 10: 255-256

Barnett P.R.O., 1971. Some changes in intertidal sand communities due to thermal pollution. Proc. Roy. Soc. Lond. B. 177: 353-364

Becker C.D. and G.B.Pauley, 1968. An ovarian parasite (protista incertae sedis) from the Pacific Oyster Crassostrea gigas (Thunberg). J. Invert. Pathol. 12 (3): 425-437

Belly R.T., B.B.Bohlhool and T.D.Brock, 1973. The genus Thermoplasma. Ann. N. Y. Acad. Sci. 225

Bernstein-Ziv R., 1969. Cell division in Mycoplasma gallisepticum. Can. J. Microbiol. 15: 1125-1123

Bernstein-Ziv R., 1971. The effect of hypotonic solutions on the morphology of cells of Mycoplasma gallisepticum. Can. J. Microbiol. 17: 1203-1205

Biberfield, G. and P.Biberfield, 1970. Ultrastructural features of Mycoplasma pneumoniae. J. Bacteriol. 102: 855-861

Bishop R.F., G.P.Davidson, I.H.Homes and B.J.Ruck, 1974. Detection of a new virus by electron microscopy of faecal extracts from children with acute gastro-enteritis. The Lancet, 74 (1) No. 7849: 150

Black F.T., A.Birch-Andersen and E.A.Freundt, 1972. Morphology and ultrastructure of human T-Mycoplasmas. J. Bacteriol. 111: 254-259

Boatman E., 1973. Morphology and ultrastructure of Mycoplasmas. Ann. N. Y. Acad. Sci. 225: 172-180.

Boatman E.S. and G.E.Kenny, 1970. Three dimensional morphology, ultrastructure and replication of Mycoplasma felis. J. Bacteriol. 101: 262-277

Boatman E.S. and G.E.Kenny, 1971. Morphology and ultrastructure of Mycoplasma pneumoniae spherules. J. Bacteriol 106: 1005-1015.

Bonami J.R. and C.Vago, 1971. A virus of a new type pathogenic to crustacea. Experentia 27: 1361-1364

Bonami J.R., C.Vago and J.L.Duthoit, 1971. Une maladie virale chez les crustaces decapodes due a un virus d'un type nouveau. C.R. Acad. Sci. 272: 3087-3091

Bonami, J.R., H.Grisel, C.Vago and J.L.Duthoit, 1972. Recherches sur une maladie epizootique de l'huître plate Ostrea edulis L. Rev. Trav. Inst. Pêches Mar. December issue

Bonami, J.R., J-C.Veyrunnes, F. Cousserans and C.Vago, 1975. Virologie des Invertebres. Ultrastructure, developpment et acide nucleique du virus S du crustace decapode Macropipus depurator. C.R.Acad. Sci. 280: 359-361

Boettger C.R., 1952. Grossenwachstum und Geschlechsreife bei schnecken und pathologischer Reisenwuchs als Folge einer gestorten Wechselwirkung beider Faktoren. Zool. Anz. Suppl. No. 17: 468-487

Bove J.M. 1976. Plant and Insect Mycoplasmas. Proc. Soc. Gen. Microbiol. III (4): 139

Bredt W. 1974. Les Mycoplasmes. Les Colloques de l'Institut National de la Sante et de la Recherche Medicale, 33: 47-52

Buchanan J.S., 1973. Electron microscope observations of virus-like particles in the digestive gland of a marine bivalve Tellina tenuis (da Costa). Proc. 5th. Int. Colloquium on Insect Pathology and Microbial Control. p 28

Caddy J.F., 1967. Maturation of gametes and spawning in Macoma balthica Can. J. Zool. 45 (6): 955-963

Cassie R.N., 1950. The analysis of polymodal frequency distributions by the probability paper method. N.Z. Sci. Rev. 8: 89-91

Cheng T.C., 1963. Histological and histochemical studies on the effects of parasitism of Musculium partumeum (Say) by the larvae of Gorgodera amplicava (Looss). Proc Helminthol. Soc. Wash. 30: 101-107

Cheng T.C., 1964. The Biology of Animal Parasites. W.B.Saunders & Co.

Cheng T.C., 1967. Marine molluscs as hosts for symbioses: with a review of known parasites of commercially important species. Advan. Mar. Biol. 5: 1-424

Cheng T.C., 1971. Enhanced growth as a manifestation of parasitism and shell deposition in parasitized molluscs. In "Aspects of the Biology of Symbiosis" (T.C.Cheng, Ed.), 103-137. University Park Press, Baltimore.

Cheng T.C., 1973. General Parasitology. Acad. Press N.Y.

Cheng T.C. and R.W.Burton, 1965. Relationships between Eucephalus sp. and Crassostrea virginica: Histopathology and sites of infection. Chesapeake Science, 6: 3-16

Christensen J.F. and D.A.Porter, 1939. A new species of coccidium from cattle, with observations on its life history. Proc. Helm. Soc. Wash. 6: 45-48

Clarke R.B. and A.Milne, 1955. The sub-littoral fauna of two sandy bays on the Isle of Cumbrae, Firth of Clyde. J. mar. Biol. Ass. U.K. 34: 161-180

Clyde W.A., 1973. On the biologic significance of Mycoplasmatales viruses. Ann. N.Y. Acad. Sci. 225: 159-160

Coe W.R., 1953. Resurgent populations of littoral marin invertebrates and their dependence on ocean currents and tidal currents. Ecology, 34: 225-229

Coe W.R., 1956. Fluctuations in populations of littoral marine invertebrates. J. Mar. Res. 15: 212-213

Coelho M.V., 1954. Acao des formas larvarias de Schistosoma mansoni sobre a reproducao de Australorbis glabratus. Publ. Avulsas Inst. Aggeu Magalhaes Recife, Brasil 3: 39-53

Cole H.A., 1956. Oyster cultivation in Britain. Min. of Agric., Fish and Food. H.M.S.O., London. 43pp.

Cole R.M., J.G.Tully, T.J.Popkin and J.M.Bove, 1973. Morphology, ultra-structure and bacteriophage infection of the helical mycoplasma-like organism Spiroplasma citri cultures from stubborn disease of citrus. J.Bact. 115: 367-86.

Cole R.M., 1976. Mycoplasma viruses. Proc. Soc. Gen. Microbiol. III (4): 139

Cooley N.R., 1962. Studies on Parorchis acanthus (Trematoda, Digenea) as a biological control for the Southern Oyster drill, Thais haemostoma Fish. Wildl. Serv., Fish. Bull. U.S., 62: 77-91

Dakin W.J., 1911. Notes on a new coccidian Merocystis kathae (n. gen. et sp.) occuring in the renal organ of the Whelk. Arch. Protist XXIII: 145

Dales S., 1963. Association between spindle apparatus and reovirus. Proc. Natl. Acad. Sci. U.S. 50: 268-275

Davies E.E. and R.E.Howells, 1971. A pathogen of the malaria parasite. Trans. Roy. Soc. Trop. Med. Hyg. 65: 13-14

Davis L.R., 1973. Techniques, ch 10: 411-458. In "The Coccidia" (ed. D.M.Hammond). University Park Press & Butterworths, London.

Davis R.E. and R.F.Whitcomb, 1971. Mycoplasmas, Rickettsiae and Chlamydiae: possible relation to Yellows Disease and other disorders of plants and insects. Ann. Rev. Phytopathology 9: 119-154

Dasgupta B., 1968. A possible virus disease of the malarial parasite. Trans. Roy. Soc. Trop. Med. Hyg. 62: 730

Dasgupta B., 1971. A virus disease of the malaria parasite. Trans. Roy. Soc. Trop. Med. Hyg. 65: 541

Dinamani P. 1957. On the stomach and associated structures in the backwater clam Villorita cyprinoides (Gray) var. cochinensis (Harley). Bull. Cent. Res. Inst. Univ. Kerala. 5: 123-148

Dobell C., 1925. The life history and chromosome cycle of Aggregata eberthi Parasitology 17:1 1-136

Dolfuss R., 1921a. Resume de nos principales connaissances pratiques sur les maladies et les ennemis de l'huitre. Notes Mem. Off. Sci. Tech. Peches Marit, 7: 46 pp.

Domermuth C.H., M.H.Nielsen, E.A.Freundt and A. Birch-Andersen, 1964 Ultrastructure of Mycoplasma species. J. Bacteriol. 88: 727-744

Edward D.G. and E.A.Freundt, 1956. The Classification and Nomenclature of Organisms of the Pleuropneumonia group. J. Gen. Microbiol. 14: 197-207

Edward D.G. and E.A.Freundt, 1969. Classification of the Mycoplasmatales p. 147-200. In L.Hayflick (ed.), The Mycoplasmatales and the L-phase of bacteria. Appleton-Century-Crofts, N.Y.

Elmhirst R., 1931. Studies in the Scottish marine fauna: The Crustacea of the sandy and muddy areas of the tidal zone. Proc. Roy. Soc. Edinb. 51: 169-175

Etges F.J. and W.Gresso, 1965. Effect of Schistosoma mansoni infection upon fecundity in Australorbis glabratus. J. Parasitol. 51: 757-760

Farley C.A., W.G.Banfield, G.Kasnic Jr., and W.S.Foster., 1972. Oyster Herpes Type Virus. Science 178: 759-760

Fawcett D.W., 1956. Electron microscope observations on intracellular virus-like particles associated with the cells of the Lucke Renal Adenocarcinoma. J. Biophys. Biochem. Cytol., 2: 725-742

Fisher Sir R.A.E. and F.Yates, 1953. Statistical tables for Biological, Agricultural and Medical Research. 4th. Ed. (Oliver and Boyd) London.

Fretter V. and A. Graham, 1964. Reproduction. In: Physiology of Molluscs. eds. K.M.Wilbur and C.M.Yonge, 127-164. New York. Academic Press.

Freundt E.A., 1970. Morphology and ultrastructure of the Mycoplasmas. p. 29-81. In: J.T.Sharp (ed.), The Role of Mycoplasmas and L-forms of Bacteria in Disease. Thomas, Springfield. Ill.

Freundt E.A., 1976. Species Differentiation of Mycoplasmas. Proc. Soc. Gen. Microbiol. III (4): 137-138

Gabbott P.A., 1975. Storage cycles in marine bivalve molluscs. A hypothesis concerning the relationship between glycogen metabolism and gametogenesis. Proc 9th. Europ. Mar. Biol Symp. ed. H.Barnes. p 191-211 Aberdeen University Press.

Galluci B., 1971. Fine structure of fertilization and ookinete formation in Haemaproteus columbae Kruse. J. Protozool. 18: (Suppl.), 29

Garnham P.C.C., R.G.Bird and J.R.Baker, 1962. Electron microscope studies of motile stages of malaria parasites II. The ookinetes of Haemamoeba and Plasmodium. Trans. Roy. Soc. Trop. Med. Hyg. 56: 116-120

Garnham P.C.C., 1969a. The structure and function of the cytostome (micropyle) in the Sporozoa. In: Progress in Protozoology, 8: (Abstracts of papers read at the IIIrd. Int. Congr. on Protozoology). Leningrad: Academy of Sciences of the USSR, Publishing House, Nauka.

Garnham P.C.C., R.G.Bird, J.R.Baker, S.S.Desser and N.M. El-Nahal, 1969. Electron microscope studies of motile stages of malaria parasites VII. The ookinete of Plasmodium berghei yoelii and its transformation into the early oocyst. Trans. Roy. Soc. Trop. Med. Hyg. 63: 187-194

Girardi A.J., V.V.Hamparian, N.L.Somerson and L.Hayflick, 1965. Mycoplasma isolates from primary cell cultures in human diploid cell strains. Proc. Soc. Exp. Biol. Med. 120: 760-770

Glude J.B., 1974. A summary report of Pacific Coast oyster mortality investigations 1965-1972. Proc. 3rd. U.S.Japan Meeting on Aquaculture at Tokyo, Japan, October 15-16, 1974. pp 1-28

Godman D.C., 1973. Picornaviruses. In: A.J.Dalton and F. Hagenau, eds., Ultrastructure of animal viruses and bacteriophages, An atlas. Vol. 5. Academic Press. N.Y.

Gomatos P.J., I.Tamm, S.Dales and R.U.Franklin, 1962. Reovirus Type 3: Physical characteristics and interaction with L cells. Virology 17: 441-454

Gourlay R.N., 1970. Isolation of a virus infecting a strain of Mycoplasma laidlawii. Nature 225: 1165

Gourlay R.N., J.Bruce and D.J.Garwes, 1971. Characterization of Mycoplasmatales Virus laidlawii 1. Nature New Biol. 229: 118-119

Gourlay R.N., 1972. Mycoplasmatales Virus-laidlawii 2. A new virus isolated from Acholeplasma laidlawii. J. Gen. Virol. 12: 65-67

Gourlay R.N., D.J.Garwes, J. Bruce and S.G.Wyld, 1973. Further studies on the morphology and composition of Mycoplasmatales Virus-laidlawii 2 J. Gen. Virol. 18: 127-133

Gourlay R.N. and S.G.Wyld, 1973. Isolation of Mycoplasmatales Virus-

laidlawii 3. A new virus infecting Acholeplasma laidlawii.
J. Gen. Virol 19: 279-283

Gourlay R.N., 1973. Mycoplasmatales viruses. Ann. N. Y. Acad. Sci 225: 145-148

Gourlay R.N., 1975. Mycoplasmatales Virus-laidlawii 3
J. Gen. Virol 29(1): 11-23

Green F. and R.P.Hanson, 1973. Ultrastructure and capsule of Mycoplasma meleagridis. J. Bacteriol. 116: 1011-1018

Gustafson P.V., H.D.Agar and D.J.Cramer, 1954. An electron microscope study of Toxoplasma. Amer. J. Trop. Med. Hyg. 3: 1008-1021.

Hammond D.M., 1964. "Coccidiosis of cattle. Some unsolved problems." Thirtieth faculty Honor lecture. The Faculty Association, Utah State University.

Hammond D.M., 1970. The development and ecology of coccidia and related intracellular parasites. In: Ecology and Physiology of Parasites - a symposium. ed., A.M.Fallis. University of Toronto.

Hammond D.M. and P.L.Long, 1973. The Coccidia. Eimeria, Isospora, Toxoplasma and related genera. University Park Press, Baltimore & Butterworths, London.

Hancock D.A., 1965. Graphical estimation of growth parameters. Journal du Conseil 29: 340-351

Harding J.P., 1949. The use of probability paper for the graphical

analysis of polymodal frequency distributions. J. mar. biol. Ass. U.K. 28: 141-153

Hedgpeth J.W., 1954. Bottom communities of the Gulf of Mexico. U.S. Fish and Wildlife Service, Fish. Bull. 89:(55): 203-214

Hedgpeth J.W., 1957. Sandy beaches. In: Treatise on Marine Ecology and Palaeoecology, volume 1. Mem. Geol. Soc. Am. 67: 587-608

Hedstrom C-E. and E Lycke, 1964. An experimental study on Oysters as virus carriers. Am. J. Hyg. 79: 134-142

Higashi N., 1965. Electron microscopic studies on the mode of reproduction of the trachoma virus and psittacosis virus in cell culture. Exptl. Mol. Pathol. 4: 24-39

Hirumi H. and Maramarosch K., 1973. Ultrastructure of the Aster Yellows agent: Mycoplasma-like bodies in sieve tube elements of Nicotiana rustica Ann. N. Y. Acad. Sci. 225: 201-221

Hill

Hill B.J., 1975. Properties of a virus isolated from the bivalve mollusc Tellina tenuis (da Costa). In: Wildlife Diseases ed., L.A.Page. Plenum Press, N.Y. and Lond. 445-452

Hoffman G.L., C.E.Dunbar, K.Wolf and L.O.Zwillenberg, 1969. Epitheliocystis, a new infectious disease of the bluegill (Lepomis macrochirus). Antonie van Leeuwenhoek 35: 146

Holme N.A., 1950. Population dispersion in Tellina tenuis (da Costa) J. mar. biol. Ass. U.K. 29: 267-280

Honigberg B.M., W.Balamuth, E.C.Bovee, J.O.Corliss, M.Godjics, R.P.Hall, R.R.Kudo, N.D.Levine, A.R.Loeblich Jr., J.Weiser and D.H.Wenrith, 1964. A revised classification of the phylum Protozoa. J. Protozool. 11: 7-20

Howard C.J. and Gourlay R.N., 1974.

Hopkins S.H., 1957. Interrelations of organisms, B. Parasitism. In: "Treatise on Marine Ecology and Palaeoecology (J.W.Hedgpeth, ed.) Vol. 1 Ch. 15. Geol Soc. Am., N.Y. Memoir 67

Hummeler K., N.Tommasine and L.Hayflick, 1965. Ultrastructure of a Mycoplasma (Negroni) isolated from human leukemia. J. Bacteriol. 90 (2): 51 -523

Hurst C. 1927. Structural and functional changes produced in the gastropod mollusc Physa occidentalis in the case of parasitism by the larvae of Echinostoma revolutum. Univ. Calif., Berkeley Publ. Zool. 29: 321-404

Jacobs L., 1967. Toxoplasma and toxoplasmosis. Adv. Parasitol 5: 1-45

Jamison R.M. and H.D.Mayor, 1965. Acridine Orange staining of purified Rat virus strain X 14. J. Bacteriol 90: 1486-1488

Jones N.S., 1950. Marine bottom communities. Biol Reviews 25: 283-313

Kahane I. and V.T.Marchesi, 1973. Orientation of proteins in mycoplasma and erythrocyte membranes. Ann. N.Y. Acad. Sci. 225: 36-45

Kahane I and J.G.Tully

Israel J. of Med. Sci. 11: 499

Kammer G.M., J.D.Pollack and A.S.Klainer., 1970. Scanning beam electron microscopy of Mycoplasma pneumoniae. J. Bacteriol 104: 499-502

Klienberger-Nobel E., 1962. Pleuropneumonia-like organisms (PPLO): Mycoplasmataceae. Academic Press Inc. N.Y.

Kudo R.R., 1966. Protozoology, 5th. Ed. C.C.Thomas, Springfield.

Kuris A.M., 1974. Trophic interactions: Similarity of parasitic castrators to parasitoids. The Quarterly Review of Biology 49 (2): 129-148

Läbbé A., 1899. Sporozoa. In: Schulze,F.E., ed. "Das Tierrich" 5: xx - 180

Langton R.W., 1975. Synchrony in the digestive diverticula of Mytilus edulis L. J. Mar. biol. Ass. U.K. 55: 221-229

Léger L., 1900. Le genre Eimeria et la classification des coccidies. C.R.Soc. Biol. 52: 576-577

Léger L. and O.Duboscq, 1915. Pseudoklossia glomerata n.g. n.sp. coccidie de lamellibranche. Arch. Zool. exper. gen. 55., (N-R): 7

Léger L. and Duboscq, 1917. Pseudoklossia pectinis n. sp. coccidie de lamellibranche. Arch. Zool. exper. gen. 56.,(N-R): 88

Lemcke R.M., 1972. Osmolar concentrations and fixation of mycoplasmas. J. Bacteriol. 110: 1154-1162

Levine N.D. 1962. Protozoology today. J. Protozool 9: 1-6

Levine N.D., 1969a. Uniform terminology for sporozoan protozoa. In: Progress in Protozoology 340. (Abstracts of papers read at the IIIrd. Int. Congr. on Protozoology) Leningrad: Academy of Sciences of the USSR, Publishing House, Nauka.

Levine N.D., 1969b. Taxonomy of the Sporozoa. In Progress in Protozoology 365-367 (Abstracts of papers read at the IIIrd. Int. Congr. on Protozoology) Leningrad: Academy of Sciences of the USSR, Publishing House, Nauka

Levine N.D., 1970 Taxonomy of the Sporozoa. J. Parasitol. 56: 208

Levine N.D., 1971. Taxonomy of the piroplasms. Trans. Am. Microsc. Soc. 90: 2-33

Levine N.D., 1973. Protozoan parasites of domestic animals and of man. 2nd. Ed. Burgess, Minneapolis

Levine N.D., 1973. Introduction, History and Taxonomy. In: The coccidia ed. D.M.Hammond. University Park Press, Baltimore.

Levine N.D. and V.Ivens, 1970. The coccidian parasites (Protozoa, Sporozoa) of ruminants. Ill. Biol. Monogr. 44: University of Illinois Press, Urbana.

Lima-de-Faria A., 1969. Handbook of molecular cytology. North Holland Publishing Co. Amsterdam and London.

Liss A. and J. Maniloff, 1971. Isolation of Mycoplasmatales viruses and characterization of MVL 1, MVL 52 and MVG 51. Science 173: 725

List T., 1902. Die Mytiliden. Fauna u. Flora Neapel. 27:

McEwen C.R., 1967

McIntyre A.D., 1970. The range of biomass in intertidal sand with special reference to the bivalve Tellina tenuis (da Costa). J. mar. biol. Ass. U.K. 50: 561-575

McIntyre A.D. and A.Eleftheriou, 1968. The bottom fauna of a flatfish nursery ground. J. mar. biol. Ass. U.K. 48: 113-142

Mackinnon and Ray, 1937. A coccidian from the eggs of Thalassema neptuni. Parasitology 29: 457

McQuiston R.W., 1969. Cyclic activity in the digestive diverticula of Lasaea rubra (Montagna) (Bivalvia: Eulamellibranchia). Proc. Malacol. Soc. Lond. 38: 483-491

Malek E.A., 1952. Morphology, bionomics and host-parasite relations of Planorbidae (Mollusca: Pulmonata) Ph.D. Thesis. University of Michigan Ann.Arbour.

Malek E.A. and T.C.Cheng, 1974. Medical and Economic Malacology. Academic Press N.Y. and L.

Manier J.F. and R.Ormieres, 1968. Ultrastructure de quelques stades de Chytridiopsis socius Schn. parasite de Blaps lethifera Marsh (coleopt. Tenebr.) Protistologica 4: 181-185

Maniloff J., 1971. Analysis of the helical ribosome structures of Mycoplasma gallisepticum. Proc. Nat. Acad. Sci. 68: 43-47

Maniloff J and A Liss, 1972. Properties of Mycoplasmatales viruses. Med. Microbiol. Immunol. 157: 172

Maniloff J. and A.Liss, 1974. The molecular biology of Mycoplasma viruses. Ann. N. Y. Acad. Sci. 225: 149-157

Maniloff J.,H.T.Morowitz and R.J.Barrnett, 1965. Ultrastructure and ribosomes of Mycoplasma gallisepticum. J. Bacteriol. 90: 193

Maniloff J. and H.J.Morowitz, 1967. Ultrastructure and life cycle of Mycoplasma gallisepticum A 5969. Ann. N. Y. Acad. Sci. 143: 59-65

Maniloff J. and H.T.Morowitz, 1972. Cell biology of the Mycoplasmas Bacteriol. Rev. 36: 263-290

Mansour K., 1946. Food and digestive processes of lamellibranchs. Nature 157: 482

Mansour K and F.G.Zaki, 1946. The digestive diverticula of Unio prasidens as organs of secretion. Proc. Egypt. Acad. Sci. 2: 38-44

Maramarosch K., R.R.Granados and H.Hirumi, 1970. Mycoplasma diseases of plants and insects. Advan. Virus Res. 16: 135-193

Marquardt W.C., 1973. Host and Site specificity. In: The Coccidia, D.M.Hammond, ed. Butterworths: Lond.

Mitsui Y., M.Fujimoto and M.Kajima., 1964. Development and morphology of trachoma agent in the yolk sac cell as revealed by electron microscopy. Virology 23: 30-45

Mix M.C. and A.K.Sparks, 1971. Repair of digestive tubulestissue of the Pacific oyster, Crassostrea gigas, damaged by ionizing radiation. J. Invert. Pathol 17: 172-177

Morowitz H.T. and J.Maniloff, 1966. Analysis of the life cycle of Mycoplasma gallisepticum. J. Bacteriol. 91: 1638-1644

Morton B.S., 1969. Studies on the biology of Dreissena polymorpha Pall. II. Correlation of the rhythms of adductor activity, feeding, digestion and excretion. Proc. Malacol. Soc. Lond. 38: 401-415

Morton B.S., 1970a. The tidal rhythm amd rhythm of feeding and digestion in Cardium edule. J. mar. biol. Ass. U.K. 50: 499-512

Morton B.S., 1970b. A note on the cytological structure and function of the digestive diverticula of Macoma balthica correlated with the rhythm of the tide. Malacol. Rev. 3: 115-119

Morton B.S., 1970c. The functional anatomy of the digestive tract of Teredo navalis L. and Lyrodus pedicellatus (Quatrefagas). Proc. Malacol. Soc. Lond. 36: 229-255

Morton B.S., 197 1 The diurnal rhythm and tidal rhythm of feeding and digestion in Ostrea edulis. Biol. J. Linn. Soc. Lond. 3: 329-342

Morton B.S., 1973. A new theory of feeding and digestion in the filter-feeding lamellibranchia. Malacologia, 14: 63-79

Morton J.E., 1956. The tidal rhythm and the action of the digestive system of the lamellibranch Lasaea rubra. J. mar. biol. Ass. U.K. 35: 563-586

Morton J.E., 1960. Mollusca, an introduction to their form and function. Harper. N.Y.

Morton J.E., A.D.Boney and E.D.Corner, 1957. The adaptations of Lasaea rubra (Montagu) a small intertidal lamellibranch. J. mar. biol. Ass. U.K. 383-405

Morton J.E. and C.M.Yonge, 1964. Classification and structure of the Mollusca. In: "Physiology of Mollusca" (K.M.Wilbur and C.M.Yonge, eds.), 1: 1-58. Academic Press, New York.

Moss L.H. and M.Gravell, 1979. Ultrastructure and sequential development of infectious Pancreatic Necrosis Virus. J. Virol 3(1): 52-58

Nicholson B.L. and C.Byrne, 1970. An established cell line from the Atlantic Salmon (Salmo salar). J. Fish. Res. Board Can. 30: 913-916

Oliver W.R.B., 1923. Marine littoral plant and animal communities in New Zealand. Trans. and Proc. N.Z. Inst. 54: 495-545

Owen G., 1955. Observations on the stomach and digestive diverticula of the Lamellibranchia I. The Anisomyaria and Eulamellibranchia. Q. J. Microscop. Sci. 97: 541-567

Owen G., 1966. Digestion. In: Physiology of Mollusca 2 ed. K.M.Wilbur and C.M.Yonge 53-96. New York: Academic Press

Owen G., 1970. The fine structure of the digestive tubules of the marine bivalve Cardium edule. Phil. Trans. Roy. Soc. B. 258: 245-260

Owen G., 1972. Lysosomes, peroxisomes and bivalves. Science Progress, Oxford 60: 299-318

Owen G., 1972. Peroxisomes in the digestive diverticula of the bivalve mollusc Nucula sulcata. Z. Zellforsch. 132: 15-24

Owen G., 1974. Feeding and digestion in the Bivalvia. In: Advances in Comparative Physiology and Biochemistry, 5: 1-35. ed. O.Lowenstein. N.Y. and Lond. Academic Press.

Pan C.T., 1965. Studies on the host-parasite relationship between Schistosoma mansonii and the snail Australorbis glabratus. J. Parasitol. 40: 1-4

Pal S.G., 1971. The fine structure of the digestive tubules of Mya arenaria L. Proc. Malacol. Soc. Lond. 39: 303-310

Patten R., 1935. The life history of Merocystis kathae in the whelk Buccinum undatum. Parasitology 27: 399

Pauley G.B. and A.K.Sparks, 1966. The acute inflammatory reaction in two different tissues of the Pacific oyster, Crassostrea gigas. J. Fish. Res. Board Can. 23: 1913-1921

Pearre S.D., 1976 Gigantism and partial parasitic castration of Chaetognatha infected with larval trematodes. J. mar. biol Ass. U.K. 56: 503-513

Petersen C.G.J., 1918. The sea-bottom and its production of fish food. A survey of the work done in connection with the valuation of the Danish waters from 1883-1917. Rep. Danish Biol. Stn., 25: 62 pp.

Platt A.M., 1971. Studies on the digestive diverticula of Mytilus edulis Ph.D. Thesis. Queen's University, Belfast.

Pohlo R., 1969. Confusion concerning deposit feeding in the Tellinacea Proc. Malacol. Soc. Lond., 38: 361-364

Porchet-Henneré E., 1970. La microgametogenèse chez la coccidie Coleotropha durchoni (Vivier-Henneré); étude au microscope electronique. Arch. Protistenk. 112: 21-29

Porchet-Henneré E. et A.Richard, 1971. La sporogenèse chez la coccidie Aggregata eberthi. Étude en microscopie electronique. J. Protozool. 18: 614-628

Purchon R.D., 1968. The biology of the Mollusca. xxv, 560 pp. Oxford. Pergamon Press.

Purchon R.D., 1971. Digestion in filter-feeding bivalves - a new concept. Proc. Malacol. Soc. Lond., 39: 235-262

Razin S., 1973.
Advances in Microbial Physiology 5: 1-80

Razin S. 1976. Ultrastructure and Physiology of Mycoplasmas. Proc. Soc. Gen. Microbiol. III pt 4: 135-136

Rees W.G., 1936. The effect of parasitism by larval trematodes on the tissues of Littorina littorea (Linne.). Proc. Zool. Soc. Lond. 357-368

Reynolds E.S. 1963. The use of lead citrate at a high pH as an electron opaque stain in electron microscopy. J. Cell Biol., 17: 208-212

Roberts W.L., D.M.Hammond, L.C.Anderson and C.A.Speer, 1970. Ultrasructural study of schizogony in Eimeria callospermophili. J. Protozool. 17: 584-592

Rodwell A.W., 1965. The stability of Mycoplasma mycoides. J. Gen. Microbiol. 40: 227-234

Rodwell A.W., J.E.Peterson and E.S.Rodwell, 1973. The nature of striated structures in Mycoplasmas. Ann. N.Y. Acad. Sci. 225: 190-200

Rothschild M., 1936. Gigantism and variation in Peringia ulvae Pennant 1777, caused by infection with larval trematodes. J. mar. biol. Ass. U.K. 30: 537-546

Rothschild M.,1938. Further observations on the effect of trematode parasites of Peringia ulvae (Pennant, 1777). Novitates Zool. 41: 84-102

Rothschild M., 1941a. The effecto of trematode parasites on the growth of Littorina neritoides (L.). J. mar. biol Ass. U.K. 25: 84-102

Rothschild M., 1941b. Observations on the growth and trematode infections of Peringia ulvae (Pennant, 1777). in a pool in the Pamar saltings, Plymouth. Parasitology 33: 406-415

Rungger D., M.Rasteli, E.Braendle and R.G.Marlsberger., 1971. A virus-like particle associated with lesions in the muscles of Octopus vulgaris. J. Invert. Pathol. 17: 72-80

Saleuddin A.S.M., 1965. The mode of life and functional anatomy of Astarte spp. (Eulamellibranchia). Proc. Malacol, Soc, Lond., 36: 229-255

Salt G. and F.S.Hollick, 1946. Studies of wireworm populations II. Spatial distribution. J. Exp. Biol., 23: 1-46

Scholtyseck E., 1973. Ultrastructure, Ch. 4 In: The Coccidia, D.M. Hammond and P.L.Long, eds., University Park Press, Baltimore and Butterworths, London.

Scholtyseck E., H.Melhorn and K.Friedhoff, 1970. The fine structure of the conoid of Sporozoa and related organisms. Z. Parasitenk. 34: 68-94

Scholtyseck E., H.Mehlhorn and D.M.Hammond, 1972. Electron microscope studies of microgametogenesis in coccidia and related groups. Z. Parasitenk. 38: 95-131

Schrével J., 1970. Recherches ultrastructurales et cytochimiques sur le paraglycogene réserve glucidique des gregarines et coccidies. J. Microsc. 9: 593-610

Sénaud J., 1967. Contribution a l'étude des Sarcosporidies et des Toxoplasmes (Toxoplasmea). Protistologica 3: 167-232

Simpson J., 1953. The major features of Evolution, Columbia University Press, N. Y.

Sindermann C.J., 1970. Principle diseases of marine fish and shellfish. Academic Press, N. Y. 369 pp.

Sindermann C.J. and A. Rosenfield, 1967. Principal diseases of commercially important marine bivalve Mollusca and Crustacea. U.S. Fish and Wildlife Service, Fish. Bull. 66: 335-385

Smith J., 1905. Note on the gregarine Aggregata inachi (sp. n.) which may cause parasitic castration of its host Inachus dorsettensis. Mitth. Zool. Sta. Neapel 17: 406-410

Smith P.F., 1971. The biology of Mycoplasmas. Acad. Press N.Y. 257 pp.

Smith K.O., J.L.Melnick and F.Rapp, 1965. Fine structure of unassembled viral subunits from human warts. J. Bacteriol 90: 278-280

Smith P.F., K.J.Mayberry-Carson, T.A.Langworthy, W.R.Mayberry and T.Sugiyama, 1974. Les Mycoplasmes, Les Colloques de l'Institut Nationale de la Santé et de la Recherche Medicale. 33: 63-68

Smith R.I., 1955. Salinity variation in interstitial water of sand at Kames Bay, Millport, with reference to the distribution of Nereis diversicolor. J. mar. biol. Ass. U.K. 34: 33-46

Snieszko S.F., 1969. Symposium on diseases of fishes and shellfishes. American Fisheries Society sp. Publn. 5, Washington D.C. 528 pp

Sparks A.K., 1972. Invertebrate Pathology (Non communicable Diseases) Academic Press N.Y., 387 pp

Sprague V., 1965. Observations on Chytridiopsis mytilovum (Field) formerly Haplosporidium mytilovum (Field) (Microsporida?). J. Protozool. 3: 385-389

Sprague V., 1970. Some protozoan parasites and hyperparasites in marine bivalve molluscs. In: Symposium on diseases of fishes and shellfishes. ed. S.F.Snieszko. Am. Fish. Soc., Sp. Publ. 5, Washington D.C.

Stephen A.C., 1928 Notes on the biology of Tellina tenuis (da Costa) J. mar. biol. Ass., Vol XV: 683-702

Stephen A.C.,1929a. Studies on the Scottish Marine Fauna - The fauna of sandy and muddy areas of the tidal zone. Trans. Roy. Soc. Edin. LVI: 291-306

Stephen A.C., 1929b. Notes on the rate of growth of Tellina tenuis (da Costa) in the Firth of Clyde. J. mar. biol Ass., Vol XVI: 117-129

Stephen A.C., 1930. Studies on the Scottish Marine Fauna. Additional observations on the fauna of the sandy and muddy areas of the tidal zone. Trans. Roy. Soc. Edin., LVI: 521-536

Stephen A.C., 1931. Notes on the biology of some lamellibranchs on the Scottish coast. J. mar. biol. Ass. N.S. 17: 277-300

Stephen A.C., 1932. Notes on the biology of some lamellibranchs in the Clyde area. J. mar. biol. Ass. (N.S.) 18: 51-68

Stephen A.C., 1933. Studies on the Scottish marine fauna: The natural faunistic divisions of the North Sea as shown by the quantitative distribution of the molluscs. Trans. Roy. Soc. Edin. 57: 601-616

Stephen A.C., 1938. Production of large broods in certain marine lamellibranchs with a possible relation to weather conditions. J. Anim. Ecol. 7: 130-143

Stephen A.C., 1953. Life on some sandy shores. In: Essays in Marine Biology, Oliver and Boyd, Edinburgh. 50-72

Stevenson R.N. and G.R.South, 1975. Observations on phagocytosis of Coccomyxa parasitica (Coccomyxaceae: Clorococcales) in Placopecten magellanicus. J. Invert. Pathol. 25: 307-311

Sturrock B.M., 1967. The effect of infection with Schistosoma haematobium on the growth and reproduction rate of Bulinus (Physopsis) nasutus productus. Ann. Trop. Med. Parasitol. 61: 321-000

Sturrock B.M. and R.F.Sturrock, 1970. Laboratory studies of the host-parasite relationship of Schistosoma mansonii and Biomphalaria glabrata from St. Lucia, West Indies. Ann. Trop. Med. Parasitol. 64: 357

Sturrock R.F. and B.M.Sturrock, 1971. Shell abnormalities in Biomphalaria glabrata infected with Schistosoma mansonii and their significance in field transmission studies. J. Helminthol. 45: 201-210

Sumner A.T., 1969a. The cytology and histochemistry of the digestive gland cells of some fresh water lamellibranchs. J.R.Microsc. Soc., 85: 201-211

Sumner A.T., 1966b. The fine structure of the digestive gland cells of Anodonta. J. R. Microsc. Soc., 85: 417-423

Tait R.V., 1968. Elements of Marine Ecology. Butterworth, London

Tammes P.M.L and A.D.G.Dral, 1955. Observations on the straining of suspensions by mussels. Archs. Neerl. Zool. 11: 87 - 112

Terzakis J.A., 1968. Uranyl acetate, a stain and a fixative. J. Ultrasruct. Res., 22: 168-184

Terzakis J.A., 1969. A protozoan virus. Mil. Med. 134: 916-921

Terzakis J.A., 1970. Transformation of the Plasmodium cynmolgi oocyst. J. Protozool. 18: 62-73

Thomson R.J., N.A.Ratcliffe and B.L.Bayne, 1974. Effects of starvation on structure and function in the digestive gland of the mussel (Mytilus edulis L.). J. mar. biol. Ass. U.K. 54: 699-712

Thorson G., 1950. Reproductive and larval ecology of marine bottom invertebrates. Biol. Reviews 25: 1-45

Thorson G., 1955. Modern aspects of marine level-bottom animal communities. J. Mar. Res 14: 387-397

Thorson G., 1957. Bottom communities (sublittoral and shallow-shelf) Ch. 17 In: Treatise on Marine Ecology and Palaeoecology, ed. J.Hedgpeth.

Trefiak W.D. and S.S.Desser, 1973. Crystalloid inclusions in species of Leucocytozoon, Parahaemoproteus, and Plasmodium. J. Protozool. 20: 73-80

Trevallion A., 1971. Studies on Tellina tenuis (da Costa) III. Aspects of general biology and energy flow. J. exp mar. Biol. Ecol. 7: 95-122

Trevallion A. and A.D.Ansell, 1967. Studies on Tellina tenuis (da Costa) II. Preliminary experiments in enriched sea water. J. exp. mar. Biol. Ecol 1: 257-270

Trevallion A., R.Johnston, D.M.Finlayson and N.T.Nicoll. 1973. Studies in the bivalve Tellina tenuis (da Costa) IV. Further experiments in enriched sea water. J. exp. mar. Biol. Ecol. 11: 189-206

Trevallion A., J.H.Steele and R.R.C.Edwards, 1969. Dynamics of a benthic bivalve. In: Marine Food Chains, J.H.Steele, ed. Oliver and Boyd. Edin.

Tripp M.R., 1958. Disposal by the oyster of injected red blood cells of vertebrates. Proc.Nat. Shellfish Ass. 48: 143-147

Trueman E.R., 1942. The structure and deposition of the shell of Tellina tenuis. J. Roy. Microsc. Soc.

Vago C., 1966. A virus disease in Crustacea, Nature 209: 1290

Vatova A., 1934. Richerche quantitative sur benthos del Golfo di Rovigno Not. 1st. Biolog. Rovigno 12: 1-12

Vatova A., 1935. Ricerche preliminari sulla biocenosi del Golfo di Rovigno. Thallassia 2: 1-30

Vatova A., 1936. Ricerche quantitative sulla fauna bentonica dell'Alto Adriatico e loro importanza per la biolia marina. Nov. 1st. Biolog. Rovigno 12: 1-15

Vatova A., 1949. La fauna bentonica dell'Alto e medio Adriatico. Nova Thalassia 1: 1-110

de Vincentiis M. and A.Renzoni, 1963. Sulla presenza di uno sporozoo in ovociti di Mytilus galloprovincialis Lam. Arch. Zoöl. Ital. 47: 21-26

Vonk H.J., 1904. "Verdauungsphagocytose bei den Austern". Z. vergl. Physiol., 1: 607

Watkin E.E., 1942. The macrofauna of the intertidal sand of Kames Bay, Millport, Buteshire. Trans. Roy. Soc. Edin. 60: 543-61

Wesenburg-Lund C., 1931. Contributions to the development of the Trematoda digenea. Part I. The biology of Leuchloridium paradoxum. Mem. Acad. Roy. Sci. Danemark, Sect. Sci. 9: 90-142

Wesenburg-Lund C., 1934. Contributions to the development of the Trematoda Digenea Part I. The biology of the freshwater cercariae in Danish waters. Kgl. Dan. Vidensk. Selsk. Skr. 5: 1-223

Whittlestone P., 1976. Isolation and detection of Mycoplasmas. Proc Soc. Gen. Microbiol. III (4): 136-137

Wilbur K.M. and C.M.Yonge, 1966. Physiology of Mollusca II. Academic Press, N.Y. and Lond.

Williams C.S., 1969. The effect of Mytilicola intestinalis on the biochemical composition of mussels. J. mar. biol Ass. U.K. 49: 161-173

Wilson M.H. and A.M.Collier, 1976. Ultrastructural study of Mycoplasma pneumoniae in organ culture. J. Bacteriol. 125: 332-339

Wolfe K. and M.C.Quimby, 1962. Established eurythermic line of fish cells in vitro. Science 135: 1065-1066

Wolfe K. and M.C.Quimby, 1967. Infectious Pancreatic Necrosis (IPN) its diagnosis, identification, detection and control. Riv. It. Piscic. Ittiopat. 2(4): 76-80

Wolke R.E., D.S.Wyand and L.H.Khairallah, 1970. A light and electron microscopic study of epitheliocystis disease in the gills of Connecticut striped Bass (Morone saxatilis) and white perch (Morone americanus). J. Comp Pathol. 80: 559

Yonge C.M., 1926b. The digestive diverticula in the lamellibranchs. Trans. Roy. Soc. Edin. 54: 703-718

Yonge C.M., 1949. On the structure and adaptations of the Tellinacea, deposit feeding Eulamellibranchia. Phil Trans. B. 234: 29-76

Zavadova M., 1972. Le genre Mycoplasma. Bull. Inst. Pasteur, 70: 5--71

Zambernard J., A.E.Vatter and R.G.McKinnell, 1966. The fine structure of nuclear and cytoplasmic inclusions in primary renal tumours of mutant leopard frogs. Cancer Res. 26: 1688-1700

Appendix 1.

Protocol for the preparation of minimum essential medium (MEM) Earles BSS with non-essential amino acids and with 15% foetal bovine serum (Flow Laboratories Ltd.)

Before use, all stock reagents were dispensed into predetermined aliquot sizes suitable for media preparation.

Storage of stock reagents

	Volume	Store at	Shelf life
Earles BSS	100 ml	Room temperature	1 year
Sodium Bicarbonate	10 ml	Room temperature	Indefinite
MEM Amino Acids	10 ml	4 C	1 year
Non-essential Amino Acids	5 ml	4 C	1 year
MEM Vit	5 ml	-20 C	1 year
Penicillin/Streptomycin	10 ml	-20 C	1 year
Foetal Bovine Serum	5 ml	-20 C	1 year
Glutamine	5 ml	-20 C	1 year

Media preparation

To eliminate the risk of cross contamination and to reduce preparation time procedures involving pipetting were avoided. This was done by dispensing reagents into suitable sized aliquots.

The following quantities were used to make 1 litre of media:

Sterile deionized water	830 ml
Earles BSS	100 ml
MEM AA	20 ml

Appendix 1.

Cell Dispersants

Phosphate Buffered Saline

NaCl	8.00 grams
KH_2PO_4	0.20 grams
KCl	0.20 grams
NaH_2PO_4	1.15 grams
Distilled water	to 1,000 ml

Dispense into 50 ml aliquots.

Autoclave at 15 P.S.I. for 15 minutes.

Store at room temperature.

Shelf life at least one year.

Phosphate Buffered Saline/EDTA

Disodium ethyl diamine tetra-acetic acid (1:5,000 EDTA)	0.20 grams

otherwise as above.

Dispense into 10 ml and 20 ml aliquots.

Autoclave at 15 P.S.I. for 15 minutes.

Store at room temperature.

Shelf life at least one year.

Phosphate Buffered Saline/EDTA/Trypsin

As Phosphate Buffered Saline/EDTA above.

Dispense into 250 ml aliquots in 500 ml bottles.

Autoclave at 15 P.S.I. for 15 minutes.

Cool - add 10 ml 2.5%, 1:250, trypsin in saline.

Check for sterility. Store at -20 degrees C.

Shelf life one year.

NE AA	10 ml
MEM Vit	10 ml
Sodium Bicarbonate	20 ml
Penicillin/Streptomycin	20 ml (500 i.u. of each/ml)

Media was divided into 44 ml aliquots and stored at 4 degrees C. Foetal bovine serum (15%) and glutamine were added just prior to use.

Appendix 2.

Kames Bay, Millport Tellina tenuis survey. Data collected during the months of April, JUly, October and December, 1974 and January, 1975.

The following abbreviations have been used in presenting these tables:-

1. wh. wt. = whole weight
2. lnth. = length of shell between the anterior and posterior x maximum.
3. wdth. = width of shell from the umbo at right angles to 2.
4. thck. = thickness of shell with both valves firmly closed.
5. wt. wt. = flesh wet weight, meat removed from the shell, blotted both sides and weighed immediately.
6. sh. wt. = shell weight (both valves)
7. dr. wt. = weight of meat after drying at 80 ^{o}C. for 3 weeks
8. sex, either male (m), female (f) or neuter, ie., undifferentiated (n). The designation (sp) signifies spent individuals.
9. prsts. = presence or absence of the coccidian parasite of the ovaries, Merocystis tellinovum on a scale of 1 for very light to 5, very heavy infection.

Weights are given in milligrams and measurements without the decimal which should be inserted after the 1st. digit to correct to centimetres.

Appendix

Kames Bay Tellina Survey, 8 April 1974, Top of Beach

lnth.	wh.wt.	wt.wt.	sh.wt.	dr.wt.	sex	prsts (1-5)
113	128	052	060	009	m	-
139	308	081	170	-	f	5
138	255	087	114	015	m	-
135	232	062	120	010	m	-
127	204	066	101	013	m	-
139	237	070	139	014	m	-
123	188	050	110	011	m	-
100	099	031	050	008	m	-
101	084	032	043	010	f	2
100	089	033	042	008	f	2
186	636	227	295	033	m	-
175	423	158	264	017	m	-
181	470	172	267	021	m	-
177	493	196	221	024	m	-
167	470	161	223	-	f	5
165	417	145	221	017	m	-
168	450	165	201	019	m	-
177	516	182	258	009	f	5
124	178	079	089	011	f	2
124	179	079	087	017	f	3
173	480	144	256	014	f	3
162	463	150	240	020	f	5
133	141	060	081	016	m	-
149	402	227	115	014	m	-
140	257	095	132	008	f	5
150	234	089	135	016	f	5
139	235	089	146	010	f	5
130	188	076	096	013	f	5
148	306	099	150	-	f	4

Appendix

Kames Bay *Tellina* Survey, 8 April 1974, Upper Middle *Tellina*

lnth.	wh.wt.	wt.wt.	sh.wt.	dr.wt.	sex	prsts (1-5)
088	051	019	129	-	f	2
084	045	020	026	016	n	-
151	299	109	164	009	f	3
156	333	160	175	014	m	-
150	252	111	140	013	m	-
130	230	093	124	010	f	5
135	167	070	100	013	f	4
130	197	108	093	013	m	-
077	039	014	022	001	n	-
121	140	051	080	010	n	-
135	205	096	095	001	m	-

Lower Middle *Tellina*, 8 April 1974

170	500	183	258	027	m	-
172	504	189	234	018	f	2
190	823	249	430	030	f	2
177	540	179	262	025	f	2
172	520	179	261	021	f	3
167	568	190	283	022	f	5
162	381	146	180	-	m	-
156	411	156	217	020	f	5
161	350	125	182	018	f	3
139	245	101	102	015	f	3
102	100	034	042	009	m	-
091	072	032	033	007	n	-
093	073	031	034	008	n	-
104	103	046	050	008	f	2
091	078	037	038	007	n	-
075	045	015	028	008	n	-
063	033	012	020	004	n	-

Appendix

Kames Bay Tellina Survey, 8 April 1974

Lower Middle Tellina continued

lnth.	wh.wt.	wt.wt.	sh.wt.	dr.wt.	sex	prsts (1-5)	
147	354	120	175	017	f	5	
148	324	137	162	017	m	-	
143	268	095	121	012	f	3	
122	164	066	074	009	n	-	
140	259	119	120	015	f	2	
146	322	139	147	020	f	3	
148	343	141	164	016	m	-	
140	280	100	140	014	f	3	
129	176	064	079	011	f	3	heavy eggs
139	296	090	150	010	f	2	
120	140	067	058	009	n	-	
131	227	079	108	010	n	-	
128	158	058	074	009	f	5	

Mean Low Water Springs, 8 April 1974

201	620	247	363	-	n	-	
179	590	204	302	027	f	3	
174	626	219	311	f	3		
165	527	171	289	014	f	3	
163	402	147	205	020	f	2	
149	304	125	149	012	n	-	
155	403	162	195	021	f	5	
147	322	121	154	017	f	1	
136	203	099	091	025	f	1	
130	191	089	085	013	f	3	
122	179	080	079	015	f	3	
139	256	104	102	009	f	3	
111	124	057	063	006	f	1	
141	267	118	121	008	f	2	
109	120	050	069	011	f	3	

Appendix

Kames Bay Tellina Survey, 8 April 1974, Mean Low Water Springs

lnth.	wh.wt.	wt.wt.	sh.wt.	dr.wt.	sex	prsts (1-5)
127	155	066	080	012	n	-
111	130	066	060	009	n	-
110	114	054	051	006	f	2
097	080	032	037	009	m	-
102	080	038	042	004	m	-
076	040	017	022	009	m	-
108	109	049	051	009	m	-
114	120	055	063	008	m	-
120	137	058	070	007	m	-
104	090	038	050	007	f	ripe2
100	080	036	043	010	m	-
100	084	041	043	010	m	-
093	059	023	039	006	f	1
089	060	025	034	006	m	-
088	055	021	032	004	m	-

Cumulative Frequencies, April 1974

200-181	2	2.08%
180-161	19	19.79%
160-141	16	16.66%
140-121	29	30.21%
120-101	12	12.50%
100-081	13	13.54%
080-061	4	4.16%
060-041	0	-

Appendix

Kames Bay Tellina Survey, 9 July 1974

Second sample, Mean Lower Water Springs

lnth.	wh.wt.	wt.wt.	sh.wt.	dr.wt.	sex	prsts (1-5)
148	295	140	149	030	m	-
150	262	132	125	030	f	2
161	310	154	144	033	m	-
161	315	139	145	037	m	-
138	209	109	089	027	f	3
150	310	158	132	038	m	-
128	177	078	080	021	f	3
145	237	104	110	021	f	2
122	170	052	070	015	f	2
123	200	094	070	021	f	2
117	140	055	069	012	f	2
130	200	044	102	020	m	-
105	099	023	038	014	m	-
104	101	025	046	010	m	-
130	231	070	090	011	m	-
109	102	047	044	008	f	2
130	211	090	100	011	f	2
100	079	038	038	014	f	2
103	079	038	041	028	f	2
099	062	025	034	017	f	2
110	110	047	050	010	m	-
101	092	049	039	009	m	-
099	068	030	036	004	f	2
098	063	025	034	004	m	-
108	074	039	039	005	m	-
080	044	-	-	-	n	-
163	020	-	-	-	n	-
054	011	-	-	-	n	-
054	008	-	-	-	n	-
040	002	-	-	-	n	-

Appendix

Kames Bay Tellina Survey, 9 July 1974, Mean Low Water Springs

lnth.	wh.wt.	wt.wt.	sh.wt.	dr.wt.	sex	prsts (1-5)
150	309	155	127	030	f	3
140	251	128	105	030	f	2
134	202	109	077	033	f	1
150	356	175	155	037	f	5
150	349	148	150	027	f	3
134	254	137	099	038	f	3
140	310	149	129	021	m	-
140	241	120	108	021	f sp	5
130	219	107	099	015	f r	1
141	293	114	111	021	m	-
140	239	111	111	020	f	1
127	140	071	056	010	f	5
107	114	055	048	012	m	-
110	123	064	049	015	m	-
150	296	140	129	034	m	-
118	162	072	068	020	m	-
128	246	121	079	025	m	-
134	207	098	090	019	m	-
112	136	061	059	011	f	2
110	108	048	050	010	f sp	2
118	175	082	081	017	m	-
102	108	049	046	010	m	-
112	123	065	055	010	m	-
124	088	102	065	015	m	-
111	134	076	054	018	m	-
077	057	031	022	011	m	-
063	025	013	012	006	n	-
072	033	013	018	004	n	-
061	022	014	017	-	n	-
061	022	013	015	-	n	-

Appendix

Kames Bay Tellina Survey, 9 July 1974, Top

lnth.	wh.wt.	wt.wt.	sh.wt.	dr.wt.	sex	prsts (1-5)
187	629	275	284	039	m	-
182	607	231	309	036	m	-
187	674	299	310	-	f	5
148	295	142	136	-	f	1
136	206	105	098	-	m	-
110	113	050	055	008	f	2
116	130	058	060	010	f	2
100	093	041	-	007	f	3
099	061	024	030	-	f	2
083	040	022	020	-	n	-

Upper Middle

157	340	189	136	029	f r	2
163	425	195	219	029	m sp	-
167	053	250	205	034	f r	2
151	262	144	112	024	f r	2
138	212	115	087	016	f r	2
138	259	140	105	019	f r	2
135	161	089	066	011	f r	2
095	079	040	037	004	f r	2
110	142	068	063	011	m	-
090	054	-	027	004	n	-

N.B. All females very ripe with light parasitization

Cumulative frequency percentages

200-181	4	4.49%
180-161	10	11.24%
160-141	16	17.97%
140-121	22	24.72%
120-101	18	20.22%
100-081	9	10.10%
080-061	6	6.74%
060-041	4	4.49%

Appendix

Kames Bay Tellina Survey, 10 October 1974, Mean Low Water Springs

lnth.	wh.wt.	wt.wt.	sh.wt.	dr.wt.	sex	prsts (1-5)
120	157	060	100	014	n	-
137	214	107	100	019	n	-
135	225	068	129	016	n	-
131	190	051	082	016	f	5
127	194	056	094	015	n	-
143	295	082	140	018	f sp	2
140	252	075	129	017	n	-
118	148	065	072	016	f r	1
118	148	040	066	013	f	5
146	270	099	120	021	f r	2
138	270	102	121	013	f r	2
128	206	069	094	009	m r	-
119	180	050	086	007	n	-
123	087	069	087	009	n sp	-
131	201	067	091	009	f sp	5
127	110	033	060	007	n sp	-
109	128	039	055	005	f so	2
112	095	029	064	005	m	-
104	109	030	048	005	f	2
125	282	057	085	005	n	-
110	119	030	051	004	n	-
111	124	036	052	006	n	-
084	065	015	029	025	f	5
067	042	008	014	002	f	3
080	057	011	125	002	n	-
068	038	008	014	002	n	-
056	029	007	012	002	n	-
050	023	006	010	002		
067	034	008	011	085	n	-
145	164	045	112	050	n	-
120	123	032	075	040	n	-

Appendix

Kames Bay Tellina Survey, 10 October 1974, Top of Beach

lnth.	wh.wt.	wt.wt.	sh.wt.	dr.wt.	sex	prsts (1-5)
172	451	121	231	013	f	5
163	503	106	300	011	n	-
141	298	071	168	007	f sp	2
129	120	150	030	099	n	-
120	150	030	099	002	n	-
117	140	021	079	002	f sp	2
105	105	026	074	003	n	-
127	129	046	079	003	n	-
080	045	008	030	003	n	-
045	006	-	-	-	n	-
Upper Middle						
142	200	081	110	011	f	3
123	170	046	092	006	n	-
156	365	105	177	018	f	5
152	330	097	185	011	f sp	5
120	153	052	081	009	m sp	-
152	300	100	157	012	m sp	-
154	370	095	157	015		
149	300	099	163	016	n	-
180	579	182	253	028	f	2
141	320	090	166	017	n	-
076	040	007	019	-	n	-
131	180	064	104	011	n	-
150	360	104	200	013	n	-
155	365	112	205	012	f sp	2
123	128	047	058	009	f	2
118	146	050	065	009	f	2
147	361	097	160	-		
143	280	086	155	012		
110	128	030	059	007	m sp	-
127	209	066	092	010	f	3
113	145	041	066	009	m sp	-
156	614	140	292	017	m sp	-
141	280	080	140	013	n	-
124	170	037	066	008	n	-

Appendix

Kames Bay *Tellina* Survey, 10 October 1974, Lower Middle

lnth.	wh.wt.	wt.wt.	sh.wt.	dr.wt.	sex	prsts (1-5)
124	163	048	085	015	n	-
170	464	120	252	014	f sp	2
149	326	104	174	012	f sp	2
163	380	120	200	019	m	-
111	113	030	063	016	m r	-
159	381	121	200	010	f r	2
136	249	072	130	012	f	3
154	365	124	180	004	m sp	-
107	105	027	056	012	m sp	-
145	309	097	179	009	m r	-
147	324	085	163	005	m r	-
116	247	048	079	007	m sp	-
132	294	053	110		n	-
057	020	-	-	-	n	-
047	010	-	-	-	n	-

Percentage cumulative frequencies of length

200-181	0	0
180-161	6	6.81%
160-141	24	27.26%
140-121	23	26.13%
120-101	23	26.13%
100-081	1	1.14%
080-061	4	4.54%
060-041	6	6.81%

Appendix

Kames Bay Tellina Survey, 23 December 1974, Mid-beach, Storm Tide

wh.wt.	lnth.	wdth.	thck.	wt.wt.	sh.wt.	dr.wt.	sex	prsts (1-5)
515	167	126	046	150	221	017	f	3
785	193	136	050	254	365	030	f	3
559	177	124	045	254	273	018	f	3
462	183	118	040	118	244	013	m	-
491	168	118	042	126	222	012	f	4
231	142	101	046	093	138	012	n	-
689	183	136	047	189	350	017	n	-
382	138	101	037	090	125	010	n	-
379	150	105	052	088	120	010	f	2
118	111	076	025	033	046	060	m	-
459	164	117	043	109	240	010	f	5
394	157	116	036	093	194	010	f	2
494	162	119	039	121	211	010	m	-
291	145	101	034	065	138	008	f	2
579	187	131	040	127	279	011	m	-
412	158	117	040	100	206	009	f	5
149	113	077	029	055	064	009	m	-
548	178	125	040	144	264	017	f	2
522	172	121	041	136	232	022	f	5
333	152	109	037	105	150	012	f	5
127	111	087	025	041	053	-	f	4
476	165	108	042	100	231	012	f	3
226	132	094	032	070	098	015	m	-
292	134	080	028	059	087	009	m	-
211	130	082	031	059	094	018	m	-
149	125	086	027	029	066	005	f	0
133	113	082	027	046	062	-	f	0
132	118	083	025	044	062	008	n	-
107	106	072	026	038	054	016	n	-
116	137	091	030	070	078	010	n	-

Appendix

Kames Bay Tellina Survey, 23 December 1974, Storm Tide

wh.wt.	lnth.	wdth.	thck.	wt.wt.	sh.wt.	dr.wt.	sex	prsts (1-5)
275	145	099	031	073	099	-	n	-
0076	093	062	021	024	047	-	f	3
111	106	071	027	033	059	009	n	-
151	121	080	027	045	073	010	m	-
099	102	070	026	031	048	008	n	-
094	110	072	027	038	050	010	f	2
238	127	094	032	078	112	011	n	-
298	144	100	035	077	144	010	f	3
209	137	090	031	055	099	010	f	5
173	122	082	030	050	094	009	f	4
300	147	100	033	089	139	012	n	-
191	120	083	030	069	090	010	f	1
186	127	086	030	051	088	010	n	-
199	130	093	031	069	091	010	f	5
181	125	087	030	049	086	009	n	-
120	113	081	027	044	057	009	n	-
057	087	060	020	018	029	004	n	-
168	121	082	028	057	077	010	f	5
139	115	077	028	045	069	-	f	0
123	106	077	027	045	060	-	f	1
108	104	072	025	042	051	019	n	-
166	126	075	028	051	080	006	f	5
149	119	080	027	046	064	006	f	4
144	117	079	027	051	062	008	n	-
062	091	061	020	022	031	009	n	-
119	110	073	026	047	052	010	n	-
197	130	091	032	053	094	004	n	-
139	119	077	027	051	059	-	n	-
160	120	083	030	057	070	010	f	1
155	127	085	038	059	077	011	f	4

Appendix

Kames Bay Tellina Survey, 23 December 1974

wh.wt.	lnth.	wdth.	thck.	wt.wt.	sh.wt.	dr.wt.	sex	prsts (1-5)
318	140	100	375	090	140	-	m	-
393	145	096	375	105	129	-	f	4
160	175	083	275	061	060	-	n	-
157	125	083	280	037	075	007	m	-
223	132	951	325	063	105	010	n	-
082	101	068	230	014	037	005	n	-
155	127	085	029	050	081	010	n	-
259	142	100	031	065	118	-	f	2
300	140	100	035	085	158	011	f	2
209	146	095	030	068	088	010	f	2
117	100	074	023	032	052	-	f	5
311	150	107	035	083	148	010	f	4
120	111	078	023	035	056	006	n	-
161	120	087	028	043	084	009	n	-
103	107	070	024	035	051	009	n	-
113	108	080	025	038	057	009	n	-
400	165	115	039	109	208	019	f	5
264	140	975	033	063	137	010	m	-
283	149	100	034	078	131	011	f	3
279	145	095	034	079	123	013	f	2
227	142	091	029	070	110	013	f	-
441	156	115	040	117	213	014	f	1
123	109	074	023	040	051	008	n	-
360	150	105	038	090	177	011	f	-
119	112	075	025	042	154	008	n	-
304	142	099	035	070	142	010	f	5
338	156	107	036	099	161	016	f	4
241	139	080	031	097	119	020	f	4
230	131	090	031	055	123	010	n	-
295	146	097	033	090	135	015	n	-
185	120	084	029	055	077	016	n	-

Appendix

Kames Bay Tellina Survey, 23 December 1974

wh.wt.	lnth.	wdth.	thck.	wt.wt.	sh.wt.	dr.wt.	sex	prsts (1-5)
112	111	077	025	031	054	012	n	-
705	193	137	047	287	337	021	f	4
489	170	123	039	142	228	017	f	3
267	142	099	032	080	124	010	m	-
282	135	098	030	080	119	010	m	-
445	162	137	039	130	225	017	m	-
304	149	106	034	187	145	010	f	3
506	172	122	041	149	247	018	n	-
168	120	083	025	054	084	009	n	-
275	143	099	033	088	134	011	n	-
339	155	113	035	088	134	011	n	-
326	150	107	037	110	222	014	n	-
151	120	083	027	047	069	007	n	-
369	155	112	036	102	182	011	n	-
439	162	115	040	129	222	019	n	-
509	170	124	090	147	253	014	n	-
237	135	094	032	063	108	009	f	2
318	150	104	033	091	143	011	n	-
361	196	108	037	099	159	006	n	-
327	148	105	035	056	151	010	m	-
355	155	107	038	100	157	004	m	-
335	149	110	036	080	158	009	m	-
139	115	079	025	042	064	009	m	-
333	149	108	035	084	164	009	f	2
382	160	114	038	092	189	011	m	-
325	147	104	037	080	159	009	m	-
333	152	105	035	095	154	011		
230	129	087	031	084	093	015	f	2
225	146	102	032	078	140	-	f	2
309	145	107	036	081	161	010	n	-

Appendix

Kames Bay Tellina Survey, 23 December 1974

wh.wt.	lnth.	wdth.	thck.	wt.wt.	sh.wt.	dr.wt.	sex	prsts (1-5)
250	147	100	035	100	115	018	f	5
352	155	109	037	104	183	020	n	-
140	112	079	027	049	068	009	n	-
210	139	090	030	085	116	011	n	-
256	140	093	034	073	133	010	n	-
279	139	097	033	080	136	010	n	-
173	120	082	028	058	087	010	f	3
154	118	084	027	040	080	009	n	-
150	114	080	027	045	064	009	f	5
099	100	069	025	033	044	009	n	-
180	120	078	029	054	064	015	n	-
156	115	080	027	041	072	010	n	-
205	142	o(0	030	057	110	009	f	2
225	132	091	031	079	112	012	m	-
157	118	077	-	037	078	-		
140	112	076	027	047	054	011	n	-
172	122	080	028	051	082	008	n	-
181	127	089	030	061	087	010	f	3
218	146	085	033	073	111	012	f	3
139	170	081	028	059	070	010	f	2
250	147	084	032	080	119	012	f	3
129	115	079	027	049	065	010	n	-
220	146	086	031	065	118	010	f	3
208	142	085	031	070	110	010	f	2
148	128	078	026	050	076	010	n	-
200	137	080	030	059	104	010	f	3
142	117	077	028	061	069	011	m	-
120	110	075	026	039	064	008	n	-
113	105	071	025	038	057	020	-	
211	130	087	031	085	084	013	-	
118	114	077	024	040	055	014	f	5

Appendix

Kames Bay *Tellina* Survey, 23 December 1974, Storm Tide - Total

wh.wt.	lnth.	wdth.	thck.	wt.wt.	sh.wt.	dr.wt.	sex	prsts (1-5)
130	115	077	027	050	063	009	n	-
144	114	079	027	041	070	009	f	5
095	102	070	022	036	049	009	m	-
122	110	076	027	039	058	008	f	3
117	111	077	025	040	058	008	f	0
115	105	073	027	040	052	008	f	0
104	107	070	025	041	044	007	n	-
127	118	077	027	051	059	009	n	-
065	092	060	022	020	033	010	n	-
104	107	073	025	039	049	005	f	2

Cumulative Frequencies

181+	3	1.96%
161-180	14	9.15%
141-160	45	29.41%
121-140	35	22.87%
101-120	42	27.45%
081-100	3	1.96%
061-080	10	6.54%
041-060	1	0.65%
021-040	-	-

Appendix

Kames Bay Tellina Survey, 24 January 1975, Mean Low Water Springs

lnth.	wh.wt.	wt.wt.	sh.wt.	dr.wt.	sex	prsts (1-5)
181	657	178	348	019	f	5
147	328	119	153	014	m r	-
174	480	149	250	016	n	-
173	520	164	268	016	f	5
173	530	208	250	019	f	4
174	518	169	247	019	n	-
143	250	096	110	011	m r	-
163	466	150	240	017	f sp	2
139	190	077	088	010	f r	5
144	249	106	115	011	f vr	5
128	205	100	090	-		
127	177	061	081	-	f sp	3
100	091	033	042	-		
123	189	067	090	-	m sp	-
135	215	072	100	-	f sp	5
167	505	138	259	015	m f	-
152	395	130	210	014	m sp	-
150	320	127	146	015	f sp	4
120	169	056	086	007	f sp	4
100	100	025	050	004	f sp	5
150	301	102	144	015	m sp	-
133	208	059	103	008	n	-
163	351	106	163	018	m sp	-
149	332	105	158	014	f sp	5
124	132	033	064	006	m sp	-
116	143	039	067	012	f sp	2
130	294	070	099	009*	f sp	1
058	017	-	-	016*	n	-
046	010	005	003	009*	n	-
102	092	030	048	003	f r	5

Appendix

Kames Bay Tellina Survey, 24 January 1975, Lower Middle

lnth.	wh.wt.	wt.wt.	sh.wt.	dr.wt.	sex	prsts (1-5)
223	186	387	606	-	f	3
180	718	223	370	018	m	-
188	723	220	400	023	m	-
169	457	178	240	017	m	-
193	811	250	484	020	n	-
180	514	203	233	022	m	-
179	640	216	332	026	n	-
174	560	229	260	020	n	-
179	646	196	350	024	n	-
170	623	160	329	025	n	-
171	512	169	239	015	f	3
165	389	135	184	014	f	5
145	287	114	136	013	m	-
172	493	188	227	025	m	-
196	855	300	391	-	f	2
147	350	149	153	021	m	-
142	290	106	143	010	m r	-
106	101	042	057	005	n	-
088	064	029	034	003	n	-
136	227	088	115	008	m r	-
136	211	098	105	-	f r	5
113	122	055	066	-	f r	5
096	094	050	047	005	n	-
094	078	026	048	004	n	-
082	050	030	029	003	n	-
085	060	021	033	003	n	-
086	060	011	039	003	n	-
080	051	009	028	-	n	-
080	045	007	020	-	n	-

Appendix

Kames Bay Tellina Survey, 24 January 1975, Top of Beach

lnth.	wh.wt.	wt.wt.	sh.wt.	dr.wt.	sex	prsts (1-5)
170	578	140	296		n	-
173	525	253	238		n	-
161	532	200	211		f sp	2
174	545	153	280		n	-
126	196	064	106		n	-
097	087	017	054		n	-
104	101	040	080		n	-
096	074	017	047		n	-
113	135	040	089		f	5
043	009	-	-		n	-
Upper Middle						
179	469	165	224	024	m	-
197	926	288	496	026	f sp	2
171	500	179	226	018	n	-
177	587	160	302	020	f	5+
160	419	100	234	012	n	-
123	167	062	086	008	f	5
123	181	040	094	009	m	-
123	174	052	097	007	f	3
105	103	037	055	005	n	-
100	099	036	054	004	n	-
056	017	-	-	-	n	-

Percentage cumulative frequencies of length

220-201	0	0
200-181	1	1.25
180-161	21	26.25%
160-141	10	12.50%
140-121	17	21.25%
120-101	12	15.00%
100-081	10	12.50%
080-061	6	7.50%
060-041	3	3.75%
040-021	1	1.25%

Appendix 3.

Protocol for the staining of sections with phloxine and tatrazine to show the presence of viral inclusion bodies.

Inclusions showed magenta to red; nuclei - blue; connective tissue - yellow.

a) Sections were cut on a BRIGHT cryostat and fixed for 5 minutes in Wolmans aqueous alcohol fixative.

b) Hydration was achieved by passing sections through 80%, 70% and 50% alcohol for 10 seconds each and rinsing in distilled water.

c) Sections were stained in 3% potassium dichromate for 30 minutes and then rinsed in distilled water

d) Sections were then stained in phloxine in M 10 calcium chloride for a further 30 minutes and rinsed in distilled water

e) Sections were treated with cellosolve for 30 seconds and transferred to tartrazine in cellosove (1st. Solution) for 5 minutes.

f) After rinsing sections were treated in haemalum for 30 seconds and rinsed again

g) Sections were 'blued' in Scott's tap water substitute, rinsed and returned to cellosolve for a further 30 seconds.

h) Finally, sections were treated with tartrazine in cellosolve, washed and mounted in D.C.M.

Appendix 4.

Acridine Orange staining for RNA (fluoresces red) and DNA (fluoresces green)

a) Sections were cut on a BRIGHT cryostat at 8 um and fixed for 5 minutes in Wolman's aqueous alcohol fixative.

b) The sections were hydrated by passing them through 80%, 70% and 50½ alcohol for 10 seconds in each bath and finally rinsed in distilled water

c) Sections were treated with 1% acetic acid for 6 seconds followed by 2 changes of distilled water

d) Sections were stained in 0.1% acridine orange for 3 minutes

e) Sections were washed in M 15 Sorensens phosphate buffer pH 6 for 1 minute

f) Sections were differentiated in M 10 calcium chloride for 30 seconds

g) Finally, the sections were mounted pH 6 phosphate buffer and examined under fluorescence optics in a Leitz Orthoplan light microscope