STUDIES ON ANIMALS CLOSELY ASSOCIATED WITH SOME NEW ZEALAND MARINE SHELLFISH.

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" Our search for understanding is
like a well without a bottom"

Neils Bohr.

ABSTRACT

Between March 1973 and September 1974, 858 Perna canaliculus (Gmelin), 150 Mytilus edulis aoteanus Powell, 237 Crassostrea glomerata (Gould) and 153 Ostrea lutaria Hutton, were surveyed for parasites. From these four commercially important shellfish species, a total of two sporozoans, three species of trematode sporocyst, and a copepod were found. A second copepod and pea-crabs were found associated with certain of the shellfish, but the nature of this association is uncertain.

During the examination of each shellfish the ratio of the meat volume to internal shell volume was measured. This provided a condition factor for the shellfish, and gave an indication of the effect of the parasite on the meat weight of the bivalve.

Perna canaliculus was collected from Ahipara, Wellington Harbour, and the Marlborough Sounds. Spores of a gregarine, Nematopsis sp., were abundant in the Ahipara mussels, common in Wellington and rare in the Sounds. The fellodistomid trematode sporocyst known as Cercaria haswelli Dollfus was found in mussels from all three locations. Laboratory infection experiments established that the cercaria from this sporocyst develops into the trematode Tergestia agnostomi (Manter). Gravid specimens of this trematode were obtained for the first time, from the mullet Aldrichetta forsteri Cuvier & Valenciennes. Two specimens of the bucephalid sporocyst described by Haswell (1903) were recovered and re-described.

The copepods Pseudomyicola spinosus Raffaele & Monticelli and Lichomolgus n. sp. were associated with the mussels, but their status is uncertain.

The post-planktonic stages of the pea-crab Pinnotheres novaezelandiae Filhol are described for the first time, and the seasonal abundance, effect of depth on abundance, and the effect of the crab on the host's condition are described. Differences between the zoea of apparently identical female crabs from different host species are noted and the significance of these is discussed. Because of the difference between the zoea of crabs from P. canaliculus and Atrina zelandica Gray, only the crabs from the former host are refered to as

P. novaezelandiae. The pea-crabs found in A. zelandica, C. glomerata, and M. edulis aoteanus, have not been assigned to a species.

Mytilus edulis aoteanus is host to Tergestia agnostomi sporocysts, Pseudomyicola spinosus, and Pinnotheres sp.

Crassostrea glomerata was collected from the Bay of Islands. Only one parasite, the copepod Pseudomyicola spinosus, was found in this host. A pea-crab Pinnotheres sp. is occasionally found associated with the oyster. A disease of this oyster, a symptom of which is the formation of necrotic pustules in the adductor mussel, could not be traced to any parasite. This disease is discussed in an appendix.

Ostra lutaria was obtained from Wellington Harbour, the Marlborough Sounds, and Foveaux Strait. Sporozoan cysts were found to occur in 10% of the oysters from Foveaux Strait, but were not observed to adversly affect the oyster. The sporocysts of the trematode Bucephalus longicornutus (Manter) occur in the areas sampled. Pseudomyicola spinosus infests the oyster in Wellington and in the Sounds, but not in Foveaux Strait.

It was concluded that there were no serious pathogens likely to infect the shellfish farms growing these species, and that there was little farmers could do at present to reduce the effect on the host of the symbionts already present in the shellfish beds.

A checklist and bibliography of all the parasites infecting New Zealand marine molluscs is included is an appendix.

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GENERAL INTRODUCTION

In Europe and in North America considerable damage has been done to the oyster and mussel industries by a variety of parasites. Haplosporidians of the genus Minchinia, and the fungus Labrynthomyxa (=Dermocystidium) marinum (Mackin Owen and Collier 1950), both cause mortalities in excess of 50% in the American oyster Crassostrea virginica (Gmelin 1791) (Sinderman 1968). In Europe the copepod Mytilicola has seriously reduced the production from the beds of Mytilus edulis Linnaeus 1758 (Cole & Savage 1951), and a fungus disease is presently devastating the oyster beds in the Aber estuary in France (Alderman 1974).

New Zealand has a thriving shellfish industry which was worth in excess of $3,700,000 in 1974. The five most valuable species harvested in that year were; the rock oyster Crassostrea glomerata (Gould 1850); the paua Haliotis iris Martyn 1784; the dredge oyster Ostrea lutaria Hutton 1873; the scallop Pecten novaezelandiae novaezelandiae Reeve 1853; and the green mussel Perna canaliculus (Gmelin 1791).

There was an extensive mortality of O. lutaria in 1958, and this has been attributed to the trematode Bucephalus longicornutus (Manter 1954), the sporocysts of which infect the mollusc (Miller 1963; Howell 1967). Other parasites could also affect our shellfish stocks, perhaps by being introduced into New Zealand, or by being transferred from one area to another. This was dramatically illustrated during the course of this study when it was found that 80% of the live Perna canaliculus brought from Ahipara and released into Wellington Harbour were infected with the spores of a gregarine (Nematopsis sp.)

Changes in the environmental conditions of either the host or the parasite are also capable of upsetting an existing host parasite relationship, causing heavy host mortality. Laird (1961) and Lipovski & Chew (1972) have demonstrated that adverse environmental conditions may weaken oysters to the extent that their resistance to disease and infection is impared. It has been suggested that the mysterious Malpeque

disease which caused a serious decline in the oyster fishery in Malpeque Bay Canada in 1915 was caused in this way (Laird 1961).

Because of the possibility of substantial expansion in the New Zealand shellfishing industry, particularly from oyster and mussel cultivation, a considerable amount of work has been done by both government and university scientists on the biology of the above species. As a contribution to this effort, a study was undertaken:

(1) to determine what parasites were present in the New Zealand oyster and mussel stocks,
(2) to determine, as far as possible, the effect of the parasites on their hosts,
(3) to gather information on the biology of the parasites.

Soon after the start of this study it became apparent that the project could not be restricted to those animals which could be demonstrated to be parasites. The accepted definition of a parasite is an animal in a "heterospecific relationship, be it permanent or temporary, during which there exists metabolic dependence of the parasite, the smaller of the two species, on its host" (Cheng 1967 p. 5)

A definition such as this works well for endoparasites such as trematode sporocysts which must absorb the nutrients necessary for growth from the tissues of the host, but it excludes other symbionts such as the pea-crab Pinnotheres, for which no metabolic dependance upon its host has been demonstrated. Nevertheless, many of the people who have studied pea-crabs are of the opinion that Pinnotheres is a parasite even although proof is lacking (see discussion at the end of the paper by Silas & Alagarswami 1967, and in Cheng 1967 pp. 98 - 100)

A similar example is Pseudomyicola spinosus (Raffaele & Monticelli 1885), which has been found on the gills of over 39 species of shellfish (Humes 1968). This apparently harmless copepod is now known to cause damage to the gut of C. glomerata (Dinamani & Gordon 1974) and it is probably metabolically dependant upon its host (Gordon pers. comm.).

The study was therefore broadened to include those species whose relationship to their molluscan host is not yet known. This includes Pinnotheres and the copepod genera Pseudomyicola and Lichomolgus, but excludes the shell boring polychaetes such as Polydora which will also bore into limestone, and the "oyster leeches" (turbellarian worms) which feed on dead or dying oysters.

The approach to this study had firstly to be qualitative, and it was necessary to describe or re-describe several species.

Later, some quantitative investigations were carried out. This latter part of the study centered around the parasites of Perna canaliculus in Wellington Harbour, and in addition, preliminary work was done to determine the seasonal pattern of infection of P. spinosus in C. glomerata from the Bay of Islands.

An experiment to determine the adult trematode of Cercaria haswelli Dollfus 1927 was conducted, and and attempt to rear the zoea of Pinnotheres novaezelandiae Filhol 1886 is described briefly.

Because of the variety of parasites and hosts delt with in this study, the thesis has been divided into three sections: Methods and materials; systematics; and results. The results section has been compiled with the shellfish biologist in mind, so the format is similar to that used by Cheng (1967) in his review of the known parasites of commercially important molluscs. The section begins with a list of the parasites and suspected parasites found in each of the host species studied. Each symbiont is then delt with in a systematic sequence, giving a summary of its biology together with relevant discussion. A general discussion follows the last section.

SECTION 1

MATERIALS AND METHODS.

MATERIALS & METHODS

(I) Extent and source of the material examined.

A total of 858 Perna canaliculus, 153 Ostrea lutaria, and 237 Crassostrea glomerata were examined for parasites. The material came from five different areas: Ahipara; Bay of Islands; Wellington harbour; Marlborough Sounds; and Foveaux Strait. (see figs. 1-3)

Table 1 shows the species, size, area, and month of collection for each sample.

The Ahipara, Bay of Islands, and Foveaux Strait samples were provided by personnel from the Fisheries Research Division, Department of Agriculture and Fisheries.

Wellington and Marlborough Sounds samples were collected by colleagues and the author.

For experiments to establish the life-cycle of the trematode Cercaria haswelli, found in P. canaliculus, it was necessary to collect samples of the yellow-eyed mullet Aldrichetta forsteri (Cuvier & Valenciennes 1836). A total of 67 fish from Wellington Harbour and 10 fish from Lake Ellesmere were examined for trematodes.

TABLE 1

Material examined

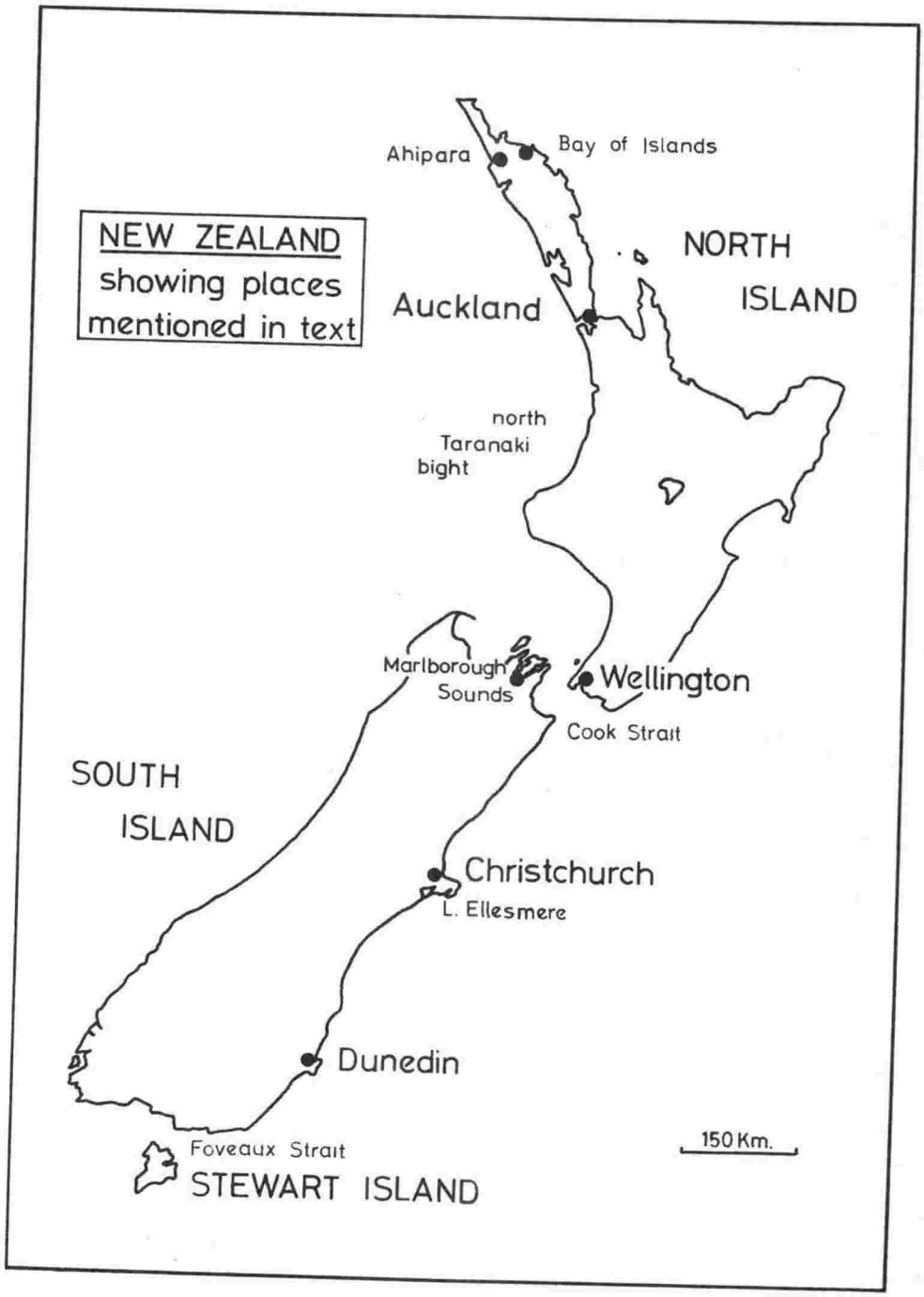
	Ahipara	Bay of Islands		Wellington			Marlborough Sounds					Foveaux Strait		
	Perna	Crassostrea		Perna Ostrea			Perna					Ostrea		
		Orongo Bay (Farm Trays)	WAITANGI	WARD ISLAND	SEATOUN	EVANS BAY	PELOROUS SD	KENEPERU SD	N.W. BAY	CRAIL BAY	KENEPERU RAFT	AREA H	AREA I	AREA ?
	A	A		B	C		B					B		
1973														
MAR				32		6		14			30			
APR				23		5								
MAY														
JUN				22					26					
JUL	25		8	35								50		
AUG		25		43					24					
SEPT				50				42						
OCT			41	42										
NOV				49										27
DEC				49						38				
1974														
JAN				41										
FEB				31										
MAR			25	56						46			32	
APR														
MAY		37			19	12								
JUN	13													
JUL		25												
AUG														
SEPT		25			39									

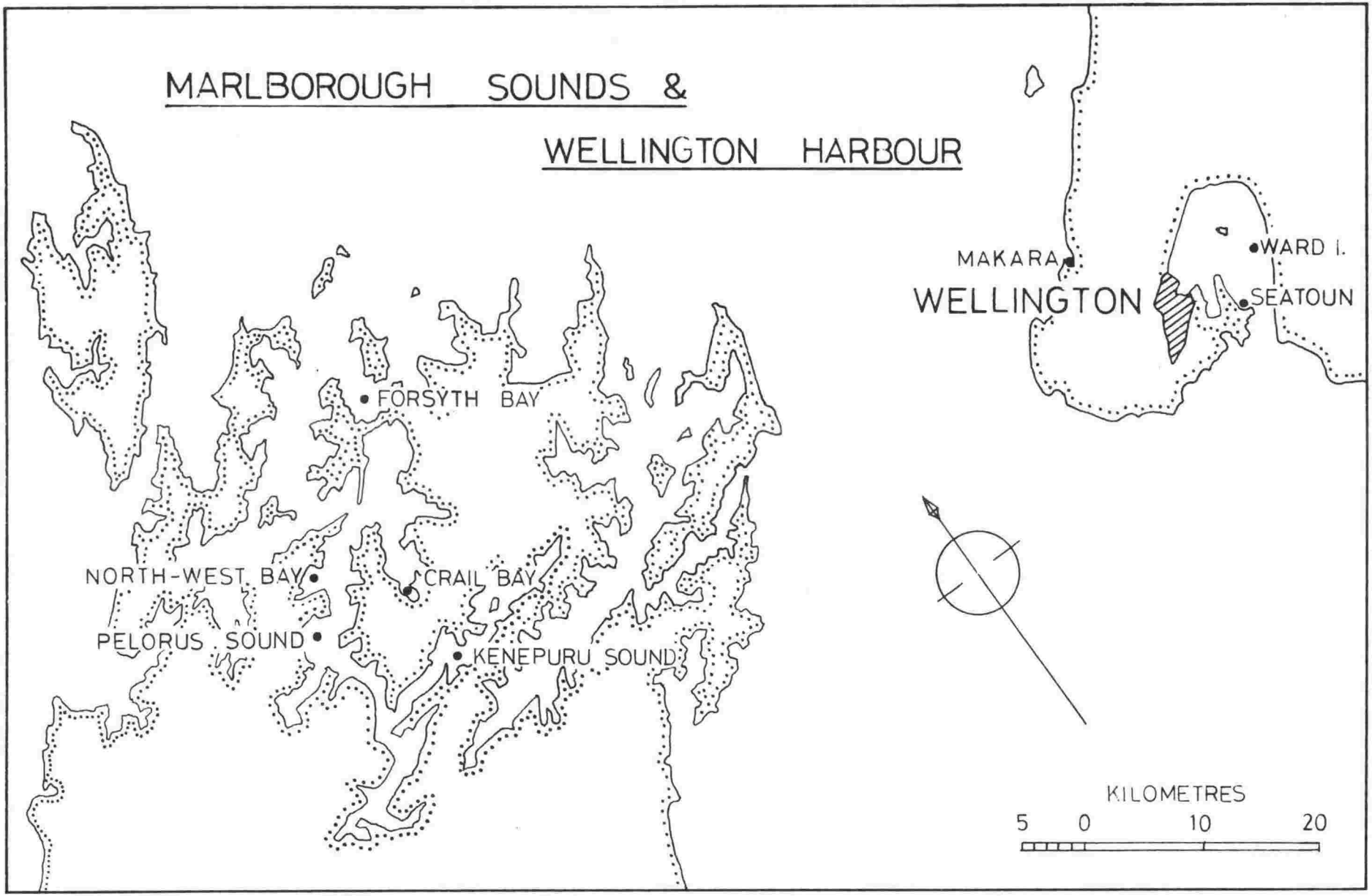
KEY

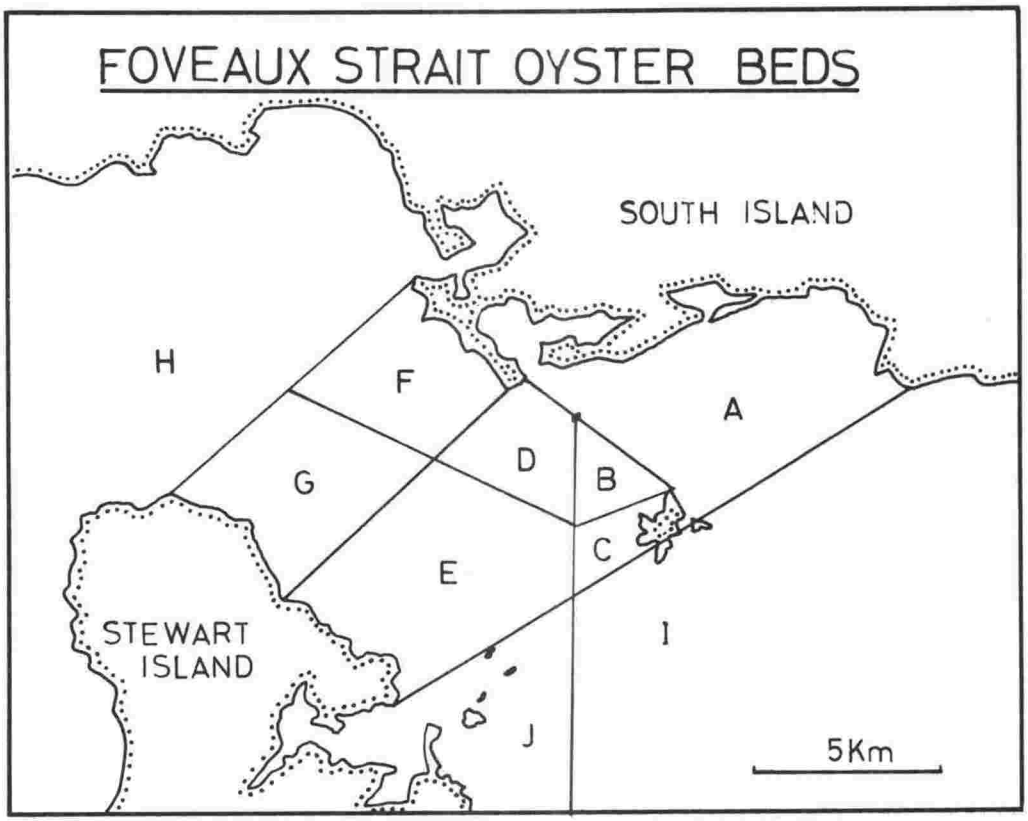
A Picked at low tide

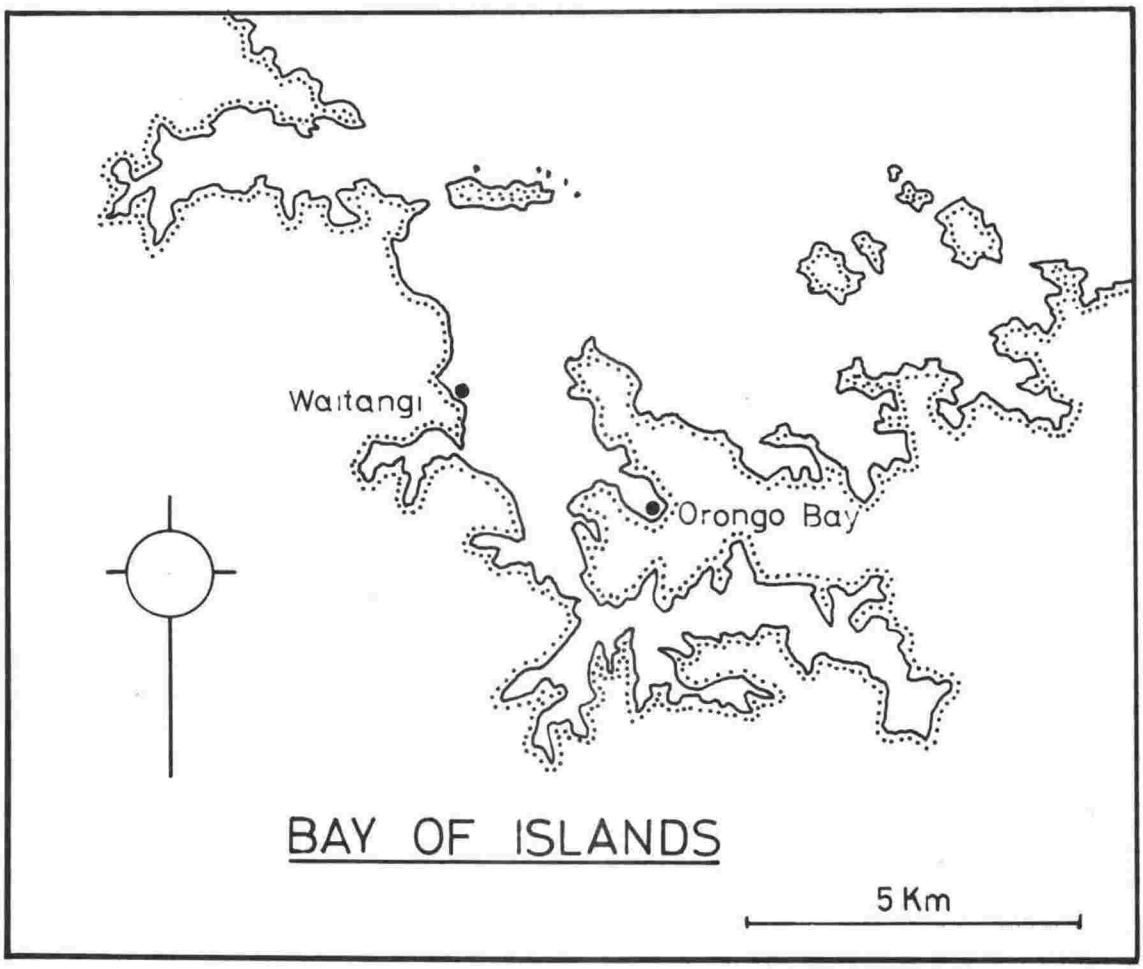
B from 4-5m depth

C from 2m depth


NEW ZEALAND
showing places
mentioned in text
Ahipara
Bay of Islands
NORTH
ISLAND
Auckland
north
Taranaki
bight
Marlborough
Sounds
Wellington
Cook Strait
SOUTH
ISLAND
Christchurch
L. Ellesmere
Dunedin
Foveaux Strait
STEWART ISLAND
150 Km.


MARLBOROUGH SOUNDS &
WELLINGTON HARBOUR
FORSYTH BAY
NORTH-WEST BAY
CRAIL BAY
PELORUS SOUND
KENEPURU SOUND
MAKARA
WELLINGTON
WARD I.
SEATOUN
KILOMETRES
5 0 10 20


FOVEAUX STRAIT OYSTER BEDS
SOUTH ISLAND
H
F
A
G
D
B
C
E
I
STEWART ISLAND
J
5Km


Waitangi
Orongo Bay
BAY OF ISLANDS
5 Km

(II) Field technique.

Shellfish were obtained in a number of ways. Table 1 shows the methods used for each sample. Samples from 4 - 5m depth include shellfish collected with an Agassiz trawl, and shellfish collected from a similar depth by skin-diving. The 2m sample was collected by skin-diving only.

Foveaux Strait samples were obtained from the commercial oyster beds, which extend from the lower shore line a depth of 50 metres. The beds have been divided into a number of regions by the Department of Agriculture and Fisheries (fig. 3). The samples examined in this study came from area H in July 1973, an unknown area in November 1973, and from area I in March 1974.

Samples of shellfish were collected live and undamaged. The shellfish were washed to remove sediment and fouling organisms before transfer to the laboratory, Samples from Ahipara were brought to Wellington by Fisheries personnel, and collected by the author from the Fisheries Laboratory tanks in Wellington. Rock oysters and dredge oysters from Foveaux Strait were flown to Wellington live in plastic bags. The samples from Marlborough were brought across Cook Strait by sea, in containers of sea-water.

Collecting samples of yellow-eyed mullet was not easy. The mullet is a schooling fish, and proved difficult to catch undamaged. The Wellington samples were caught using a baited hook, or with a small box net. The Lake Ellesmere fish were caught by Fisheries Research personnel using a beach seine. Lake Ellesmere is separated from the sea by a sand-bar, and the sample was taken at the point where the lake emptied over the sand-bar into the sea.

All the fish were frozen until they could be examined. In addition to the above samples, twelve mullet from Wellington Harbour were caught in a box net and were transferred live to laboratory tanks for use in the infection experiments.

(III) Laboratory techniques.

(a) Examination of the shellfish.

On arrival at the laboratory, any shellfish which could not be examined within 24 hours were preserved in either 10% formalin or Davidsons fixative. Each shellfish was assigned a unique code so that the measurements, slides, and parasites could be related to one another. The shell was measured from the hinge to the bill, before opening by severing the adductor muscle. The shellfish was examined macroscopically, and the condition, macroparasites and abnormalities were recorded. Individually the meats were removed from the shell, washed in clean sea-water to remove sand, blotted dry and immersed in a measuring cylinder of water. The change in volume of the water in the cylinder registered the volume of the shellfish meat. The internal volume of the shells was measured in one of two ways. For small shellfish and rock oysters, the cleaned but still unopened shellfish were immersed in a measuring cylinder of water. The change in volume of the water gave the total volume of the shellfish. The procedure was repeated using the empty shells, the volume of which was subtracted from the total volume to give the internal volume.

This method was unsatisfactory for large shellfish, and for preserved shellfish which had gaped open, so after the meat was removed, the shells were filled with a known quantity of water, thus giving the internal volume directly (Anderson, 1975). The error in this method was approximatly 5% when compared to the first method, which was used as the standard.

It is generally agreed (Galtsoff, 1964; Wilber & Gilchrist, 1965) that body component indicies are reliable indicators of the general health of many animals.
A measure of the condition factor of the shellfish in this study was obtained using the formula:

$$\frac{\text{meat volume}}{\text{internal volume}} \times \frac{100}{1} = \text{C.F.}$$

A section of tissue was next cut from the shellfish meat. The first transverse cut was made where the palps and gills meet. The second cut was made 5-6mm behind the first cut. The section of tissue removed was placed in a small vial of Davidsons fixative together with a label bearing the code of that shellfish. After at least 24 hours the tissue was embedded in paraffin wax and sectioned at 7 µm. Slides were stained with haematoxylin and eosin, cleared in xylol, and mounted in DePex.

After the discovery of Nematopsis spores in the tissue of P. canaliculus, a technique to rapidly diagnose for the infection was developed. The tip of one of the palps was removed and squashed onto a slide, A drop of sea-water and a coverslip were added. The spores could then be seen under a compound microscope fitted with phase contrast of Normaski differential interference contrast.

All scissors, dishes and scalpels used for cutting the shellfish were rinsed between shellfish to avoid any contamination.

(b) Preservation of parasites.

Copepods were washed and preserved in 70% isopropyl alcohol. Drawings were made from specimens dissected with mounted needles, or from whole mounted specimens. The mounting medium used was either lactic acid or Berlese fluid.

The carapace width of each pea-crab was measured using vernier calipers, or with a sterio-microscope fitted with a calibrated occular micrometer. The stage of development of the gonad in mature female crabs was determined using the scale shown in the table 2.

TABLE 2.

Development stages of crab gonad

1. no eggs in abdomen, abdomen has an orange stripe.
2. abdomen bears eggs, but is not full.
3. abdomen distended with bright orange eggs which do not have eye-spots

4. eggs brown-orange with eye-spots.
5. pre-zoeal stage (zoea in egg membrane) or hatching out.

The post-planktonic development stage of all the crabs was determined using the table given by Christensen & McDermott (1958 p. 154)

Crabs were preserved in 5% formalin for one week, then washed and transferred to 70% isopropyl alcohol.

It was necessary to soak the crabs in lactic acid for one week before dissecting out the mouthparts in order to clear the chitin and prevent the parts breaking during the dissection. Mouthparts and pleopods were mounted in lactic acid for microscopical examination.

Trematode material was fixed in warm formalin-acetic acid, stained with acetic acid alum-carmine, cleared in beechwood creosote and xylol, and then mounted in DePex.

(IV) The ultrastructure of Nematopsis spores.

The electron microscope material was prepared in the Victoria University electron microscope unit by Mr M.N. Loper. Three live Perna canaliculus from Ahipara were cut open and a 2mm portion removed from the tip of each palp. The six pieces of tissue were fixed for 1½ hours in a 1% solution of osmic acid in sea-water, and embedded in Araldite. Sectioning was done with an ultramicrotome fitted with a glass knife, the section was stained with an acid-lead stain, and examined in a Zeiss EM9A electron microscope.

(V) Life history studies on Cercaria haswelli Dollfus 1927

(a) Behaviour of the cercaria:

(1) Movement of cercaria and response to gravity.

A two-litre measuring cylinder was filled with sea-water. Ten cercariae were added and examined each hour until they ceased to move.

(2) Response to light.

A horizontal glass tube was filled with sea-water and 25 cercariae were added. These were distributed as equally as possible along the tube, half of which was covered in

black paper to exclude the light. The cercariae in the light side were counted every 10 minutes over a one hour period, after which the light side was blacked out, the dark side uncovered and the experiment repeated.

(3) Response to temperature.

A horizontal glass tube was filled with sea-water and cercariae as in (2) above, but a thermometer was inserted at each end of the tube. One end of the tube was heated on a hotplate while the other was cooled with ice.

Gray (1965), and Schiff (1974) using troughs instead of a glass tube, found that a temperature gradient free of convection currents was formed provided that the water in the trough was less than 2 cm deep.

Assuming that an even temperature gradient formed between the hot and cold ends of the tube, it was possible to estimate the temperature of the area in which the cercariae concentrated.

(b) Infection experiments.

Six fish (A. forsteri) were collected live from Wellington Harbour, and placed in a tank to acclimatise. After about seven days the fish began to take food (minced cooked meat and bread). They were fed for a further twenty days before they were removed from the tank, anasthesitised with tricane methanesulphonate (MS-222) and then each was dosed with 100mg/Kg of 2-4 di-n-butyl tin oxide (an anthelmintic). The anthelmintic was introduced using a stomach tube connected to a syringe. The fish were allowed to recover and were separated into two tanks, each of three fish. Both tanks had a filtered sea-water supply to prevent accidental entry of cercariae.

After two weeks three fish were fed with live cercariae of C. haswelli. The remaining fish were kept as controls.

Four weeks post-infection all the fish were killed and examined for trematodes.

This experiment was repeated using six fish, without controls, but after dosing they were put into two separate tanks, three fish in each tank. The fish were dosed with 0.3g anthelmintic mixed with 4.3 gm minced meat moistened with vegetable oil (the anthelmintic is soluble in oil),

fed over a seven day period. It was decided not to introduce the drug directly into the stomach, because the small size of the fish made injury, and the subsequent introduction of fungus disease, likely.

(VI) Examination of Pinnotheres zoea

(a) Rearing of the zoea.

Ovigerous crabs were removed from freshly opened mussels and placed in individual aquaria, without food, but with a constant supply of fresh sea-water. When the eggs hatched, the zoea swam to the surface and concentrated in the area of the aquarium nearest a light source. The zoea were then pipetted into 100mm diameter finger bowls filled with sterilised and millipore filtered sea-water. The maximum number of zoea in each bowl was 25. Each day the live zoea were transferred to sterile bowls containing fresh sterilised and filtered sea-water.

In this way eight separate hatches of zoea were obtained from pea-crabs removed from P. canaliculus, and three hatches of zoea were recovered from crabs inhabiting Atrina zelandica Gray 1835.

Attempts to feed the zoea with Isochrisis, Artemia larvae, and freshly hatched barnacle nauplii were apparently unsucessfull. The zoea could not be induced to moult into the second zoel stage.

Mouthparts and appendages of zoea were dissected and mounted in Berlese fluid for microscopical examination.

(b) Scanning electron microscope examination of zoea

Live zoea were placed in a drop of sea-water in a metal watchglass. This was dipped into liquid freon, and then liquid nitrogen for about five minutes. The watchglass was then placed in a vacuum overnight. Salt crystals left on the zoea were washed off with distilled water, and the zoea returned to a vacuum for a further four hours. The dry brittle zoea were mounted, with the aid of a sterio-microscope, onto metal stubs which had previously been coated with double-backed sellotape as an adhesive. The zoea were earthed to the metal stub using

silver paint, then coated with gold-palladium. The scanning electron microscope used was a Cambridge S600.

Initially four zoea were examined, two from P. canaliculus derived crabs, and two from Atrina crabs.

A second pair of zoea from each host (as above) were examined, but these had previously been killed and fixed in 5% formalin, which was rinsed off prior to freeze drying.

SECTION II

SYSTEMATICS

"So out of the ground the Lord God formed every beast of the field and every bird of the air, and brought them to man to see what he would call them; and whatever man called every living creature, that was its name." Genesis 2:19

NEMATOPSIS N. SP. (SPOROZOA : GREGARINIDAE) IN *PERNA CANALICULUS* (NOTE)

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ABSTRACT

Spores of a new species of Nematopsis have been found in the green-lipped mussel Perna canaliculus Gmelin. This is the first record of a sporozoan parasite of a New Zealand shellfish.

INTRODUCTION

The genus Nematopsis belongs to the Porosporidae Labbé, a family of gregarines which have an alternation of hosts, where marine molluscs serve as intermediate hosts and decapods as primary hosts. Nematopsis is believed to be harmless to the host shellfish, even when present in large numbers (Sprague 1970).

Spores belonging to the genus Nematopsis were discovered in Perna canaliculus collected at Ahipara (35°05'S, 173°10'E) where approximately 80% of the mussels are infected. The sporozoan has since been found in Wellington Harbour (41°06'S, 174°50'E) and the Marlborough Sounds (41°06'S, 174°00'E), but in these two areas it is rare.

DESCRIPTION

The cyst (Fig. 4) is usually spherical, 30-40 µm in diameter, with a heavy cyst wall 1-3 µm thick. The cyst may contain from 2 to 10 spores, but the average number is 4.

The cysts may be found in any of the organs of P. canaliculus but they are found most frequently in the connective tissue, especially in the palps and at the base of the gills.

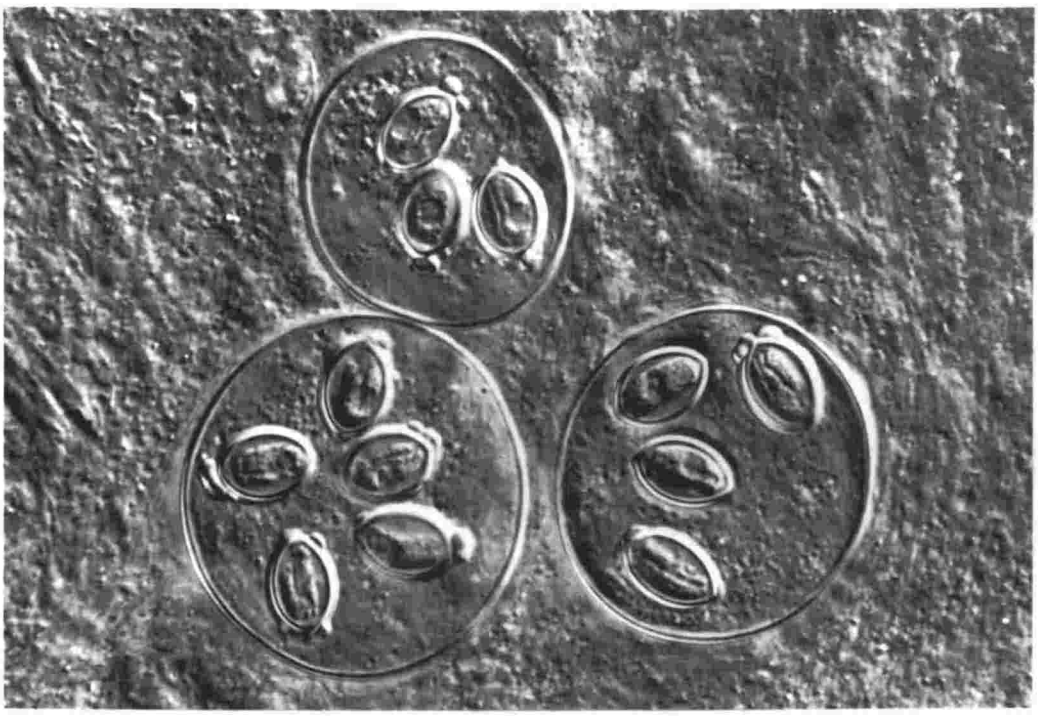
The spores are hard shelled, ellipsoidal and circular in cross-section. The spores all measure 7 X 12 µm, and each spore contains a single vermiform sporozoite.

DISCUSSION

The size and shape of the spores separate this species of Nematopsis from all other species so far described. The cyst wall is thicker than is usually found for Nematopsis spores (Sprague, pers. comm.), but electron microscope studies (author, unpublished) reveal that most of this

FIGURE CAPTION

Figure 4: Nematopsis n. sp. cysts. Normaski differential interference contrast, X 500.



wall is laid down by the host shellfish.

The presence of Nematopsis in freshly opened P. canaliculus can be determined by cutting 2-3 mm off the tip of a palp then squashing the removed portion on a slide. After adding a drop of sea-water and a cover-slip, the slide may be examined under a compound microscope. The spores can be seen with transmitted light, but best results are obtained using Normaski differential interference contrast, or phase contrast.

ACKNOWLEDGEMENTS

I wish to thank Dr. V. Sprague for identifying the spores and Mr R.W. Hickman for the shellfish from Ahipara.

LITERATURE CITED

SPRAGUE, V. 1970: Some protozoan parasites and hyper-parasites in marine bivalve molluscs. pp 511-526 in Snieszko (ed.) A symposium on diseases of fishes and shellfishes. Special publication 5, American Fisheries Society, Washington., i-viii, 526pp.

A RE-EXAMINATION OF THE CERCARIA AND SPOROCYST OF THE "GASTEROSTOMUM" DESCRIBED BY HASWELL (1903)

INTRODUCTION

Two specimens of the green mussel Perna canaliculus recovered during 1973 from Wellington Harbour, and preserved in formalin, were later found to be infected with bucephalid sporocysts. These were identified as the sporocysts of the "gasterostomum" described by Haswell (1903). Cercariae were obtained from the mantle cavity of the shellfish, and by teasing open the sporocysts. The sporocysts and cercariae are here re-described in more detail than is given in the original description.

The sporocyst: Bright red in colour, elongate and branching at irregular intervals. The sporocyst is more or less uniform in diameter. It is impossible to determine the number of individual sporocysts infecting a mussel, since the branches lie in a tangle throughout the gonad tissue. The sporocyst contains cerceriae in all stages of development.

In transverse section (Fig. 5b) the sporocyst is rounded and hollow, covered with a cuticle 2-3 μm thick. Within the cuticle is a nucleated layer of cytoplasm 8-16 μm wide, although in places it may appear considerably wider where the section has been cut at an angle to the sporocyst. Individual cell membranes appear incomplete and indistinct.

There are three types of nuclei visible in the cytoplasm layer, Type one is an ovoid to spherical vesicular nucleus, 5-6 μm in diameter, with a distinct densly staining nucleus.

The second type is an ovoid nucleus 4-8 μm in diameter, containing scattered chromatin globules. Type three is a germinal nucleus found near the terminal regions of the sporocyst. This type is spherical, 5-7 μm in diameter, with a nucleolus and many granules of chromatin in the nucleoplasm which stain more densly than in the other two types of nuclei.

No muscle fibres were observed and no feeding or nutrative branches were seen.

The cercaria: (Fig. 5a) consists of a body, tail stem and long furcae. The body is small (0.25mm length), elongate and cylindrical. The anterior half is covered with fine cuticular spines. At the anterior end is a large ovoid

cystogenous organ measuring 40 x 50 µm. The cystogenous organ opens anteriorly through a small pore surrounded by four lips. There is an "H" shaped ganglionic mass just posterior to the cystogenous organ.

The oral sucker is situated about 60 µm from the posterior end of the body. The pharynx is spherical, 24 µm in diameter.

Intestine sacculate, up to 45 µm long by 22 µm wide, but may contract to 48 µm x 4-5 µm. The intestine is lined with cuboidal epithelium.

Genital anlagen consist of two lobed masses of densly staining nuclei posterior to the pharynx, each 30 µm long by 16 µm wide. The genital pore is situated at the posterior extremity of the body.

The excretory vesicle is saccular, beneath the genital anlagen. The excretory tubules and flame cells were not seen.

The tail is un-ornamented. A paranchymatous layer within the cuticle of the tail stem is continuous with an axial strand of paranchyma. The furcae are circular in cross-section, and taper distally. They measure 1.75 to 2 times the length of the body.

DISCUSSION

The red colour of the sporocysts was remarked upon by Haswell. He knew of no other case where red colouring was present in the sporocysts. No other bucephalid of a comparable colour is known to the author, although the sporocysts of Cercaria mytili Cole 1935 are a pale yellow (Cole 1935). It is probable that the colour in the bucephalid described by Haswell is caused by the same factor which causes the red colouration in the sporocysts of Tergestia agnostomi (see section III, p. 114)

Haswell did not realise that the anterior cystogenous organ was not a sucker, and refers to it as an "anterior sucker or proboscis". The "pair of relatively long and slender cilia" which Haswell described as extending forwards from the anterior end of the cercaria, were not seen.

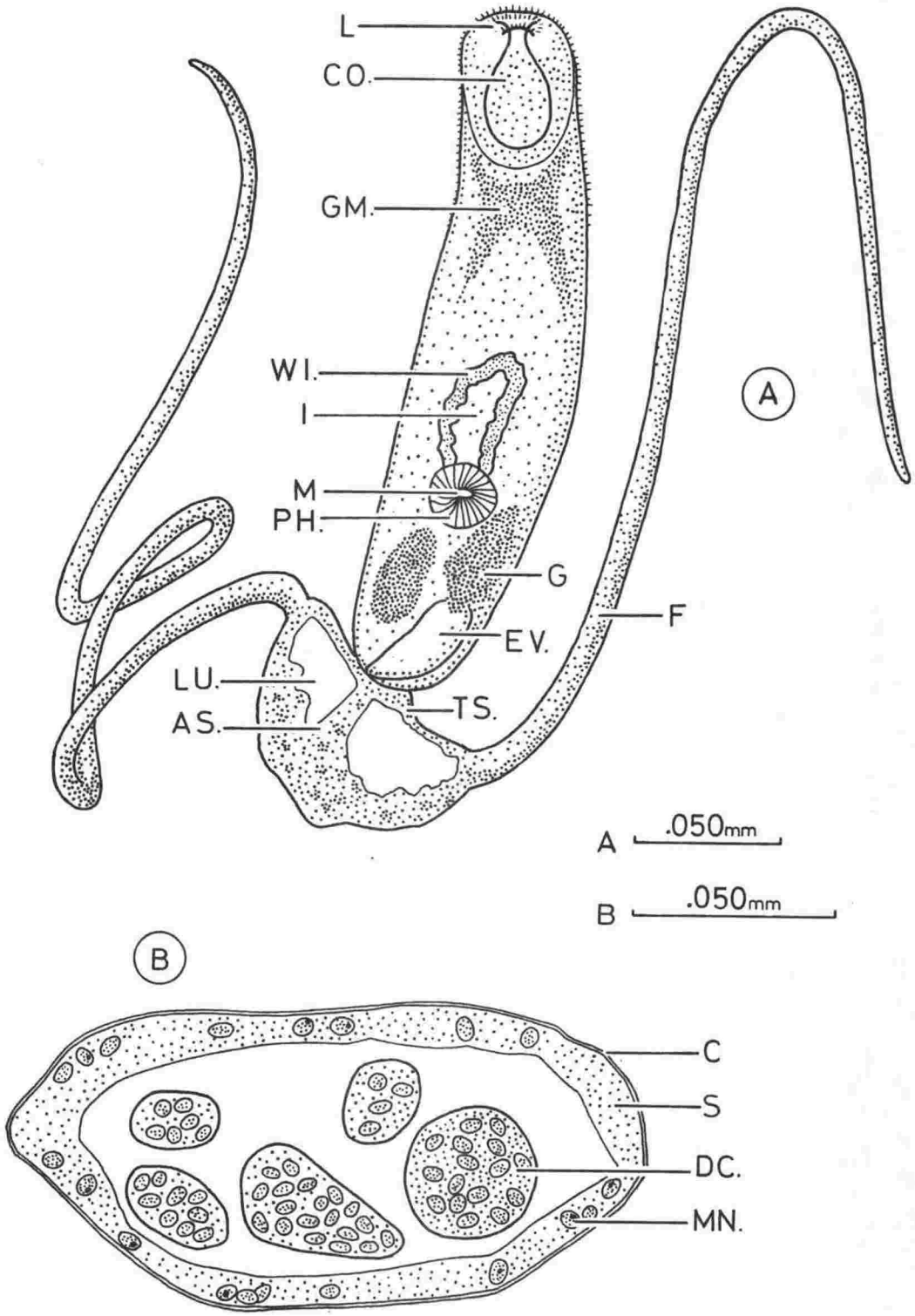
FIGURE 5

(a) Cercaria sp. Haswell 1903

(b) Transverse section of a sporocyst

List of abbreviations

AS., axial strand; C., cuticle; CO., cystogenous organ; DC., developing cercariae; EV., excretory vescicle; F., furca; G., genital anlagen; GM., ganglionic mass; I., intestine; L., four lipped anterior extremity; LU., lumen; M., mouth; MN., mesenchymal nuclei; PH., pharynx; S., syncytial layer; TS., tail stem; WI., wall of intestine.


L
CO.
GM.
WI.
I
M
PH.
G
F
EV.
LU.
TS.
AS.
A
A .050mm
B .050mm
B
C
S
DC.
MN.

Haswell inferred that the entire cellular layer of the sporocyst was a germinal epithelium, and that any part was capable of producing germ cells. As germinal nuclei (similar to those described by Howell, 1966) were found only in the tips of the sporocysts, Haswells inference is incorrect.

A RE-DESCRIPTION OF *TERGESTIA AGNOSTOMI* MANTER 1954, BASED ON GRAVID SPECIMENS

INTRODUCTION

Experimental evidence (see section III, p.106) shows that Cercaria haswelli, found in P. canaliculus, develops into Tergestia agnostomi Manter (1954) in the yellow-eyed mullet Aldrichetta forsteri. Tergestia agnostomi was described by Manter (1954) from A. forsteri caught in Wellington Harbour. As none of Manter's specimens were gravid he suggested that A. forsteri might not be the definative host.

Yamaguti (1958) suggested that T. agnostomi might be an immature T. laticollis Rudolphi (1819) which is widely distributed in the Mediterranean, Caribbean, and Japan.

The discovery of gravid T. agnostomi in the mullet A. forsteri shows that T. agnostomi is a valid species, and that A. forsteri is a definative host.

Tergestia agnostomi Manter 1954
(see figure 31 , section III, p107)

Material. Three fish containing a total of four gravid Tergestia were obtained from a sample of 14 A. forsteri caught at Days Bay , Wellington Harbour. A further 8 gravid Tergestia were recovered from a specimen of A. forsteri caught in Lake Ellesmere. Lake Ellesmere is a freshwater lake separated from the sea by a sand-bar. Aldrichetta is amphidromous, moving freely from fresh to salt water (Woods, 1963). The Lake Ellesmere fish containing Tergestia had a parasite fauna identical to A. forsteri caught in a marine environment, while the other fish in the sample had a freshwater or estuarine parasite fauna dominated by the nematode Hedruris spinigera Baylis 1931.

Description. (Measurements quoted are averages from 8 Lake Ellesmere specimens) Body elongate, length 2.12mm, greatest width 0.29mm, Oral sucker 0.25 x 0.29mm diameter. Acetabulum 0.20 x 0.18mm in diameter, being smaller than the oral sucker in the ratio 5:4. Oral sucker surrounded by a semicircle of lobes dorsally and laterally. There are six pairs of "flanges" posterior to the oral sucker, as

TABLE 3

Sizes of *Tergestia* from different locations

	Source of data, or sample origin		
	Manter, 1954	L. Ellesmere	Days Bay
Number of specimens	5	8	4
length	1.218-1.554	1.95-2.60 (2.125)	0.92-1.60 (1.204)
width	0.230-0.292	0.26-0.31 (0.29)	0.208-0.32 (0.295)
oral sucker	0.169-0.232	0.208-0.260 (0.243)	0.246-0.164 (0.196)
acetabulum	0.131-0.161	0.184-0.208 (0.196)	0.144-0.205 (0.168)
sucker ratio	-	5:4	5:4
ovary	-	0.082	0.082
testis	-	0.082	0.082
egg sizes	-	32 x 24 µm 37 x 22 µm 32 x 21 µm 40 x 28 µm	36 x 24 µm 36 x 28 µm 32 x 24 µm 40 x 28 µm
pharynx length	0.176-0.192	0.205-0.246 (0.219)	0.143-0.195 (0.169)
pharynx width	0.092-0.108	0.143-0.156 (0.147)	0.082-0.113 (0.095)

All measurements in mm unless otherwise stated. Figure in brackets is the mean.

as recorded by Manter, (each flange is 0.205mm long, 0.143 - 0.102mm wide). Oesopagus long, forking just posterior to acetabulum. Caecae end blindly just posterior to testis.

Testis ovoid, diagonal and intercaecal, each 0.125 x 0.35mm. Anterior half of cirrus sac contains a pars prostica and wide cirrus, the narrower posterior part contains a straight seminal vesicle. Ovary circular, 0.082mm in diameter, situated above the testis. Uterus distended with eggs from point near acetabulum to posterior tip of trematode.

Eggs ovoid, and measure 0.021 x 0.032mm to 0.022 x 0.035mm.

The excretory system was not observed. Vitellaria extend from between the acetabulum to the anterior testis.

DISCUSSION

The specimens conform well to the description given by Manter (see table 3). The size of the oral sucker which is larger than the acetabulum, separates *T. agnostomi* from all the *Tergestia* so far described, all of which have an acetabulum which is larger than, or as large as the oral sucker.

A NEW SPECIES OF *LICHOMOLGUS* (COPEPODA : CYCLOPOIDA) ASSOCIATED WITH THE MUSSEL *PERNA CANALICULUS* GMELIN

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Victoria University, Wellington.

ABSTRACT

A new species of cyclopoid copepod belonging to the genus Lichomolgus Thorell, which was obtained from the gills of the bivalve Perna canaliculus, is described and illustrated.

INTRODUCTION

During a study of animals closely associated with some New Zealand marine shellfish, a new copepod was discovered on the gills of Perna canaliculus a common edible mussel occuring both intertidally and below low tide level around much of the New Zealand coastline. The copepod, described below, belongs to the genus Lichomolgus as re-defined by Humes and Stock (1973).

FAMILY: LICHOMOLGIDAE

GENUS : Lichomolgus Thorell 1859

Lichomolgus uncus n. sp.

Female: (Figs 6-9): Body cyclopoid, length (excluding ramal setae) 1.25mm (1.0 - 1.67mm). Greatest width 0.42mm (0.30 - 0.50mm), based on ten specimens in alcohol. Length to width ratio of prosome 1.45:1. Ratio prosome to urosome 1.14:1. Urosome five-segmented. First segment is 82x102.5 μ and bears the fifth legs. Next segment is the greatly expanded genital segment which is heart shaped, and bears the eggstrings. The width at the anterior end of the genital segment is 164 μ. The three post-genital segments are 102, 152, and 49 μ in length.

Rostrum: (Fig. 7b) has its posterio-ventral margin in the shape of a parabola.

Antennule: (Fig. 6a) seven-segmented. Lengths of segments 32, 56, 18, 40, 60, 28 and 16 μ (measured along posterior non-setiferous margins). The formula for the armature is 4, 13, 3, 3, 4+1, 2+1, 7+1. All setae naked.

Antenna: (Fig. 7c) four-segmented, the first two segments are short, the second bears a seta. The third segment bears two setae on the medial inner surface, and three setae on the distal apex. The tiny fourth segment bears three curved claws, and a claw-like spine at the apex. One claw is 152 μ in length, the other two are approximately 64 μ long. All setae naked.

Mandible: (Fig. 6d) has on its concave margin a row of long slender spinules. The convex margin bears a row of fine spinules extending onto the long terminal lash.

First Maxilla: (Fig. 6c) bears two terminal setae. The distal margin is distinctly pointed.

Second Maxilla: (Fig. 6b) has an unarmed first segment. The second segment bears two short setae, one on the posterior surface and one inserted beneath the lash. On its convex margin the lash has a row of slender spinules, on the concave side a few barbules.

Maxilliped: (Fig. 7a) three segmented. First segment unarmed. The second segment bears two naked setae, and numerous spinules. The third segment bears a terminal spine and a tiny setule.

Legs 1-4 (Figs. 7d, 8c, 8d, 9c) have the following armature (Roman numerals indicate spines, arabic numerals represent setae)

P1	coxa 0-1	basis 1-0	exp. I-0	I-1	III,	I, 4
			end. 0-1	0-1		I, 5
P2	coxa 0-1	basis 1-0	exp. I-0	I-1	III,	I, 5
			end. 0-1	0-2	III,	3
P3	coxa 0-1	basis 1-0	exp. I-0	I-1	III,	I, 5
			end. 0-1	0-2	III,	2
P4	coxa 0-1	basis 1-0	exp. I-0	I-1	II,	I, 5
			end. 0-1	0-II		

Leg 5 (Fig. 9a) the inner margin of the free segment is expanded so that the greatest width is $\frac{3}{4}$ of the distance along the axis from the base of the segment. There are two terminal setae, almost equal in length, and a short seta near the base of the free segment.

Leg 6 is represented by two setae and a spiniform process near the attachment of the eggsack.

Caudal ramus. Ratio length to width 10:1. There is a short seta on the outer surface approximately halfway along the ramus. A short setule, also on the outer surface, is just back from the apex which bears two short naked setae.

Colour in life: Light golden yellow, with a red eyespot.

Male: (Figs. 8b, 9b, d) smaller than the female, with a more pointed prosome and a six-segmented urosome. Length, excluding ramal setae, is 1.0mm (0.90 - 1.20mm). Greatest width 0.38mm (0.30 - 0.45mm) based on ten specimens in alcohol. Ratio of prosome to urosome is 1.14:1.0. The only appendages showing marked sexual dimorphism are the maxilliped and legs 5 and 6.

Maxilliped: (Fig. 8b) slender and three-segmented. (four-segmented if the proximal part of the claw represents a segment.) The second segment bears two setae and a row of setules. The strongly curved claw, 168 μ in length, bears a proximal seta.

FIGURE 6

Lichomolgus uncus n. sp., female

(a) dorsal view

(b) second maxilla

(c) first maxilla

(d) mandible

(e) antennule

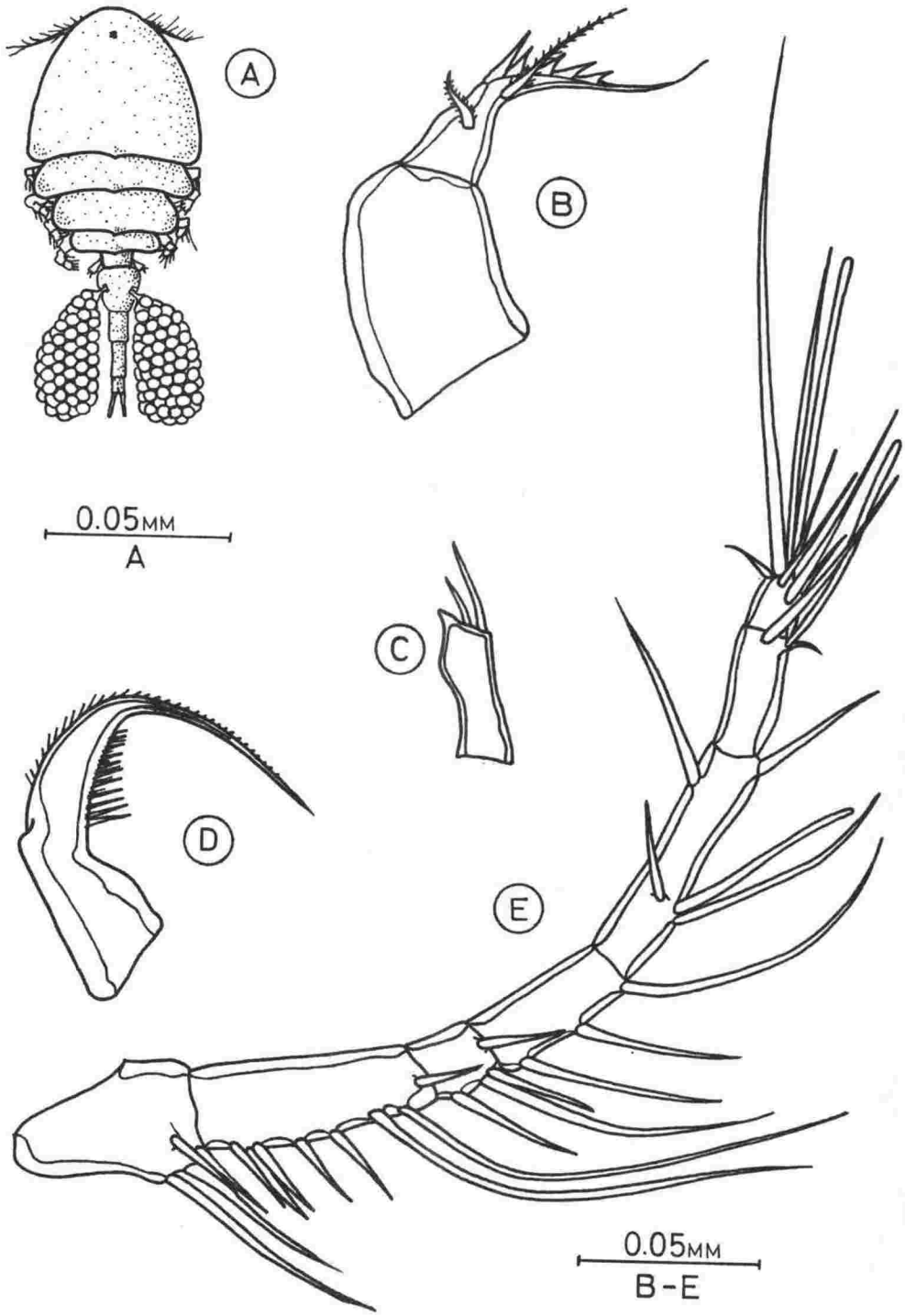

A
B
0.05мм
A
C
D
E
0.05мм
B-E

FIGURE 7

Lichomolgus uncus n. sp., female

(a) maxilliped

(b) rostral area

(c) antenna

(d) first leg.

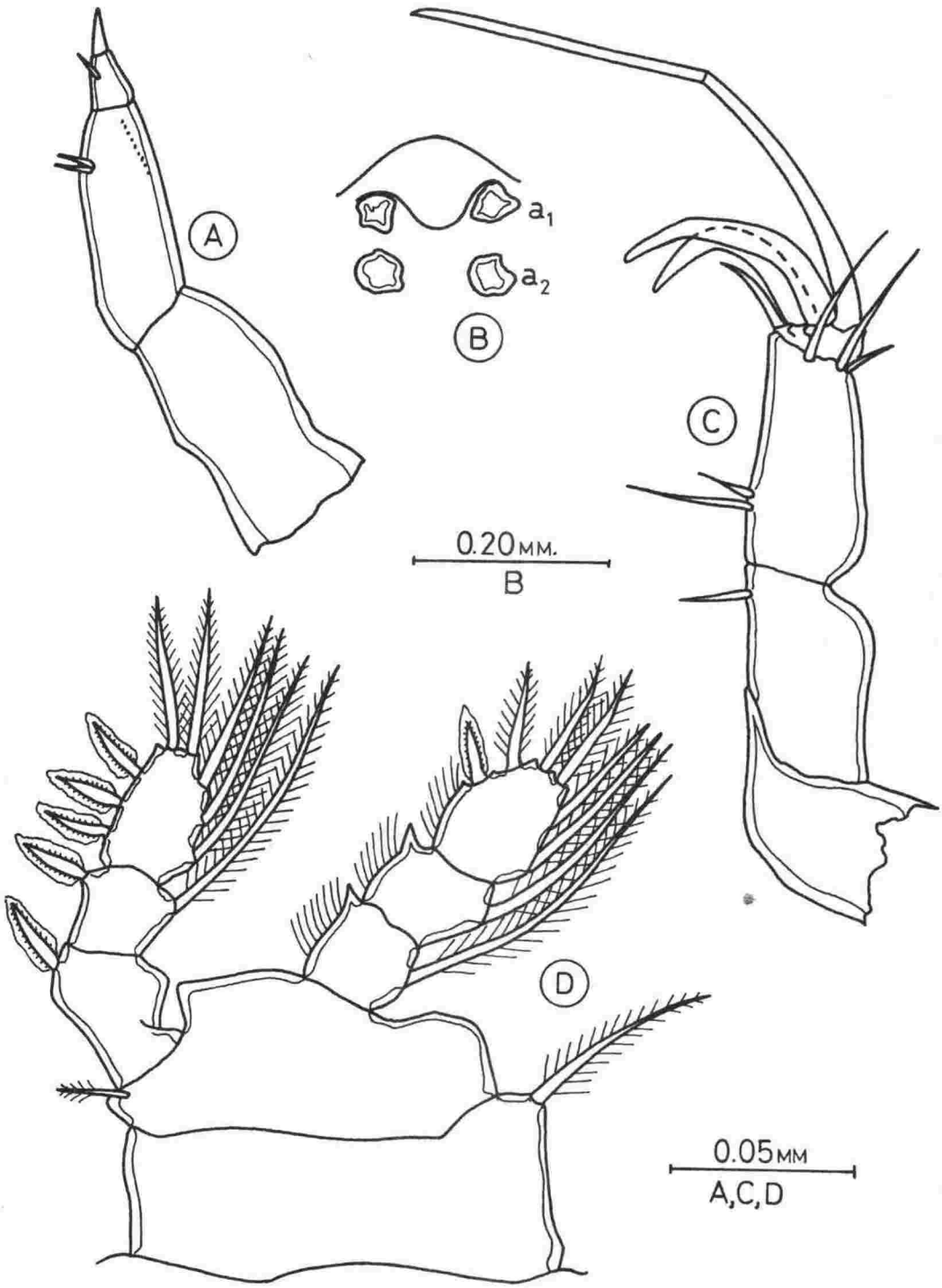

A
a1
a2
B
C
0.20мм.
B
D
0.05мм
A,C,D

FIGURE 8

Lichomolgus uncus n.sp.

(a) male, fifth leg

(b) male, maxilliped

(c) female, second leg

(d) female, endopod of third leg

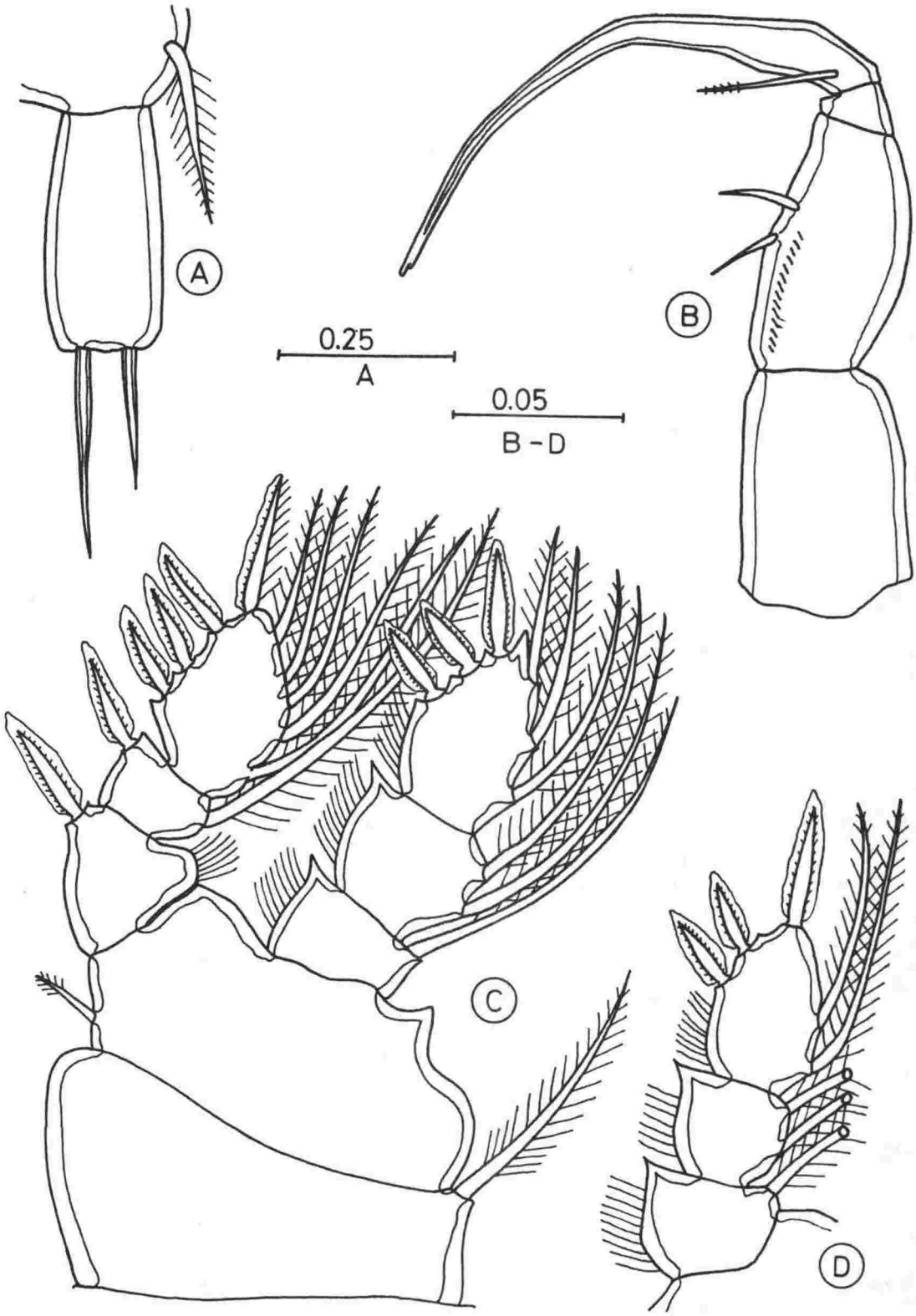
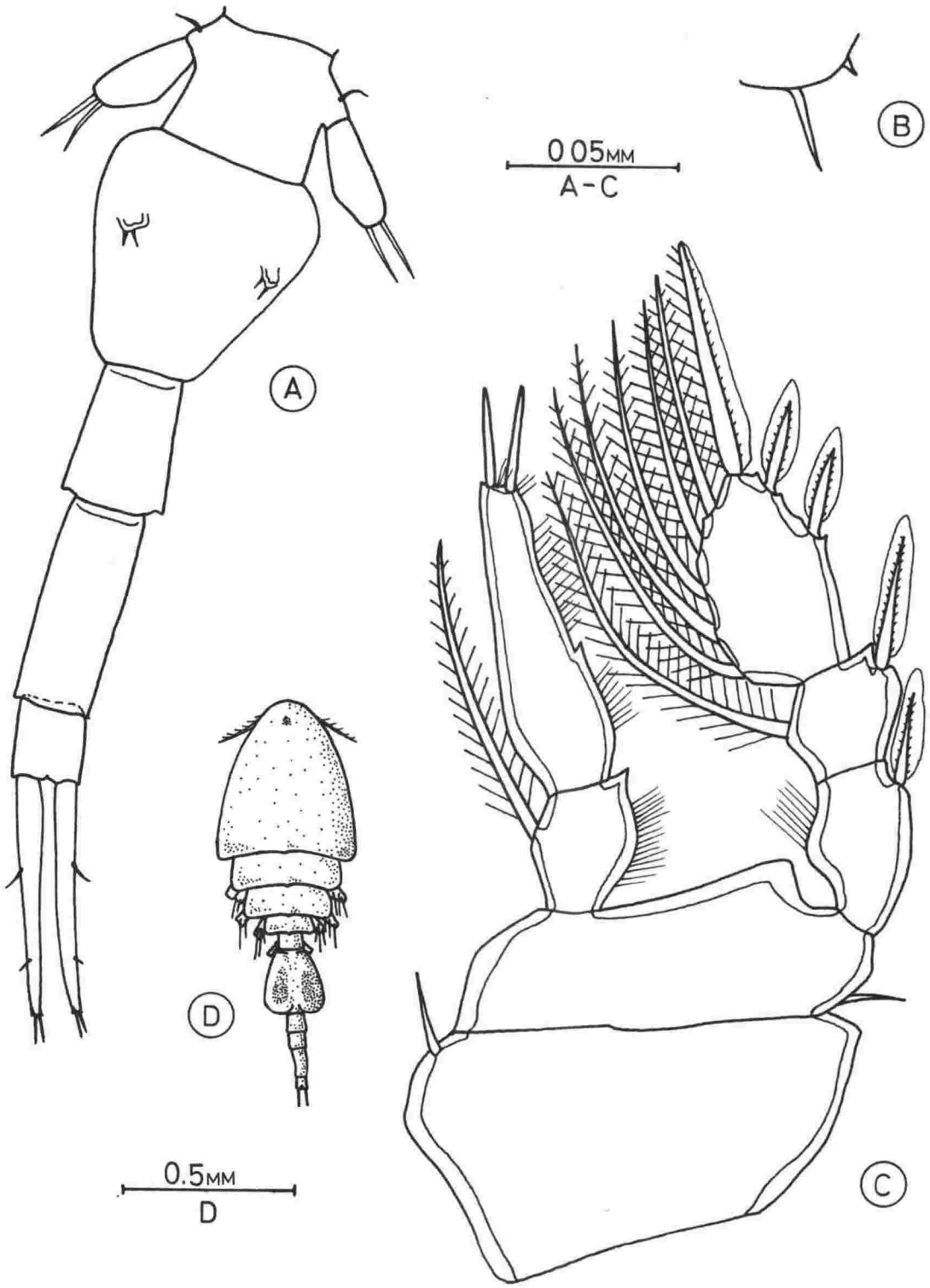

A
B
0.25
A
0.05
B - D
C
D

FIGURE 9

Lichomolgus uncus n. sp.,

(a) female, urosome and fifth leg
(b) male, sixth leg
(c) female, leg four
(d) male, dorsal view


A
B
0 05MM
A-C
C
D
0.5MM
D

Leg 5 (Fig. 8a) bears a rectangular free segment with two almost equal terminal setae.

Leg 6: (Fig. 9b) is represented by a naked seta and a setule or very short spine near the posterior end of the segment.

Colour in life: resembles that of the female.

Type material: 47 females, 25 males, and 2 copepodids from Perna canaliculus Gmelin, collected in Wellington Harbour (41°10'S, 173°55'E) in December 1973 and March 1974. Holotype female from Perna canaliculus Gmelin dredged from a depth of 4m off Ward Island, Wellington Harbour, November 1973. Deposited in the National Museum, Wellington, (Z.Cr.1974).
Paratypes: 8 females and 5 males from Perna canaliculus Gmelin dredged from a depth of 4m. off Ward Island, Wellington Harbour, November 1973. Deposited at the National Museum, Wellington (Z.Cr 1975-6). Remainder in collection of author.

The name chosen (uncus), the latin for 'hook', refers to the long curved claw on the antenna.

DISCUSSION

Of the seventeen described species of Lichomolgus (see Humes and Stock 1973) only two have more than two claws on the antenna. These are L. leptodermatus Gooding 1957, with three claws, and L. elegantulus Stock 1960, with four claws. In neither of these two species does a claw on the antenna exceed twice the length of the other claws, as one does on the antenna of L. uncus.

Lichomolgus uncus shows no trace of an articulation on the third segment of the antenna, where the setae are inserted, as described for L. leptodermatus and L. elegantulus but articulates with a tiny fourth segment below the base of the claws.

The genital segment of L. leptodermatus is wider at the posterior end than at the anterior end, unlike that of L. uncus, which is wider at the anterior end than at the posterior.

The caudal ramus of L. elegantulus bears five setae on the apex. Two of these setae are longer than the ramus. There are four setae on the caudal ramus of L. leptodermatus, the longest being 5/8 the length of the ramus. Lichomolgus uncus has two short setae on the apex of the caudal ramus. Both setae are less than $\frac{1}{4}$ the length of the ramus.

Lichomolgus uncus occurs on the surface of the gills of P. canaliculus, and also inside the gill water tubes, where the copepods can be seen as tiny opaque lumps under the surface. The copepods exhibit little movement unless disturbed, when they move quickly away. The females lose their eggsacks very quickly after being disturbed. The presence of the pea crab Pinnotheres novaezelandiae does not appear to affect the numbers of copepods present in a mussel, and gravid female copepods were found in mussels containing large pea crabs.

Despite a careful search, no early copepodid stages were ever found, although two late instar copepodids were found in November 1973.

Lichomolgus leptodermatus was described by Gooding (1957) from the gills of Laevicardium where it occurs in cysts or small swellings of the gill tissues. This copepod also showed little movement until released from the gill cyst, and the females shed their eggsacks soon after removal from the host. Gooding also failed to find any early copepod instars and postulated that the infection of the shellfish took place in one of the last copepodid stages. The nature of the association between L. uncus and P. canaliculus remains unknown. There is no observable effect on the mussel due to the presence of the copepod.

ACKNOWLEDGEMENTS

I am grateful for the helpful advice of my supervisor, Dr G.C. Hewitt, and I wish to thank Mr. W.B. MacQueen and Mr. R.H. Nicol, the skipper and crew of the "Tirohia", who dredged the mussel samples for me.

REFERENCES CITED.

Gooding, R.V. 1957
On some copepoda from Plymouth, mainly associated with invertebrates, including three new species.
Journal of the Marine Biological Association of the United Kingdom, 36: 195-221, figs. 1-6

Humes, A.G. & Stock, J.H. 1973
A revision of the family Lichomolgidae Kossman 1877, cyclopoid copepods mainly associated with marine invertebrates.
Smithsonian contributions to Zoology, 127: i-v 368pp.

Stock, J.H. 1960
Sur quelques copépodes associés aux invertébrés des côtes du Roussillon.
Crustaceana, 1(3): 218-257 figs. 1-20

THE POST-PLANKTONIC DEVELOPMENT STAGES OF *PINNOTHERES NOVAEZELANDIAE* FILHOL 1885

(BRACHYURA : PINNOTHERIDAE)

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ABSTRACT

The post-planktonic developmental stages of Pinnotheres novaezelandiae Filhol 1885 are described and illustrated. Development of P. novaezelandiae was found to be similar to that of the other members of the Pinnotheridae whose developmental stages have been published. The re-description of P. schauinslandi Lenz 1901 given by Bennett (1964) is compared to the descriptions of P. novaezelandiae. Bennett's description is considered to be that of a hard-shell stage P. novaezelandiae, not P. schauinslandi. On the basis of the description by Lenz (1901) P. schauinslandi must remain a valid species.

INTRODUCTION

Prior to a review of the New Zealand Pinnotheridae by Scott (1961) five species of pinnotherid crab had been recorded from New Zealand waters. Scott showed that records of P. pisum Linnaeus 1759, and P. latipes Jacquinot & Lucas 1853 were erroneous, and that a Pinnixia zoea identified by Lebour (1928) from a drawing by Gurney (1924) probably belonged to the family Hymenosomidae. Scott supported the view of Chilton (1911) that P. schauinslandi Lenz 1901 was a male P. novaezelandiae, and stated that " the family Pinnotheridae is represented in New Zealand by a single polymorphic species, Pinnotheres novaezelandiae".

In 1964 Bennett published a description of both male and female P. schauinslandi, but for reasons given in this paper, his description must be considered to be that of a male and an immature hard shell female P. novaezelandiae.

The taxonomy of the Pinnotheridae has been complicated by the complex series of post-planktonic moulting stages, in which both hard and soft shell stages occur, the hard-shell female being identical to the male except for the genital opening and the structure and number of pleopods.

These moulting stages were first described for P. pisum by Atkins (1926), and have since been described for P. ostreum Say 1817 (Stauber 1945; Christensen & McDermott 1958), P. maculatus Say 1818 (Pearce 1964),

Fabia subquadrata Dana 1851 (Pearce 1966), and Pinnotheres casta Antony & Kuttyamma 1971 (Silas & Alagarswami 1965; Antony & Kuttyamma 1971).

The post-planktonic development stages of P. novaezelandiae are described in this paper. The nomenclature for the development stages is that used by Stauber (1945), with the modifications which were made by Christensen & McDermott (1958). This terminology has been adopted by subsequent authors.

The material for this study was obtained by sampling a population of the green mussel Perna canaliculus (Gmelin 1791) in Wellington Harbour (41°06'S, 174°50'E) over the period March 1973 - March 1974.

DEVELOPMENT STAGES

The planktonic zoel and megalopal stages are followed by a series of crab instars. It is believed that one of these early crab instars is the invasive stage which, after entering a mussel, moults to become a pre-hard stage crab. The invasive stage has not been found for P. novaezelandiae. Pre-hard stages: (Figs. 10, 11a-f). These stages were first described by Christensen & McDermott (1958) for P. ostreum, and have since been found to occur in the other pinnotherid species whose development has been studied. The number of moults a crab undergoes before the final moult into the hard-shell form, is unknown.

Only two pre-hard stage P. novaezelandiae were found, a male and a female crab in March 1973. Morphologically these resemble stage II female crabs. The carapace is rounded and soft-shelled, with a length to width ratio of 0.95: 1 in both specimens (Fig. 10a). The width of the carapace was 2.2mm in the male, and 2.3mm in the female. Abdomen narrow in both sexes, fitting closely into the sternal groove. The abdomen is held in place by a rudimentary locking apparatus similar to that described by Atkins (1926) for P. pisum, and by Stauber (1945) for P. ostreum. A pair of chitinous nobs on the fifth thoracic segment fit into two sockets on the sixth abdominal segment (Fig. 10b). When these are fitted together, the abdomen is extremely hard to dislodge from the thorax.

Periopods are slender and bear rows of long swimming hairs

FIGURE 10

Pinnotheres novaezelandiae, pre-hard stage

(a) dorsal view of female

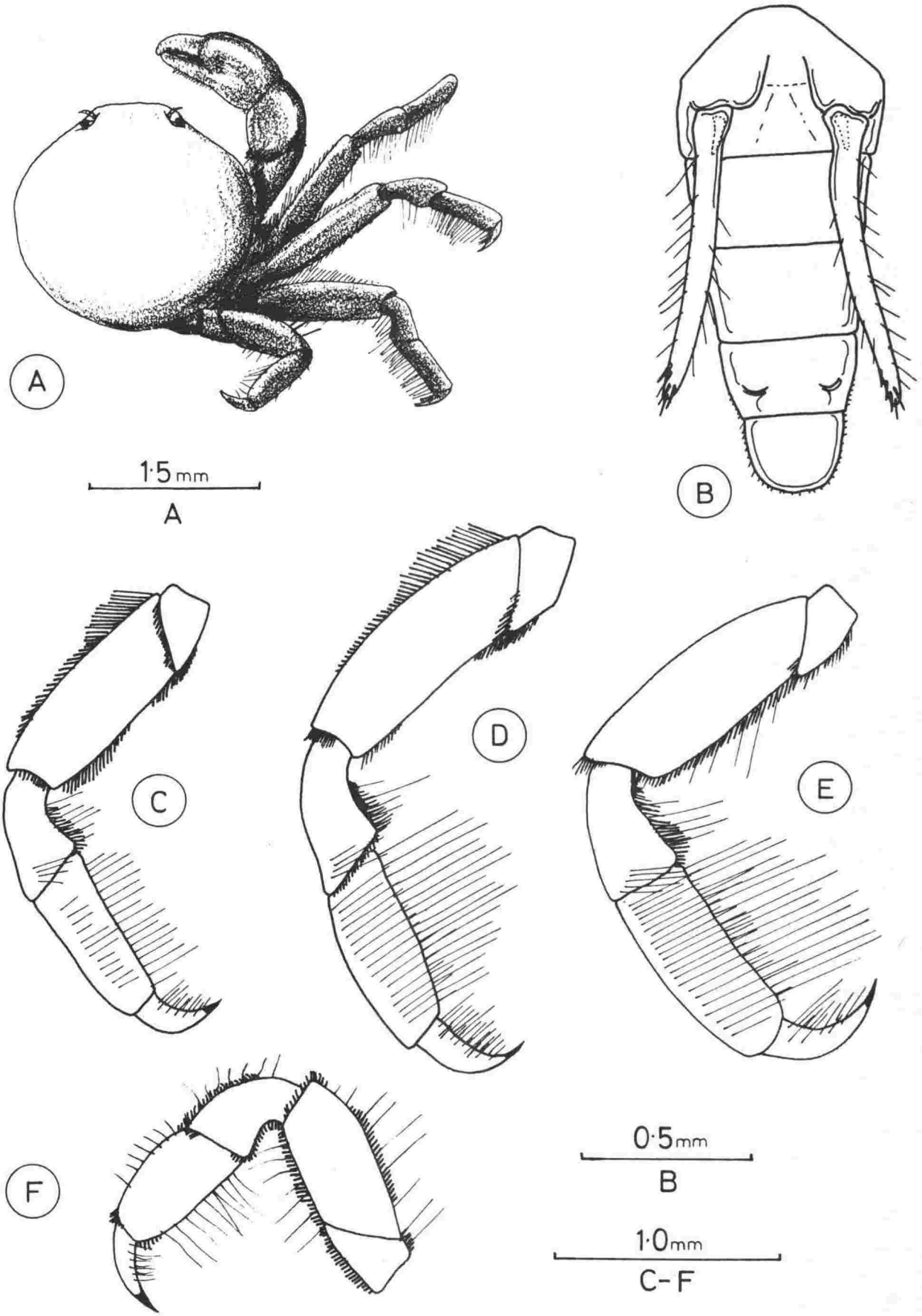
(b) abdomen of male, ventral surface

(c) second periopod

(d) third periopod

(e) fourth periopod

(f) fifth periopod


A
1·5 mm
A
B
C
D
E
F
0·5 mm
B
1·0 mm
C–F

(Figs. 10c-f). Sexes are indistinguishable except for differences in the number and shape of the pleopods. The female has four pairs of abdominal appendages (Figs. 11b, d-f). The first two pairs are biramous, while the third and fourth pairs are minute and uniramous. The male has only two pairs of abdominal appendages, the first pair are long and slender, while the second pair are rudimentary nobs under the base of the first pair.

Hard-stage male: (Figs. 11g,h, 12, 13). This stage was described by Stauber (1945) as stage I, and is the stage at which the male crab becomes sexually mature.

Carapace width 3.4 -11.3mm (mean of 34 crabs = 8.22± 0.81mm). Length to width ratio of carapace 1 : 0.96 ± 0.04. The shell is as well calcified as in free living species of crabs. Colour creamy white with distinctive orange markings. Carapace is arched dorsally and is oval to circular in shape with a protruding front. Posterior margin straight. Eyes well developed and prominent from above (Fig. 12a). Mouth-parts as for the stage V female.

Merus and carpus of cheliped (Fig. 12b) are stouter than in the later female stages. Propodus flattened on the inside, convex on the outside, with convex margins. The fingers are stout, tips cross when closed. Each finger bears a stout blunt tooth. Teeth bite together when fingers close.

The other periopods are flattened in cross section (Figs. 12c-f). Merus bears a row of long setae on the dorsal margin, and a short fringe of setae on the ventral margin. There is a row of setae on the ventral margin of the carpus and dactylus. The second and third periopods have two rows of short setae on the propodus, the other periopods have one row.

The abdomen is seven segmented (Fig. 13a) and tapers from segment three to seven. The terminal segment is arcuate. The abdomen fits into a grooved sternum, and the locking mechanism is now well developed and strongly calcified.

Copulatory appendages are large. The first pair are blade like, very setose, with a closed groove on the inner side (Figs. 11h, 13a). The second pair of appendages are rod like, but have a swollen base (Figs. 11g, 13a). The distal portion of the second copulatory appendage fits into the groove of the first appendage.

FIGURE 11

Pinnotheres novaezelandiae, pre-hard stage

(a) cheliped

(b) third pleopod

(c) abdomen of female, ventral surface.

(d) second pleopod

(e) first pleopod

(f) fourth pleopod

Stage I male.

(g) second pleopod

(h) base of first pleopod

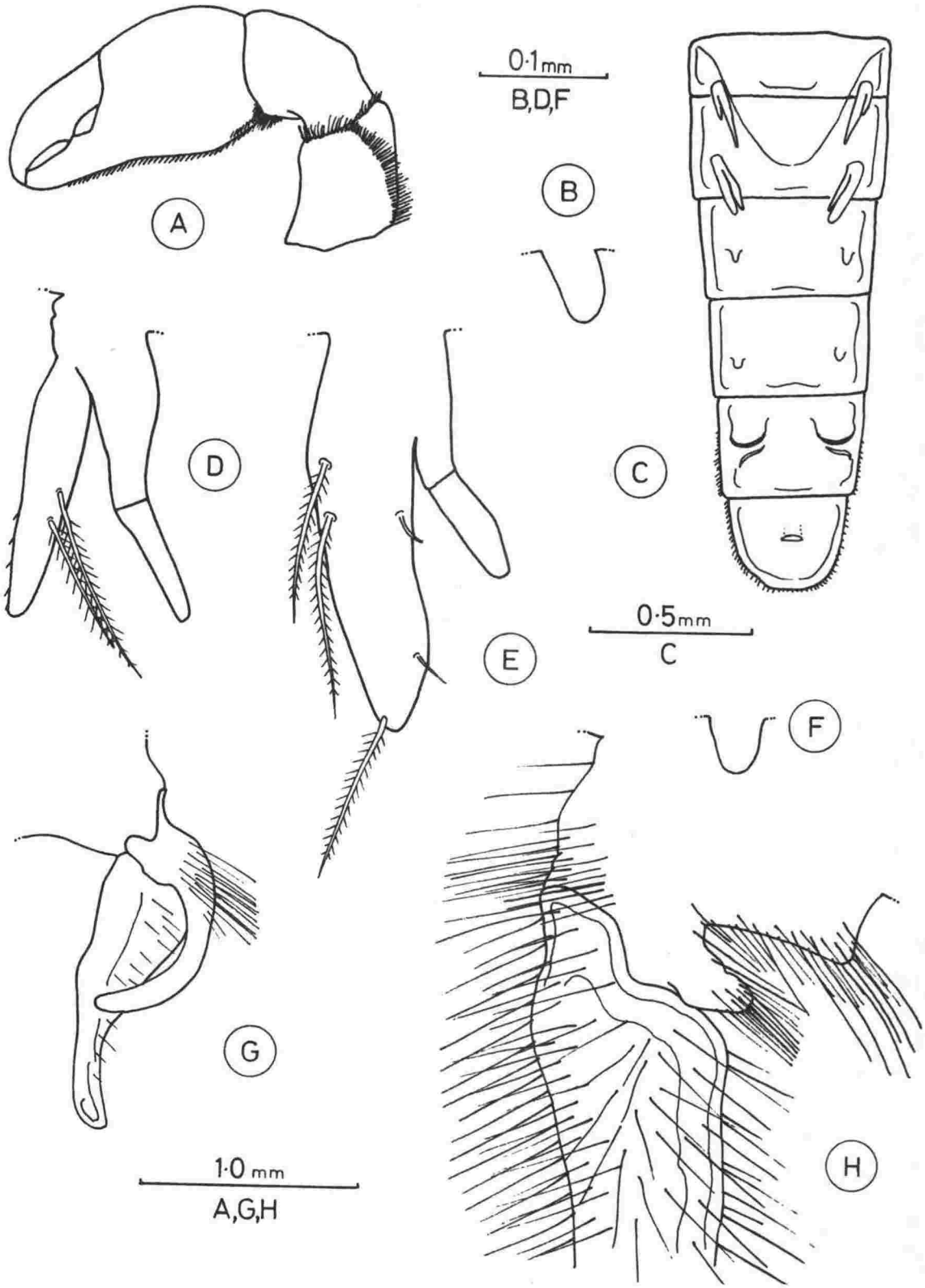
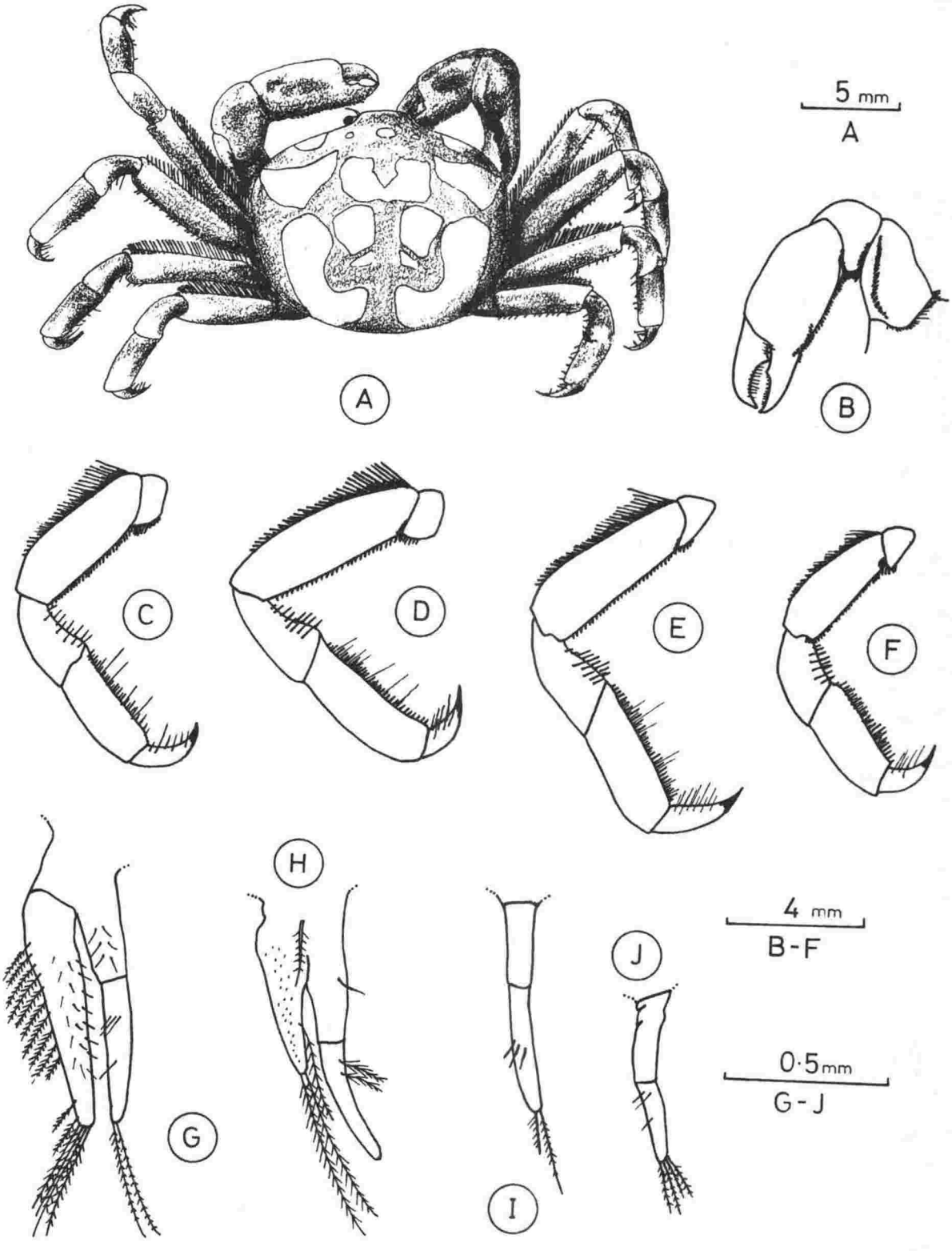

A
0·1mm
B,D,F
B
C
D
E
0·5mm
C
F
G
1·0 mm
A,G,H
H

FIGURE 12

Pinnotheres novaezelandiae, stage I

(a) male, dorsal view
(b) cheliped
(c) second periopod
(d) third periopod
(e) fourth periopod
(f) fifth periopod
(g) second pleopod
(h) first pleopod
(i) third pleopod
(j) fourth pleopod


5 mm
A
A
B
C
D
E
F
G
H
I
J
4 mm
B-F
0·5mm
G-J

Hard-stage female: (Figs. 12g-j, 13c). This stage is similar to the hard-stage male. Carapace width 1.3 - 4.8mm. Abdomen similar in shape to male (Fig. 13c) but segments one and two are longer than the corresponding male segments. The ratio of the abdomen width to the carapace width is 1:3.32 ± 0.23.

The four pairs of pleopods (Figs. 12g-j) are more developed than in the pre-hard stages. The third and fourth pairs are now two segmented.

Stage II female: (Figs. 13d-h, 14a-d). Carapace width of five specimens 3.2 - 7.00mm, mean width 5.2. ± 1.24mm. Width to length ratio of carapace 1:0.93. Front does not project as far forward as in stage I form. Exoskeleton of carapace thin, smooth and yielding to the touch. Chelipeds not as stout as in stage I instar. Propodus widest near base of fingers.

Periopods slender and sub-cylindrical in cross-section. There are delicate hairs on the last three segments.

Ratio of carapace width to abdominal width 1:2.6. Locking mechanism vesigial or absent. The endopod of the first pair of pleopods is now more elongate and has become more setose (Fig. 14b). The second pleopod (Fig. 14a) is larger and more setose than in the hard stage. The endopod has also elongated slightly, The third and fourth pleopods are now three segmented.

Stage III female: (Figs. 14e,f, 15a-e). Carapace width of six specimens 7.6 - 10.3mm (mean width 8.5 ± 0.78mm). Ratio of carapace width to abdominal width 1 : 1.46 ± 0.4. The edges of the abdomen have extended beyond the edge of the sternal groove (Fig. 14e). The lateral edge of the abdomen has developed a row of fine hairs. The locking mechanism is absent.

The first pleopod (Fig. 15c) is now very setose but the endopod shows little sign of segmentation. The second pleopod is also more setose with an elongated, segmented endopod. The third and fourth pleopods have developed a bend at the apex of the first segment, and both pleopods show further development of segments. As the crab matures, the setae on the pleopods become less randomly distributed, and form dense rows at the apex of each segment.

Stage IV female: (Figs. 16a-d, 17a-e). Carapace width

FIGURE 13

Pinnotheres novaezelandiae

(a) abdomen, ventral view, stage I male
(b) ventral surface, stage I male
(c) abdomen, stage I female
(d) second periopod
(e) third periopod
(f) fourth periopod
(g) fifth periopod
(h) female, stage II abdomen, ventral view

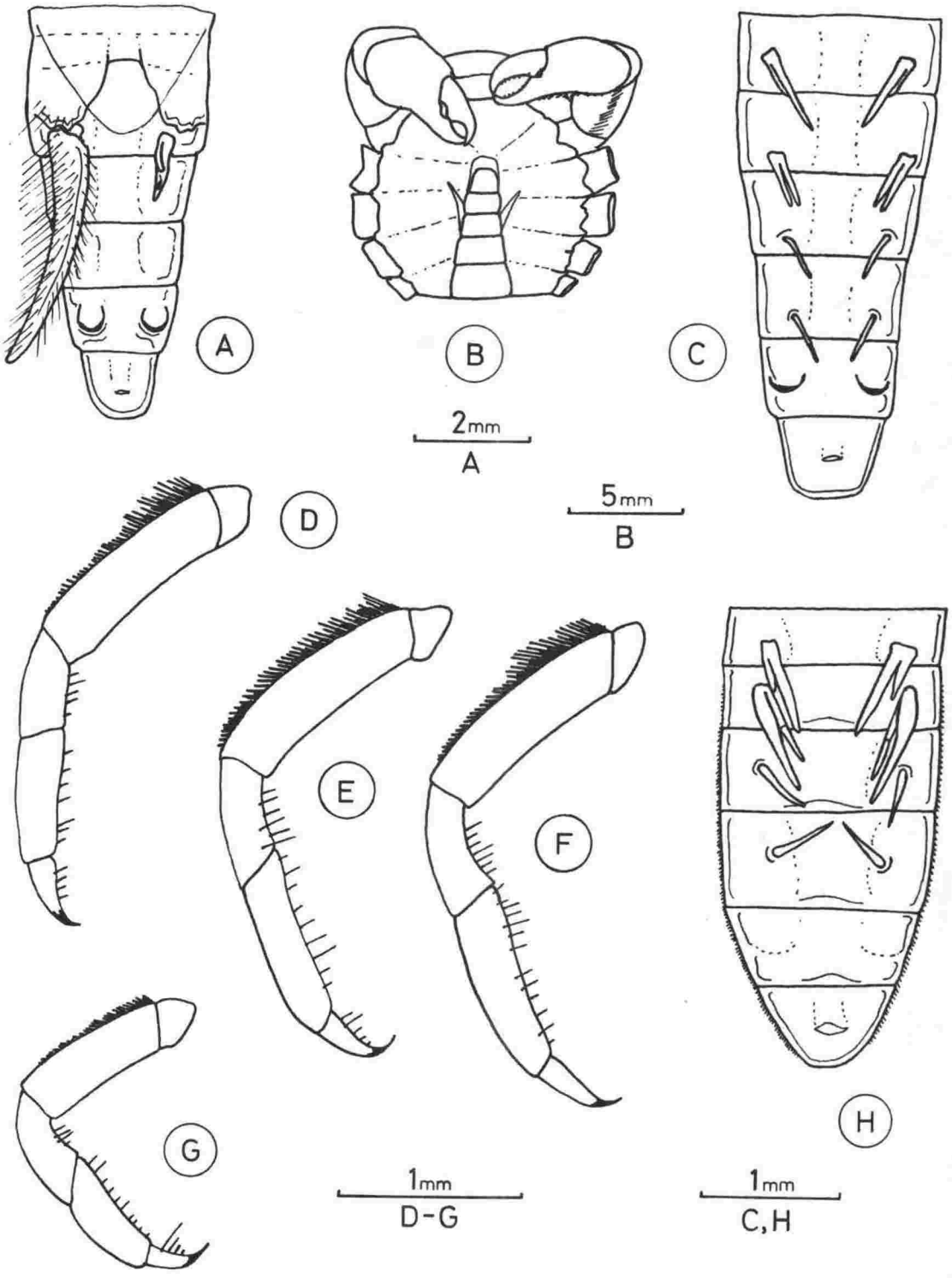

A
B
C
2mm
A
5mm
B
D
E
F
G
H
1mm
D-G
1mm
C,H

FIGURE 14

Pinnotheres novaezelandiae, stage II female

(a) second pleopod
(b) first pleopod
(c) third pleopod
(d) fourth pleopod
(e) stage III female, ventral surface
(f) stage III female abdomen, ventral view.

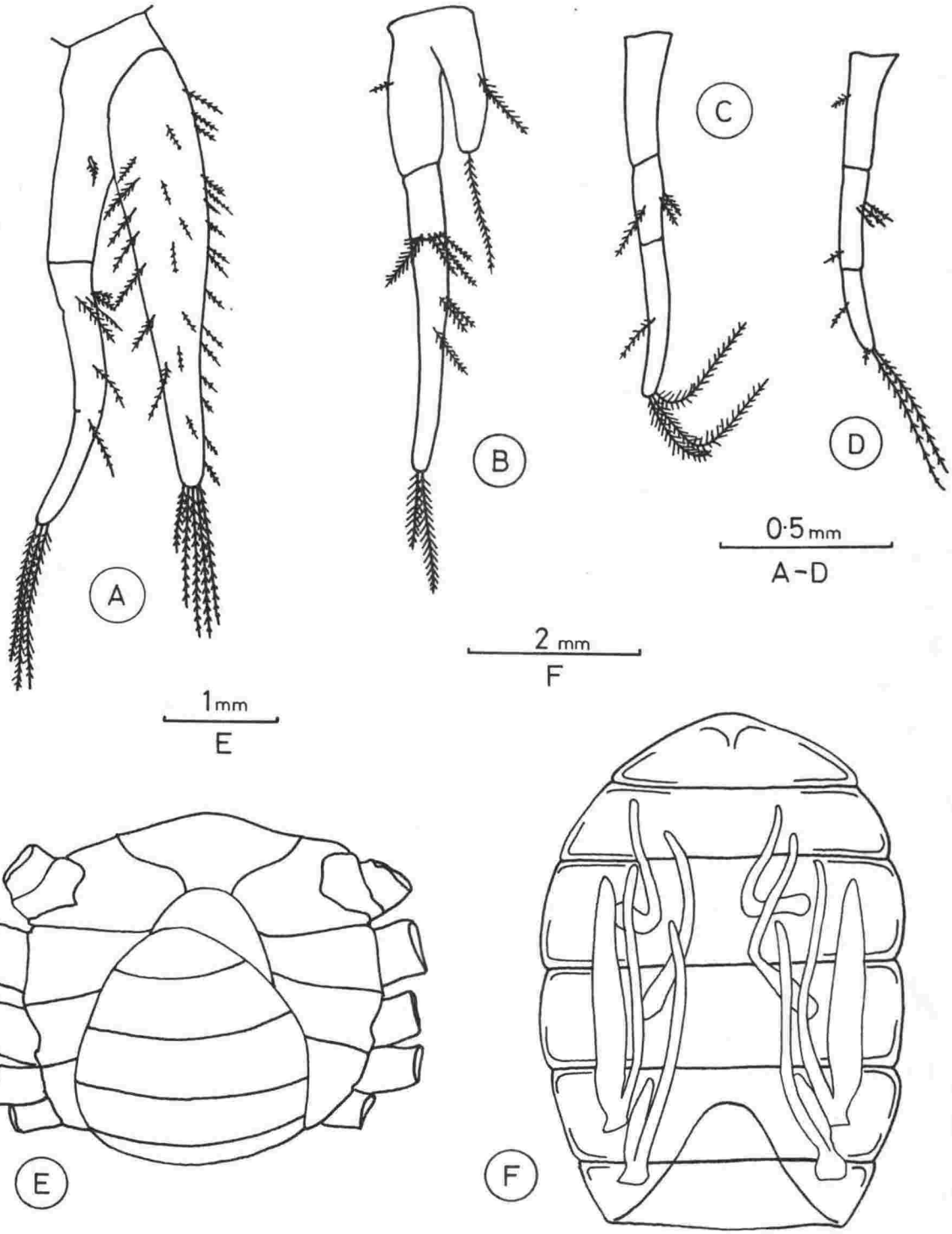

A
B
C
D
0·5 mm
A–D
2 mm
F
1 mm
E
E
F

FIGURE 15

Pinnotheres novaezelandiae stage III female

(a) first pleopod
(b) dorsal view
(c) fourth pleopod
(d) third pleopod
(e) second pleopod

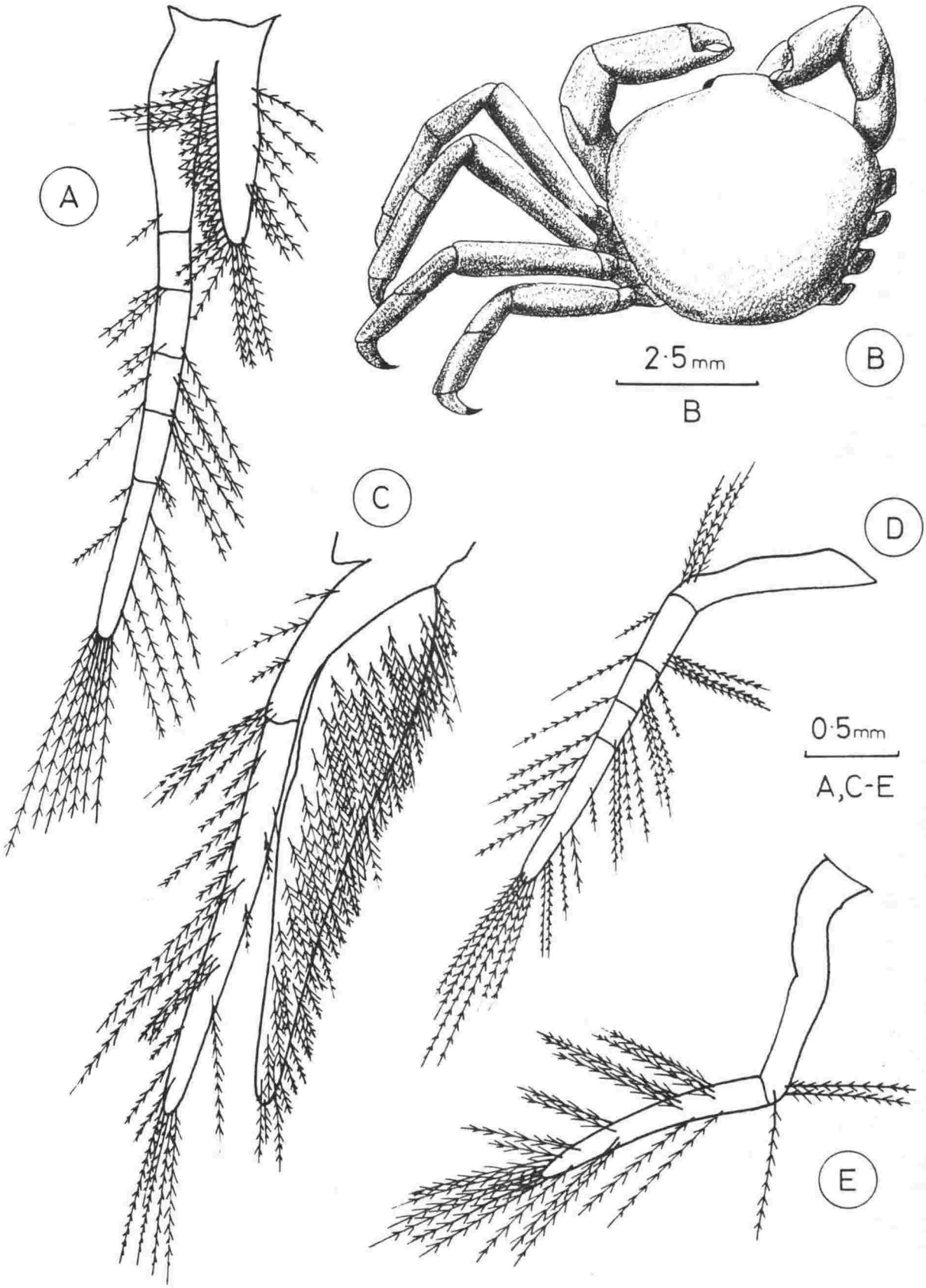
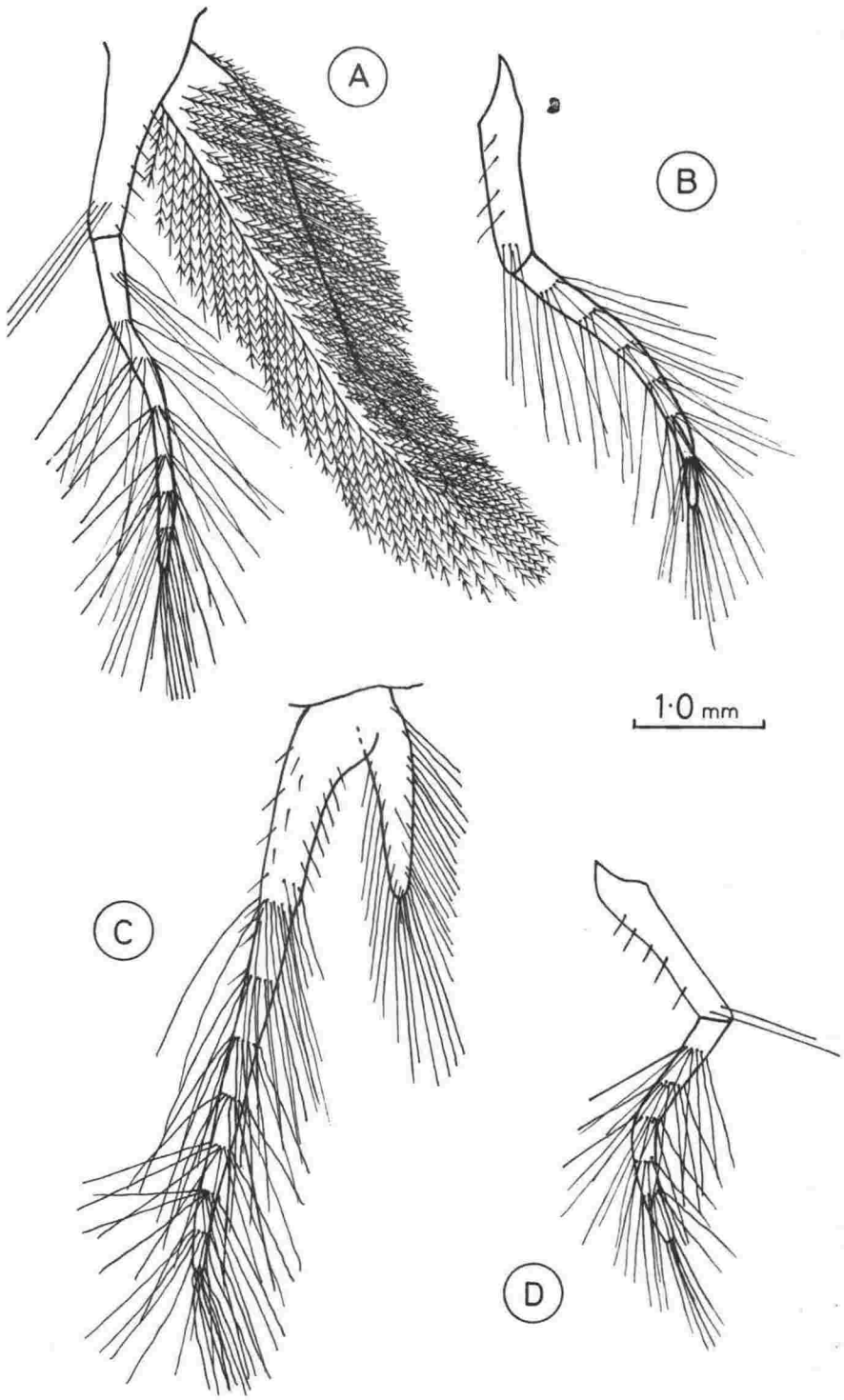

A
2·5mm
B
B
C
D
0·5mm
A,C-E
E

FIGURE 16

Pinnotheres novaezelandiae stage IV female

(a) second pleopod
(b) third pleopod
(c) first pleopod
(d) fourth pleopod


A
B
1·0 mm
C
D

7.5 - 13.0mm (mean width of 10 specimens 9.77 ± 1.10mm). Width to length ratio of carapace 1 : 0.91 ± 0.02. Ratio of carapace width to abdomen width 1 : 1.20 ± 0.075.

This stage is similar to the stage V female. The abdomen just reaches the coxa of the periopods, and extends forward to the posterior edge of the mouth. Periopods sub-cylindrical in cross-section. All bear a fringe of long setae on the merus, and a few scattered setae on the posterior margin of the propodus and dactylus (except for the fifth periopod, which has a bare propodus). The pleopods are densly setose. The first pleopod (Fig. 16c) has a small setose exopod and a long endopod, which is apparently eight segmented, the segments delineated by the rows of dense setae. The second pleopod has a stout exopod, and an eight segmented endopod of similar length to the exopod. The third and fourth pleopods are eight and seven segmented respectively. The number of segments can vary slightly among the crabs, some specimens may have one more or one less segment.

Stage V female: (Figs. 17f-j, 18, 19). This is the mature female crab. Carapace smooth, membranous and oval in outline. Carapace width 8.9 - 19.2mm. Mean width of 12 specimens 14.8 ± 0.6mm. Ratio of the carapace width to the length 1 : 0.93 ± 0.02mm. Frontal region slightly rounded. Posterior margin straight. Eyestalks usually hidden from above, Cornea very small and lightly pigmented.

Antennule (Fig. 19g) biramous. Outer ramus two segmented. Second segment bears numerous short setae. Inner ramus four segmented. The three terminal segments all bear long setae which form a dense brush.

Antenna (Fig. 19e) short, four segmented and reaching to the tip of the eyestalk. Terminal segment bears three long plumose setae and a short lateral seta.

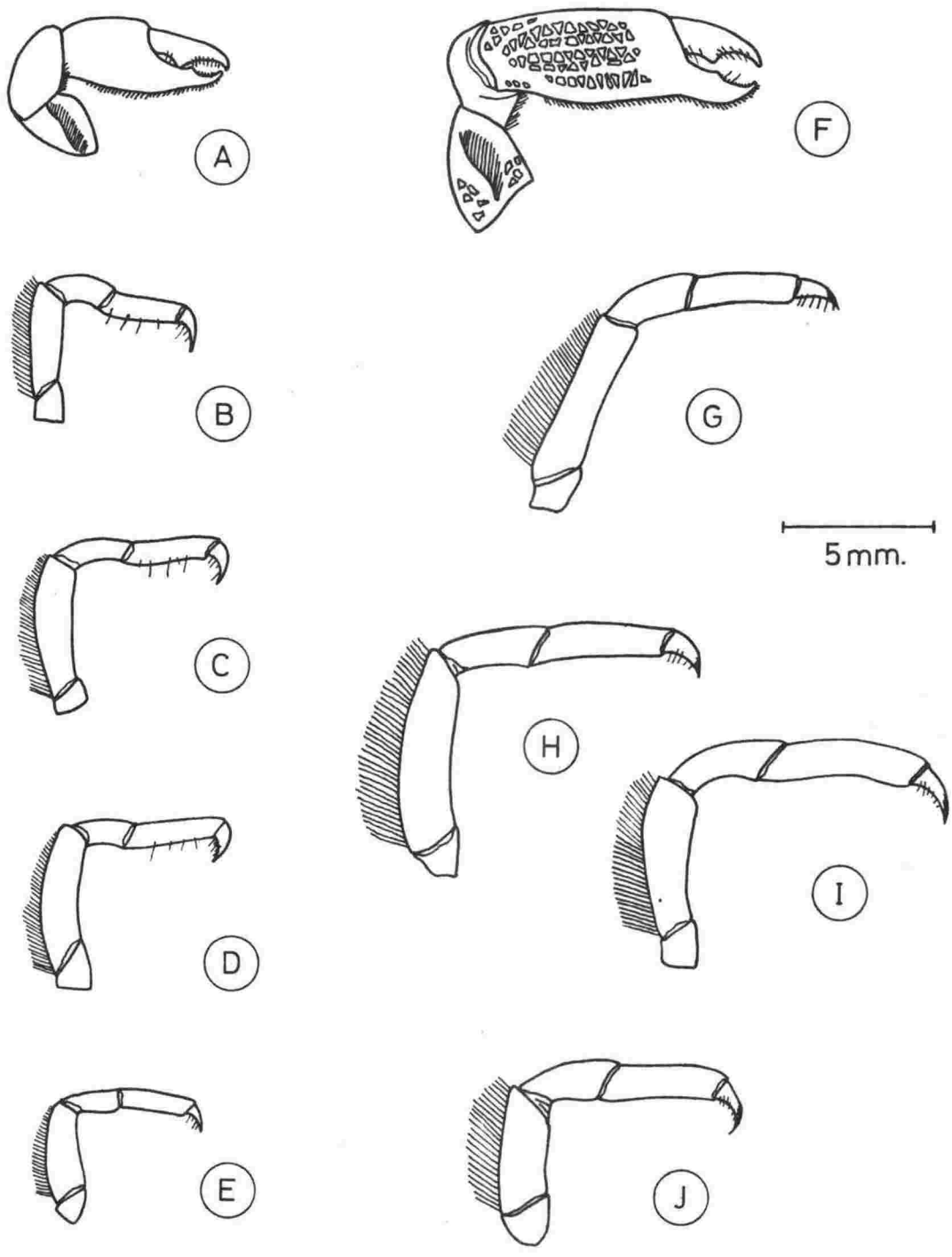
Third maxilliped (Fig. 18e) has a broad long setose merus. Carpus short, wider than long in the ratio 2 : 1. Propodus setose, bearing a setose dactylus on the inner margin.

Second maxilliped (Fig. 18c) setose, with three segmented exopod, The joint between segment two and segment three is indistinct. Endopod three segmented. Terminal segment born on interio-lateral margin of sub-terminal segment.

FIGURE 17

Pinnotheres novaezelandiae

(a) - (e) stage IV female, periopods 1-5
(f) - (j) stage V female, periopods 1-5


A
B
C
D
E
F
G
5 mm.
H
I
J

First maxilliped (Fig. 18f), exopod two segmented. Endopod one segmented and flat in cross section. Both the exopod and endopod are setose. Most of the setae on the dorsal lobes are naked.

Maxilla (Fig. 18d). Exopod has a plumose setae around the entire border. Endopod is also setose and two lobed, but setae on distal lobe are naked.

Maxillule (Fig. 18a) is tri-lobed and setose. Most setae are naked. The distal lobe is elongate and two segmented. The terminal segment is pointed distally and bears two plumose setae.

Mandible (Fig. 18b). The mandibular appendage is two segmented. The mandible usually has three teeth on the cutting edge, but one or more teeth may be absent.

Periopods (Fig. 17f-j). Chelipeds are stouter than in stage IV and comparable with those of a stage I male. The propodus is longer than wide in the ratio 1 : 2.5 (range of 12 specimens 1 : 2,2 - 1 : 2.7). There is a fringe of setae from midway on the ventral margin to the tip of the claw. The fingers are shorter than the palm. The tips of the fingers are curved, and cross when closed. The carpus and merus both have a fringe of setae on the dorsal margin.

Remaining periopods sub-cylindrical in cross-section. Relative lengths 6:7:7:5 (but see discussion). All bear long setae on the merus, and a few scattered setae on the posterior margin of the dactylus.

Abdomen is as large, or larger than the carapace and usually the abdominal edges cover the coxae. The edge of the abdomen has a setose fringe.

The pleopods (Figs. 19a-d) are now covered with a dense mat of setae, similar to stage IV, but the endopod usually has an extra one or two segments.

The egg diameter is 0.35mm at the time the eyespots form. Young eggs are bright orange in colour but darken to an orange brown when about to hatch. When in berry, the female crab is almost spherical (Fig. 19f) and quite helpless.

FIGURE 18

Pinnotheres novaezelandiae, stage V female

(a) maxillule

(b) mandible

(c) second maxilliped

(d) maxilla

(e) third maxilliped

(f) first maxilliped

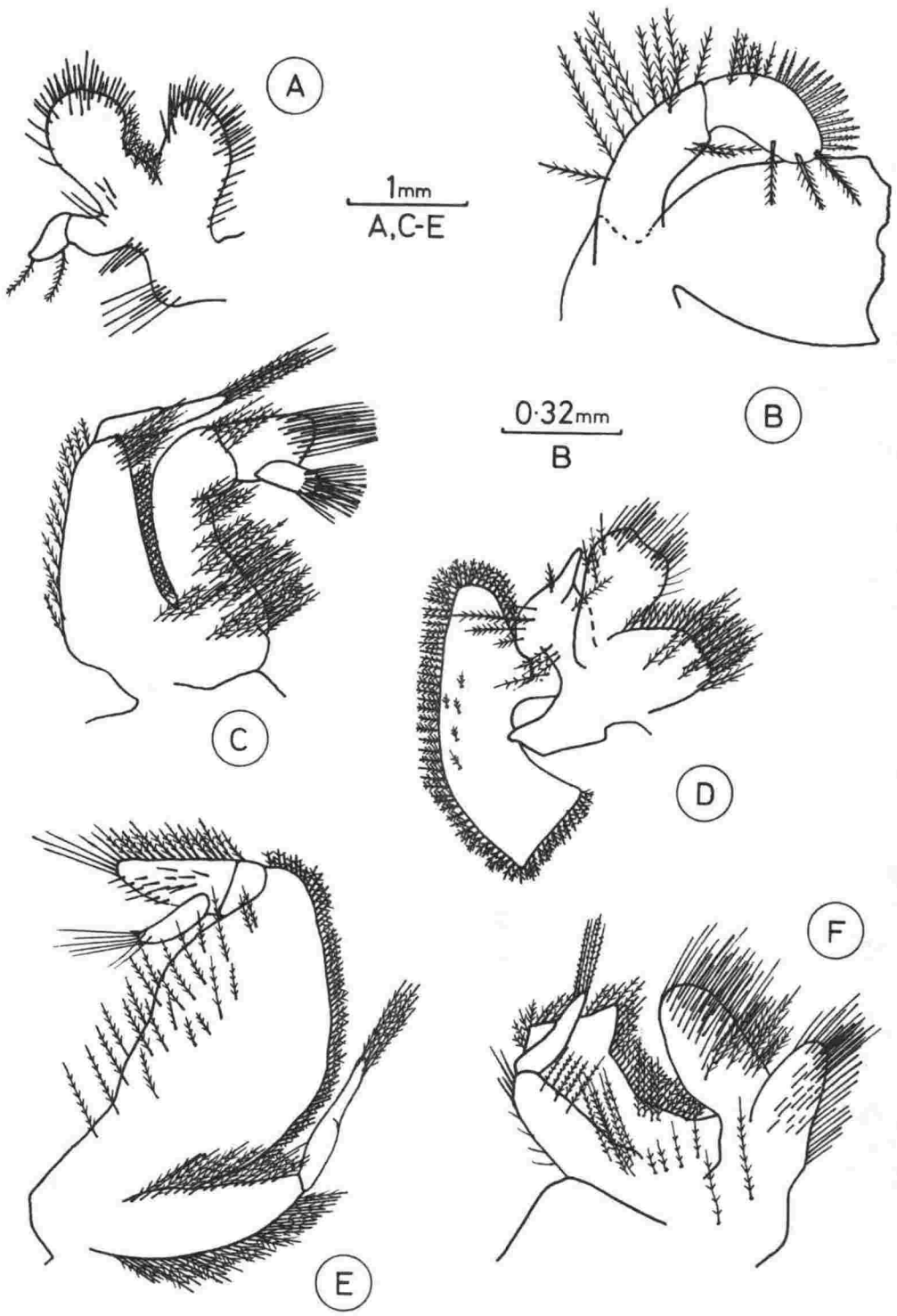

A
1mm
A,C-E
B
0·32mm
B
C
D
E
F

FIGURE 19

Pinnotheres novaezelandiae, stage V female

(a) first pleopod

(b) third pleopod

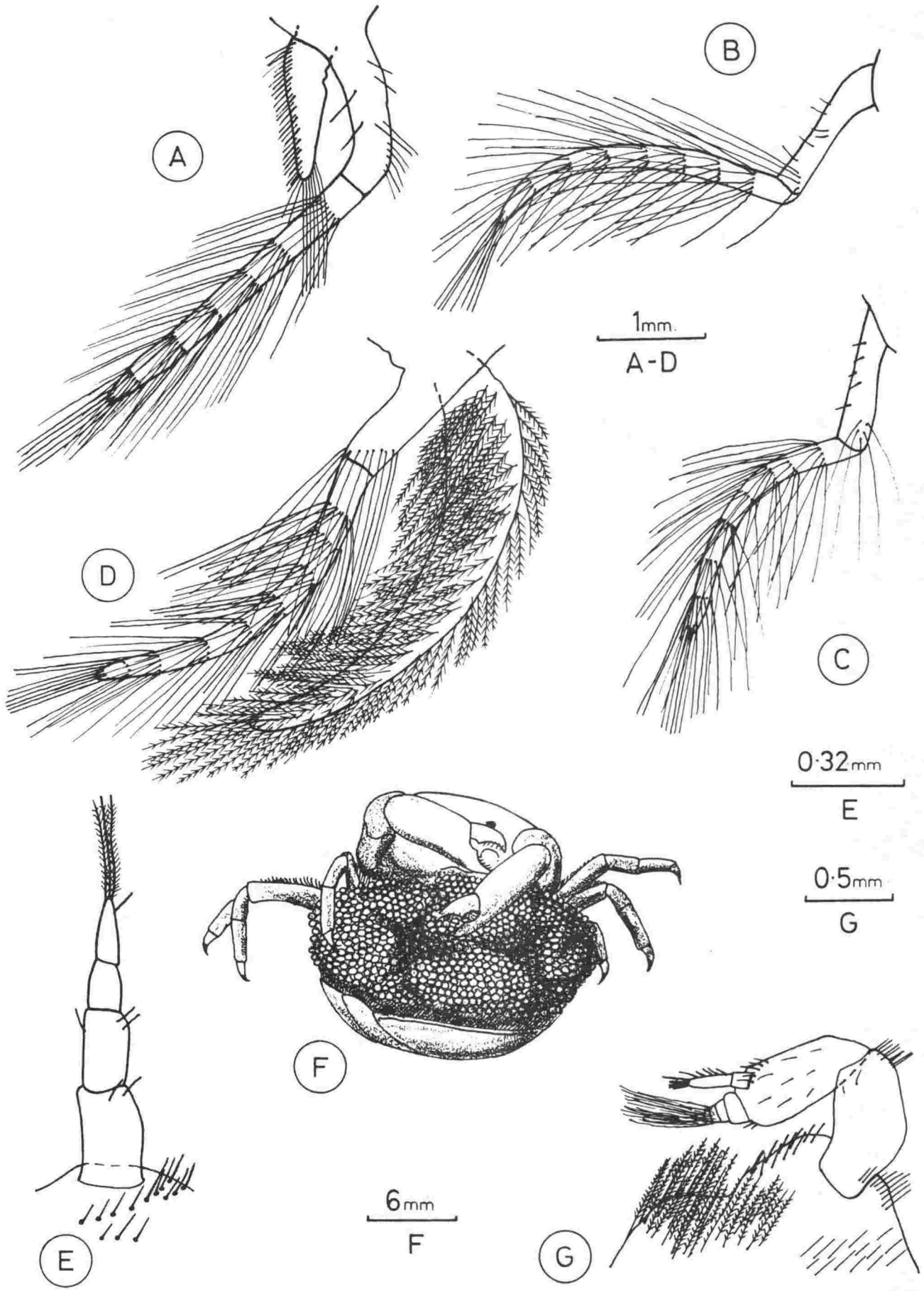
(c) fourth pleopod

(d) second pleopod

(e) antenna

(f) gravid female crab

(g) antennule


A
B
1mm.
A-D
D
C
0·32mm
E
0·5mm
G
F
E
6mm
F
G

DISCUSSION

The complex and unusual development of the Pinnotheridae seems to be quite fixed within the family. The post-planktonic development of P. novaezelandiae is very similar to that described for P. ostreum, P. maculatus, and Fabia subquadrata.

Atkins (1958) reported the occurence of soft-shell post-hard stage males of P. pisum. The significance of this post hard-stage is unknown. It is not a male which has just moulted and is still in the soft stage, but is a true thin carapace stage. No such stage has been observed for P. novaezelandiae, but in all other respects the development of P. novaezelandiae is similar to P. pisum.

There is considerable variation in the size of P. novaezelandiae as has been indicated in the descriptions. The shape of the carapace can vary from oval to circular, and can overhang the coxae of the periopods, but in other specimens the coxae are quite visible in dorsal view.

The relative lengths of the legs is one of the characters most often used to identify pea-crabs. The key given by Tesh (1918) is an example of this. De Mann (1888), however, noted that pea-crabs are often asymmetric, the legs of one side of the crab being longer than the legs on the other. Griffin & Campbell (1969) point out that it is possible in some cases for each side of a specimen to key out as a different species, when using the relative length of the dactyl as a specific character. Specimens of P. novaezelandiae can also be asymmetric, and long legged and short legged forms do occur.

Probably the best way to determine the identity of a pea-crab is to rear the zoeal stages, which tend to exhibit marked differences between the species, but this is only possible with live stage V crabs.

The description given by Bennett (1964) for male P. schauinslandi also fits male P. novaezelandiae. The main differences recorded by Bennett were that the merus of the cheliped had a stronger terminal tooth than in P. novaezelandiae and that the periopods were much longer than in P. novaezelandiae. As has been mentioned, these characters are

variable in P. novaezelandiae.

Bennett was apparently unaware of the unusual development of the Pinnotheridae, and of the presence of hard-shell narrow tailed female specimens. Bennett's description, and the sizes he gives, are consistant with his specimens being P. novaezelandiae. It is significant that he was unable to find any gravid female "P. schauinslandi" and that there are differences between his description and the original description of Lenz (1901).

Although it is generally accepted that Lenz was describing hard-shell P. novaezelandiae (Chilton 1911; Scott 1961), this is by no means certain. There are differences between his description and P. novaezelandiae (Lenz 1901; Bennett 1964) so P. schauinslandi may still prove to be a valid species.

Wear (1965) recovered two species of Pinnotheres zoea from Wellington Harbour. One was P. novaezelandiae, the other he suggested was the zoea of P. schauinslandi. Unfortunatly Wear did not describe or draw his specimens, so the status of the zoea he found must remain in doubt.

ACKNOWLEDGEMENTS

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REFERENCES

Atkins, D. 1926: The moulting stages of the pea-crab (Pinnotheres pisum). Journal of the Marine Biological Association of the U.K., 14: 475-493

_____ 1958: British pea-crabs (Pinnotheres). Nature, London, 181: 1087

Antony, A. & Kuttyamma V.J. 1971: A new species of the pea-crab Pinnotheres Latreille (Crustacea: Brachyura: Pinnotheridae) from the clam Meretrix

casta (Chemnitz). Bulletin of the Department of Marine Biology & Oceanography, University of Cochin 5: 59-68

Bennett, E.W. 1964: The marine fauna of New Zealand: Crustacea: Brachyura. New Zealand Department of Scientific and Industrial Research, Bulletin 153: 120pp

Chilton, C. 1911: Scientific results of the N.Z. government trawling expedition 1907. Crustacea. Records of the Canterbury Museum 1: 285-312

Christensen, A.M. & McDermott, J.J. 1958: Life-history and biology of the oyster crab Pinnotheres ostreum Say. Biological Bulletin, Marine Biological laboratory, Woods Hole, Mass., 114(2): 146-179

De Mann, J.G. 1888: Report on the podophthalmous crustacea of the Mergui Archipelago, collected for the trustees of the Indian Museum. Journal of the Linnean Society, Zoology, 22: 1-312

Griffin, D.J.G. & Campbell, B.M. 1969: The sub-littoral Goneplacidae & Pinnotheridae (Crustacea:Brachyura) of Morton Bay. Memoirs of the Queensland Museum 15(3): 141-164

Gurney, R. 1924: Crustacea, part IX, Decapod larvae. Natural history report, Terra Nova expedition Zoology, 8(2): 37-202

Lebour, M.V. 1928: The larval stages of the Plymouth Brachyura. Proceedings of the Zoological Society, London. 1928: 473-560

Lenz, H. 1901: Crustaceen in Ergebnisse einer Reise nach dem Pacific (Schauinsland 1896-97). Zoologishe jahrbücher, 14: 429-482

Pearce, J.B. 1964: On reproduction in Pinnotheres maculatus (Decapoda:Pinnotheridae). Biological Bulletin: Marine Biological laboratory, Woods Hole, Mass., 127: 384

_____ 1966: The biology of the mussel crab, Fabia subquadrata, from the waters of the San Juan

Archipelago, Washington, Pacific Science 20: 3-35

Scott, M.S. 1961: A review of the N.Z. Pinnotheridae. Transactions of the Royal Society of New Zealand Zoology 1: 303-309

Silas, E.G. & Alagarswammi, K. 1965: On an instance of parasitisation by the pea-crab (Pinnotheres sp.) on the backwater clam Meretrix casta (Chemnitz) from India, with a review of the work on the systematics, ecology, biology, and ethology of pea-crabs of the genus Pinnotheres Latreille. Symposium on Crustacea. Marine Biological Association of India III, pp. 1161-1227

Stauber, L.A. 1945: Pinnotheres ostreum, parasitic on the American oyster Ostrea (Gryphaea) virginica. Biological Bulletin, Marine Biological laboratory Woods Hole, Mass., 88: 269-291

Tesh, J.J. 1918: The Decapoda Brachyura of the Siboga expedition. V. Goneplacidae & Pinnotheridae. Siboga Expedition XXXIXC livre 84: 149-295 plates 7-17

Wear, R.G. 1965: Zooplankton of Wellington Harbour, New Zealand. Zoology publications of Victoria University of Wellington 38: 1-31

DIFFERENCES BETWEEN THE FIRST ZOEA OF PINNOTHERES (BRACHYURA:PINNOTHERIDAE) FROM DIFFERENT HOST SHELLFISH.

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ABSTRACT

The pea crab Pinnotheres novaezelandiae Filhol 1886, which is found in the green mussel Perna canaliculus (Gmelin 1791), is slightly smaller and has smaller eggs than the otherwise identical Pinnotheres which lives in the horse mussel Atrina zelandica Gray 1835.

First stage zoea reared in the laboratory from these two crabs were examined with a scanning electron microscope. It was found that the zoea of the crab from A. zelandica differs from the zoea of P. novaezelandiae in possessing a row of four minute pits on the labrum, and in the absence of a rostrum. The significance of this is discussed.

INTRODUCTION

In Wellington Harbour the pea-crab Pinnotheres novaezelandiae Filhol 1886 is commonly found in the green mussel Perna canaliculus(Gmelin 1791). Almost all the horse mussels (Atrina zelandica Gray 1835) also contain pinnotherid crabs, which have been classified as P. novaezelandiae (Scott 1961; Bennett 1964).

The crabs from A. zelandica grow to a larger size and have larger egg than their counterparts from P. canaliculus (table 4). Examination of the crabs reveals no significant differences in the mouthparts or pleopods which could not be attributed to the generally larger size of the crabs from A. zelandica. This difference in size can be related to the amount of space within the host, since A. zelandica are commonly found with a length exceeding 300mm while P. canaliculus rarely exceeds 200mm. The size of the pea-crab eggs, however,

TABLE 4

Size differences between crabs from *Atrina zelandica* and *Perna canaliculus*

	A. zelandica	*P. canaliculus*
Range in carapace width of mature female crab	7.5–21.3mm	8.9–19.2mm
mean carapace width	17.8 ± 3.35mm*	14.4 ± 2.09mm *
sample size	13	136
number bearing eggs which were measured	3	6
range in egg size	0.48–0.54mm	0.35–0.40mm
mean egg size	0.51mm	0.35mm
size of zoea (greatest diameter of carapace)	0.56–0.69mm	0.44–0.50mm
number of zoea measured	30	30

* mean ± one standard deviation

should remain approximatly the same size and not vary with the size of the crab (Dr. Pike, pers. comm.; but see Lebour (1931) who uses egg and zoeal sizes to distinguish between Hyas araneus and H. coarctatus). It is this difference in egg size which indicates that the two crab types may be different species or subspecies.

If the crabs inhabiting these two hosts are different species then there are likely to be distinct differences in the zoea. This situation exists with Pinnotheres pisum Linnaeus 1758, which is so similar to P. novaezelandiae that the adults of the two crabs have often been confused (Scott 1961), although there are distinct differences between the zoea of the two species (Bennett 1964).

It is known that more than one species of pinnotherid crab zoea occurs in the plankton of Wellington Harbour (Wear 1965), and since A. zelandica and P. canaliculus are the two most common hosts for pinnotherid crabs in the harbour, it was decided to rear and examine the zoea of crabs from both of these hosts.

MATERIALS & METHODS

Perna canaliculus and Atrina zelandica were both dredged from a depth of 4m in Wellington Harbour (41° 20'S, 174° 50'E). Seven ovigerous female crabs were removed from P. canaliculus and four from A. zelandica. The crabs were kept in separate aquaria until their eggs hatched. The zoea were pipetted into sterile fingerbowls containing sterilised and filtered sea-water which was changed daily. Attempts to feed the zoea with Isochrisis, Artemia larvae, and freshly hatched barnacle nauplii were unsuccessful and the zoea did not moult into the second stage.

Day old zoea were killed in 5% formalin and dissected in Berlese fluid. The appendages were mounted in Berlese fluid on glass slides for examination with a light microscope.

Six live day-old zoea from the A. zelandica crabs, and from the P. canaliculus crabs, were freeze dried using liquid nitrogen. The dry brittle zoea were then mounted on metal stubs, coated with gold-palladium, and examined using a Cambridge S600 scanning-electron microscope.

RESULTS

The crabs from P. canaliculus had an egg diameter of 0.35mm by the time that the eyespots began to develop on the eggs. The crabs derived from A. zelandica had a diameter of 0.51mm by this stage. When the zoea hatched, those of P. novaezelandiae measured 0.44-0.50mm carapace length, while the other zoea measured 0.56-0.69mm carapace length.

An examination of the appendages of 30 zoea from each of the two crab types did not reveal any significant differences. Whole mounts of the zoea, however, revealed the presence of a minute rostrum on the P. novaezelandiae zoea (Fig.20a) which was absent from the A. zelandica derived crabs.

Under the scanning electron microscope it was found that this rostrum has a distinct pit on each side (Fig. 20c). There is no sign of any similar organ on the zoea from the horse mussel (Fig. 21a). This zoea has a plate-like fold of the carapace instead of the rostrum, and four definate pits on the labrum (Fig. 21b). These pits on the labrum are absent from P. novaezelandiae (Fig. 21c).

FIGURE 20

Pinnotheres zoea from the mussel P. canaliculus

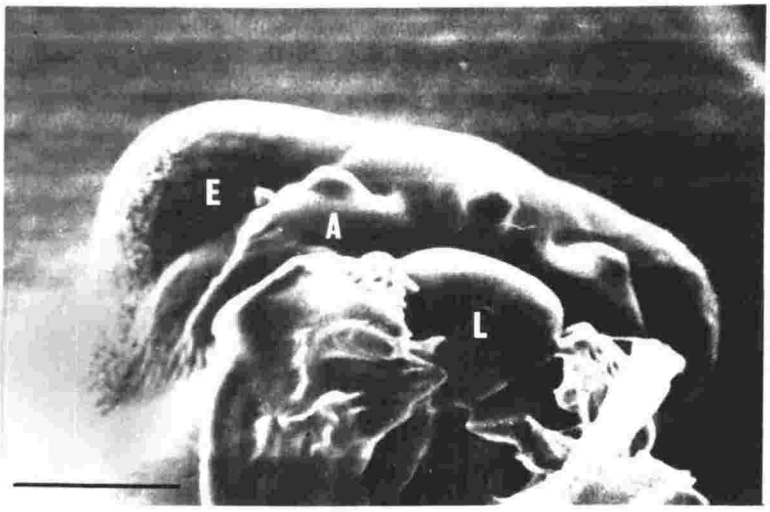
(a) head region showing small rostrum above the labrum. Scale bar = 100µm.

(b) rostrum. Scale bar = 40µm.

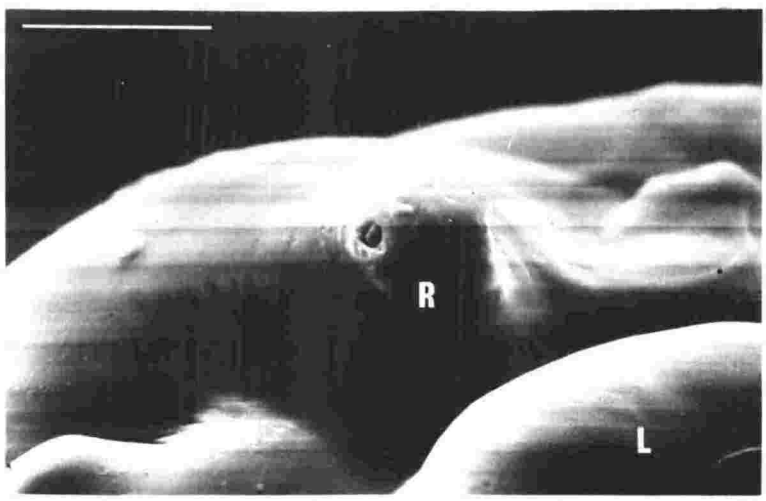
(c) detail of rostral pit. Scale bar = 4µm.

List of abbreviations for Figs. 20-22

A, antenna; E, eye; L, labrum; M, mandible; Ma, maxilliped; R, rostrum, Rp, rostral plate.



A



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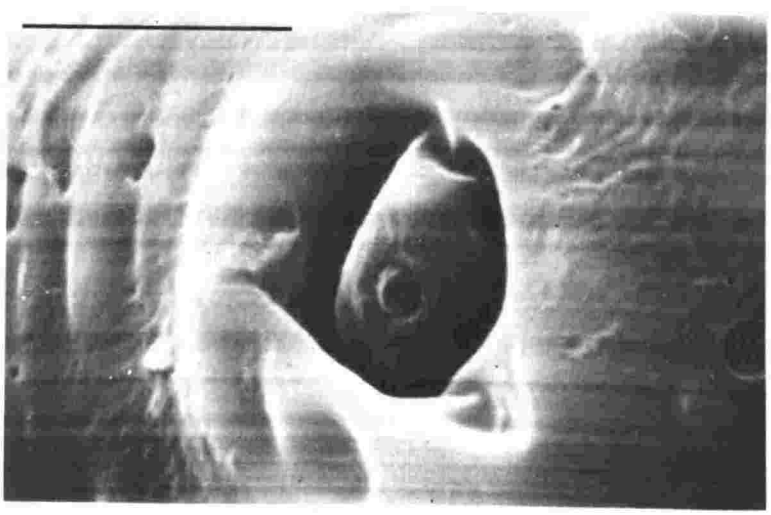

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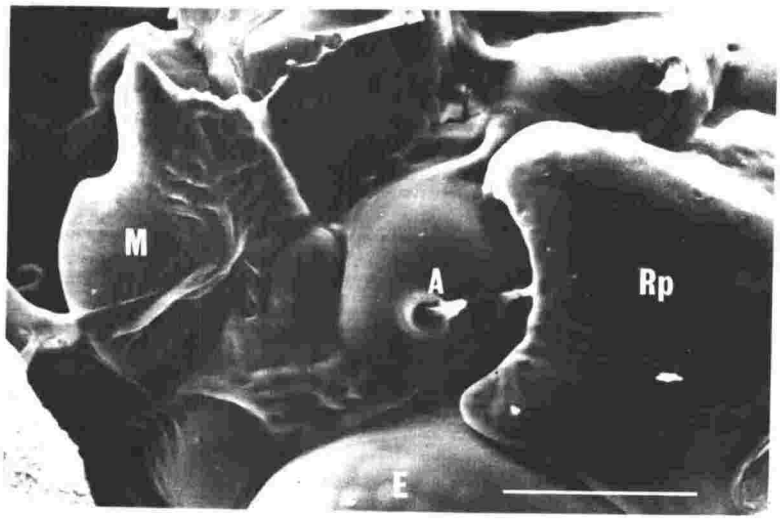
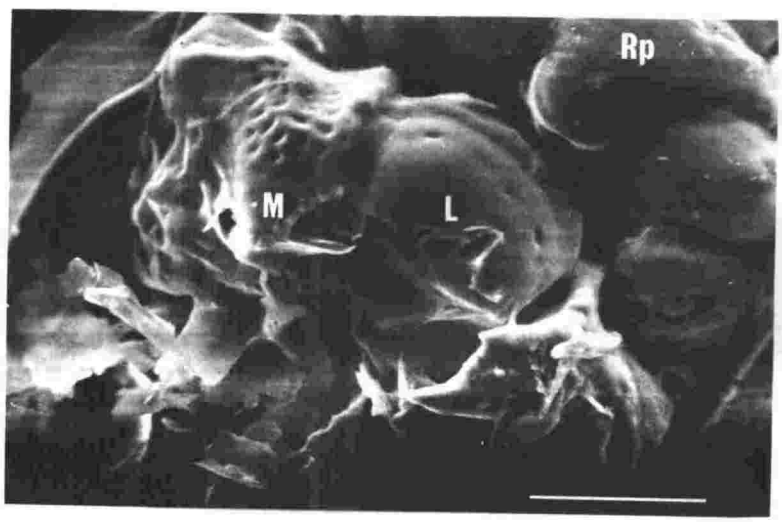
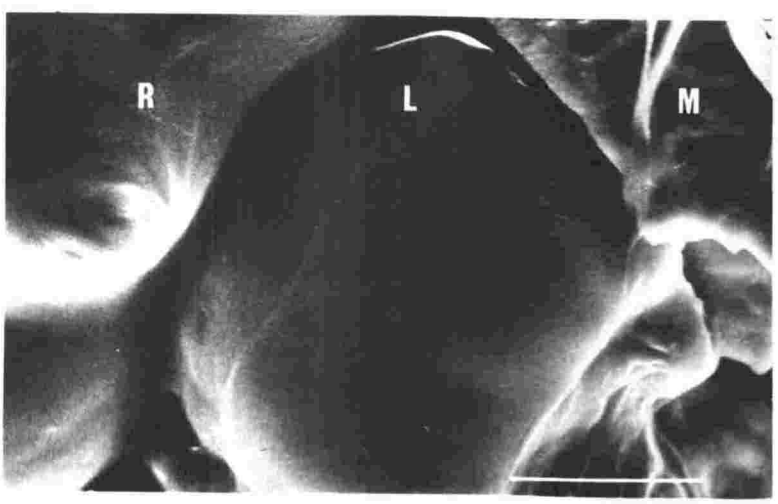


A



B



C

Of the appendages, only the mandibles could be seen with the scanning microscope. The mandible of P. novaezelandiae zoea has three teeth (Fig. 22a) while the other zoea has a four or five tooth mandible (Fig. 22b).

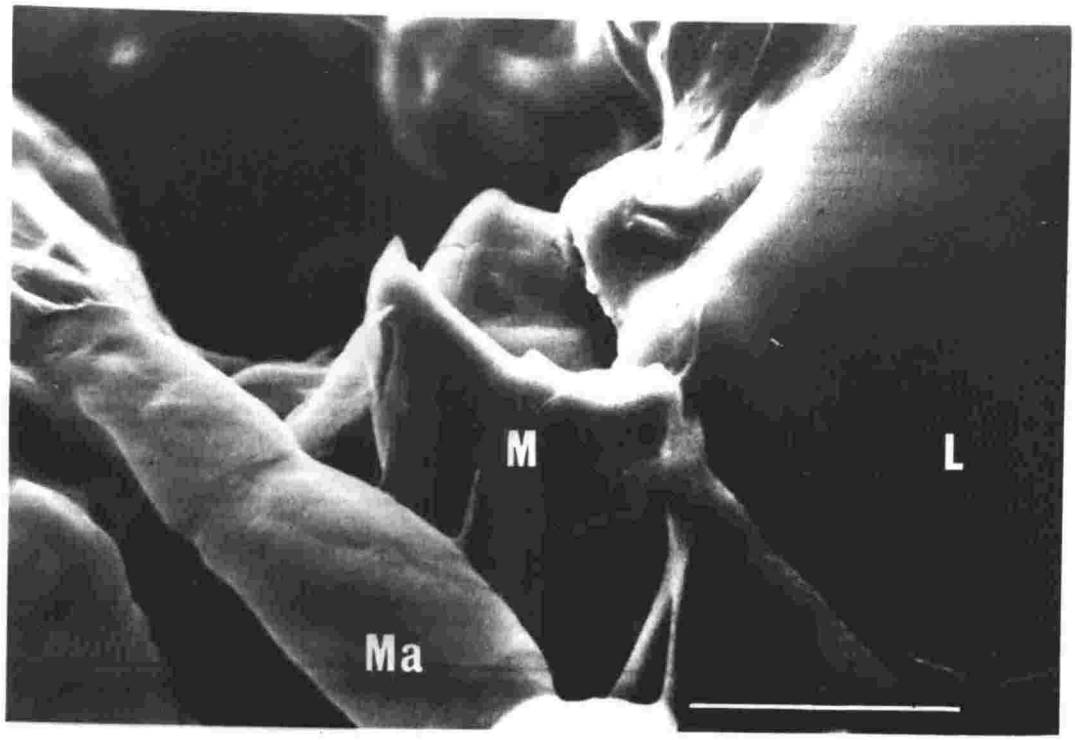
No other differences were seen.

DISCUSSION

The differences between the two types of zoea are quite consistant. The rostrum on the P. novaezelandiae zoea has been found on every crab zoea known to come from this host. It is, however, difficult to see unless a careful examination is made of the head of the zoea.

While the differences are almost certainly indicative of a specific difference between the adult crabs, it would be advisable to study the biology of the crab from A. zelandica before describing it as a new species. There appears to be no way of distinguishing the non-gravid female P. novaezelandiae from the A. zelandica derived crabs, and the male of the latter is unknown. The taxonomy of the Pinnotheridae is rather confused, and it would be unwise to add any new species which cannot be readily identified (Griffin & Campbell 1969).

The discovery of differences between the zoea of these two New Zealand crabs will make it necessary to re-examine a number of established pea-crab species. Pearce (1962) states that the pea-crabs are generally restricted to a single definative host, but that exceptions are known. A good example is P. maculatus Say 1818 which has a range extending from Martha's Vinyard in Massachusetts to the



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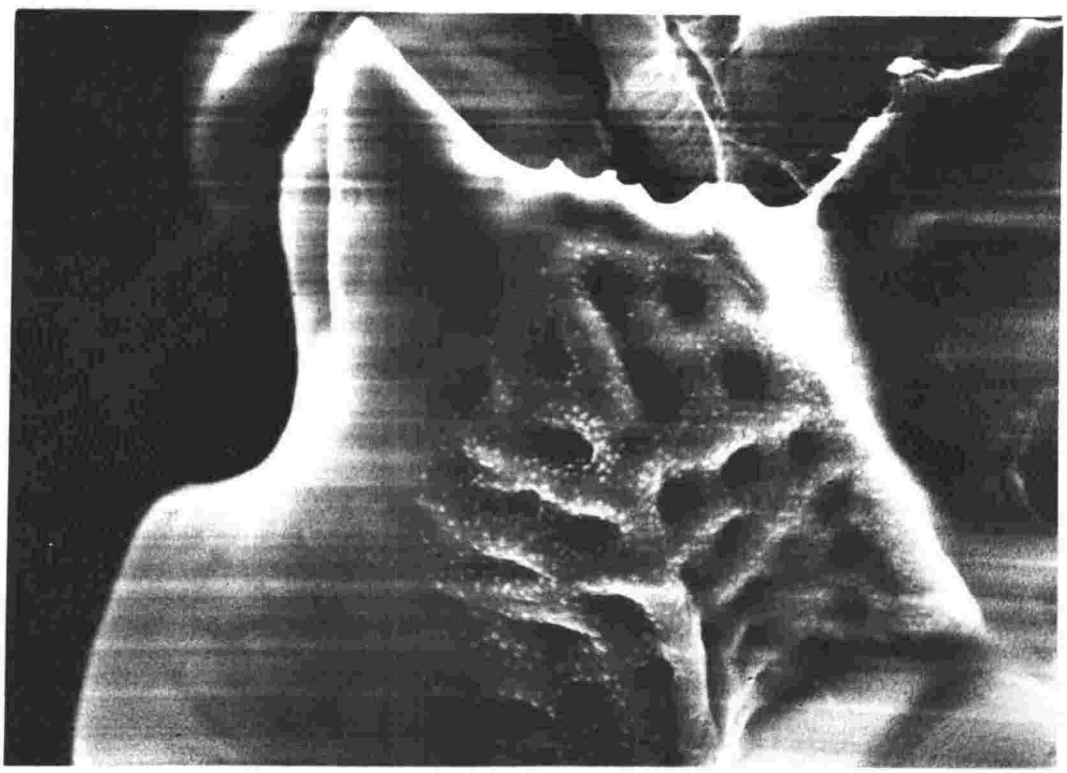


Mar del Plata in Argentina (Williams 1965). Its hosts include Mytilus edulis, Modiolus modiolus, M. americanus, Mya arenaria, Aequipecten irradians, A. gibbus, Placopecten magellanicus, Atrina serrata, the tubes of Chaetopterus variopedatus, from Molgula robusta, in the pharynx of Bostrichobranchus pilularis and on Asterias vulgaris (Cheng 1967).

Though the planktonic stages of this crab have been described (Costlow & Bookhout 1966), they have not been reared from crabs taken from all these hosts, and specifically from the non-molluscan hosts. An examination of these zoea may well reveal the presence of a number of new species or subspecies.

ACKNOWLEDGEMENTS

I gratefully acknowledge the help of Mr M.N.Loper of the Victoria University electron microscope unite, who helped to prepare the material for examination, and I would also like to thank Dr. G.C.Hewitt for his criticism of the manuscript.

REFERENCES CITED

Bennett, E.W. 1964: The marine fauna of New Zealand: Crustacea, Brachyura. N.Z. Department of Scientific and Industrial Research, Bulletin 153:120pp.

Cheng, T.C. 1967: Marine molluscs as hosts for symbioses with a review of known parasites of commercially important species. Advances in marine biology 5: i-xiii,1-423

Costlow, J.D. & C.G.Bookhout, 1966: Larval stages of the crab Pinnotheres maculatus under laboratory conditions. Chesapeake Science 7(3):157-163

Griffin, D.J.G. & B.M.Campbell, 1969: The sub-littoral Goneplacidae & Pinnotheridae (Crustacea:Brachyura) of Morton Bay. Memoirs of the Queensland Museum 15(3): 141-164

Lebour, M.L. 1931: Furthur notes on larval Brachyura. Proceedings of the Zoological Society of London 1931: 93-96, 2 plates.

Pearce, J.B. 1962: Adaption in symbiotic crabs of the family Pinnotheridae. Biologist 45:11-15

Scott, M.S. 1961: A review of the New Zealand Pinnotheridae. Transactions of the Royal society of N.Z., Zoology 1: 303-309

Wear, R.G. 1965: Zooplankton of Wellington Harbour, New Zealand. Zoology publications of Victoria University of Wellington 38:1-31

Williams, A.B. 1965: Marine decapod crustaceans of the Carolinas. Fishery Bulletin. Fish Wildlife Service of the U.S. 65:1-298

HOST-PARASITE LIST

MYTILIDAE:

Mytilus edulis aoteanus Powell 1958 (Blue mussel)

Digenea:

Tergestia agnostomi Manter 1954, sporocysts

Crustacea:Copepoda

Pseudomyicola spinosus (Raffaele & Monticelli 1885)

Crustacea:Malacostraca

Pinnotheres sp.

Perna canaliculus (Gmelin 1791)(Green mussel)

Protozoa:Sporozoa

Nematopsis sp., spores

Digenea:

Cercaria sp. Haswell 1903, sporocysts

Tergestia agnostomi Manter 1954, sporocysts

Crustacea:Copepoda

Lichomolgus uncus n. sp.

Pseudomyicola spinosus (Raffaele & Monticelli 1885)

Crustacea:Malacostraca

Pinnotheres novaezelandiae Filhol 1885

OSTREIDAE

Crassostrea glomerata (Gould 1850)(Rock-oyster)

Crustacea:Copepoda

Pseudomyicola spinosus (Raffaele & Monticelli 1885)

Crustacea:Malacostraca

Pinnotheres sp.

Ostrea lutaria Hutton 1873 (dredge oyster)

Protozoa:Sporozoa

Unidentified sporozoan from Foveaux Strait

Digenea:

Bucephalus longicornutus (Manter 1954), sporocysts

Crustacea:Copepoda

Pseudomyicola spinosus (Raffaele & Monticelli 1885)

PECTINIDAE

Pecten novaezelandiae novaezelandiae Reeve 1853

Crustacea:Copepoda

Pseudomyicola spinosus (Raffaele & Monticelli 1885)

Crustacea:Malacostraca

Pinnotheres sp.

Unidentified sporozoan parasite from Foveaux Strait oysters

Figure 23.

Stained sections of the oyster Ostrea lutaria revealed the presence of cysts in the connective tissue surrounding the epithelium of the gut. These cysts were oval or spherical in shape and measured 50-70 µm in diameter. Each cyst contained in excess of 500 spores. Each spore measured approximatly 2 x 7 µm. Identification of the spores was complicated by this small size. The spores appear to be truncate, with long dorsal and ventral processes. Each spore is covered with a transparent biconical or navicular envelope.

When stained with giemsa, the central body of the spore turns violet in colour, while the dorsal and ventral processes appear light blue.

The spores do not appear to have any polar capsules and no lid, such as occurs on spores of the Haplosporidia, was seen.

Occurence: During the present survey, 23 oysters were examined from Wellington Harbour, and 109 from Foveaux Strait. The only samples which were infected came from Foveaux Strait. The incidence of infection was as follows:

July 1973, from area H, 10.0% infected
November 1973, from an unknown area, 12% infected
March 1974, from area I, 9.4% infected.

This shows a remarkably consistant pattern, since the incidence of B. longicornutus sporocysts in the same samples was 4%, 0, and 2% respectively.

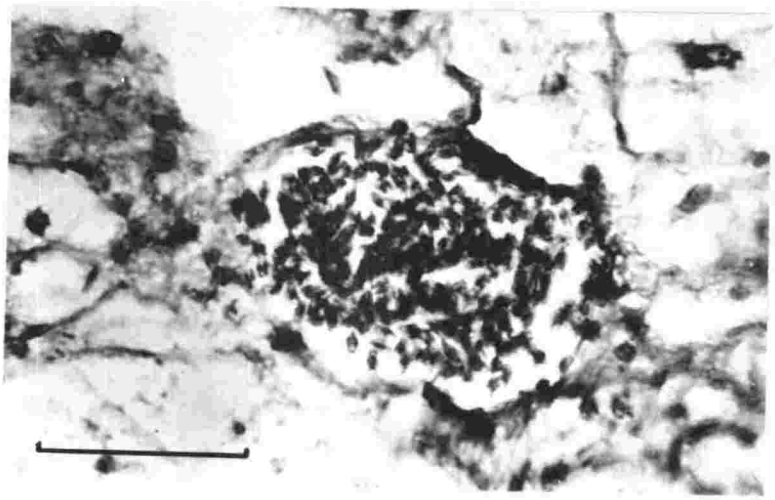
Pathology: No pathological effects were observed. The cysts must occur in low numbers within an infected oyster since the maximum number appearing in a section was two. It is unlikely that this parasite is a significant cause of mortality among O. lutaria at this time.

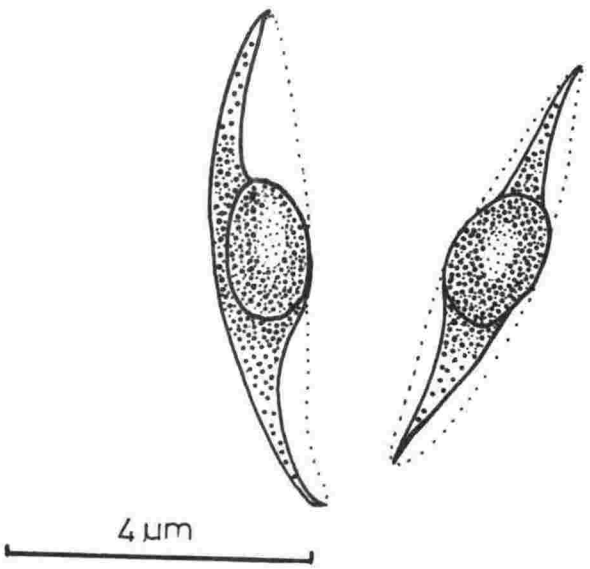
FIGURE 23

Unidentified sporozoan parasite from *O. lutaria*

(a) Cyst containing spores, stained with Giemsa. Scale bar = 0.05mm

(b) Spores, stained with Giemsa, as seen under oil immersion.




4 µm

Nematopsis sp.

(Class Telosporea; subclass Gregarinia; order Eugregarinia; suborder cephalina; family Porosporidae)

Adult gregarines are found in arthropods, and the majority spend their entire life-cycle in the one individual. The family Porosporidae is an exception in that it has an alternation of hosts. The life-cycle of a member of this family (*Nematopsis portunidarum* Frenzl 1885) was first determined experimentally by Léger & Duboscq (1913).

Only one marine gregarine, belonging to the family Porosporidae, is known to occur in New Zealand waters.

Shellfish host (the intermediate host):

Perna canaliculus (Gmelin 1791)

Arthropod host (the definative host):

unknown at present

Description of stage in mollusc:

Not all the development stages which occur in the mollusc have been found. The only stage so far discovered is the cyst containing the spore stage, and this has been described in section II, p. 19.

Life-cycle:

The life-cycle of the New Zealand species is unknown, but it is probably similar to that of other members of this genus, whose life-cycle is as follows:

The adult gregarine occurs in the intestine of a marine arthropod, usually a crab. Two or more gregarines become associated and encyst. Repeated nuclear and cytoplasmic division results in the formation of an enormous number of gymnospores in the hind gut of the arthropod. The clump of gymnospores is voided with the faeces, and if it comes in contact with the molluscan host, the gymnospores enter the epithelial cells of the gills, mantle or digestive system. Individual gymnospores pair and fuse, the resulting zygote developing encapsulated sporozoites (spores). When eaten by a crustacean host, the sporozoite develops as an adult gregarine. (adapted from Kudo 1954).

TABLE 5

Occurence of *Nematopsis*

Locality	Date	% of infection	number in sample
Ahipara	Jul 1973	96.0	25
	Dec	88.5	26
	Jul 1974	81.8	11
Wellington	Mar 1973	0	32+
	Apr	0	23+
	May	-	-
	Jun	9.1	22
	Jul	0	35+
	Aug	0	43+
	Sep	0	50+
	Oct	4.8	42
	Nov	0	49+
	Nov 1974	0	75+
	Mar 1975	56.25	16
Makara	Sept 1973	11.1	8
N.W. Bay (Marlborough Sounds)	Jun 1973	4.0	22

+ No infected mussels in sample

Distribution:

Spores of Nematopsis have been found in every locality from which the author has examined P. canaliculus. The results are summarised in table 5.

The percentage of infection in the Ahipara samples is consistantly high. In Wellington and the Marlborough Sounds the percentage of infection is much lower. This probably reflects the abundance of the arthropod in which the adult gregarine lives. The Cook Strait region is known to be the southerly limit of a number of species (see Morton & Millar 1973 pp. 295-302), and it may be that this region is the southern limit for the host arthropod.

It is also possible that Nematopsis sp. has only recently spread south to the Cook Strait region, giving an initial low rate of infection, which may be rising (see table). Distribution of the gregarine, however, is known to be patchy, and to vary with time. Perna canaliculus removed from a rock on the low-water line in Wellington Harbour during 1974 were found to have an infection rate of 30%. The same rock, when sampled six months later, was found to be free of infection. (This does not indicate that the mussels are able to lose the infection since intertidal P. canaliculus are known to have a short life due to overcrowding by M. edulis aoteanus, and to predation by fishermen collecting bait.)

The possibility that Nematopsis sp. has been introduced into Ahipara from overseas cannot be discounted. Crassostrea gigas (Thunberg 1793) has recently appeared in New Zealand (Dinamani 1971) although the method by which it arrived is unknown. Skerman (1960) documents the transport of ship fouling organisms to New Zealand from Australia, and Chilton (1910) gives examples of crustacea brought to New Zealand on the hull of the "Terra nova".

Pathology:

Prytherch (1940) believed that Nematopsis was responsible for the common and extensive mortalities of Crassostrea virginica on the Atlantic coast of America. Sprague and Orr (1955) produced heavy experimental infections in the oyster, and concluded that Nematopsis, while detrimental to the host did not cause appreciable mortality, Later

Feng (1958) provided evidence that N. ostrearum Prytherch 1940 spores could be eliminated by the oyster, and that a dynamic equilibrium existed between elimination and re-infection.

The effect of Nematopsis on P. canaliculus is not known but in view of the heavy infection rate at Ahipara and the apparent health of the mussel stocks there, the effect of the spores on their hosts cannot be serious.

THE ULTRASTRUCTURE OF A *NEMATOPSIS* SP. CYST (SPOROZOA : EUGREGARINIDAE)

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ABSTRACT

A Nematopsis cyst from the palp of the New Zealand green mussel Perna canaliculus (Gmelin) was examined using elecron microscope methods. The cyst, which contained four spores, was surrounded by a thin membrane on the outside of which was a 1.4 - 2.8mm thick fibrous wall that had apparently been laid down by the host shellfish. In addition to the four spores, the highly vacuolate cytoplasmic matrix of the cyst was found to contain a golgi complex, mitochondria, and four nuclei, one of which contained a distinct nucleolus.

INTODUCTION

The Nematopsis sporocysts found in the green mussel Perna canaliculus are unusual in possessing a thick cyst wall instead of a thin membranous cyst wall as in other members of the genus (Sprague pers. comm.). A cyst was therefore examined using electron microscope techniques, in order to determine the nature of the cyst wall.

Attempts to locate literature on the ultrastructure of other Eugregarine cysts and spores was unsuccessful, and so it was concluded that this was the first time such cysts had been examined. Accordingly, serial sections of the cyst were cut so that the complete structure could be observed.

Unfortunatly, the glass knife used was unable to section the contents of the spores within the cysts, due to the hard nature of the spore wall, so the ultrastructure of the developing sporozoite was not observed. A diamond knife may have been more successful, but none was available.

MATERIALS & METHODS

See section I, page 14, for the methods used.

OBSERVATIONS

The cyst (Figs. 24-29) is 46 µm in diameter, and contains four spores. In a cyst of this species, the number of spores is variable, and other spores have been seen with as few as two or as many as ten spores, but the mean number is four.

The cyst is enclosed by a cyst wall 1.4 - 2.8 µm thick,

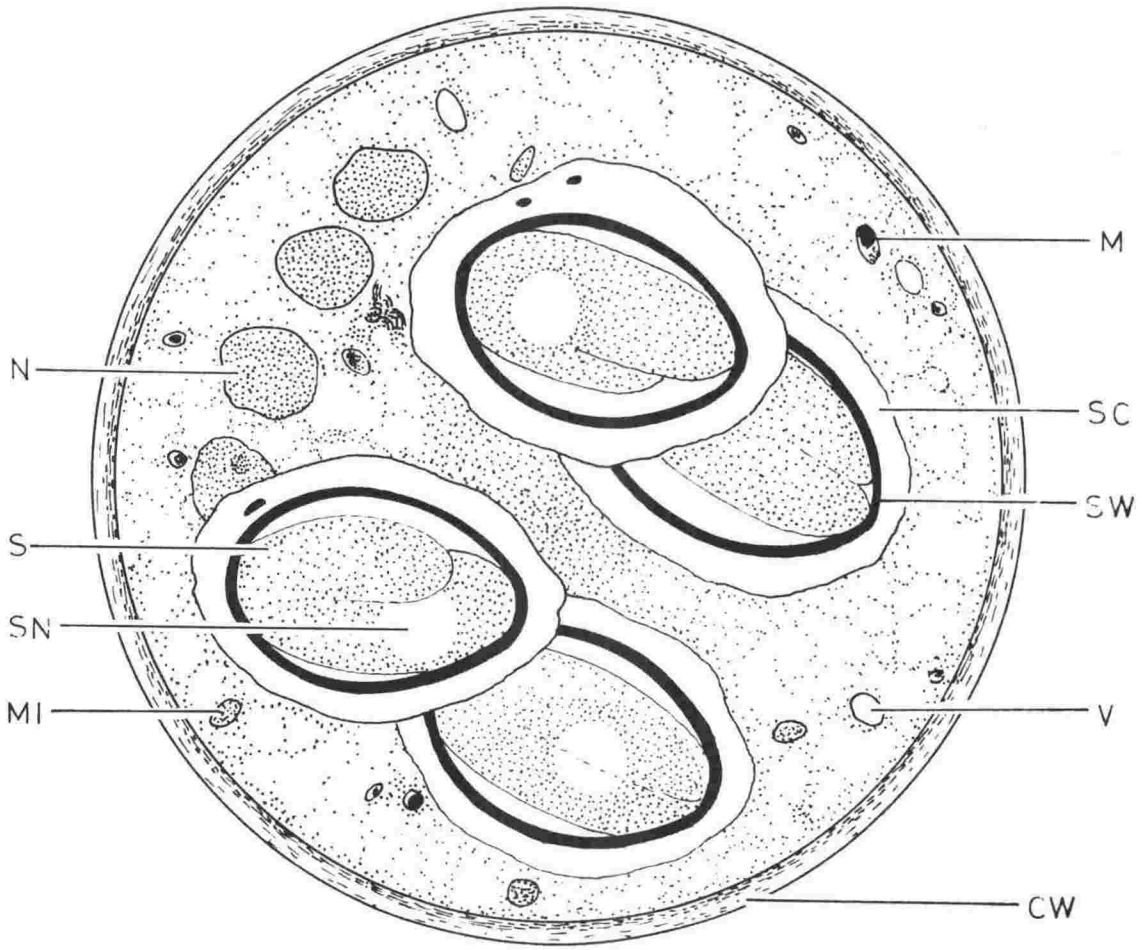
FIGURE 24

Nematopsis sp.

Diagrammatic transverse section through cyst and spores.

List of abbreviations for Figs. 24-30

Cm, cyst membrane; CW, cyst wall; M, microbody; MI, mitochondria; N, nucleus; Nm, nuclear membrane; Np, nuclear pore; S, sporozoite; SC, spore coat; SN, nucleus of sporozoite; SW, spore wall; V, vacuole.


M
N
SC
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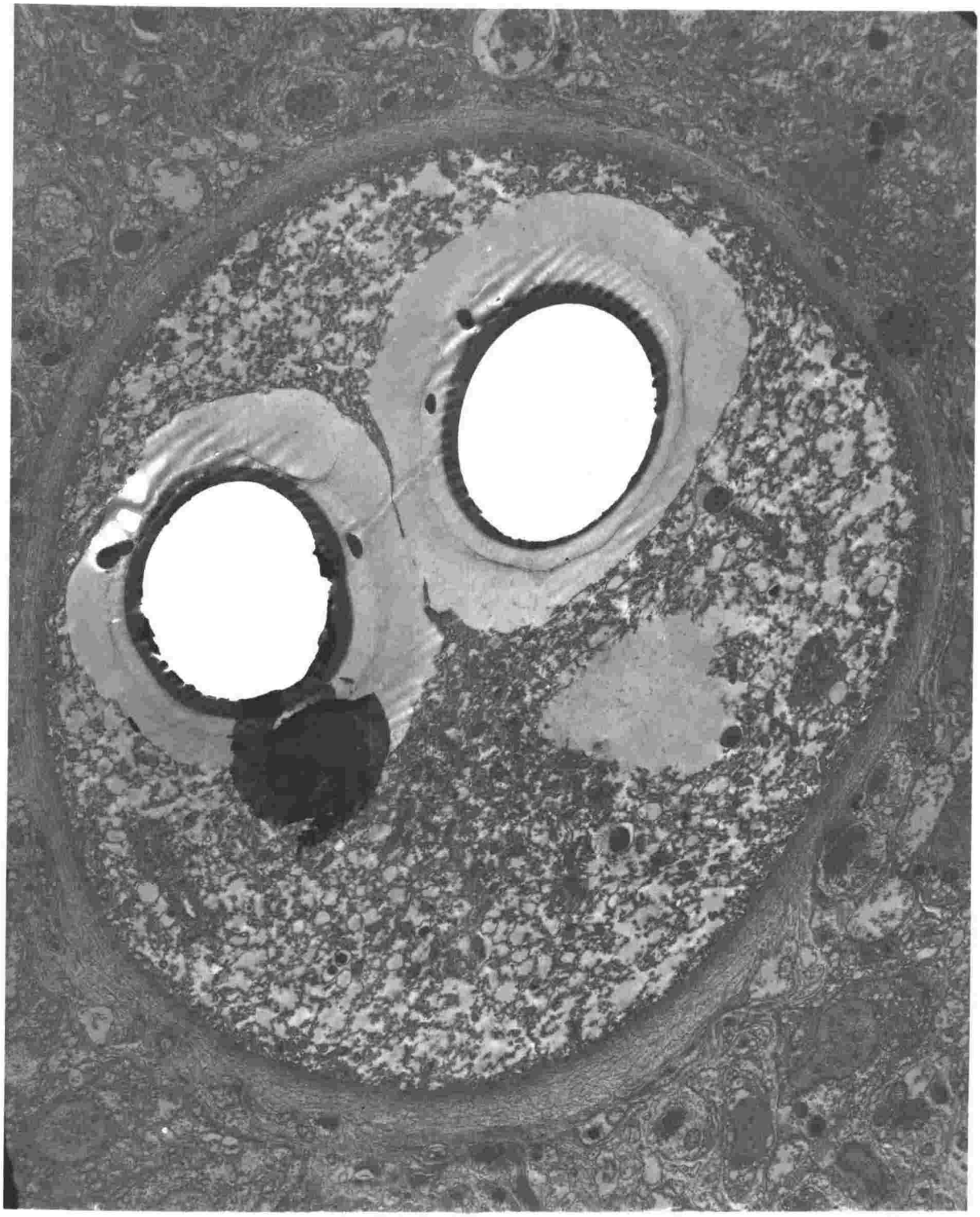
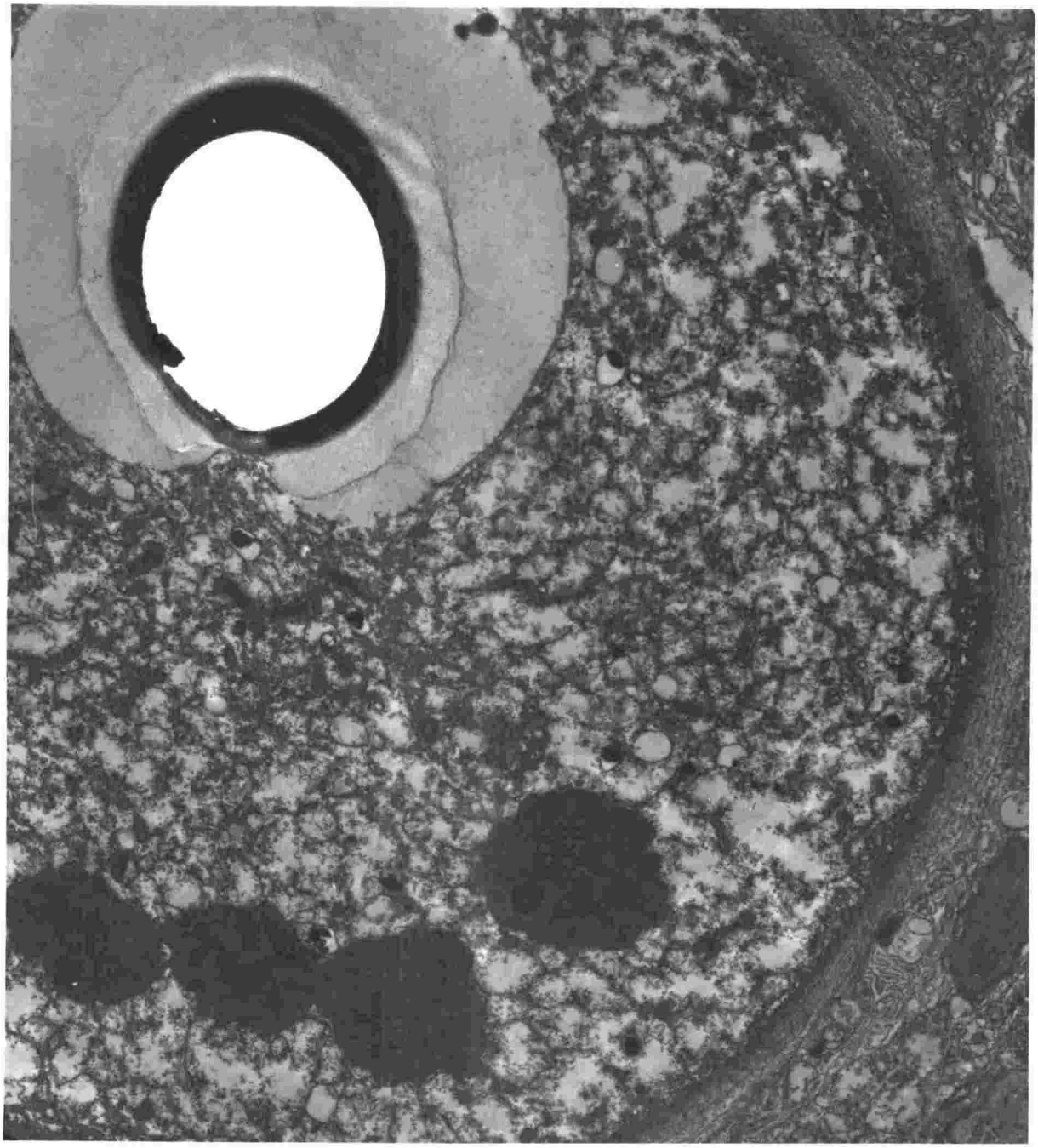
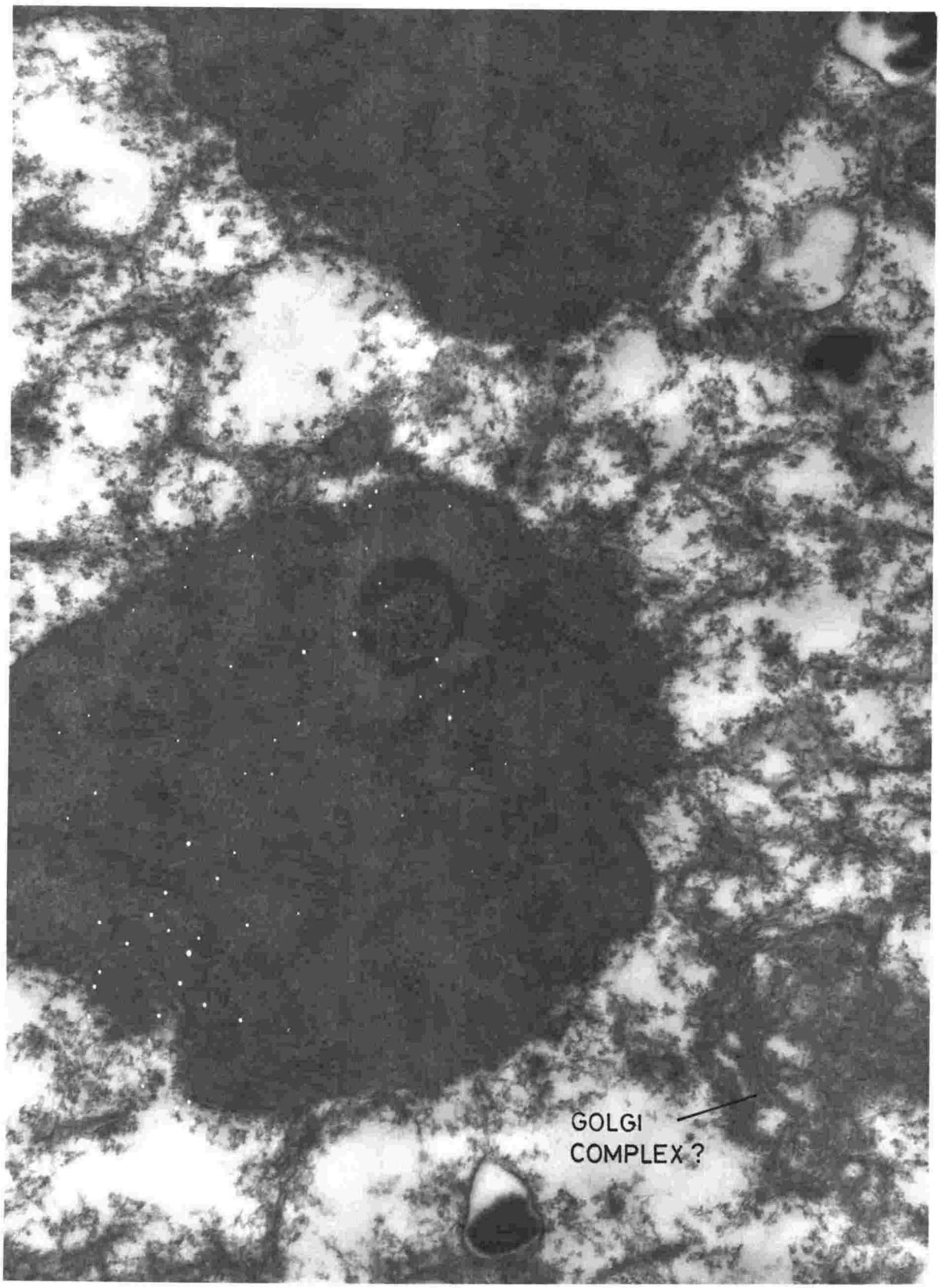
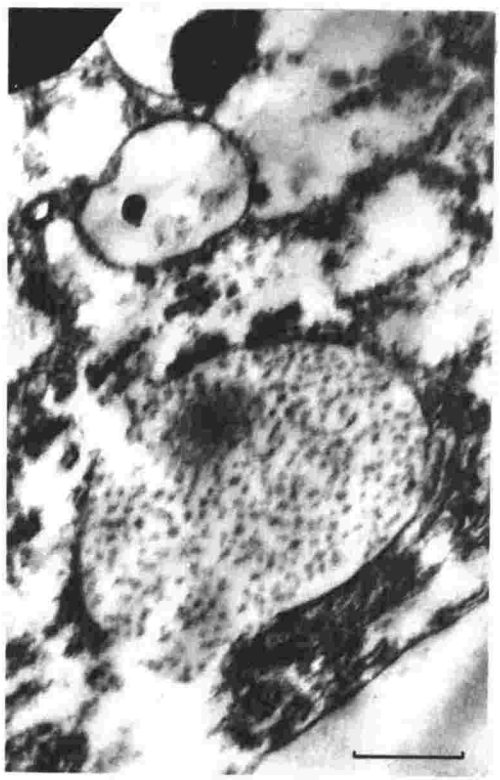
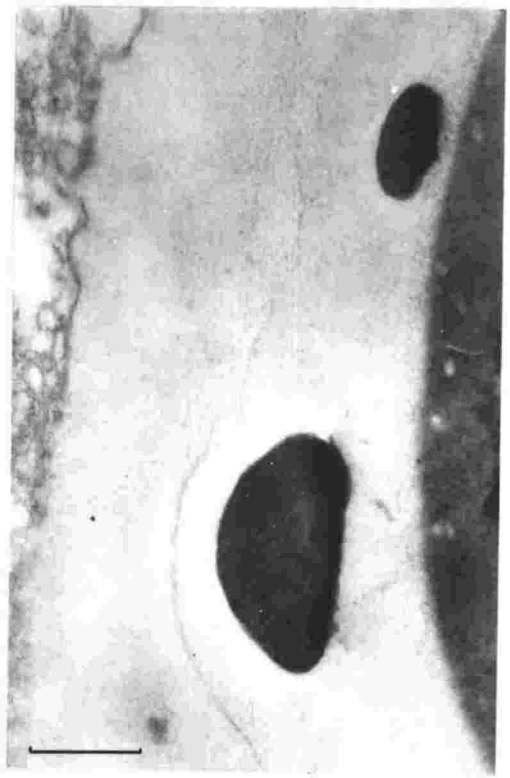





GOLGI
COMPLEX ?



A



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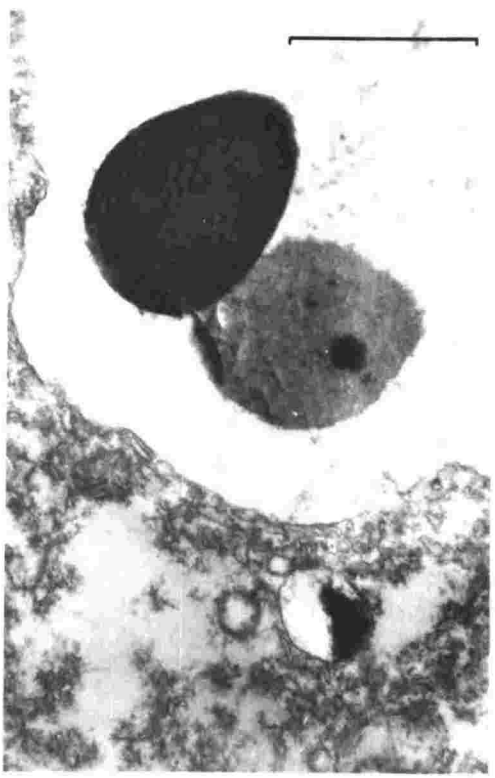

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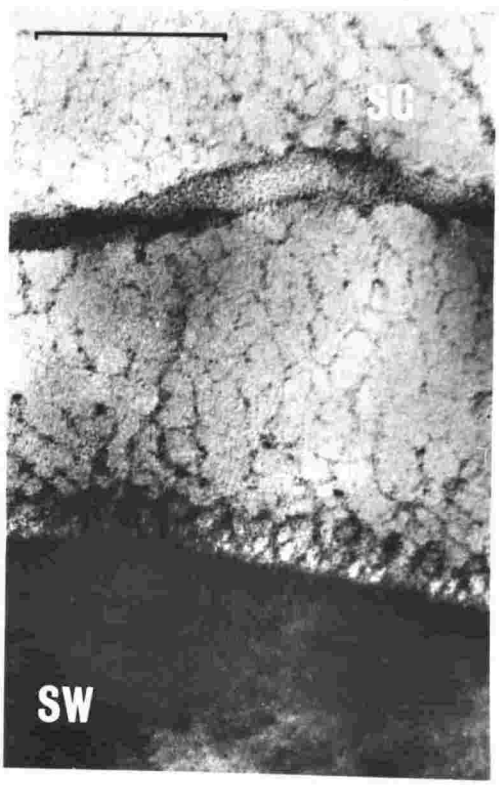

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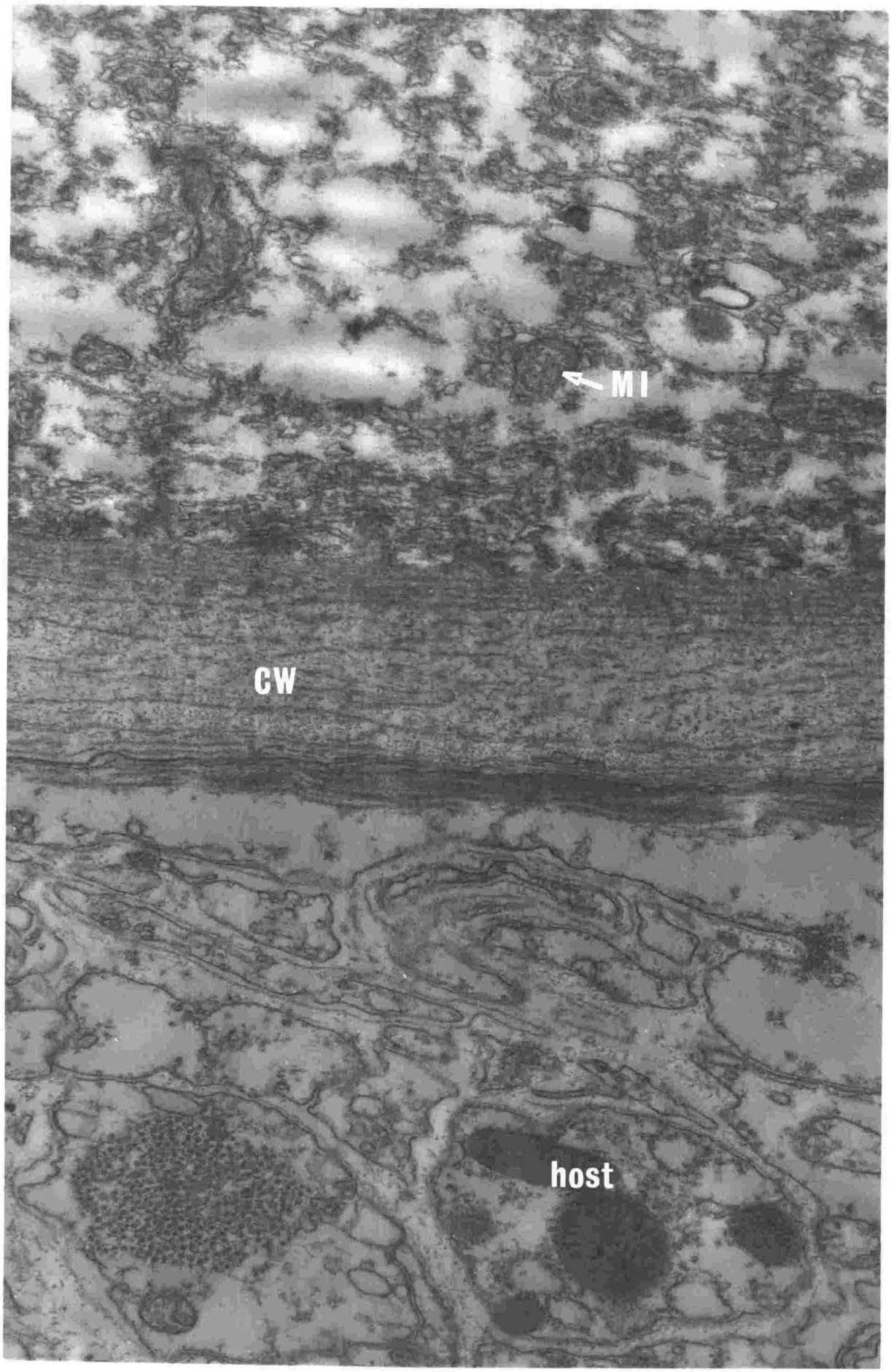
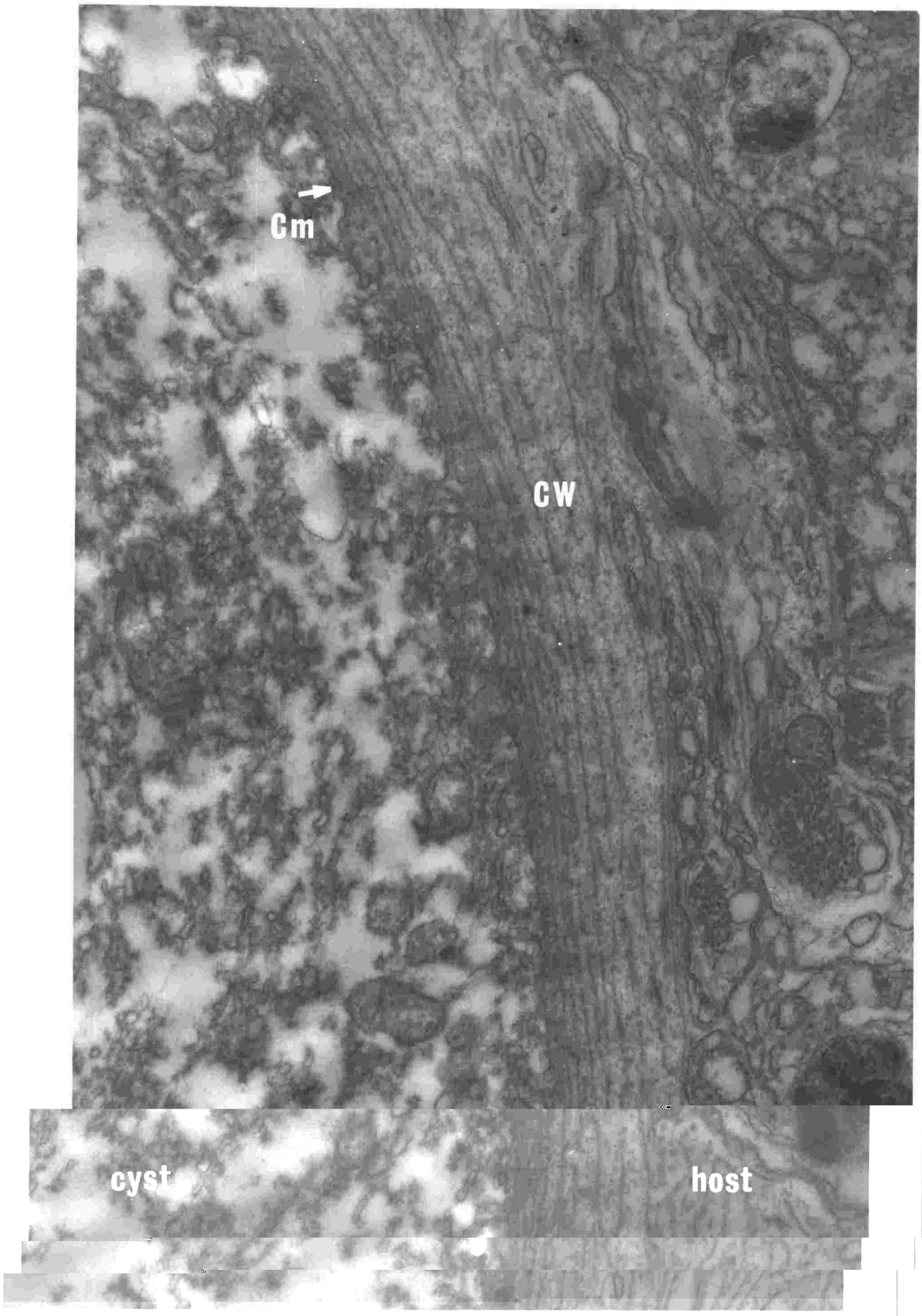

MI
CW
host


Cm
CW
cyst
host

and has a granular and highly vacuolate cytoplasmic matrix. Many of the vacuoles contain an electron dense material (Figs. 25, 26, 28a). Four nuclei were found in the cytoplasm of the cyst (Figs. 26, 27), one of which has a prominent nucleolus. The nuclei are each surrounded by a nuclear envelope 18.7 µm thick, with nulear pores of 47 nm diamter. Rough endoplasmic reticulum can be traced around the nuclei (Fig. 27).

What appears to be a golgi complex was observed near one of the nuclei (Fig. 27). This consists of a series of tightly packed smooth vesicles, many of which are heavily stained with osmium.

Numerous mitochondria occur, These are similar to other protozoan mitochondria in that the inner membrane forms tubules, not the christae found in vertebrate mitochondria (Loewy & Siekevitz 1963) (Fig. 30).

The cyst wall: It appears that most of the cyst wall is laid down by the host shellfish, since a highly invaginated double membrane 9.4 nm thick was found on the inside of the cyst wall. On the outside of the membrane the dense fibrous layers of the cyst wall thin out gradually to merge with the host tissues (Fig. 29). In places, however, the wall appears to be bound by a second outer layer of dense fibrous material, but this is uncommon. This second layer is apparently caused by host cells actively involved in the encapsulation of the parasite (Fig. 30).

The spore wall: This is in three main layers, an outer opaque coat divided into two layers by a granular zone 50 nm thick. The inner layer of the opaque coat is separated from the hard inner spore wall by a zone 70 nm thick. This zone appears to be fibrous in nature (Fig. 28a).

Ine inner spore wall is extremely hard and electron dense. Deposits of a similar substance occur in the outer opaque wall of the spore (Fig. 28b, c).

There is a micropyle between the spore and the cyst (Fig. 26) plugged by a less dense area of the spore wall. The opaque spore coat is only 72 nm thick at the micropyle.

DISCUSSION

The cyst contents appear very confused and disordered in appearance, which is not surprising considering

that the cyst is a syncytial mass with four nuclei.

The cytoplasmic matrix of the Nematopsis cyst bears a superficial resemblance to the matrix of young malarial oocysts. Duncan et al (1960) found that malarial oocysts from the stomach wall of mosquitoes have a dense cytoplasmic matrix which subsequently loses density and becomes more vacuolate. The authors provide evidence that the development of the sporozoites in the malarial oocyst is not entirely dependent upon the substances within the oocyst capsule, but that much of the nutrient material required must be passing in some way through the cyst wall from the host. For the same reasons as those advanced by Duncan et al for malaria, it is apparent that Nematopsis must also rely on nutrient material derived from the host. The invaginated cyst membrane of Nematopsis will greatly increase the absorbative surface of the cyst, since absorbtion must be retarded by the thick fibrous nature of the wall. It would be interesting to know whether the membrane of Nematopsis species which do not have this wall, is also invaginated.

The oocyst wall of malaria blends indiscernably with the host tissues, which suggested to Duncan et al (1960) that this wall was formed by the host. A similar effect was noticed in the wall around the vegetative stages of the oyster fungus Labrynthomyxa marina Mackin Owen & Collier by Perkins (1969), but in this case the wall contained parallel arrays of host membranes embedded in the wall, similar to those observed in the wall around Nematopsis.

Encapsulation of a parasite is a common defense mechanism of molluscs (Cheng 1967) and so it is not surprising that the wall around L. marina is so similar to the wall around Nematopsis.

From the observations made by Duncan et al, and the author, it seems that encapsulation does not neccessarily isolate the parasite from the host, but probably reduces the rate of exchange of material with the host. Duncan et al noted the resemblance of the malarial capsule to the basement membrane of the mammalian glomerulus, and suggested that any material for protein synthesis was unlikely to be in the form of whole protein molecules. A selectively premiable capsule would prevent parasite proteins coming into contact with the host, and may force the parasite to

store certain unwanted by-products. This would explain the vacuolate nature of the cytoplasmic matrix.

The function of the extra nuclei in the Nematopsis cyst is unknown. No cysts have been found with spores at different stages of development, so it is unlikely that they are destined to become extra spores. Sections cut through other Nematopsis spores, for light microscope examination, often reveal free nuclei within the cyst, but it has not been possible to determine the number which occur in a cyst by this method.

Other sporozoans are known to be multinucleate. One example is Nosemia, the spores of which have been found to contain two nuclei (Huger 1960). It is possible that in some types of cell, the control of the cell functions is divided between the nuclei present, instead of being under the control of the one nucleus as in most animal cells.

ACKNOWLEDGEMENTS

I gratefully acknowledge the help of Mr M.L. Loper, of the Victoria University electron microscope unite, who fixed, cut, and stained the sections, and who introduced me to the science of electron microscopy.

REFERENCES

Cheng, T.C. 1967: Marine molluscs as hosts for symbioses, with a review of known parasites of commercially important species. Advances in Marine Biology 5: i-xii, 424pp.

Duncan, D.; Eades, J; Julien, S.R; & Micks, D. 1960: Electron microscope observations on malarial oocysts (Plasmodium cathemerium). Journal of protozoology 7(1): 18-26

Huger, A. 1960: Electron microscope study on the cytology of a microsporidian spore by means of ultrathin sectioning. Journal of insect pathology 2: 84-105

Loewy, A.G. & Siekevitz, P. 1963: Cell structure and function. Holt, Rinehart & Winston; New York. i-x, 516 pp (2nd. edn.)

Perkins, F.O. 1969: Ultrastructure of vegetative stages in Labrynthomyxa marina (=Dermocystidium marinum)

a commercially significant oyster pathogen.
Journal of invertebrate pathology 13: 199-222

Cercaria sp. Haswell 1903

(Superorder Anepitheliocystidia; order Strigeatoidea; suborder Brachylaimata; superfamily Bucephaliodea; family Bucephalidae)

The bright red sporocysts of this bucephalid trematode wereonly found on two occasions during this survey, and as a result, the sporocyst must be considered to be rare.

Shellfish host:

Perna canaliculus (Gmelin 1791)

Other hosts:

unknown at present

Description of stages in mollusc:

See section II, p. 23.

Distribution: Tasman Bay
Wellington Harbour
? Christchurch (Haswell's specimen)

Pathology:

Nothing is known of the effect that this parasite has on the mussel. Parasitic castration of the host is caused by all other bucephalid larvae known to date (Hopkins 1957), and this bucephalid is probably no exception. The low numbers of mussels infected suggests that the infection is lethal to P. canaliculus, or that this might not be the normal intermediate host.

Bucephalus longicornutus (Manter 1954)

(Superorder Anepitheliocystidia; order Strigeatoidea; suborder Brachylaimata; superfamily Bucephaloidea; family Bucephalidae)

The trematode Bucephalus longicornutus was originally described by Manter (1954) as Alcioornis longicornutus. Manter based his description on a single adult specimen retrieved from the intestine of the Monk-fish Kathetostoma giganteum Haast 1873 which had been caught in Wellington Harbour.

In 1966 Howell demonstated experimentally that Alcioornis longicornutus, which he transferred to the genus Bucephalus was the trematode whose sporocysts were infecting the dredge oyster Ostrea lutaria.

This parasite is known to cause mortality among infected oysters (Miller 1963; Howell 1967).

Shellfish host (intermediate host):

Ostrea lutaria Hutton 1873

Second intermediate hosts:

Acanthoclinus quadridactylus (Bloch & Schneider 1801)

Tripterygion spp.

Definative host:

Kathetostoma giganteum Haast 1873

Scorpaena cardinalis (Richardson 1842)

Description of stages in the mollusc;

A complete description of the sporocyst and cercaria is given by Howell (1966). The sporocysts are milky white, branching and swollen at irregular intervals. The parasite forms a mat of sporocyst tubules ramifying throughout the gonad tissue, viscera, and palps of the oyster.

This is the only sporocyst known to occur in O. lutaria.

The cercaria is small and opaque, about 3mm in body length when relaxed, with long furcae which are approximatly 4 mm in length when expanded.

Life cycle:

This was determined experimentally by Howell (1966), and his results are summarised here.

The adult trematode occurs naturally in the monk fish K. gigantæum. Scorpæna cardinalis was successfully infected in the laboratory, but has not been found to contain B. longicornutus naturally. The miracidia infects the oyster O. lutaria, and develops into a dense network of sporocysts in the gonad. From these, cercariae are released which penetrate a second intermediate host. The natural second intermediate host is unknown, but Trigla spp. and A. quadridactylus were experimentally infected with cercariae, and viable metacercariae developed. Metacercaria which were 80 days or older in development were fed to S. cardinalis where they developed into gravid trematodes.

Data from present study:

Unfortunatly only three samples of dredge oysters were examined. These were collected in July and November 1973, and March 1974. The areas from which the July and March samples came (H and I respectively) (see Fig. 3, p. 10) were areas not covered by Howell's (1967) survey. The November sample came from an unknown area of the beds. No direct comparison with Howell's figures was therefore possible, but the 4% infection of the July sample and the 22% infection of the March sample fit the pattern of infection given by Howell. He found a low percentage of infection on the eastern and western sides of the commercial beds, with a maximum infection of 47% in area B in August 1963. The average infection in areas A and B during 1963 and 1964 was 40%.

The mean condition factor of the oysters infected with B. longicornutus was higher in both the July and March samples than the mean condition factor of the healthy oysters.

area	date	% infected	condition factor	
			infected	healthy
H	July 1973	4	58.7	41.3
?	November	-	-	45.6
I	March 1974	22	49.9	49.3

Hopkins (1957) noted that Bucephalus infected oysters were fat looking and full of glycogen, when compared with uninfected oysters. It is almost certain that the degree

of difference between the normal and infected oyster condition will vary with the spawning cycle of the oyster in a similar manner to that observed for P. canaliculus infected with Pinnotheres novaezelandiae (see p.144)

Pathology:

From the data given by Howell (1967) it appears that B. longicornutus infects the oysters sometime between November and February. The infected oysters develop a heavy infection of sporocysts by September of the following year. Infection is gradual, beginning in the dorsal region of the visceral mass and later spreading to the pyloric caeca and pericardium of the host. It appears that a well developed gonad is necessary to support the infection, but that parasitic castration eventually occurs (Howell 1967). This would explain why the infection of the oysters follows a seasonal cycle, since the definative host is not known to leave the oyster beds and there is no reason why the miracidia should not occur throughout the year.

Howell found that oysters were approximatly two years old before they were subject to infection, and once infected they lived at most for 12 months from the time a light infection could be recognised.

The infection eventually affects the ability of the adductor muscle to close the shell, and it is this which causes the death of the oyster.

The decline in catch per unite effort which first became noticable in Foveaux Strait from 1958, when the maximum yield dropped from 14 sacks/hour to 6 sack/hour has been attributed to B. longicornutus, as this parasite was especially common on the beds at this time (Unpublished Marine Department records). It is now known that there is a sporozoan parasite present on the beds, and this may have been partially responsible for the sudden mortality in 1958 from which the beds have not yet recovered.

Additional information:

Hopkins (1957) found that Bucephalus infected Crassostrea virginica had an "excellent taste" and were pleasing to look at, so he facetiously suggested that some beds should be artificially infected in order to supply

caponised oysters to a premium market.

The presence of the bucephalid parasite in New Zealand does not prevent the sale of the infected oysters to the public, who are probably unaware of its presence. The parasite is quite harmless to man.

Tergestia agnostomi Manter 1954

(Order Strigeatoidea; suborder Brachylaimata ; superfamily Fellodistomatoidae; family Fellodistomatidae)

The trematode Tergestia agnostomi was described by Manter (1954) from the yellow-eyed mullet Aldrichetta forsteri caught in Wellington Harbour. As none of Manter's specimens were gravid he suggested tha A. forsteri might not be the definative host.

Yamaguti (1958) suggested that T. agnostomi might be an immature T. laticollis, which is widely distributed in the Mediterranean, Caribbean and Japan. He also noted the similarity between the adult T. agnostomi and a cercaria described by Haswell (1903) from the mussel Perna canaliculus. This similarity was noted also by Angel (1960) and by Linzey (1971). Linzey believed that Manter's specimens were not adult trematodes, but viable tail-less cercariae of the same species as the cercaria Haswell had described.

Both Linzey and Angel accepted Manter's view that A. forsteri was not the true host, Angel, because the mullet "is obviously a bottom feeding fish" and Linzey because nobody had been able to find mature T. agnostomi in A. forsteri, although it had been searched for on several occasions.

There is, however, evidence that T. agnostomi does occur in A. forsteri. Howell (pers. comm.) obtained a gravid specimen from this host in 1966, and subsequent collections by Veal (pers. comm.) and the author revealed several gravid T. agnostomi from A. forsteri, both from Wellington Harbour and a sea-run specimen from Lake Ellesmere (see section II, p. 28).

The cercaria describe by Haswell (1903) was subsequently named Cercaria haswelli by Dollfus (1927). It occurs widely around the New Zealand coast.

Shellfish hosts (first intermediate host);

Mytilus edulis aoteanus Powell 1958
Perna canaliculus (Gmelin 1791)

? Second intermediate host:

Pleurobrachia pileus Muller

Definative host:

Aldrichetta forsteri (Cuvier & Valenciennes 1836)

Description of stages in mollusc:

A detailed description of the sporocyst and cercarial stages can be found in Linzey (1971)

The sporocysts (Fig. 31d, e) are bright orange, sack-like, rounded in cross-section, and hollow. They are muscular and move by elongation or contraction. The sporocysts may contain either a new generation of sporocysts, or cercaria which escape via a terminal "birth pore". Sporocysts can also increase their numbers by binary fission. Sizes range from 0.5 X 0.5mm to 2.0 X 1.0mm although large elongated sporocysts may reach 3mm in length.

The cercaria (Fig. 31f) is free swimming but is often encountered inside the mussel, soon after hatching from the sporocyst. The cercaria is opaque, but may have a reddish material in the intestinal caeca if newly hatched. Total length including the tail is 2-3mm. The anterior sucker is capable of being completely everted, giving the cercaria a spherical appearance, and hiding the 12 distinctive cuticular ridges around the neck and the 13 lobed "crown" around the anterior sucker. The pharynx is large, with a length to width ratio of 2:1. There is a rudimentary genital atrium just above the ventral sucker, The ventral sucker is smaller than the anterior sucker, the ratio being 5:4 (Angel 1960), or 11:7 (author). The genital anlagen consists of two densely staining masses between the intestinal caeca.

The unique feature of the cercaria is the tail, which projects laterally, and bears between the tail stem and the cercaria body, a muscular crested region capable of being inflated or contracted. Fluid forced from this organ extends the tail making it rigid. When the organ relaxes and expands the tail becomes limp. Tail furcae are as long as the tail stem. The flame cell formula was not observed in the present study, but is reported by Linzey (1971) to be $2\left[(2+2)+(2+2)\right] = 16$, as suggested by Angel (1960).

Life cycle:

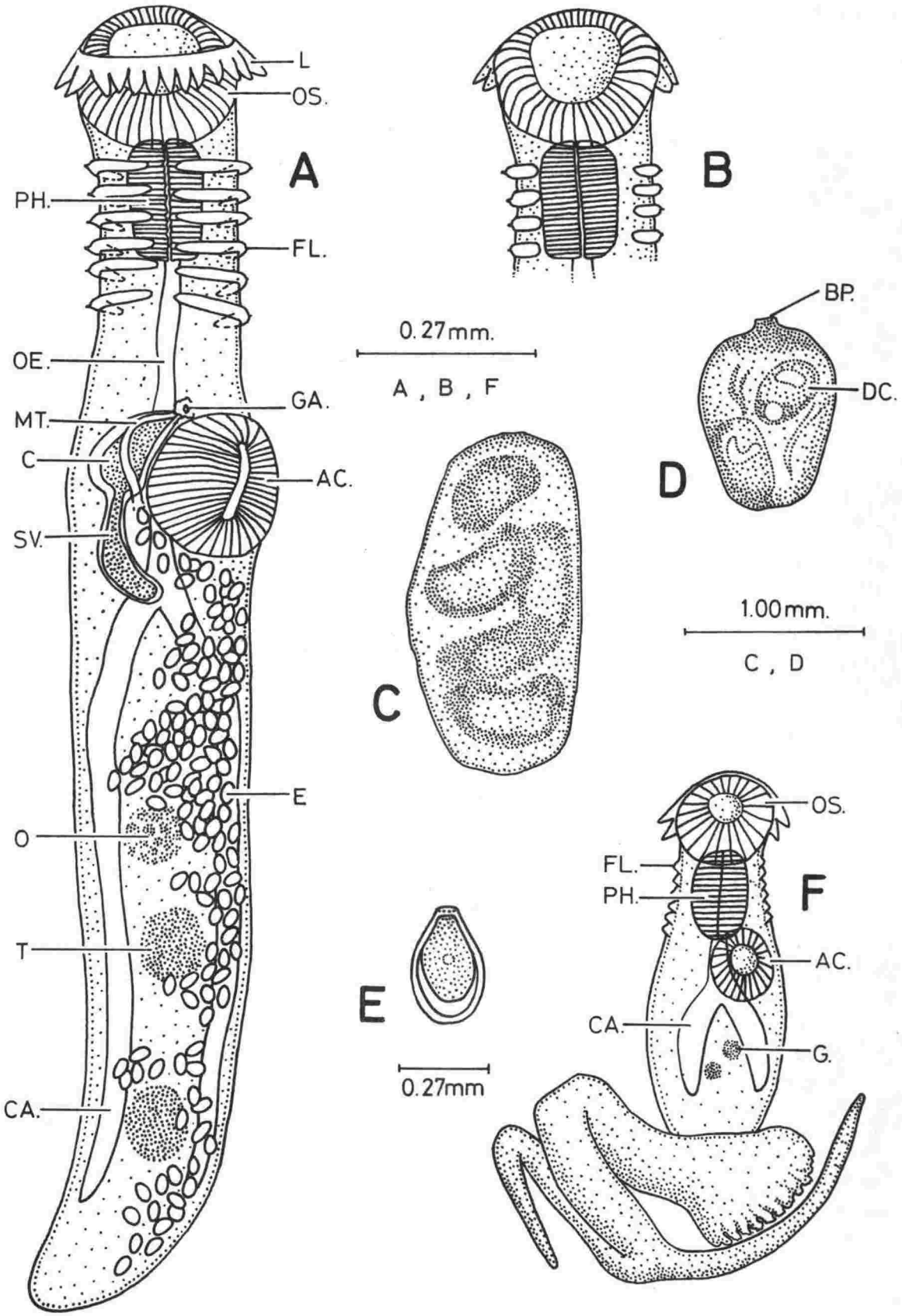
The life cycle of I. agnostomi has not previously been studied. Due to the behaviour of the cercaria, and the large number of immature I. agnostomi in A. forsteri it

FIGURE 31.

(a) gravid trematode
(b) reverse side of oral sucker region
(c), (d) sporocysts
(e) egg
(f) cercaria

List of abbreviations

AC., acetabulum; BP., birth pore; C., cirrus sack; CA., intestinal caeca; DC., developing cercaria; E., egg; FL., flange on neck; G., genital anlagen; GA., genital atrium; L., lobes around oral sucker; MT., metraterm; O., ovary; OE., oesophagus; OS., oral sucker; PH., pharynx; Sv., seminal vescicle; T., testis.


L
OS.
A
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OE.
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C
AC.
SV.
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O
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CA.
B
0.27mm.
A , B , F
BP.
DC.
D
1.00mm.
C , D
C
E
0.27mm
OS.
FL.
PH.
F
AC.
CA.
G.

was hypothesised that the life cycle involved only one intermediate host (Linzey 1971), and that the definative host was A. forsteri.

(a) Results of experimental work

From the first group of 6 A. forsteri experimentally infected with C. haswelli (see section I, p.15), live immature Tergestia identical to those described by Manter (1954) were obtained.

One of the fish had no trematodes, the other two had three and twenty-seven I. agnostomi respectively. None of the control fish were infected. On repeating the experiment, all the fish were free from trematodes despite having eaten cercariae four weeks previously.

The failure of the second infection experiment to confirm the results of the first may have been due to contamination of the tank by the anthelmintic. Unfortunatly a shortage of large tanks at the time the second experiment was run meant that the dosing tank was also used as one of the experimental tanks. It is known that the drug is passed out with the faeces, and that the tank sediment may show high tin levels (Mitchum & Moore 1969). Aldrichetta forsteri has been seen taking food from the floor of the tanks.

It is also possible that the cercariae failed to develop in the smaller fish used in the second experiment. The fish used in the first experiment were all over 120mm (length to caudal fork), while those of the second experiment were between 84 and 93mm (L.C.F.). At this size the fish would be about two years old (Manikiam 1963) (see table 6).

The absence of any trematodes from the fish in the second experiment at least shows that the anthelmintic and the filtration system were effective.

(b) Discussion of the life cycle:

The proposed life cycle of I. agnostomi is shown in Fig. 32. The sporocyst is found in the mantle and gonad tissue of Perna canaliculus or Mytilus edulis aoteanus. The cercariae are released from the sporocysts, which rupture the mantle of the host. The cercariae swim to the surface, and remain swimming there for up to 8 hours. It is while the cercariae are near the surface that they come into contact with the next host.

TABLE 6

Sizes of *Aldrichetta* which contain *Tergestia*

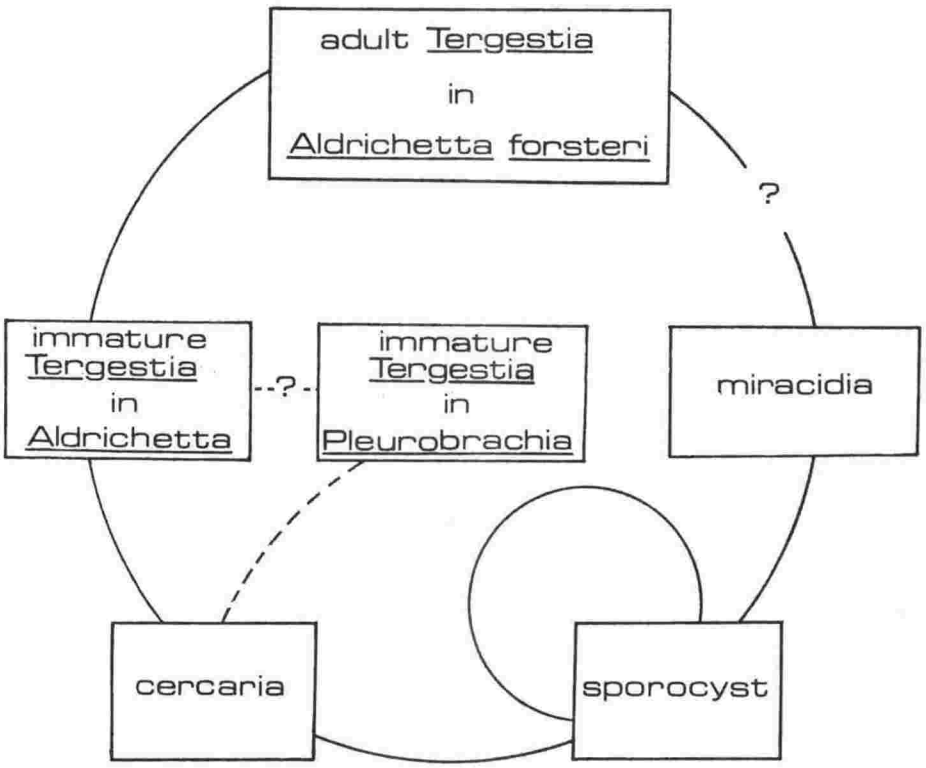
	total no. in sample	no. infected with *Tergestia*	no. fish over 100mm	infected fish: range in L.C.F.*	infected fish: mean L.C.F.
Wellington					
a. Days Bay	40	8	37	113mm to 188mm	161mm
b. Owhiro Bay	23	1	23		
c. Seatoun	3	1	2		
Lake Ellesmere	1	1	1	--	236mm

The 95% confidence interval for the size of the fish infected with *Tergestia* is 143.8 - 179.1mm (L.C.F.)

* L.C.F. = length to caudal fork.

FIGURE 32

Proposed life cycle for *Tergestia agnostomi* (? indicates that the relationship has not been verified).


adult Tergestia
in
Aldrichetta forsteri
?
immature
Tergestia
in
Aldrichetta
--?--
immature
Tergestia
in
Pleurobrachia
miracidia
cercaria
sporocyst

There is a record of un-encysted metacercarial stages of *T. agnostomi* occuring in the ctenophore *Pleurobrachia pileus* both in Wellington and in the North Taranaki Bight (Boyle 1965). Due to the low incidence of infection, Boyle suggested that the ctenophore was a fortuitous intermediate host rather than an inevitable host in the life cycle. From Boyle's drawings it seems that the "metacercariae" are tail-less cercariae, but if the cercariae (metacercariae ?) can exist for a time in a host such as *P. pileus* it could greatly increase its chances of being eaten by *A. forsteri*, which is known to eat ctenophores (Graham 1956).

Förch (pers. comm.) was unable to find any infected *Pleurobrachia* in plankton samples collected from Wellington Harbour during 1974. This suggests that infection by this route is unlikely.

The cercariae have been shown to be capable of infecting the mullet *A. forsteri* in the laboratory (see above) and gravid *T. agnostomi* are known to occur naturally in this fish, so *T. agnostomi* probably does not have a metacercarial stage.

Linzey (1971) drew a miracidium found on the gills of *P. canaliculus*, and which she described as the miracidium of *Cercaria haswelli*. However, she had no way of knowing where the miracidium had come from or what it would develop into. *Perna canaliculus* is known to be host to at least one other sporocyst (see p. 23) so until a miracidium can be hatched from an egg known to come from *T. agnostomi*, the status of Linzey's miracidium must remain in doubt.

There is no other life-cycle of a *Tergestia* known with which the life-cycle of *T. agnostomi* can be compared. The only marine fellodistomid whose life-cycle has been described is *Proctoeces maculatus* Odhner 1911. This trematode develops a tail-less cercaria from sporocysts in *Mytilus edulis* Linnaeus 1758. The cercariae develop as progenic metacercariae on the mantle of the host shellfish. The definative host is a fish which eats the shellfish (Stunkard & Uzmann 1953).

Other trematodes are known to have life-cycles in which the cercariae develop directly within the definative host. The best known are the schistosomes, and members of the Sanguinicolidae. The Spirorchiidae also have this type of life history.

Spirorchis parvus lives in the arterioles in the wall of the gut of a turtle. The miracidia develop in a snail giving rise to sporocysts, and cercariae which swim about until they contact a turtle, when they penetrate the soft tissue and migrate to the gut arterioles (Wall 1941).

La Rue (1957) included the Fellodistomatidae in the order Strigeatoidea, the families of which include the Schistosomatidae and Spirorchiidae. If the life cycle of Tergestia does contain only one intermediate host, then it will provide furthur evidence that these families are related.

Occurence:

Sporocysts of I. agnostomi probably occur wherever P. canaliculus or M. edulis are found. Infected mussels have been recovered from Ahipara, Auckland, Wellington, Tasman Bay, the Marlborough Sounds, and Christchurch.

Linzey (1971) found that the parasite density of C. haswelli differed between mussel populations inhabiting different environments, and this she attributed to variations in the duration of host exposure to the miracidia.

The percentage of mussels infected in Wellington Harbour is about 4%, but in the Marlborough Sounds the incidence may be much higher. Steed (1971) found that approximatly 15-20% of the mussels dredged in Pelorous Sound contained C. haswelli. In 1973, 33% of the mussels in Nydia Bay were found to be infected (Mead, pers. comm.). Linzey (1971) reported that between 1 and 16% of the mussels at Scarborough, near Christchurch, were infected but Allison (pers. comm.) has found that the percentage can be much higher than this.

In Wellington there was little difference in the abundance of the parasite over the year; until the marked increase in December 1973 and January 1974 (Fig. 33b). Three year old Aldrichetta forsteri are known to swim into shallow water between December and March, during their spawning season (Manikiam 1963), thereby increasing the exposure of the host mussel to the miracidia over this period. It is also possible that the increasing water temperatures are causing an increase in egg laying, by the adult trematode, during spring and summer.

Linzey (1971) reported a similar peak in the incidence of infection of the Christchurch mussels during the spring and autumn.

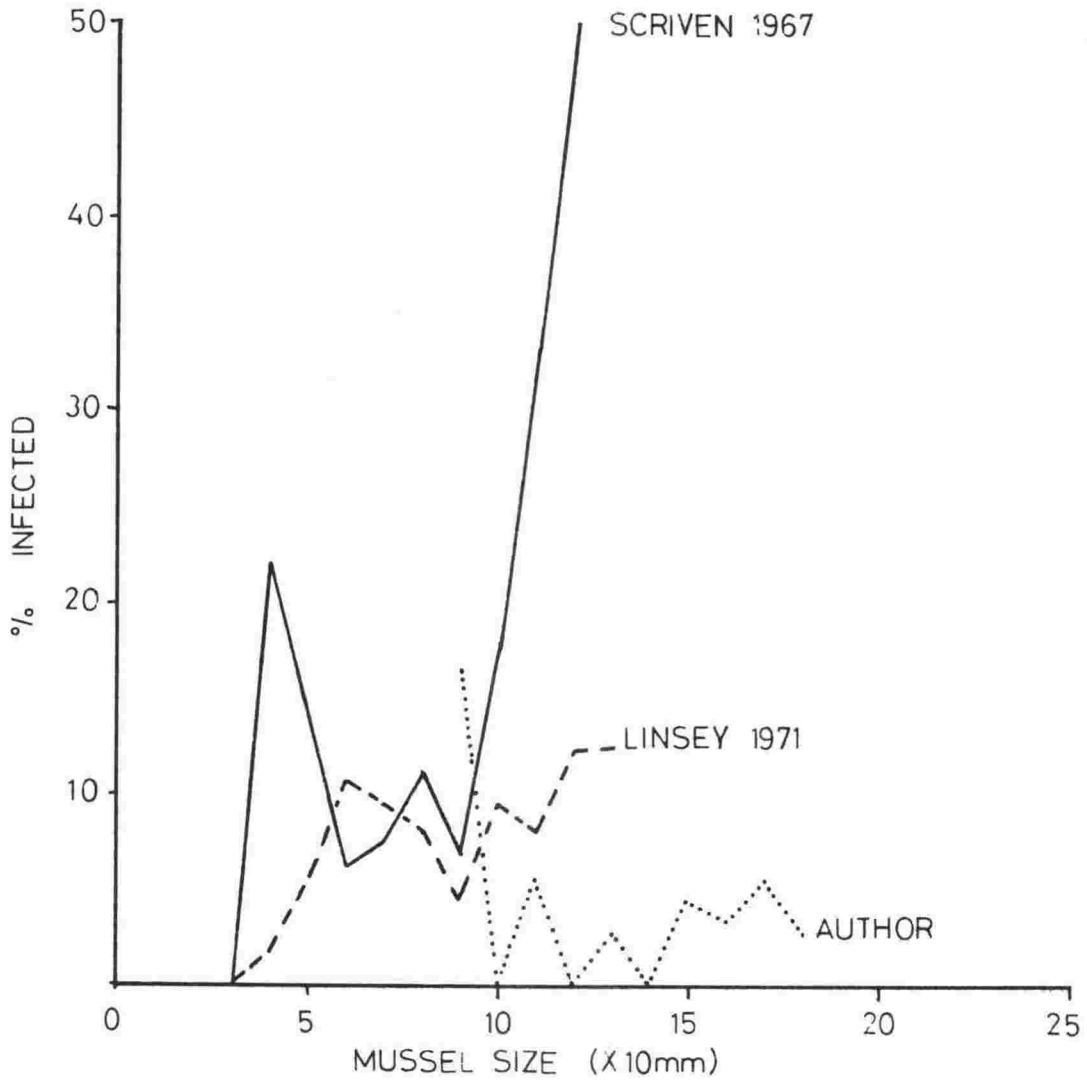
FIGURE 33.

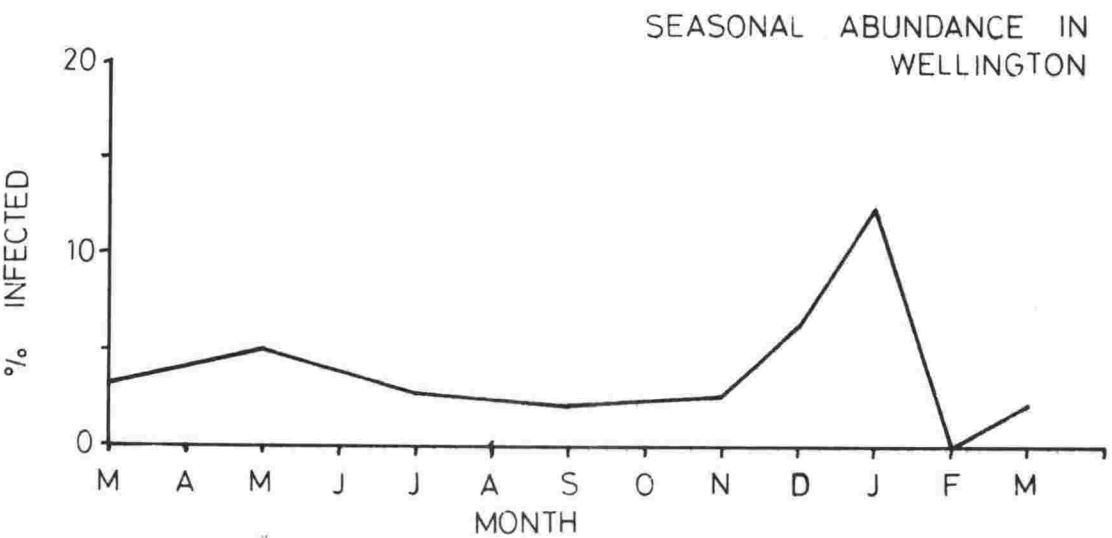
Tergestia agnostomi Manter 1954

(a) Percentage infection related to size of host. (Scriven and Linzey obtained their mussels from Canterbury, authors samples came from Wellington).

(b) Percentage of *P. canaliculus* infected over a 13 month period.

CERCARIA HASWELLI





Physiology:

(Results of experimental work on Cercaria haswelli)

Cercaria haswelli can move by swimming vertically upwards with its tail; sinking with its tail held vertically over the body and the furcae spread out; or by crawling along the substrate using muscular contraction, if the tail is lost.

The cercariae swim to the surface of the water and there alternate between vigorous swimming and relaxed sinking. When timing the swimming and sinking against the graduations on the measuring cylinder it was found that cercariae swim upwards twice as fast as they sink. Average times at a temperature of 18°C were 8 seconds to sink 20mm, 4 seconds to rise 20mm, and 2 seconds stationary just below the meniscus.

Cercaria show no response to light. Their life-expectancy is about 36 hours at 20°C but only in the first 8-10 hours do they exhibit continuous swimming and sinking movements, after this period they remain on or near the substrate.

These results confirm those previously obtained by Linzey (1971).

The cercariae appear to prefer a temperature of around 20°C. This temperature is reached around Wellington and the sheltered harbours of the South Island only on a few days in the height of summer (Skerman 1958). This preference may be linked with the behaviour of the host fish (A. forsteri), or it may be simply the optimum temperature for swimming. Bevelander (1933) working with the cercariae of Bucephalus elegans found that the frequency of contraction of the tail was at a maximum at 28°C.

Pathology:

Sporocysts of T. agnostomi cause mechanical damage and displace the potential gonad tissue of the host (Linzey 1971), and the absence of infected female mussels suggests that the parasite prevents ova formation. Linzey (1971) suggested that this was achieved by reducing the available lipid below that required for ova formation, and she also suggested that the red colour of the sporocyst was derived from the orange ova pigments. This would mean that the parasite was infecting only female mussels, and then by using the host lipids and pigments, the parasite was causing

a sex change to occur in the host. There is no evidence from this survey with which to support or refute this hypothesis, but the restriction that this would impose on on the parasite (the miracidia only being able to develop in a female mussel) suggests that this method of development is unlikely. While both sterile and male mussels have been found with the infection, no mussel has been found with any trace of ova. It is more likely that both host sexes are susceptable to infection, and that once infected the reduction in lipid levels cause the host to produce the "less expensive" sperm, or in severe cases to prevent any gonad development.

Linzey (1971) found a strong correlation between increasing mussel size and the number of mussels infected, thus indicating that the infection does not kill the mussel. This is not supported by the evidence from the Wellington mussels, which were of a larger size than those available to Linzey (Fig.33a). Mussels below 100mm in length were examined by the author, but the sample numbers were too low to show meaningful percentages. The smallest Wellington mussel found which was infected with C. haswelli was 49.7mm.

The low percentages of infection shown by the mussels over 120mm in length indicate that the trematode sporocysts are lethal to the host, and that the miracidia will develop in any size of shellfish.

Commercially harvested mussels which are found to be infected with the sporocysts of T. agnostomi are thrown away. This means a direct loss to the processor, although the numbers of mussels infected are usually too low in number to cause any significant reduction in profits.

Lichomolgus uncus n. sp.

(Order Cyclopoida; superfamily Lichomolgoidea; family Li^chomolgidae)

In their review of the Lichomolgidae, Humes & Stock (1973) list seventeen species belonging to the genus Lichomolgus. These are, with one exception (L. minor Scott 1902), associated with either molluscs or ascidians. Lichomolgus minor was found in a plankton sample.

The males and females of L. uncus occur on or inside the gills of Perna canaliculus.

Shellfish host:

Perna canaliculus (Gmelin 1791)

Description of stage in mollusc:

See description given in section II, p. 32

Life cycle:

This is at present unknown. Female copepods bearing eggstrings were found from August 1973 to January 1974, and copepodids were found in November 1973.

Occurence in Wellington Harbour:

The occurence of the copepod in samples of P. canaliculus from Wellington Harbour was monitored over a one year period (March 1973 to March 1974). The results are recorded in table 7.

Except in November, the percentage of shellfish with copepods was low. The copepods appear to begin breeding during August, numbers reaching a peak in the middle of September, followed by a larger peak in November. Numbers then drop off rapidly to winter levels.

The female copepods lose their eggstrings very easily, making if difficult to count accuratly those with eggs, so no attempt was made to determine the percentage of females carrying eggs in each sample. It was noticed, however, that most of the females from September and October carried eggs, but in November less than half of the females were gravid.

The maximum number of lichomolgids in one shellfish increased from 5 in August 1973 to 13 in September, then

TABLE 7

Monthly occurence of L. uncus in Wellington

(+ = females carrying eggstrings)

MONTH	No. SHELLFISH EXAMINED	No. SHELLFISH WITH LICHOMOLGUS	% SHELLFISH WITH LICHOMOLGUS
Mar 1973	32	1	3.1
Apr	23	1	4.3
May	NO SAMPLE		
Jun	22	0	0
Jul	30	0	0
Aug	43	3+	7.0
Sep	50	7+	14.0
Oct	43	1+	2.3
Nov	50	16+	32.0
Dec	50	2+	4.0
Jan 1974	41	1+	2.4
Feb	50	2	4.0
Mar	50	0	0

dropped to 1 in October, rose to 8 in December, and finally dropped to fluctuate between 1 and 2 for the other months.

Males were present in large numbers in September (10 recovered) and in November (17 recovered), otherwise the numbers fluctuated between 1 and 0. No males were recovered during March to July inclusive.

Occurence in Marlborough Sounds:

Fewer samples were collected from the Sounds, and these were from different areas. The results are recorded in Table 8.

Four gravid females and one male were recovered in December 1973 from Crail Bay. In March 1974 one male and two females, one of which was gravid, were recovered from the same area. Either the copepod is confined to Crail Bay, which is unlikely, or it is rare in the Sounds. The Cook Strait region appears to be the southern limit of Nematopsis and of Pseudomyicola spinosus, so L. uncus may also be absent from the colder southern regions.

Occurence at Ahipara:

Live samples of intertidal P. canaliculus collected in July and December 1973, and June 1974, were examined for L. uncus (see table 1, p.7, for sample size). No L. uncus were recovered.

Other information:

There are no published studies on the seasonal abundance of any copepod species from Wellington or the Marlborough Sounds with which the abundance of L. uncus may be compared. The planktonic copepod abundance in Wellington Harbour was recorded by Wear (1965), who found copepods abundant in June, July, and November.

Lichomolgus uncus occurs on the gills of P. canaliculus and also inside the gill water passages, where the copepods exhibit little movement unless disturbed, when they move quickly away. The females lose their eggstrings very quickly after being disturbed. The presence of the pea-crab Pinnotheres novaezelandiae does not appear to affect the numbers of copepods present, gravid female copepods having been found in mussels containing large pea-crabs.

Despite a careful seardh, no early copepodid stages

TABLE 8

Occurence of L. uncus in Marlborough Sounds

(+ = females carrying eggstrings)

SAMPLE SITE	DATE	No. SHELLFISH EXAMINED	No. SHELLFISH WITH LICHOMOLGUS	% SHELLFISH WITH LICHOMOLGUS
Pelorous Sound	Mar 1973	14	0	-
North West Bay	Jun	22	0	-
North West Bay	Aug	24	0	-
Keneperu Sound	Sep	42	0	-
Crail Bay	Dec	48	5+	10.4
Crail Bay	Mar 1974	46	2+	4.3

were found, although two late instar copepodids were found in a mussel in November 1973.

Lichomolgus leptodermatus which is very similar to L. uncus, was described by Gooding (1957) from the gills of Laevicardium, where it occurs in cysts or small swellings of the gill tissues. Gooding found that L. leptodermatus showed little movement until it was released from the gill cyst, and that the females shed their eggstrings soon after removal from the host shellfish. Because he was unable to find any early copepodid stages, Gooding postulated that the infection of the shellfish took place in one of the last copepodid stages. It is probable that this is also true of L. uncus.

Lichomolgus uncus is known only from P. canaliculus, and does not occur in Mytilus edulis aoteanus, Pecten novaezelandiae novaezelandiae, or Ostrea lutaria, all of which occur in Wellington, and have been examined for copepods (sample sizes 50, 16, and 28 respectively).

Pathology:

The nature of the association between L. uncus and P. canaliculus in unknown. There is no observable effect on the mussel due to the presence of the copepod.

Pseudomyicola spinosus (Raffaele & Monticelli 1885)

(order Cyclopoida; family Myicolidae)

The family name Pseudomyicolidae, which appeared in Humes & Cressey 1958, and which was subsequently used by Cheng 1967, is a lapsus and should be ignored (Humes 1968).

This is an extremely widespread species, having been recorded from : the Atlantic coast of France, the Mediterranean, the Adriatic, the Black sea, the Atlantic coast of the United States, the Caribbean, Brazil, Senegal, Madagascar, and New Zealand.

It is probable that the species has a world-wide distribution within the tropical and temperate zones, and since it is already known from over 40 hosts representing 12 families of Bivalva, it is probable that the copepod will eventually be recorded from almost all the bivalve shellfish in the areas in which it occurs.

Gordon (Pers. comm.) has collected detailed information on: the seasonal occurence, population changes, geographical distribution, and aspects of the life history including the nauplii and some copepodid stages which he has reared. This information was not available to me, and will probably not be published for several years. With his approval the work which I have done on Pseudomyicola spinosus is recorded here.

Shellfish hosts in New Zealand:

Family Mytilidae

Mytilus edulis aoteanus Powell 1958

Perna canaliculus (Gmelin 1791)

Family Pectinidae

Pecten novaezelandiae novaezelandiae Reeve 1853

Family Ostreidae

Crassostrea glomerata (Gould 1850)

Ostrea lutaria Hutton 1873

Family Veneridae

Chione stutchburyi Gray 1828

Description:

A detailed description of the species, and of the variation within the species, may be found in Humes (1968).

A brief description is given below of specimens obtained from Crassostrea glomerata found at Walkworth, near the Bay of Islands.

Female: (Fig. 34a) length 2.03 - 2.20mm (excluding caudal furcae). Greatest width 0.64 - 0.72mm, based on 10 specimens in alcohol. Ratio of prosome to urosome 1.91:1. Prosome and urosome are both five segmented.

First antenna is six-segmented, first segment bears a distinctive posteriorly directed medial spine in addition to the five setae. Second antenna is three segmented with a terminal claw. Setal formula for legs 1-4 is as follows: (Roman numerals indicate spines, arabic numeral setae).

P1	coxa	0-1	basis	1-I	exp.	I-0	I-1	IV 4
					end.	0-1	0-1	II 4
P2		0-1		1-I	exp.	I-0	I-1	IV 5
					end.	0-1	0-2	III 3
P3		0-1		1-I	exp.	I-0	I-1	IV 5
					end.	0-1	0-2	IV 2
P4		0-1		1-I	exp.	I-0	I-1	IV 5
					end.	0-1	0-2	IV 1

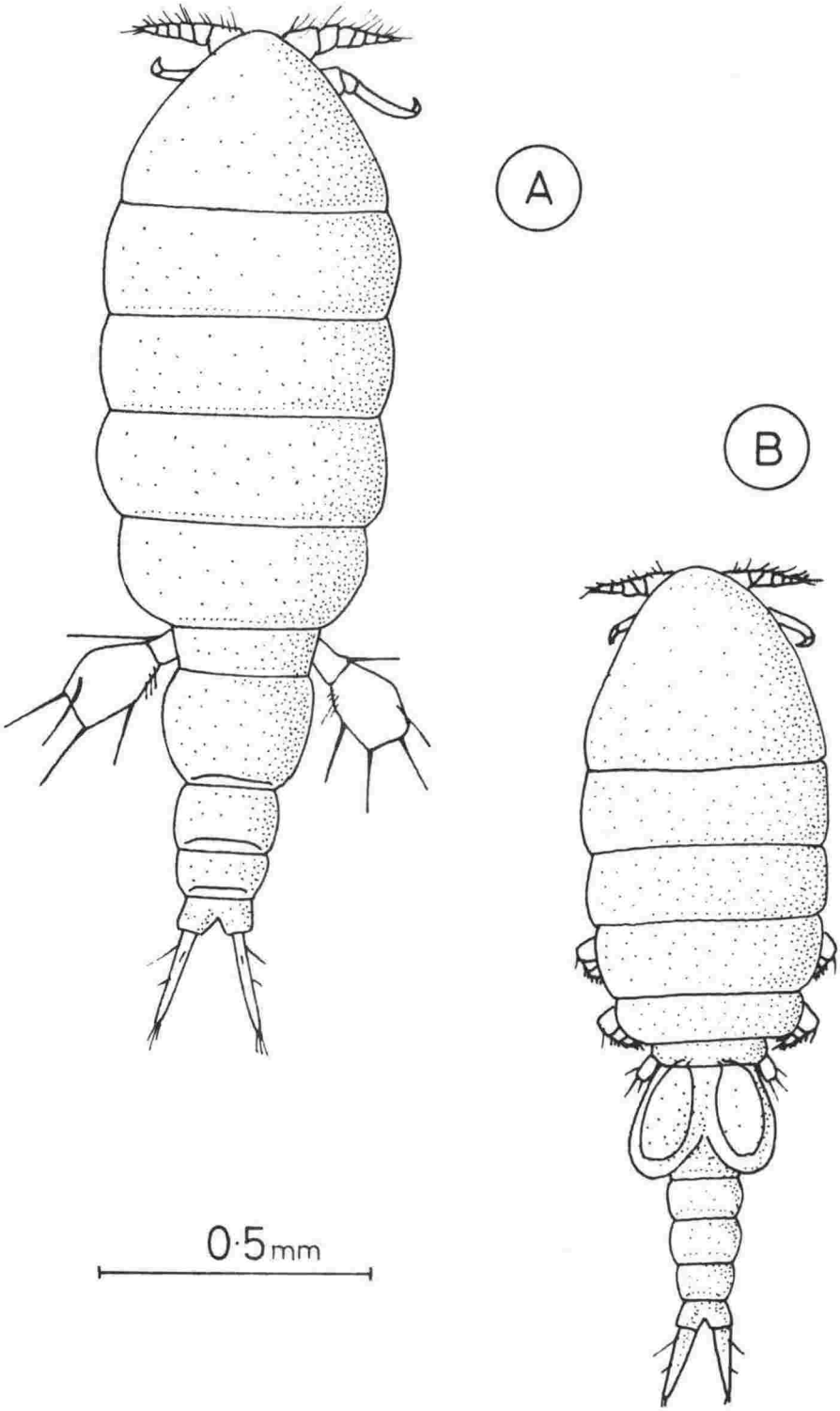
Leg 5 has a sub-cicular distal segment about 184.5 - 205 µm in diameter, and bears four naked setae and a row of small spinules.

Colour is opaque cream, gray or black with a red eyespot and orange eggsacks (The black colour is due to a hyperparasitic fungus or protozoan under the cuticle).

Male: (Figure 34b). Body resembles that of female, but is smaller and more slender. Length 1.55mm (excluding caudal furcae). Greatest width 0.51mm. Ratio of prosome length to urosome length 2.0:1. Prosome five segmented, urosome six segmented. Maxilliped of male bears a stout curved claw 153 µm in length.

Legs 1-4 have same setal formula as female. Fifth leg has a sub-rectangular free segment and bears three fringed spines and a naked seta.

Colour in life resembles female.


A
B
0·5mm

Life-cycle:

Unknown at present. There is evidence to suggest that an increase in breeding activity occurs between May and September. North of Cook Strait, female P. spinosus carrying eggstrings may be found throughout the year.

Occurence:

Pseudomyicola spinosus is known to occur around the coastline north of Auckland, and in Tasman Bay and the Marlborough Sounds. It has not been found below latitude 42°S.

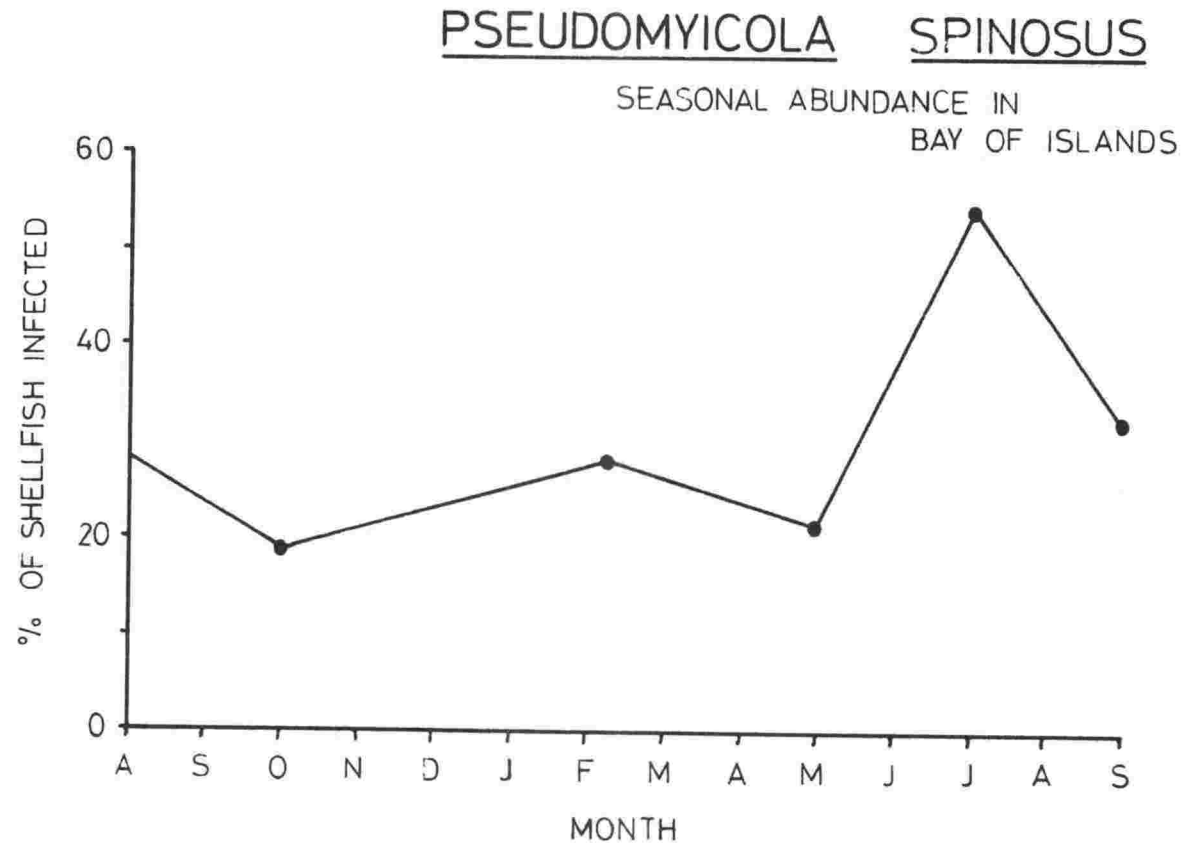
The copepod is found on the gills, between the labial palps, and in the oesophagus and stomach of all the host shellfish except P. canaliculus, where the copepod has not been found to occur in the stomach.

Seasonal occurence in the Bay of Islands: Samples of C. glomerata from the Bay of Islands were examined on six occasions between August 1973 and September 1974. The incidence of infection of this shellfish with P. spinosus is shown in figure 35a. A peak was observed in July 1974 when 54% of the shellfish were infected. Levels of infection can, however, be much higher than those recorded here (up to 100%, Gordon, pers. comm,).

Unfortunatly the oysters came from farm trays in Orongo Bay. The farm there obtains the oyster sticks from a number of locations, so that the origin of the host shellfish is not known. All the shellfish examined had been at the Orongo Bay farm for a number of months.

Copepodids first appeared in the samples in May, and were absent by August (Figure 35b). Over this period there was an increase in the number of male P. spinosus, and of females without eggstrings. The relative number of females with eggstrings dropped during the same period, suggesting that the eggs were hatching and producing the increasing number of copepodids.

Seasonal occurence in Wellington Harbour: Although Perna canaliculus was collected over a 12 month period (see table 1, p. 7), only four samples revealed the presence of P. spinosus (see table 9). No P. spinosus were found in P. canaliculus during the winter months, although the


PSEUDOMYICOLA SPINOSUS
SEASONAL ABUNDANCE IN
BAY OF ISLANDS
% OF SHELLFISH INFECTED
60
40
20
0
A S O N D J F M A M J J A S
MONTH

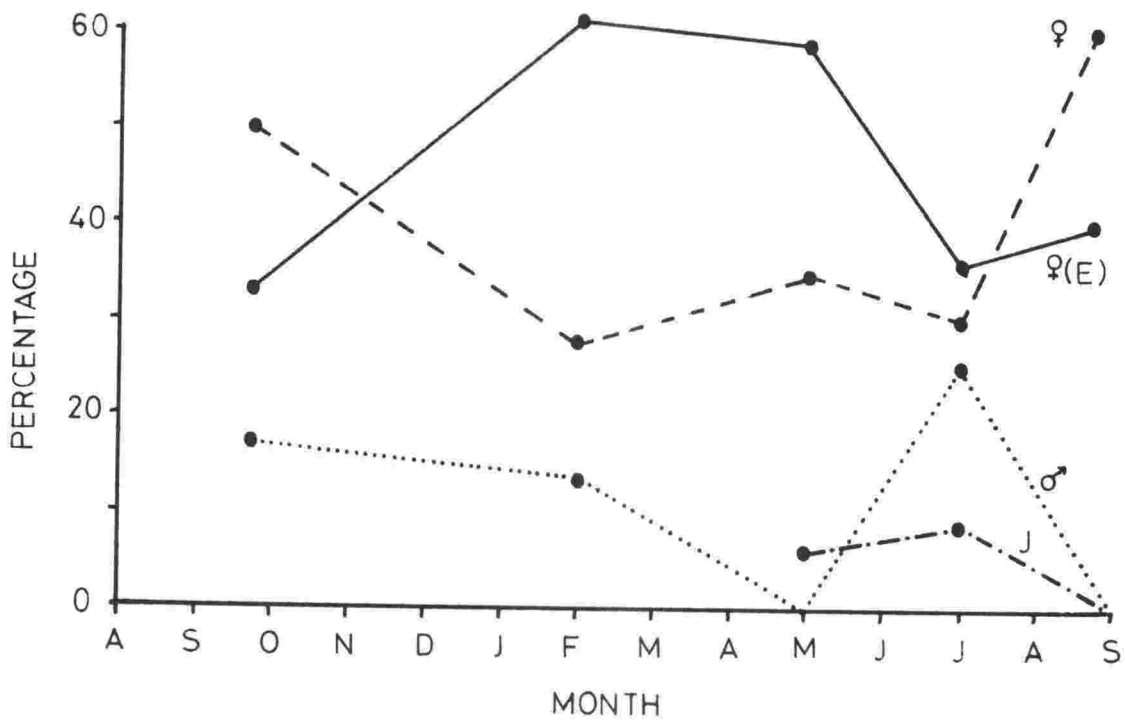

PERCENTAGE
60
40
20
0
♀
♀(E)
♂
J
A S O N D J F M A M J J A S
MONTH

TABLE 9

Pseudomyicola in *Perna canaliculus*

Date	% of sample infected	*Pseudomyicola spinosus* no. of males	no. of females
1973			
September	4.0	1	1
October	2.4	1	1
December	2.0	-	1
1974			
March	4.0	-	2

TABLE 10

Mean condition factors of *C. glomerata*

Month	Number of oysters		mean condition factor	
	I	U	I	U
Jul 1973	22	39	42.57	50.13
Dec 1973	8	29	41.15	46.18
Mar 1974	7	18	48.46	46.11
Totals	37	86	43.45	47.96

I = infected oysters

U = uninfected oysters

copepod does occur in other shellfish species during this time. In May 1975, 12 Ostrea lutaria from Wellington were opened. Ten of the oysters were found to contain P. spinosus: a total of six males, four gravid females, 21 other females and two copepodids. In July 1973, three Pecten novaezelandiae novaezelandiae were opened and two were found to contain one female P. spinosus each.

It is likely that P. canaliculus is not a favoured host, and that P. spinosus only inhabits the mussel during the summer (a period of high population density), or in areas where preferred hosts are not available. The copepod seems to occur throughout the year in O. lutaria, both in Wellington and in the Marlborough Sounds, where three of the four oysters opened in August 1973 contained P. spinosus.

The European copepod parasite Mytilicola intestinalis Steuer 1902 demonstrates a preference for M. edulis, but if this mussel is not available it will parasitize O. edulis (Cheng 1967, p.289). This is similar to the observed behaviour of P. spinosus.

Occurence in Foveaux Strait: None of the oysters from Foveaux Strait were found to contain P. spinosus.

Physiology:

Although P. spinosus is common in O. lutaria from Wellington it is absent from Foveaux Strait. Four female P. spinosus obtained from C. glomerata collected in the Bay of Islands, were placed in a fingerbowl containing a fresh oyster (O. lutaria) from Foveaux Strait. Three days later, two P. spinosus were still in the fingerbowl, and upon dissection the other two were found to be in the oyster, one on the mantle and the other in the stomach.

This demonstrates that P. spinosus will invade the Foveaux Strait oyster. Whether the absence of the copepod from Foveaux Strait is due to environmental factors, or whether it has not yet been introduced, remains to be determined.

When disturbed, the copepods on the gills and labial palps retreat into the stomach of the shellfish (Dinamani & Gordon 1974)

Pathology:

Attempts to measure the condition factors of the rock oysters were not very successful. The volumetric method used proved unsuitable for the small gnarled rock oysters, and the error in some cases approached 10%.

The mean condition factor of oysters infected with P. spinosus was usually less than for uninfected oysters over the three months measured (table 10). This difference, when tested using an analysis of variance, was not found to be significant either for individual months, or over the whole three months. A more accurate method for determining the condition of the oysters may well alter these results.

Dinamani & Gordon (1974) found that the copepod was causing damage to the epithelium of the gut of C. glomerata. This damage was similar to the mechanical damage and metaplastic changes recorded by Sparks (1962) in the gut of the Pacific oyster Crassostrea gigas parasitised by Mytilicola orientalis Mori 1935.

Odlaug (1946) demonstrated that O. lurida Solander infected with M. orientalis, had a lower condition factor than the uninfected oysters. Cole & Savage (951) working with British Mytilus edulis infected with M. intestinalis Steuer 1902 found a serious reduction in the condition of the infected mussels, confirming that Mytilicola can have deleterious effects on the host shellfish.

Though Bernard (1969) found no reduction in condition or metaplastic changes in the oyster C.gigas infected with M. orientalis in British Columbia, it is generally accepted that Mytilicola does affect the condition of the host, and may be capable of inhibiting the production of neurosecretory material by the host (Williams 1969).

Pseudomyicola spinosus has a similar habitat to Mytilicola (but in the stomach, not the intestine) and causes a similar damage to the shellfish. It is therefore reasonable to suppose that the copepod will also affect the condition of the shellfish host, although no evidence for this is presently available.

THE BIOLOGY OF *PINNOTHERES NOVAEZELANDIAE* FILHOL (BRACHYURA:PINNOTHERIDAE) IN WELLINGTON HARBOUR, NEW ZEALAND

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ABSTRACT

The development stages of post-planktonic P. novaezelandiae were found to be similar to those described for other pinnotherid crabs. The crabs were found to breed throughout the year, the female crabs living for three to four years in the host shellfish Perna canaliculus (Gmelin). Shellfish below the low tide level were most heavily infected with pea-crabs. Damage to the host caused by the crab is described. The crabs were found to adversly affect the condition factor of inhabited shellfish. There is some evidence that there were more crabs in male mussels than female mussels.

INTRODUCTION

There are at present two recognised species of pinnotherid crab in New Zealand: P. schauinslandi Lenz, which has not been found since its original description, and P. novaezelandiae Filhol. Although Scott (1961) stated that P. novaezelandiae was the only pea-crab in New Zealand, Wear (1965) recorded two species of pinnotherid zoea in plankton samples from Wellington Harbour, and studies by the author have shown that zoea hatched by pea-crabs from Atrina zelandi a Gray are different from the zoea of crabs from P. canaliculus

Pea-crabs commonly occur in three species of bivalve in New Zealand: Mytilus edulis aoteanus Powell; Perna canaliculus; and Atrina zelandica.

The present paper deals with the biology of P. novaezelandiae from the green mussel P. canaliculus in Wellington Harbour (41° 16'S, 174° 51'E).

MATERIALS & METHODS

Each month, from March 1973 to March 1974 (except May 1973), a sample of between 25 and 50 Perna canaliculus was collected from Wellington Harbour. Samples were dredged from a depth of 4m using an Agassiz trawl, or were collected from the same depth by skin diving.

Every mussel in a sample was measured, opened by severing the adductor muscle, and examined under a sterio-microscope, after first removing any visible crabs. A measure of the condition factor of the mussel was obtained using the formula:

$$\frac{\text{meat volume}}{\text{internal volume}} \times \frac{100}{1} = \text{C.F.}$$

Volumes were measured using the method described by Anderson (1975).

The carapace width of each crab was measured using a vernier calipers, or under a sterio-microscope fitted with a calibrated occular micrometer. The post-megalopal development stage of each crab was determined using the table prepared by Christensen & McDermott (1958) for Pinnotheres ostreum Say. Mature female crabs were classifed according to the stage of development of the eggs under the abdomen, using the scale shown in table 2 (section I, page 13).

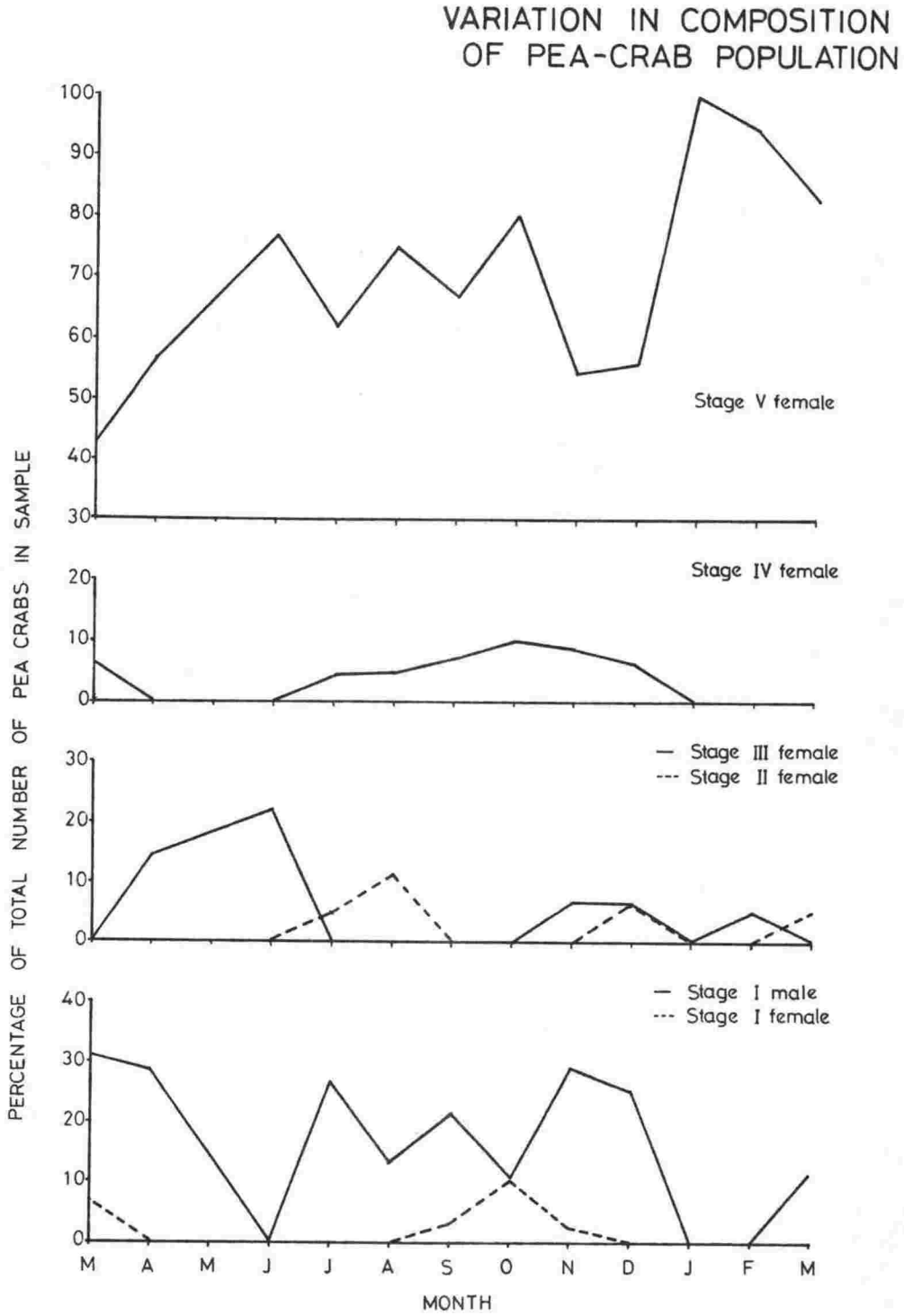
Post-planktonic development

The development of P. novaezelandiae is similar to that described for P. pisum Linnaeus (Atkins 1926); P. ostreum Say (Stauber 1945; Christensen & McDermott 1958); and Fabia subquadrata Dana (Pearce 1966)

The planktonic and megalopal stages are followed by a series of crab instars, one of the first of which is the invasive stage. Upon entering a host, this stage is believed to moult into the first of a series of pre-hard stages, with a soft membranous exoskeleton. This series is followed by the stage I instar, with a hard chitinous exoskeleton. The male and female are indistinguishable except for the number and structure of the pleopods. Male crabs range in size from 3.4 - 11.3mm (carapace width). They may continue to moult and grow, but do not moult beyond the stage I form.

Soft shell stage I males such as those described by Atkins (1958) for P. pisum, have not been observed for P. novaezelandiae.

The hard shell stage I male is believed to copulate with the immature stage I female, which has a size range of 1.3 - 4.8mm (carapace width). The spermatheca of female P. novaezelandiae were not examined for sperm, so the stage at which the female is fertilised is not known. There is no reason to believe that in this matter P. novaezelandiae differs from the other pinnotherids which have been studied.


VARIATION IN COMPOSITION
OF PEA-CRAB POPULATION
PERCENTAGE OF TOTAL NUMBER OF PEA CRABS IN SAMPLE
100
90
80
70
60
50
40
30
Stage V female
20
10
0
Stage IV female
30
20
10
0
— Stage III female
--- Stage II female
40
30
20
10
0
— Stage I male
--- Stage I female
M A M J J A S O N D J F M
MONTH

The stage I female moults into a soft membranous stage II crab, then through stages III to V, at which stage the female becomes sexually mature. Subsequent moults result largely in an increase in size, there being little morphological change. The largest stage V crab caught had a carapace width of 19.2mm.

The life cycle.

The only previous studies of the complete post-planktonic life cycles of pinnotherid crabs are those made by Christensen & McDermott (1958) for P. ostreum, and by Pearce (1966) for Fabia subquadrata. The reproductive cycle for P. pisum was recorded by Christensen (1958, 1962), and for P. maculatus Say by Pearce (1964). All of these Northern Hemisphere species have a distinct spawning season, when ovigerous stage V females occur. For the three Pinnotheres species this is during the northern summer, but for F. subquadrata it is in the northern winter.

The pattern for P. novaezelandiae is not so clear. Ovigerous females may be found throughout the year, although they form a higher percentage of the pea-crab population in January and February than in the other months (Fig. 36)

When the egg development condition of the stage V females is plotted (Fig.37c) it is apparent that ovigerous females, with eye-spots on the eggs, are predominant from August to March. Non-ovigerous females, or those with few eggs, are most common in June and July, dropping to a low in January, although they occur all year round.

It takes about 15 to 20 days in the laboratory to develop from non-ovigerous to ovigerous (stage 3) female, and the high incidence of non-ovigerous females in August to March is almost certainly due to the increase in the number of spent females. Wear (1965) recorded Pinnotheres zoea in the plankton of Wellington Harbour over the period August to March, the zoea being most common in November.

The percentage of pea-crabs at the pre-hard stage through to stage V are shown in figure 36 . As ovigerous females occur throughout the year it is likely that the developmental stages will also occur throughout the year. The scarcity of immature stages suggests that the female passes through the moults rapidly, and probably reaches sexual maturity within one year, as Atkins (1926) suggested for P. pisum.

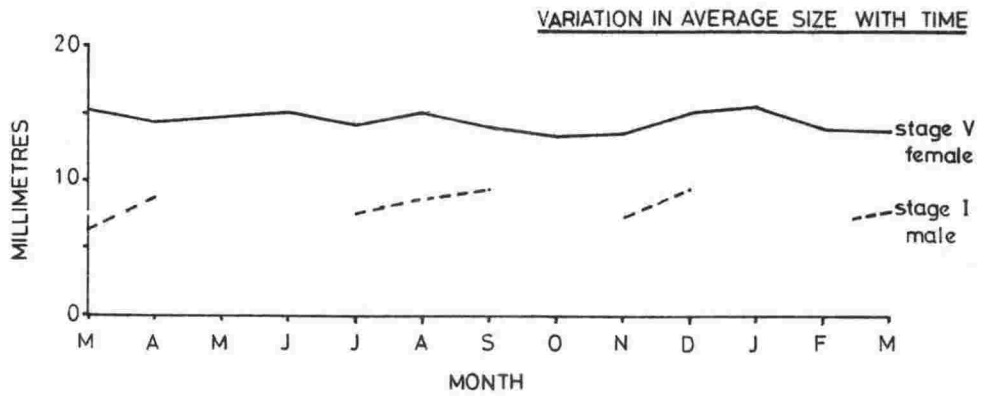
Males were obtained in each month sampled, except for June, October, January and February. It is probable that

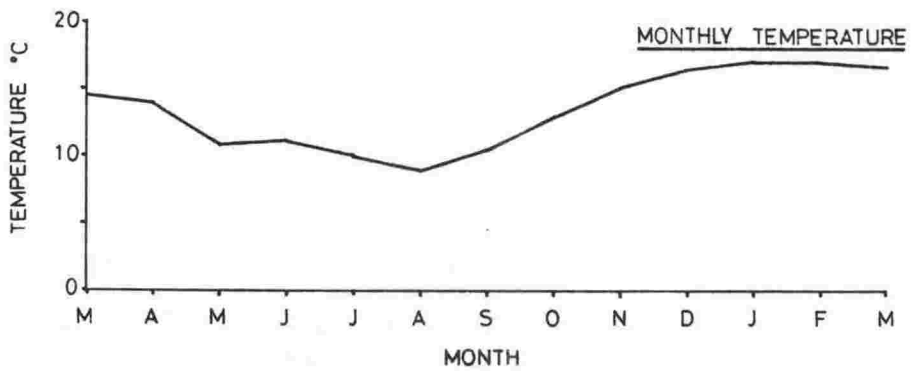
FIGURE 37

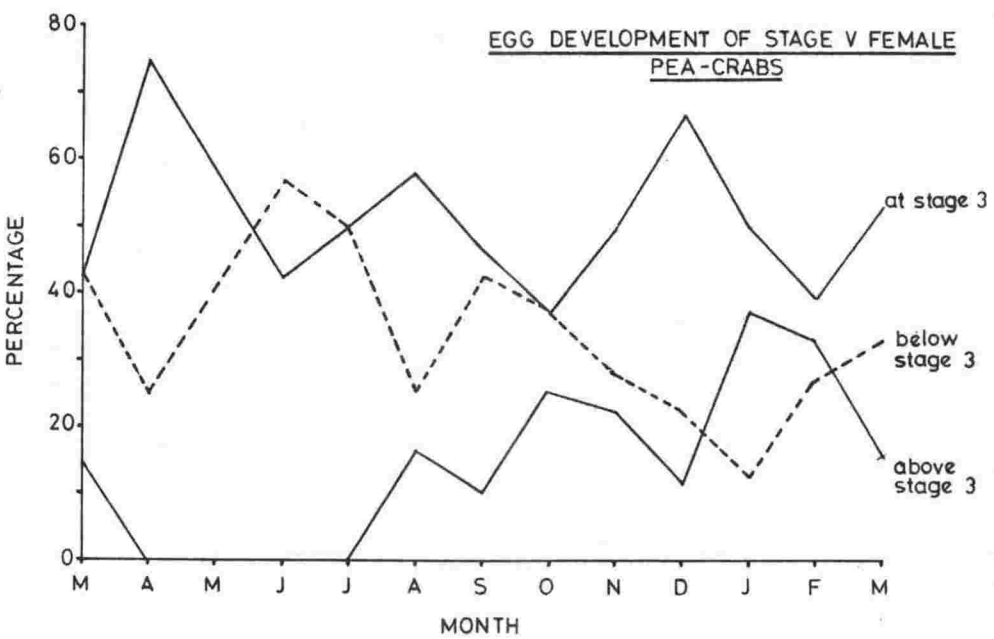
(a) Variation in the mean size of the mature pea-crabs over the 13 month period sampled.

(b) Surface water temperature in Wellington Harbour over the 13 month period sampled.

(c) The egg development of the stage V female pea-crabs, for each month sampled, expressed as a percentage of the total number of stage V females caught in that month.


VARIATION IN AVERAGE SIZE WITH TIME
MILLIMETRES
20
10
0
stage V female
stage I male
M A M J J A S O N D J F M
MONTH


MONTHLY TEMPERATURE
TEMPERATURE °C
20
10
0
M A M J J A S O N D J F M
MONTH


EGG DEVELOPMENT OF STAGE V FEMALE PEA-CRABS
PERCENTAGE
80
60
40
20
0
at stage 3
below stage 3
above stage 3
M A M J J A S O N D J F M
MONTH

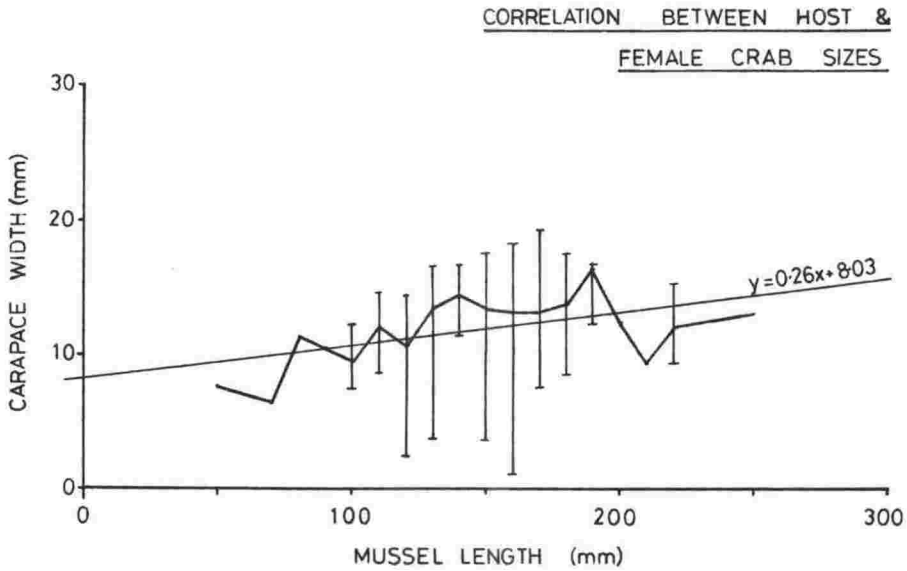
males occur throughout the year but that the percentage of mussels infected in these months was too low to show up in the mussel samples examined. The sizes of the male crabs shew no sign of seasonal peaks. (Fig. 38b).

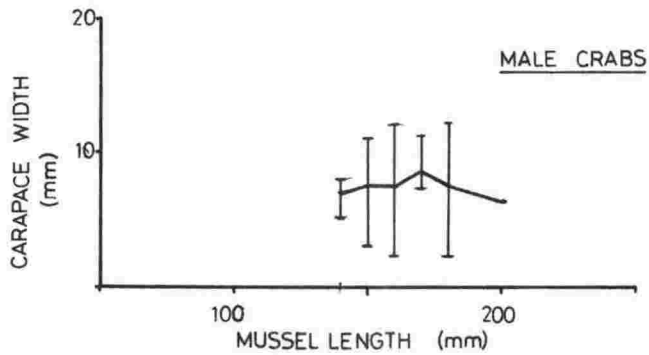
The large size of some of the male P. novaezelandiae (up to 11.3mm carapace width) suggests that several moults must have taken place while the crab was in the stage I form. Three males, kept in the laboratory, lived for 41 days before they died, one moulting on the twelfth day but remaining in the hard shell stage I form. The crabs were not seen to feed during the 41 days.

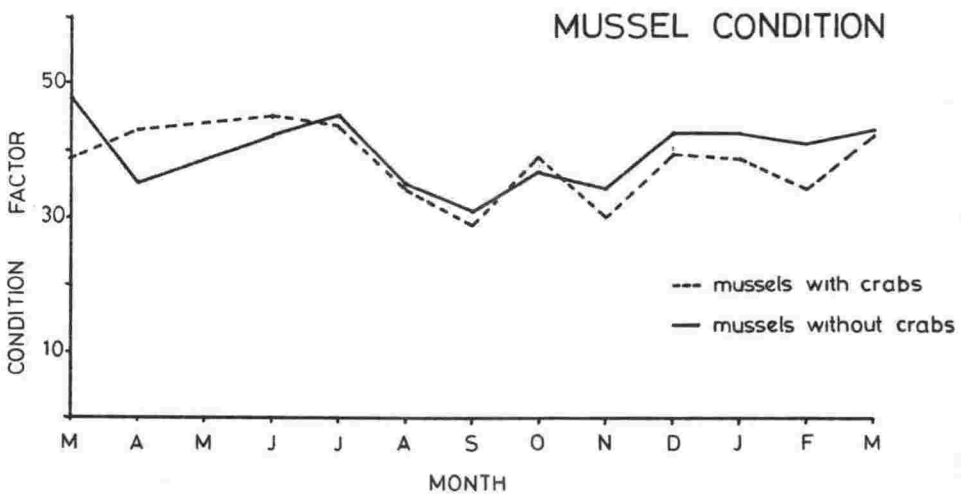
Copulatory swarming of the males and females of P. novaezelandiae such as was described by Pearce (1964, 1966) for P. maculatus and F. subquadrata, is not known to occur. Mating behaviour in P. novaezelandiae is probably similar to P. pisum and P. ostreum, the males of which seek out the female, which remains in the host.

On three occasions, male crabs were recovered crushed from the aquaria, presumably after attempting to enter mussels containing females. Stauber (1945) tested the ability of male P. ostreum to enter oysters, and found that about 13% were crushed in the process, but 70% of the males were successful in gaining entry.

Atkins (1955) found that for P. pisum one batch of sperm was sufficient for at least two batches of eggs, and this is also true for P. novaezelandiae since females kept in the laboratory have produced a second batch of viable eggs after successfully hatching a first batch.


CORRELATION BETWEEN HOST &
FEMALE CRAB SIZES
CARAPACE WIDTH (mm)
30
20
10
0
y=0·26x+8·03
0
100
200
300
MUSSEL LENGTH (mm)


MALE CRABS
CARAPACE WIDTH (mm)
20
10
100
200
MUSSEL LENGTH (mm)


MUSSEL CONDITION
CONDITION FACTOR
50
30
10
--- mussels with crabs
— mussels without crabs
M
A
M
J
J
A
S
O
N
D
J
F
M
MONTH

Growth and development correlated with host size:

There is a statistically significant trend for large female crabs to inhabit larger mussels (fig.38a), but this relationship does not apply to male P. novaezelandiae. The immature crabs are spread equally among the size classes of mussels. This suggests that the female crabs are surviving longer than one year, and are growing with the host.

Atkins (1926) and Houghton (1963) found a similar size correlation between P. pisum and its host. Wells (1940) and Pearce (1966) found the same for F. subquadrata. Christensen & McDermott (1958) established a similar relationship for P. ostreum in Crassostrea virginica (Gmelin), and while working with one year class of oyster, found that the factors which limit the individual host also directly affect the growth of the crab, but that the development of the crab was not retarded.

The absence of a correlation between the size of male P. pisum and the host length was attributed by Houghton (1963) to the male P. pisum dying after copulation. Atkins (1958) however, found that the male P. pisum moults a number of times and continues to grow after reaching stage I, although whether the life-expectancy of the male is affected by copulation is not known. While male crabs do disappear from the European host shellfish at certain times of the year, it has not been shown that they die.

The female crabs reach the largest mean size in mussels between 180 - 190mm, before the mean size of the crab drops off (Fig.38a). These mussels are 3 to 4 years old, so this is probably the age at which the female crabs die. The youngest stage V female crab was found in a 90mm mussel (which would be about 10 months old).

Multiple infestation of the host:

Very few of the mussels examined had more than one pea-crab. Of the nine cases of a multiple infestation, all were of two crabs. Only in one case were there two male crabs in a host, the other eight cases were mussels containing a male and a female crab. Two mussels each contained a stage I male and a stage II female, three mussels each had a stage I and a stage III female, and three mussels contained stage I males and stage V females.

Pearce (1966) found only three examples of multiple infestation by F. subquadrata of the mussel Modiolus modiolus Linnaeus. (sample size 2088 mussels). Pearce suggested that an infestation by two adult crabs would reduce the food gathering ability of the shellfish to a level below that required to sustain three organisms.

Stage V female P. novaezelandiae are extremely hostile towards one another, and in the laboratory, the stronger female will harass and eventually kill the weaker female. This is probably why females are not found together in the same host. This hostility does not occur between males and females.

Juvenile females of P. maculatus which enter a host with a mature female are retarded in development, and do not reach sexual maturity (Pearce, 1964)., Stauber (1945), and Christensen & McDermott (1958) found that multiple infestations of oysters, including spat, by immature P. ostreum were very common during certain periods of the life cycle. Hard shell males were found with stage II females, and Christensen & McDermott suggested that this was because the male remained in the oyster for a time after fertilising the stage I female. However, of the six stage II crabs they collected, only one had sperm in the spermatheca, so it is probable that the males also copulate with later stages. Berner (1952) stated that P. pisum copulates at stage V but this has never been experimentally verified. The occurence of stage I males with female crabs of stages I, II, III, and V suggests that males of P. novaezelandiae copulate with females at any stage of development beyond the pre-hard stage.

Effect of depth on P. novaezelandiae.

The depth of the host shellfish has a marked effect upon the pea-crabs. Shellfish on or above the low tide level are rarely infested with crabs, while a large number of crabs may be recovered from samples of mussels collected in deep water (see Table 11)

Houghton (1963) and Seed (1969) found that P. pisum was more abundant in the lower shore and sub-littoral zone mussels, than in those higher on the shore. The invasive stage of P. pisum is photonegative so Houghton (1963) suggested

TABLE 11

Showing percentage of mussels with pea-crabs at different depths in Wellington Harbour.

Area 1 (off Ward Island)

Depth (m)	Number of mussels	Mean size of mussels (mm)	Number of crabs	Percentage
2	37	165.74	6	16.21
5	37	161.82	26	70.27

Area 2 (off Seatoun Beach

2	35	105.03	5	14.28
-0.2	76	75.23	2	0.026

(above low tide level)

that the crab was unlikely to invade a host high on the shore.

Fenucci (1973) while studying P. maculatus in Mytilus platensis off Argentina in 40-50 metres of water, found that the percentage of mussels infected with crabs was not related to depth. This is evidence that the effect occurs only in the upper few metres of water, since the depth of the host is known to affect P. maculatus in M. edulis (Kruczynski, 1974) (0-20m depth), and in Argopecten irradians concentricus (Say) (Kruczynski, 1972) (0-1m depth).

Effect of pea-crab on host.

(a) Morphological effects.

That some members of the Pinnotheridae damage their hosts has been know for a number of years, and has often been cited in papers as a reason for classing Pinnotheres as a parasite. Dean (1892) noted that oysters infected with P. ostreum showed thickened outgrowths, or were malformed and stunted in their growth. Stauber (1945), and Christensen & McDermott (1958), noted injuries to the gills of Crassostrea virginica caused by P. ostreum. Stauber recorded two types of injury:

1. Small crab type characterised by local sharply delimited erosion of one or more demibranchs
2. Large crab type, characterised by an extensive shortening of one or more demibranchs reaching from the anterior end of the gills to a point usually ventral to the adductor muscle.

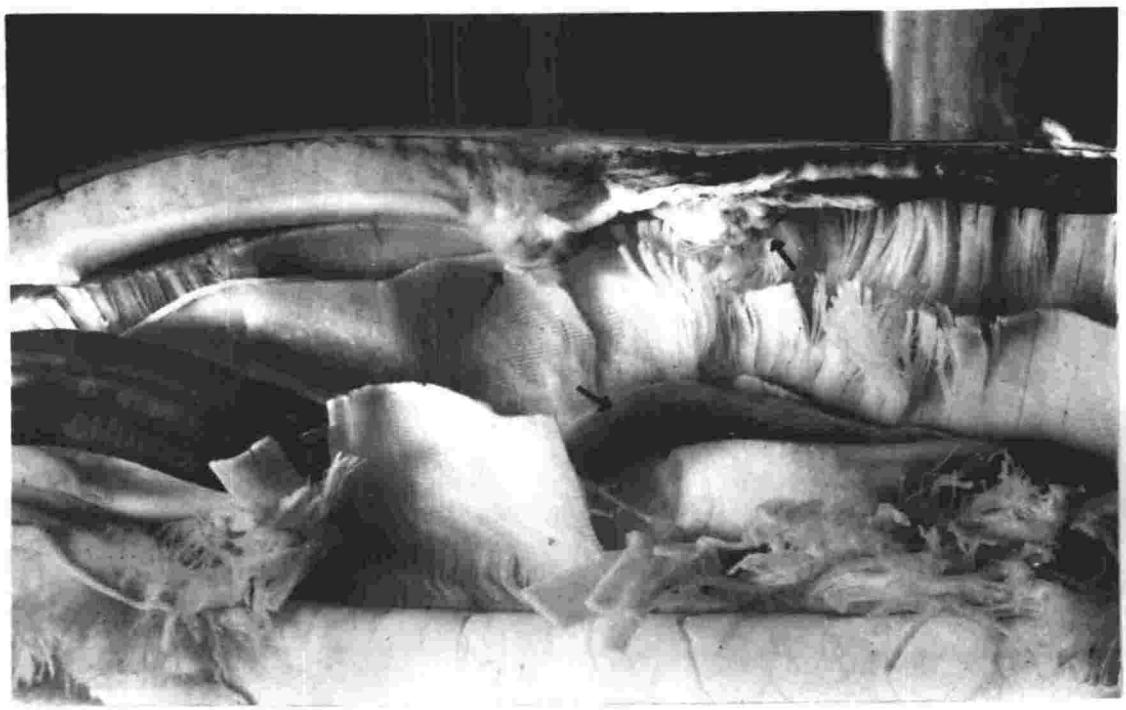
This damage is caused by the crabs while picking food strings off the gills with the chelipeds and legs (Orton, 1921; Stauber, 1945).

The erosion of the gill demibranchs in the anterior part of the mussel, just posterior to the labial palps, is a common feature of P. canaliculus inhabited with P. novaezelandiae The damage is similar to that described by Stauber (1945) as "small crab type". Large crab type damage has not been seen in P. canaliculus.

In addition to the gill damage, the mussels often develop a fibrous lump or nodule on the mantle (Fig. 39) where the carapace of the crab rubs. This nodule can be up to 1 cm diameter, and has occasionally been found in mussels which

FIGURE 39

Damage caused to *Perna canaliculus* by *Pinnotheres novaezelandiae*. Arrows show fibrous nodules on the mantle. The proximal pair of gills have been partially cut away to reveal the damage done to the distal pair of gills by the pea-crab.



have no pea-crab. Presumably the crab has died, the gills have been repaired (Stauber, 1945), but the nodule remains.

Transverse sections cut through such nodules show that the thickening consists of a mass of irregularly orientated muscle fibres.

Dix (1973), recorded similar nodules from Pinctada maxima Jameson, the pearl oyster, infected with Pinnotheres villosulus Guerin.

(b) Effect on the condition of P. canaliculus

For each monthly sample, the variation between the condition factors of female mussels with crabs; female mussels without crabs; male mussels with crabs; and male mussels without crabs, was tested for statistical significance.

Although a difference could be seen on a graph (Fig. 38), this was found not to be significant, due to the variation in condition factor within each sample, together with the small size of the sample.

When differences between the same four groups were examined for the whole year, it was found that the difference between the male mussels without crabs, and the male mussels with crabs was not significant, but for the two female mussel groups there was a significant variation. The female mussels with crabs were lower in condition than those without crabs.

It can be seen from the graph (Fig. 38) that from about September, when the mussels increase in condition as the gonads ripen, the mussels with pea-crabs take longer to reach spawning condition. This applies for both male and female mussels. Statistical analysis of the data confirmed this. Between March and September inclusive, the variation between the mussels with crabs and those without, is not significant. From October to March the variation between mussels with and without crabs was quite significant, even at the $\alpha = 0.01$ level. This was caused by a very significant difference between the female mussels, those containing crabs being very much poorer in condition in the second half of the year than female mussels which were free of crabs.

Male mussels with crabs were also poorer in condition in the second half of the year, but only slightly so.

The crabs are almost certainly starving the mussels by feeding on the mussel food-strings. As the mussels put on weight after September, in preparation for spawning, those mussels which are short of food will take much longer to reach spawning condition. Sugiura et al (1960), found a seasonal change in condition factor in Tapes japonicus infected with Pinnotheres sinensis, which is similar to that described above. Kruczynski (1972) found that Bay scallops infected with P. maculatus were smaller, weighed less, and grew less quickly than uninfested scallops. Anderson (1975) found that F. subquadrata reduced the gamete production of the testis of Mytilus californianus Conrad, and Berner (1952) suggested that large pea-crabs (over 10mm) had an adverse effect upon the gametogenesis of M. edulis, but no evidence for this was found by Seed (1969). Awati & Rai (1931) showed Ostrea cucullata infected with pinnotheres sp. had a much higher percentage of male oysters than would normally occur amohg un-infected oysters. Since the oysters could be made to change sex by starvation, the authors concluded that the pea-crab was interfering with the food intake of the oyster enough to induce it to produce sperm and not the more "expensive" eggs.

An examination of the data for P. canaliculus containing pea-crabs showed that there were more male mussels with crabs than females with crabs, but this was only just significant at the $\alpha = 0.05$ level.

When the data was divided into two groups, March to September inclusive, and October to March inclusive, it was found that there were more female mussels with pea-crabs in the first half of the year than in the second half. The numbers of male mussels containing pea-crabs in the second half of the year were also lower than would be expected, but this was not statistically significant.

The reason for the variation in the numbers of female mussels affected with P. novaezelandiae is not known. It is unlikely to be due to a sex change in the mussels, but probably reflects a seasonal change in the abundance of the pea-crabs.

Discussion

Pinnotheres novaezelandiae, in common with the Northern Hemisphere pinnotherids whose biology has been studied, shows a complex post-planktonic life-cycle. The crab has stages of development similar to those described for other pinnotherids, but there are a number of differences in the life-cycle.

As is to be expected, P. novaezelandiae has a peak in the number of ovigerous females during the southern summer, but ovigerous females can also be found throughout the year. All the other pinnotherids which have been studied cease egg laying during the winter.

Pinnotheres pisum, P. ostreum, P. maculatus, and F. subquadrata are all known to have hosts which are infected by the invasive stage crab, but which are left at the stage I instar, to continue development in another species of shellfish (Christensen, 1958; McDermott, 1962; Pearce, 1966). This has not been observed to occur in P. novaezelandiae.

New Zealand has a number of shellfish which are host to pea-crabs, presumed to be P. novaezelandiae, and perhaps P. schauinslandi, but what part these shellfish may play in the life-cycle of P. novaezelandiae has not been determined.

The association between the crab and the mussel is well established. The soft membranous shell of the female is well adapted to the life within the shellfish. Christensen & McDermott (1958) hypothesised that the males originally had post-hard stages comparable to those of the female, but that these have since been lost. Pearce (1966) suggested that the hard stage crab was the original adult, and that the soft post-hard females are a later development. Atkins (1958) recorded that hard shell males of P. pisum may moult into an additional soft shell stage. This is evidence which supports Christensen & McDermott's proposal.

The only fossil pinnotherids so far known, were found in fossil Tresus capex Gould by Zullo & Chivers (1969) and identified by them as Pinnixa faba Dana. The sediments in which the fossils were found has been dated as late Pleistocene.

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REFERENCES

Anderson, G.L. 1975: The effects of intertidal height and the parasitic crustacean Fabia subquadrata Dana on the nutrition and reproductive capacity of the Californian sea mussel Mytilus californianus Conrad. Veliger, 17 (3): 299 - 306

Atkins, D. 1926: The moulting stages of the pea-crab (Pinnotheres pisum). Journal of the Marine Biological Association of the U.K., 14: 475-493

_____ 1955: The post-embryonic development of British Pinnotheres (Crustacea). Proceedings of the Zoological Society, London, 124: 687-715

_____ 1958: British pea-crabs (Pinnotheres). Nature, London, 181: 1087

Awati, P.R., & Ray, H.S. 1931: Ostrea cucullata. Indian Zoological Memoirs, 3: 107pp

Bennett, E.W. 1964: The marine fauna of New Zealand: Crustacea, Brachyura. New Zealand Department of Scientific and Industrial Research, Bulletin 153: 120pp

Berner, L. 1952: Biology de Pinnotheres pisum Penn. (Décapode, Brachyoure). Bulletin de la Société zoologique de France, 77: 344-349

Christensen, A.M. 1958: On the life history and biology of Pinnotheres pisum. Proceedings, 15th. International congress of Zoology: 267-270

_____ 1962: Nogle parasitiske krabbers biologi. Naturens Verden : 1-7

Christensen, A.M., & McDermott, J.J. 1958: Life history and biology of the oyster crab, Pinnotheres ostreum Say. Biological Bulletin, Marine Biological laboratory, Woods Hole Mass., 114 (2): 146 - 179

Dean, B. 1892: Bulletin of the U.S. fish commission for 1890: 351

Dix, T.G. 1973: Mantle changes in the pearl oyster Pinctada maxima induced by the pea-crab Pinnotheres villosulus. Veliger, 15(4): 330-331

Fenucci, J.L. 1973: Comensalism de Pinnotheres maculatus Say (Decapoda, Brachyrua, Pinnotheridae) en el mejillon Mytilus platensis (Campana exploratoria "mejillon 1"). Physis, revista de la Sociedad argentina de ciencias naturales. Section A, 32(84): 203 -210

Houghton, D. 1963: The relationship between tide level and the occurence of Pinnotheres pisum (Pennent) in Mytilus edulis L. Journal of animal ecology, 32: 253 - 257

Kruczynski, W.L. 1972: The effect of the pea-crab Pinnotheres maculatus Say on the growth of the Bay Scallop Argopecten irradians concentricus (Say). Chesapeake Science, 13(3): 18 - 220

_____ 1974: Relationship between depth and occurence of pea-crabs Pinnotheres maculatus in Blue mussels Mytilus edulis in the vicinity of Woods Hole, Mass. Chesapeake Science, 15(3): 167-169

McDermott, J.J. 1962: The incidence and host parasite relations of pinnotherid crabs (Decapoda, Pinnotheridae). Prodeedings, 1st National coastal shallow water research conference: 162-164

Orton, J.H, 1921: The mode of feeding and sex phenomena in the pea-crab (Pinnotheres pisum). Nature, London, 106: 533-534

Pearce, J.B. 1964: On reproduction in Pinnotheres maculatus (Decapoda:Pinnotheridae). Biological Bulletin, Marine Biological laboratory, Woods Hole, Mass.127: 384

_____ 1966: The biology of the mussel crab, Fabia subquadrata from the waters of the San Juan Archipelago, Washington. Pacific science, 20: 3-35

Scott, M.S. 1961: A review of the New Zealand Pinnotheridae Transactions of the Royal society of New Zealand, Zoology, 1: 303-309

Seed, R. 1969: The incidence of the pea-crab, Pinnotheres pisum in the two types of Mytilus (Mollusca:Bivalvia) from Padstow, South West England. Journal of Zoology 158: 413-420

Sugiura, Y.; Sugita, A., & Kihara, M. 1960: The ecology of pinnotherid crabs as pests in culture of Tapes japonica 1. Pinnotheres sinensis living in Tapes japonica and the influence of the crab on the weight of the hosts flesh. (in Japanese with English abstract). Bulletin of the Japanese society of scientific fisheries 26 (2): 89-94.

Stauber, L.A. 1945: Pinnotheres ostreum, parasitic on the American oyster Ostrea (Gryphaea) virginica. Biological Bulletin, Marine biological laboratory Woods Hole, Mass. 88: 269-291

Wear, R.G. 1965: Zooplankton of Wellington Harbour, New Zealand, Zoology publications of Victoria University of Wellington, 38: 1-31

Wells, W.W. 1940: Ecological studies on the pinnotherid crabs of Puget Sound. University of Washington publications in oceanography, 2: 19-50

Zullo, V.A., & Chivers, D.D. 1969: Pleistocene symbiosis: Pinnotherid crabs in pelecypods from Cape Blanco, Oregon. Veliger, 12(1): 72-73

In the mussels and oysters examined, there were not as many symbionts as had been expected. A similar survey of the American oyster Crassostrea virginica from Connecticut (United States) revealed seven parasites, including haplosporidians, Nematopsis, ciliates, and an amoiboid organism (Newman 1971).

No amoeboid, ciliate, or fungal parasites were found in the New Zealand shellfish examined, although a Paramoecium like ciliate was found on the gills of Perna canaliculus. This ciliate was found to thrive in aquaria long after the removal of the host shellfish, and so could not be considered to be in a parasitic relationship with the mussel.

Most of the known parasites in New Zealand shellfish are trematode sporocysts, of which ten have been recorded (see appendix I). This is only a small percentage of the total number which must infect shellfish, since all known digenean life-cycles involve a sporocyst or redial stage in a mollusc, and Hewitt & Hine (1971) list over 60 species of Digenea in New Zealand marine fishes. This list is certainly not exhaustive.

The discovery of only two copepods associated with two of the four shellfish studied,was surprising, since invertebrates generally make good hosts for copepods. The ascidians Cnemidocarpa nisiotis (Sluiter), Corella eumyota Traustedt, and Pyura spinossissima Michaelsen from Wellington Harbour are between them host to five copepod species. (Jones 1974, 1975).

It is certain that more copepods will be found associated with New Zealand marine molluscs, once other shellfish species are examined.

The pea-crab Pinnotheres sp. is found in Atrina zelandica Perna canaliculus, Mytilus edulis aoteanus, Pecten

novaezelandiae novaezelandiae, Chione stutchburyi and Crassostrea glomerata. It has been generally acknowledged that there is only one pea-crab in New Zealand, P. novaezelandiae (Scott 1961; Morton & Millar 1973, p.292). This may be a false assumption (see this thesis, p. 65), and it will be necessary for much more information to be gathered about the crabs from all of these hosts before the total number of pea-crab species in New Zealand can be ascertained.

There is a need for similar surveys of the other shellfish species which are common around the New Zealand coast. Cockles (Chione stutchburyi) and pipis (Amphidesma australe(Gmelin 1791)) are both edible species, and both have been observed to suffer occasional mass mortalities. Before environmental conditions are blamed for these, it would be wise to determine whether parasites are also present on the beds.

Both P. canaliculus and the oyster O. lutaria have been studied by biologists, and both species have been extensively sectioned to determine the cycle of gonad development. Despite this, the presence of Nematopsis and the unidentified sporozoan was not suspected. This indicates that only a careful search for abnormalities is likely to uncover the presence of a foreign organism, and that although a shellfish has been examined previously, failure to find parasites does not mean that they are not there. Presumably some of the oysters that Howell (1966) sectioned for Bucephalus were also infected with the sporozoan which was found during this survey, but the cysts were not seen at that time.

Viral and bacterial diseases were not searched for during this study, as their detection involves techniques beyond

the scope of this survey. Viruses are known to occur in shellfish, however, (Tubiash et al 1970; Farley et al 1972) and have been suspected of causing shellfish mortalities, particularly when no other cause can be found (Laird 1961).

Bacterial diseases are also difficult to detect, since bacteria will be found in any dying oyster. A bacterial disease of oysters was recorded in Matsushima Bay, in Japan and this disease has since spread to the United States (Rosenfield 1969).

With the establishment of a fish diseases diagnostic centre in New Zealand by the Fisheries Research Division of the Department of Agriculture and Fisheries, the necessary techniques for isolating and identifying pathogenic strains of bacteria will eventually become available to shellfish biologists in this country.

Once parasites have been found, it is important to survey the shellfish stocks in order to determine the incidence of infection, and what areas are free from the infection. Localities which are free from the disease should be protected from introductions of shellfish from diseased areas. Apart from the obvious consequences of introducing a diseased stock into a disease free area, it is always possible that the introduced shellfish will dilute the gene pool of the indigenous resistant stock, and increase the susceptibility of new generations (Rosenfield 1969).

It is also important to collect basic biological and ecological data on the disease organism and its host. Knowledge of the vulnerable stages of the life-cycle, any restrictive environmental conditions, and the effects of stress on the host, may be useful in determining control measures.

The eradication of a disease, once it becomes established, is probably impossible. Measures can, however, be taken to limit the effects of the disease. In North America it is possible to grow Crassostrea virginica which is free of Minchinia nelsoni provided that the salinity is below 15 ‰. The disease is apparently non-infective below this salinity level (Sindermann, 1968). In Europe, Korringa (1952) found that shell disease, which is caused by a fungus, was especially severe in areas where cockle shells were used as spat collectors. He found that dead shells were a reservoir for the disease, and that dredging the beds to remove the dead shell reduced the incidence of infection. Dipping infected oysters in dilute mercuric chloride was found to kill the fungus. Fortunatly there is no similar disease among O. lutaria in Foveaux Strait since empty oyster shells are returned to these beds to provide settlement sites for oyster spat.

It has been found th t pea-crabs may be removed from oysters (and presumably other shellfish) by dipping the shellfish in a dilute solution of the pesticide "Sevin". This kills the crab but not the oyster (Andrews et al 1968). This is still an experimental technique, since the accumulation of the pesticide by the oysters has not been examined, but in future it may be possible to use chemicals to remove pea-crabs from commercial shellfish stocks in the same way that a farmer doses his cattle for parasites.

Theoretically it is possible to develop resistant strains of shellfish by breeding the survivors of an epidemic. This has happened naturally in Prince Edward Island, Canada where the oyster stock has developed a resistance to Malpeque disease (Korringa 1952; Rosenfield 1969). Efforts to

artificially breed oyster strains which are resistant, have so far been unsuccessful and are not likely to produce results for many years to come, so that the industry will continue to use resistant environments, and to adapt planting and harvesting shedules as their main defence against parasites.

None of the parasites so far recorded from the New Zealand shellfish appear to be causing severe losses to the shellfish industry, except perhaps for the mortality among the Foveaux Strait oysters. From the limited biological information presently available on these parasites, it is not possible (with one exception) to suggest ways of reducing any effect they may presently be having.

Mussels are known to drop off mussel rafts and accumulate on the sea bed beneath the raft. These accumulated mussels may act as a reservoir for parasites. Aldrichetta forsteri schools beneath floating structures (Manikiam 1963) and it would be interesting to know if the mussels under the rafts have a higher incidence of Tergestia agnostomi than in other areas.

The pea-crab P. novaezelandiae was originally absent from the raft mussels, but recently it has begun to appear in the harvested crops (Tortell pers. comm.). It is probable that the crab zoea, after rising to the surface from infected mussels near (under ?) the raft, are caught among the mussel ropes when they become photonegative and attempt to sink to the bottom. If the rafts are becoming infected in this way, then it may prove economic to periodically move the rafts, or to remove the piles of mussels, in order to reduce the incidence of infection among the raft mussels.

Other points which need furthur investigation are the identification of the sporozoan parasite from the Foveaux Strait oyster, and the determination of its life cycle together with that of Nematopsis since although the latter at least, is harmless, without any knowledge of the other hosts and whether the spores are killed when the shellfish are processed, it may well be that foreign governments will place restrictions on New Zealands shellfish exports. Such a ban is often applied as a means of subtle import control rather than from any genuine desire to prevent the spread of a parasite, and in this case it is the exporting country which has to demonstrate that its shellfish are free from viable disease organisms.

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Alderman, D. 1974 :Foreign diseases hazard British shellfish New Scientist 18 July 1974:122-124

Anderson, G.L. 1975 :The effects of intertidal height and the parasitic crustacean Fabia subquadrata Dana on nutrition and reproductive capacity of the Californian sea mussel Mytilus californianus Conrad. Veliger 17(3):299-306

Andrews, J.D.;Turgeon, D.;Hreha, M. 1968 :Removal of pea-crabs from live oysters by using "Sevin"®. Veliger 11(2) 141-143

Angel, L.M. 1960 :Cercaria haswelli Dollfus 1927: A re-examination of Haswell's material, with discussion of the genus Tergestia. Libro Homenaje al Dr. Eduardo Caballero y Caballero.Mexico 1960:75-86

Bernard, F.R. 1969 :The parasitic copepod Mytilicola orientalis in British Columbia bivalves. Journal of the Fisheries Research Board of Canada 26(1):190-191

Bevelander, G. 1933 :Response to light in the cercaria of Bucephalus elegans. Physiological zoology 6:289 - 305

Boyle, M.S. 1965 :Trematode and nematode parasites of Pleurobrachia pileus O.F. Muller in New Zealand waters. Transactions of the Royal Society of N.Z., Zoology 8(6):51-62

Cheng, T.C. 1967: Marine molluscs as hosts for symbioses with a review of the known parasites of commercially important species. Advances in Marine Biology,5: xiii,424pp.

Chilton, C. 1910 :Note on the dispersal of marine crustacea by means of ships. Transactions of the New Zealand Institute 1910:131-133

Christensen, A.M. & McDermott, J.J. 1958 :Life-history and biology of the oyster crab, Pinnotheres ostreum Say. Biological Bulletin, Marine Biological laboratory, Woods Hole, Mass. 114(2):146-179

Cole, H.A. & Savage, R.E. 1951 :The effect of the parasitic copepod Mytilicola intestinalis (Steuer) upon the condition of mussels. Parasitology. 41:156-161

Cole, H.A. 1935 :On some larval trematode parasites of the mussel (Mytilus edulis) and the cockle (Cardium edule). Parasitology 27:276 -280

Dinamani, P. & Gordon, D.B. 1974 :On the habits and nature of association of the copepod Pseudomyicola spinosus with the rock oyster Crassostrea glomerata in New Zealand. Journal of invertebrate pathology 24:305-310

Dimamani, P. 1971 :Occurence of the Japanese oyster Crassostrea gigas(Thunberg)in Northland, New Zealand. New Zealand journal of marine and freshwater research 5(2):325-357

Dollfus, R.P. 1927 :Notules sur des cercaires atlantiques (1-2): Bulletin société zoologique de France 52(2):111-118

Farley, C.A.;Banfield, W.G.;Kasnic Jr., J.;Forster, W.S. 1972: Oyster Herpes-type virus. Science 178:759-760

Feng, S.Y. 1958 :Observations on distribution and elimination of spores of Nematopsis ostrearum in oysters. Proceedings of the National Shellfisheries Asso Association 48:162-173

Galtsoff, P.S. 1964:The American oyster Crassostrea virginica Gmelin. Fishery Bulletin of the U.S. Fish and Wildlife Service, 64:1-480, 400 figs. 46 tables.

Gooding, R.V. 1957:On some copepods from Plymouth, mainly associated with invertebrates including three new species. Journal of the Marine Biological Association of the U.K. 36 : 195-221, 6 figs, 4 tables

Graham, D.G. 1956 :A treasury of New Zealand fishes. A.H. & A.W. Reed, Wellington 424pp.

Gray, J.S. 1965:The behaviour of Protodrilus symbioticus (Giard) in temperature gradients. Journal of Animal Ecology, 34:455-461

Haswell, W.A. 1903 :On two remarkable sporocysts occuring in Mytilus latus on the coast of New Zealand. Proceedings of the Linnean Society of N.S.W. 27 (4):497-515

Hewitt, G.C. & Hine, P.M. 1971 :Checklist of parasites of New Zealand fishes and their hosts. New Zealand journal of marine and freshwater research 6(1 & 2) 69-114

Hopkins, S.H. 1957 :Our present knowledge of the oyster parasite Bucephalus. Proceedings of the National Shellfisheries Association 47:58-61

Howell, M. 1966 :A contribution to the life-history of Bucephalus longicornutus (Manter 1954). Zoology publications from Victoria University, Wellington 40:1-42

_____ 1967 :The trematode Bucephalus longicornutus (Manter 1954) in the New Zealand mud oyster Ostrea lutaria. Transactions of the Royal Society of New Zealand, Zoology 8(22):221-237

Humes, A.G. & Cressey, R.F. 1958 :Copepod parasites of molluscs in West Africa. Bulletin de l'Institute française d'Afrique Noire (A) 20(3): 921 - 942

Humes, A.G. & Stock, J.H. 1973 :A revision of the family Lichomolgidae Kossman 1877, cyclopoid copepods mainly associated with marine invertebrates. Smithsonian contributions to Zoology 127:i-v, 368pp.

Humes, A.G. 1968 :The cyclopoid copepod Pseudomyicola spinosus (Raffaele & Monticelli) from marine pelecypods chiefly in Bermuda and the West Indies. Beaufortia 14:203-226

Jones, J.B. 1974 :New Notodelphyidae (Copepoda:Cyclopoida) from solitary ascidians. New Zealand journal of marine and freshwater research 8(2):255-273

_____ 1975 :Lichomolgidium tupuhiae a new cyclopoid copepod associated with an ascidian from New Zealand. New Zealand journal of marine and freshwater research 9(2):245-251

Korringa, P. 1952 :Recent advances in oyster biology. The Quarterly Review of Biology 27:266-308;339-365

Kudo, R. 1954 :Protozoology.4th. edition: Thomas; Springfield, Illinois. 966pp.

Laird, M. 1961 :Microecological factors in oyster epizootics Canadian Journal of Zoology 39:449-485

LaRue, G.R. 1957 :The classification of digenetic trematodes, a review and a system. Experimental Parasitology 6: 306-344

Légér, L. & Duboscq,O. 1913 :Le cycle évolutif de Porospora portunidarum Frenzel Compte Rendu de l'Academie des Sciences. Paris 3:115-130

Linzey, M.C. Unpublished 1971 :The biology of Cercaria haswelli (Dollfus 1927) Larval digenean parasite of the mussel Perna canaliculus (Gmelin 1791). MSc.thesis, Canterbury University library.

Lipovski, V.P. & Chew, K.K. 1972 :Mortality of Pacific oysters (Crassostrea gigas):the influence of temperature and enriched seawater on oyster survival. Proceedings of the National Shellfish Association 62:72-82

Manikiam, J.S. Unpublished 1963 :Studies on the yellow-eyed mullet Aldrichetta forsteri Cuv. & Val. (Mugilidae). MSc. thesis, Victoria Univesity library

Manter, H.W. 1954 :Some digenetic trematodes from fishes of New Zealand. Transactions of the Royal Society of N.Z. 82(2):475-568

Miller, R.H. 1963 :Oysters killed by trematode parasites. Nature 197:616

Mitchum, D.L. & Moore, T.D. 1969 :Efficacy of di-n-butyl tin oxide on an intestinal fluke Crepidostomum farionis in golden trout. The progressive fish culturist 31:143-148

Morton, J. & Millar, M. 1973 :The New Zealand sea shore. Collins, London. 653pp. 32 plates.

Newman, M.W. 1971 :A parasite and disease survey of Connecticut oysters. Proceedings of the National Shellfisheries Association 61:59-63

Odlaug, T.C. 1946 :The effect of the copepod Mytilicola orientalis upon the Olympia oyster Ostrea lurida. Transactions of the American Microscopical Society 65:311-317

Prytherch, H.F. 1940 :The life cycle and morphology of Nematopsis ostrearum and N. prytherchi (Eugregarinina:Porosporidae) with special reference to the host parasite relations. Journal of parasitology 41:89-104

Rosenfield, A. 1969 :Oyster diseases in North America and some methods for their control. Proceedings of the conference on artificial propagation of commercially valuable shellfish, College of marine studies, University of Delaware.:67-78

Schiff, C.J. 1974 :Seasonal factors influencing the location of Bulinus (Physopsis) globosus by miracidia of Schistosoma haematobium in nature. Journal of parasitology 60:221-260

Scott, M.S. 1961 :A review of the New Zealand Pinnotheridae. Transactions of the Royal Society of New Zealand, Zoology 1(22):303-309

Scriven, M.C. Unpublished 1967:Host parasite relationships between Perna canaliculus and Cercaria haswelli. BSc (Hons) project, Canterbury University library

Silas, E.G. & Alagarswami, K. 1967 :On an instance of parasitism by the pea-crab (Pinnotheres sp.) on the backwater clam (Meretrix casta (Chemnitz)) from India, with a review on the work on the systematics, ecology, biology and ethnology of the pea-crabs of the genus Pinnotheres Latreille. Proceedings of the symposia on crustacea held at Ernakulam.Marine Biological Association of India, part III, pages 1163-1227

Sinderman, C.J. 1968 :Oyster mortalities, with particular reference to Chesapeake Bay and the Atlantic Coast of North America. U.S. Fish and Wildlife Service, special scientific report, Fisheries 569: 1-10

Skerman, T.M. 1958 :Seasonal variations in seawater temperature within New Zealand Harbours. New Zealand journal of geology and geophysics 1(1):197-218

_____ 1960 :Ship fouling in New Zealand waters: A survey of marine fouling organisms from vessels of the coastal and overseas trades. New Zealand journal of science 3(4):620-648

Sparks, A.K. 1962 :Metaplasia of the gut of the oyster Crassostrea gigas (Thunberg) caused by infection with the copepod Mytilicola orientalis Mori. Journal of insect pathology 4:57-62

Sprague,V. & Orr, P.E. 1955 :Nematopsis ostrearum and N. prytherchi (Eugregarinina:Porosporidae) with special reference to the host parasite relations Journal of parasitology 41:89-104

Steed, D.H. 1971 :A preliminary survey of mussel stocks in Pelorous Sound. N.Z. Marine Department Fisheries technical report 61:28pp.

Stunkard, H.W. & Uzmann, J.R. 1959 :The life cycle of the digenetic trematode Proctoeces maculatus (Looss 1901) Ohdner 1911. Biological Bulletin, Marine Biological laboratory, Woods Hole, Mass.116(1): 184-193

Tubiash, H.S.;Colwell, R.R.;Sakazaki,R. 1970 :Marine vibrios associated with bacillary necrosis, a disease of larval and juvenile bivalve molluscs. Journal of bacteriology, 103(1):272-273

Wall, L.D. 1941 :Spirorchis parvus (Stunkard), its life-history and the development of its excretory system (Trematoda:Spirorchiidae). Transactions of the American Microscopical Society 60:221-260

Wear, R.G. 1965 :Zooplankton in Wellington Harbour, New Zealand. Zoology publications, Victoria University of Wellington 38:1-31

Wilber, C.G. & Gilchrist, R.D. 1965 :Organ weight: body weight ratios in the Mongolian gerbil Meriones unguiculatus Chesapeake Science 6(2):109-114

Williams, C.S. 1969 :The effect of Mytilicola intestinalis on the biochemical composition of mussels. Journal of the Marine Biological Association of the United Kingdom 49(1):161-173

Woods, C.S. 1963 :Native and introduced freshwater fishes. A.H. & A.W. Reed, Wellington. 64pp.

Yamaguti, S. 1958 :Systema helminthum. Interscience, New York Vol 1(parts 1&2)1575pp.

A CHECKLIST AND BIBLIOGRAPHY OF THE PARASITES OF NEW ZEALAND MARINE SHELLFISH.

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ABSTRACT

Parasites are listed for fourteen species of New Zealand marine shellfish (8 Bivalva, 4 Gastropoda, 2 Cephalopoda). No parasites are known from the Amphineura or the Scaphopoda. Five new host records for Pseudomyicola spinosus are recorded in the checklist and twenty seven published papers, theses, or honours projects are listed in the bibliography.

References to the Pinnotheridae and the boring polychaete worms are omitted.

INTRODUCTION

Very few species of New Zealand marine invertebrate fauna have been examined for parasites, and much of the information on the parasites which have been found, is in unpublished projects or theses, many of which do not appear in library catalogues.

This bibliography covers all the parasites recorded in marine shellfish from New Zealand coastal and estuarine waters. It does not include references to pea-crabs (family Pinnotheridae) which are found in the mantle cavity of many marine bivalves , or the boring polychaete worms which are often found in the shells of marine shellfish.

Arrangement of the checklist

The host species are divided into the five classes of the phylum Mollusca. Within these classes the hosts are arranged under their respective families in the alphabetical order of

the genus.

Against each host are listed the parasites of that host, together with the numbers of the references in the bibliography which refer to the parasite.

Parasites marked + are new host records.

ACKNOWLEDGEMENTS

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PHYLUM MOLLUSCA
BIVALVA

MYTILIDAE:

Mytilus edulis aoteanus Powell 1958

Digenea:

Cercaria haswelli Dollfus 1927:2,8,9,10,11,19,22,24.

Copepoda:

+ *Pseudomyicola spinosus*(Raffaele & Monticelli 1885)

Perna canaliculus (Gmelin 1791)

Protozoa:Sporozoa

Nematopsis sp.:18

Digenea:

Cercaria sp.(Bucephalidae) Haswell 1903:9,10,11.

Cercaria haswelli Dollfus 1927:2,8,9,10,19,22,24.

Copepoda:

+ *Pseudomyicola spinosus* (Raffaele & Monticelli 1885)

PECTINIDAE:

Pecten novaezelandiae novaezelandiae Reeve 1853

Copepoda:

+ *Pseudomyicola spinosus* (Raffaele & Monticelli 1885)

OSTREIDAE:

Crassostrea glomerata Gould 1850

Copepoda:

+ Pseudomyicola spinosus (Raffaele & Monticelli 1885)

Ostrea lutaria Hutton 1873

Digenea:

Bucephalus longicornutus (Manter 1954):11,13,14,21

Copepoda:

+ Pseudomyicola spinosus (Raffaele & Monticelli 1885)

TEREDINIDAE:

Bankia australis Calman 1920

Copepoda:

Teredicola typicus Wilson 1942:17,20

Lyrodus pedicellatus Quatrefages 1849

Copepoda:

Teredicola typicus Wilson 1942:20

VENERIDAE:

Chione stutchburyi Gray 1828

Digenea:

Cercaria chiltoni Dollfus 1927:4,8,10

Copepoda:

+ Pseudomyicola spinosus (Raffaele & Monticelli 1885)

GASTEROPODA

AMPHIBOLIDAE:

Amphibola crenata Martyn 1784

Digenea:

Cercaria sp. :3

BUCCINIDAE:

Cominella glandiformis Reeve 1847

Digenea:

Cercaria sp. A:3,23

Cercaria sp. B:3,23

Cercaria sp. C:3,23

POTAMIDIDAE:

Zeacumantus subcarinatus Sowerby 1855

Digenea:

Philophalmus sp.:14

Cercaria sp.'ZI':3

TROCHIDAE:

Melagraphia aethiops Gmelin 1790

Digenea:

Cercaria melagraphia Clark 1958:5,6

AMPHINEURA

(No parasites recorded)

SCAPHOPODA

(No parasites recorded)

CEPHALOPODA

OCTOPODIDAE:

Octopus maorum Hutton 1880

Dicyemidae :

Dicyema knoxi Short 1971:25

Dicyema maorum Short 1971:25

Dicyemennea kaikouriensis Short & Hochberg 1969:26

Digenea:

Plagioporus maorum Allison 1966:1,27

Robsonella australis Hoyle 1885

Dicyemidae:

Dicyema robsonellae Short 1971:25

Dicyemennea rostrata Short & Hochberg 1969:26

Digenea:

Plagioporus maorum Allison 1966:1,27

BIBLIOGRAPHY

1. Allison, F.R. 1966:A new species of adult Allocreadiide (Trematoda) from Octopus maorum Hutton. Records of the Canterbury Museum 8(2):81-85

2. Angel, L.M. 1960:Cercaria haswelli Dollfus 1927: a re-examination of Haswells material, with discussion of the genus Tergestia. Libro Homenaje al Doctor Edwardo Caballero y Caballero, Mexico 1960: 75-86

3. Andrews, E. Unpublished 1966:Some aspects of structure and biology of larval trematodes developing in certain estuarine molluscs. BSc.(hons.) project, Canterbury University library

4. Chilton, C. 1904:On the occurence of a species of cercaria in the cockle. Transactions of the N.Z. Institute 37:322-324

5. Clark, W.C. Unpublished 1957:Studies on some littoral trochid gastropods belonging to the genera Melagraphia Gray and Zediloma Finlay. MSc thesis Canterbury University library.

6. _____ 1958: A new cotylocercous cercaria from Melagraphia aethiops (Gm.)(Gastropoda). Transactions of the Royal Society of N.Z. 85(4):681-683

7. Dinamani, P. & Gordon, D.B. 1974: On the habits and nature of association of the copepod Pseudomyicola spinosus with the rock oyster Crassostrea glomerata in New Zealand. Journal of invertebrate pathology 24(3): 305-310

8. Dollfus, R.P. 1925: Liste critique des cercaires marines à queue sétigère signalées jusqu'à présént. Travaux station zoologique 9:43-65

9. _____ 1927: Notules sur des cercaires atlantiques (1-2). Bulletin Societe Zoologique de France 52(2):111-118

10. Haswell, W.A. 1903:On two remarkable sporocysts occuring in Mytilus latus on the coast of New Zealand. Proceedings of the Linnean society of N.S.W. 27(4): 497-515

11. Holliman, R.B. 1961: Larval trematodes from the Apalachee Bay area Florida with a checklist of the known marine cercariae arranged in a key to their superfamilies. Tulane studies in Zoology (1):1-74

12. Howell, M.J. 1963:Preliminary identification made of trematode parasite infecting Foveaux Strait oysters. Commercial Fisheries 1(11):16-18

13 _____ Unpublished 1965:Studies on the life history of Bucephalus longicornutus (Manter 1954)(Trematoda: Bucephalidae) and the biological control of its sporocysts in Ostrea lutaria Hutton. MSc thesis,

Victoria University library.

14. _____ 1965 :Notes on a potential trematode parasite of man in New Zealand. Tuatara 13(3):182-184

15. _____1966 :A contribution to the life history of Bucephalus longicornutus (Manter 1954). Zoology publications, Victoria University Wellington 40: 1-42

16. _____1967 :A contribution to the life history of Bucephalus longicornutus (Manter 1954) in the New Zealand mud oyster, Ostrea lutaria. Transactions of the Royal Society of New Zealand 82(2):221-237

17. Humes, A.G. & Turner, R.D. 1972:Teredicola typicus C.B. Wilson, 1942 (Copepoda, Cyclopoida) from shipworms in Australia, New Zealand and Japan. Australian journal of marine and freshwater research 23:63-72

18. Jones, J.B. (In press):Nematopsis n. sp. (Sporozoa: Gregarinidae) in Perna canaliculus (Note). New Zealand journal of marine and freshwater research.

19. Linzey, M.C. Unpublished 1971 :The biology of Cercaria haswelli (Dollfus 1927) larval digenean parasite of the mussel Perna canaliculus (Gmelin 1791). MSc thesis, Canterbury University library.

20. McKoy, J.L. Unpublished 1973 :Studies on the biology, distribution and ecology of New Zealand Teredinidae. Phd. thesis, Victoria University library.

21. Millar, R.H. 1963 :Oysters killed by trematode parasites. Nature 197:616

22. Milne, R.A. Unpublished 1966 :Host parasite relationships of Cercaria haswelli and Perna canaliculus. BSc (hons) project, Canterbury University library.

23. Ryburn, M.I. Unpublished 1972 :Larval trematode infections in Cominella glandiformis Reeve (Gastˆeropoda: Cominellidae) at the Avon Heathcote estuary. BSc (hons) project, Canterbury University library.

24. Scriven, M.C. Unpublished 1967 :Host parasite relationships between Perna canaliculus and Cercaria haswelli BSc (hons) project, Canterbury University library.

25. Short, R.B. 1971 :Three new species of Dicyema (Mesozoa: Dicyemidae) from New Zealand. Antarctic research series 17:231-249

26. Short, R.B. & Hochberg, F.G. 1969 :Two new species of Dicyemennea (Mesozoa:Dicyemidae) from Kaikoura, New Zealand. Journal of parasitology 55(3):583-596

27. Short, R.B. & Powell, E.C. 1968: Mature digenetic trematodes from New Zealand octopusses. Journal of parasitology 54(4):757-760

- APPENDIX 2 -

"WINTER MORTALITY IN CRASSOSTREA GLOMERATA (GOULD)

The occurence of mass mortalities of the New Zealand rock oyster Crassostrea glomerata was first noted by Curtin (1971) who named the phenomenon "winter mortality" because of its similarity to a disease of that name which occurs in New South Wales, Australia.

The extensive mortality of the oysters (up to 100% in some areas) is often accompanied by yellow necrotic pustules on the mantle and adductor muscle, and by brown rubber-like warts and spots on the inner surface of the shell. These symptoms are common to a number of oyster diseases: Malpeque disease (Laird 1961); Dutch shell disease (Korringa 1952); Minchinia nelsoni infections (Farley 1968); and Labrynthomyxa marina (= Dermocystidium marinum) infections (Mackin 1951).

Samples of Crassostrea glomerata taken from areas where "winter mortality" is known to occur, were examined for parasites. The results of this search were inconclusive, as no parasites were recognised as causing the disease, but a definate seasonal pattern was established for the presence of the necrotic pustules (Table 12).

Microscopical examination of the affected adductor muscles revealed extensive haemocytosis, with a gradual reduction of the muscle fibres (Fig. 40a). The muscle fibres eventually break down, and the individual fibres lie in a haphazard and twisted mass (Fig. 40 b). It is this destruction of the adductor muscle which causes the death of the oyster.

TABLE 12

Occurence of "Winter mortality" in the Bay of Islands

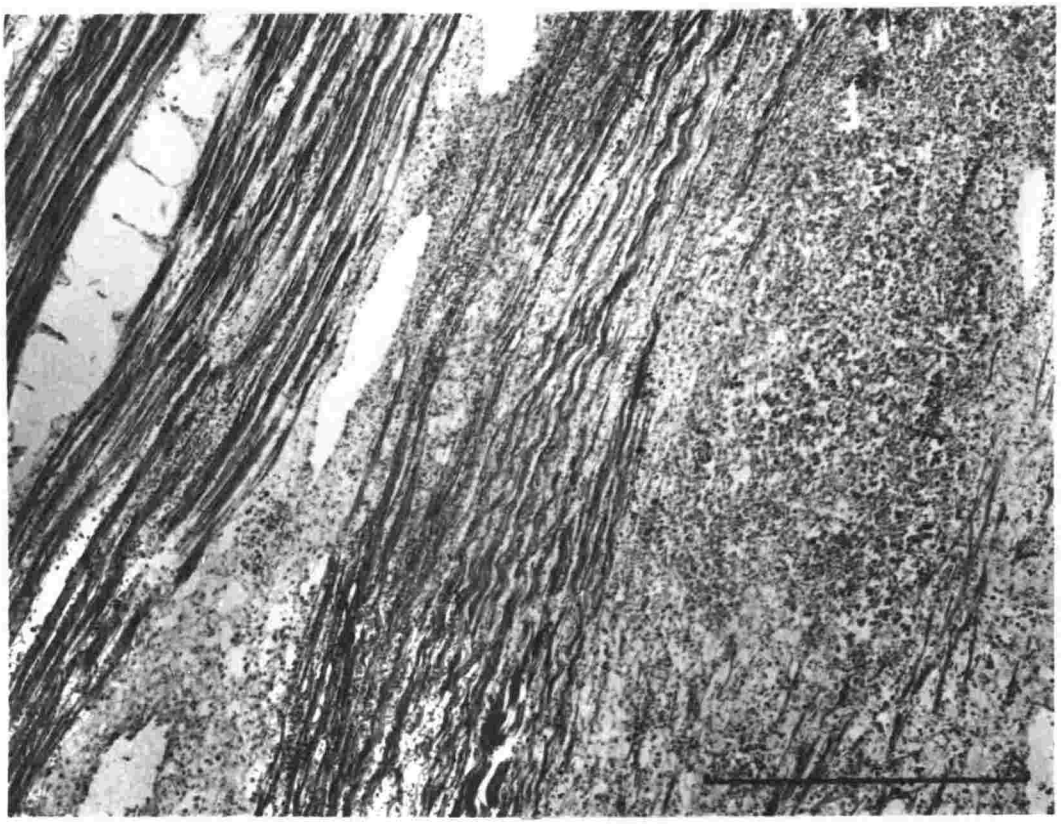
DATE	AREA	No WITH NECROSIS / TOTAL No.	%
August	Marine farm, Orongo Bay.	2/26	7.7
	Wairoa Bay	3/50	6.0
October	Wairoa Bay	3/41	7.3
February	Wairoa Bay	1/25	4.0
May	Orongo Bay	1/37	2.7
July	Te Tii Bay	7/26	26.9
September	?	3/25	12.0

FIGURE 40

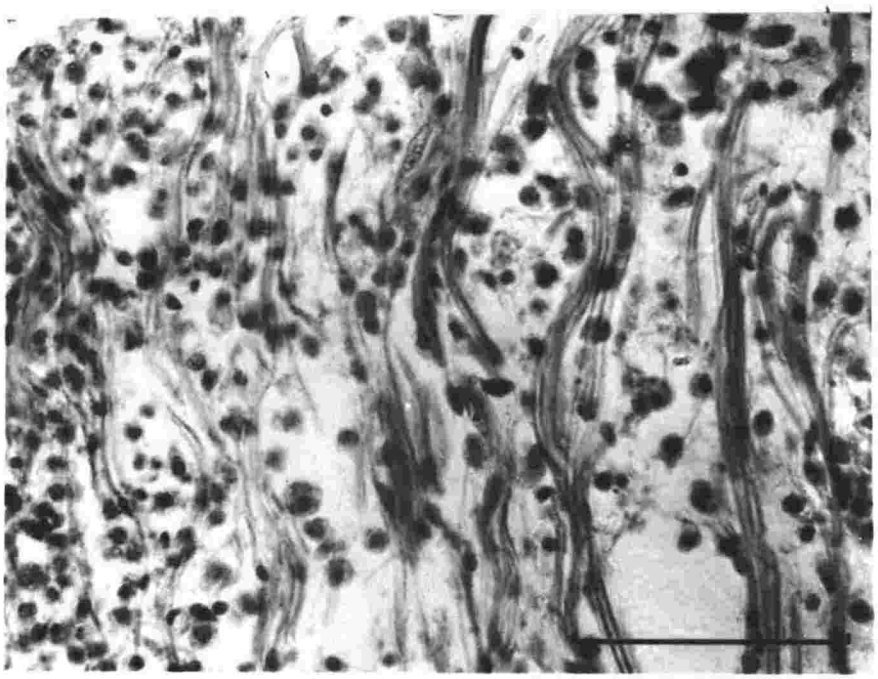
"Winter mortality" in Crassostrea glomerata

(a) Muscle fibres from the edge of a necrotic area. Fibres degenerating from left to right, with an increase in haemocytosis. Scale bar = 0.5mm

(b) Degenerating muscle fibres, and large numbers of haemocytes in a necrotic region. Scale bar = 0.05mm



A



B

DISCUSSION

Oysters have been found to develop necrotic pustules after injection with foreign substances such as turpentine (Pauley & Sparks 1965). Oyster drills (Lepsiella sp.) are known to attack oysters in the Bay of Islands, and it could be that the necrotic areas are formed by foreign substances entering the holes that they drill. The shells of affected oysters were examined for evidence of drilling by these gastropod molluscs, but no holes were found in the vicinity of the necrotic areas.

The pustules are known to be associated with pathological agents, as mentioned earlier, but no protozoa or fungi were detected in the New Zealand oysters.

Bacterial infections are also thought to be capable of forming necroses of the type under discussion (Laird 1961), but Grams stained sections through the necrotic area of one of the affected oysters failed to reveal more bacteria than could be expected from an injured oyster. Even if bacteria did occur in large numbers, their presence does not indicate that they are the primary cause of the disease, since bacteria and ciliates are known to invade "sick" oysters (Laird 1961).

Viral infections have been recorded in Crassostrea virginica (Farley et al 1972), but the symptoms do not include those found in C. glomerata. The detection of viral infections was beyond the resources of the author.

Laird (1961) and Lipovski & Chew (1972) believe that the oyster mortalities such as that at Malpeque Bay, are caused by temperature and enriched (i.e. polluted) seawater. The pollution need not be man-made, since Ho & Imai (1955) recorded that a Japanese oyster raft may produce from 6-10

tons of pseudofaeces per annum. This is highly organic, and in decomposing during hot still weather, it may be capable of reducing the amount of dissolved oxygen in the water, stressing the oysters to the stage where their normal resistance to infection is reduced. Bacteria and protozoa may then invade the oyster and kill it. Laird(1961) suggested that the organic debris brought down to the estuary by freshets may also bring about this effect.

The occurence of "winter mortality" in New Zealand does not fit this pollution theory. Mortality occurs no earlier than August, and extends over September to October, though the highest water temperatures are not until January (Booth 1974). (While winter mortality does not seem to occur in winter, however, the necrotic pustules may still be found). The mortality occurs in some harbours and not in others, and in any one harbour it will be worse the closer one goes towards the entrance. Severity varies and is always worse after dry winters (Curtin pers. comm.).

If a lack of oxygen in the water, and the presence of "sewerage bacteria" were causing the mortality, then the effect should be greatest among the oysters at the furthurest point from the sea. Likewise the severity of the mortality after dry winters rules out low salinity caused by freshets as a contributing factor. The evidence suggests that a low salinity alleviates the effects of the disease (Curtin 1971).

A high salinity may well prove to be one of the factors which triggers the mortality.

Another possible cause of the mortality could be a genetic muscular distrophy in the oyster. Weinstock & Iodice (1969) give the symptoms for muscular distrophy as: muscular degeneration, necrosis, and disruption of the normal muscle

structure. If this is the cause, then an electron microscope examination of the infected tissues should show lysosomal abnormalities. What triggers muscular distrophy is unknown.

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I wish to acknowledge the help of Dr Dinamani (Fisheries Research division), who first brought the problem to my attention, and Mr L. Curtin (Fisheries Management division) who provided the samples for examination, and who gave me much valuable information on the occurence of the disease. Dr T.W. Jordan (V.U.W. Biochemistry department) suggested muscular distrophy as a cause of the symptoms, and for this suggestion I am very grateful.

REFERENCES

Booth, J.D. 1974: Observations on the hydrology of the Bay of Islands, New Zealand. New Zealand journal of marine and freshwater research 8(4):671-690

Curtin, L. 1971: Oyster farming in New Zealand. New Zealand Marine Department, fisheries technical report 72: 99pp.

Farley, C.A.; Banfield, W.G.; Kasnic, G.; Foster, W.S. 1972: Oyster Herpes-type virus. Science 178:759-760

Ho, S. & Imai, T. 1955: Ecology of oyster bed. I: On the decline of productivity due to repeated cultures. Tohoku journal of agricultural research 5:251-268

Korringa, P. 1952: Recent advances in oyster biology. The quarterly review of biology 27:266-308;339-365

Laird, M. 1961: Microecological factors in oyster epizootics. Canadian journal of zoology 39:449-485

Lipovski, V.P. & Chew, K.K. 1972: Mortality of Pacific oysters (Crassostrea gigas): the influence of temperature and enriched seawater on oyster survival. Proceedings of the National Shellfish Association 62:72-82

Mackin, J.G. 1951: Histopathology of infection of Crassostrea virginica (Gmelin) by Dermocystidium marinum Mackin Owen and Collier. Bulletin of marine science of the Gulf & Caribbean 1:72-87

Pauley, G.B. & Sparks, A.K. 1965: Preliminary observations on the acute inflammatory reaction in the Pacific oyster Crassostrea gigas (Thunberg). Journal of invertebrate pathology 7:248-256

Weinstock, I.M. & Iodice, A.A. :Acid hydrolase activity in muscular distrophy and denervation atrophy. In Dingle J.T. & Fell, H.B. (editors) Lysosomes in biology and pathology. North Holland: Amsterdam. pp. 450-468 (vol. 1)

- APPENDIX 3 -

STATISTICAL ANALYSIS OF THE DATA FROM THE *PERNA CANALICULUS* SAMPLES

(1) Condition factors:

TABLE 13

Mean condition factor of *P. canaliculus* for each month

Date		♀(n)	♀(p)	♂(n)	♂(p)	♂(H)
Mar	1973	52.16	39.46	43.65	38.10	52.50(1)
Apr		42.96	45.19	27.90	41.58	-
May			No sample			
Jun		-	45.30	42.07	46.40	41.20(1)
Jul		43.16	45.88	46.98	42.08	51.00(1)
Aug		35.83	32.20	35.35	35.81	-
Sep		32.11	31.42	30.12	26.80	38.60(1)
Oct		38.14	38.05	35.91	46.35	-
Nov		35.50	28.98	33.60	31.19	24.40(1)
Dec		43.13	40.83	42.44	39.09	56.33(3)
Jan	1974	45.03	35.15	40.52	43.53	47.62(5)
Feb		39.52	35.46	42.77	33.67	-
Mar		41.83	45.88	44.40	38.39	45.10(1)

n = 488

key

(n) = mussels without pea-crabs
(p) = mussels with pea-crabs
(H) = mussels with *C. haswelli*
( ) = number in sample, if less than 10

(i) Variation in the C.F. between the five groups in any one month:

Using the analysis of variance (Mendenhall 1967, page 274) ($\alpha = 0.05$), it was found that for each month, the variation between the mean condition factors of the five groups was not significant. An exception was the ♂(H) group, which in some months did prove to have a higher condition factor than the mean, but the number of mussels in this group was always very small.

(ii) Variation in C.F. between the five groups over the twelve month period:

Ho : That there is no significant difference between the mean condition factors of the five groups.

n (the number of shellfish examined) = 488

p (the number of groups) = 5

	♂(n)	♂(p)	♂(H)	♀(p)	♀(n)
T	6509.51	4168.70	698.30	2597.45	4939.10
n_i	166	112	15	72	123
$\bar{x}_i$	39.21	37.22	46.55	36.68	40.16
Σx_i^2	270,822.13	164,968.68	34,662.01	101,308.33	214,480.11

where: T = total of the condition factors.(C.F.).

n_i = number in each group.

$\bar{x}_i$ = mean C.F. of each group.

Σx_i^2 = sum of the square of each C.F.

Correction for the mean (CM) = $\frac{(\Sigma T)^2}{n}$ = 732 999.67

Total sum of squares for treatments (TSS) = $\Sigma \Sigma x_i^2$ – CM = 53,241.59

Sum of squares for treatments (SST) = $\Sigma \frac{T}{n}$ – CM = 1968.93

Mean square for treatments (MST) = $\frac{SST}{p-1}$ = 492.23

Sum of squares for error (SSE) = TSS – SST = 51,272.66

Mean square for error (MSE) = $\frac{SSE}{n-p}$ = 106.15

$$F_{(p-1,n-p)} = \frac{MST}{MSE} = 4.64$$

reject if $F \geq F_{\alpha}$

from precomputed tables: $F_{0.05} = 2.37$

$F_{0.01} = 3.32$

Thus the evidence is sufficient to reject the Ho at both the $\alpha = 0.05$ and $\alpha = 0.01$ levels. There is a significant difference between the mean C.F's of the five groups.

If the male mussels containing Cercaria haswelli are excluded, is there any difference in this result ?

$p = 4 \quad n = 473$

$F_{(3,469)} = 3.37$

Which is not significant at the $\alpha = 0.01$ level, but which is significant at the $\alpha = 0.05$ level

Is there any difference between ♂(p) and ♂(n) over the whole year ? $p = 2 \quad n = 278$

$F_{(1,276)} = 2.89 \qquad F_{(\alpha = 0.05)} = 3.07$

Which is not significant.

Is there any difference between ♀(p) and ♀(n) over the whole year ? $p = 2 \quad n = 195$

$F_{(1,193)} = 6.30 \qquad F_{(\alpha = 0.05)} = 3.07$

Therefore the difference is significant, and the ♀(p) group does have a lower mean C.F. than ♀(n) mussels.

How significant is the difference between male mussels and ♂(H) over the whole year ? $p = 3 \quad n = 303$

$F_{(2,300)} = 86.06 \qquad F_{(\alpha = 0.01)} = 3.78$

The difference is significant even at the $\alpha = 0.01$ level.

(iii) If the results for the first six months (March to September inclusive) are combined, is there a difference between the groups ?

Ho : There is no difference between the five groups.

	♀(n)	♀(p)	♂(n)	♂(p)	♂(H)
n_i	52	41	68	44	5
$\bar{x}_i$	39.58	35.96	37.69	38.38	44.22

$p = 5 \qquad n = 210$

$F_{(4,205)} = 0.97 \qquad F_{(\alpha = 0.05)} = 2.37$

The Ho is not rejected, and there is no significant difference between the mean C.F. of the five groups.

(iv) If the results for the second six months (October to March inclusive) are combined, is there a difference between the groups ?

Ho : There is no difference between the five groups.

	♀(n)	♀(p)	♂(n)	♂(p)	♂(H)
n_i	71	31	98	68	10
$\bar{x}_i$	40.52	37.39	39.94	38.70	43.36

$p = 5 \qquad n = 278$

$F_{(4,273)} = 27.12 \qquad F_{(\alpha = 0.01)} = 3.32$

The Ho is rejected. The difference between the five mean C.F's is significant.

Is the difference between the ♀(n) and ♀(p) groups significant ?

$p = 2 \qquad n = 102$

$F_{(1,100)} = 22.94 \qquad F_{(\alpha = 0.01)} = 6.85$

The difference is significant. The ♀(n) mussels have a higher C.F. during the second six months, than the ♀(p) group.

Is the difference between the mean C.F's of the male mussels significant in the second six month period ?

$p = 3 \qquad n = 176$

$F_{(2,173)} = 6.37 \qquad F_{(\alpha = 0.01)} = 4.61$

The difference is significant.

Is the difference between the male mussels still significant if the mussels with C. haswelli are removed ?

$p = 2 \qquad n = 166$

$F_{(1,164)} = 4.72 \qquad F_{(\alpha = 0.01)} = 6.63$

$F_{(\alpha = 0.05)} = 3.84$

The difference is just significant at the $\alpha = 0.05$ level, but not at the $\alpha = 0.01$ level.

(2) Effect of crabs on the sex of the host:

TABLE 14

Number of shellfish with and without crabs, for each month

		♀(n)	♀(p)	♂(n)	♂(p)
Mar	1973	6	5	11	10
Apr		13	4	5	3
May					
Jun		0	4	12	4
Jul		8	2	11	14
Aug		11	7	17	8
Sep		8	8	14	18
Oct		9	0	23	8
Nov		7	8	10	22
Dec		14	4	18	11
Jan	1974	9	3	20	3
Feb		11	6	17	14
Mar		13	8	10	11
T		109	59	168	126
$\bar{x}_i$		9.08	4.91	14.0	10.5
n_i		12	12	12	12
$\leq x_i$		1151	363	2638	1704

Is there any evidence to suggest that one sex of mussel is more heavily parasitised than the other ?

$\frac{126}{294}$ male mussels contain crabs = 42.8[illegible] %

$\frac{59}{168}$ female mussels contain crabs = 35.12 %

Although there are fewer female mussels than male mussels in the population, if the crabs were randomly distributed through the mussel population, the ratio of infested to uninfested mussels should be about the same for both male and female mussels.

Using a one tailed test:

$$\text{Ho}: \quad \frac{♂(p)}{n} = \frac{♀(p)}{n} = 0.3512$$

$$\text{Ha}: \quad \frac{♂(p)}{n} > 0.3512$$

$$Z = \frac{y/n - p}{\sqrt{\frac{p-q}{n}}} = \frac{126/294 - 0.3512}{\sqrt{\frac{(0.3512)(0.6488)}{294}}}$$

$$= 1.6503$$

reject Ho if $Z > 1.645$ $\alpha = 0.05$

(Mendenhall 1967, p.172)

95 % confidence interval:

$$0.4286 \pm 1.645\sqrt{\frac{(0.4286)(0.5714)}{294}}$$

$$0.4286 \pm 0.0466$$

There is a slight difference between the mussel sexes, more male mussels contain pea-crabs than would be expected if the crabs were distributed at random through the population.

(3) Sex of crabs in mussels:

	number of ♀crabs		number of ♂crabs	
	♂mussels	♀mussels	♂mussels	♀mussels
Totals	92	63	20	8

4 ♀crabs were in hosts of uncertain sex.

Total ♀crabs = 159

Total ♂crabs = 28

The number of female mussels in a sample is less than half the total number of mussels in a sample. From table 14 the ratio of female mussels without crabs to the total number of mussels without crabs, is: $\frac{109}{277} = 0.3935$

Is the ratio of female mussels with female crabs, to the total number of mussels with female crabs, the same as this ?

$\frac{♀(♀\text{crabs})}{n_a} = \hat{p}$ Ho : $\hat{p} = 0.3935$

Where n_a = the total number of mussels with crabs.

$$Z = \frac{63/155 - 0.3935}{\sqrt{\frac{(0.3935)(0.6065)}{155}}} = 0.3359$$

reject Ho if $-1.96 < Z > 1.96$ $\alpha = 0.05$

Thus female crabs are not found more frequently (or less frequently) in female mussels than in male mussels.

Is this true for female mussels with male crabs ?

$\frac{8}{28} = \hat{p}$ Ho : $\hat{p} = 0.3935$

$$Z = \frac{8/28 - 0.3935}{\sqrt{\frac{(0.3935)(0.6065)}{28}}} = -1.1692$$

reject Ho if $-1.96 < Z > 1.96$ $\alpha = 0.05$

Thus male crabs do not occur more (or less) frequently in female mussels than in male mussels.

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REFERENCE

Mendenhall, W. 1967: Introduction to probability and statistics. 2nd. edition. Wadsworth Publishing Co.:California. i-xii, 1-393