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The Trematode, *Bucephalus longicornutus* (Manter, 1954) in the New Zealand Mud-oyster, *Ostrea lutaria**

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Abstract

THE incidence of infection of the sporocysts of *Bucephalus longicornutus* in the New Zealand mud-oyster is given for seven localities. Particular reference is made to the Foveaux Strait oyster beds where a 40% incidence occurred in areas A and B.

Initially the sporocysts infect the oyster gonad. Extensive growth of the sporocysts causes parasitic castration. Experiments show that the majority of infected oysters die, but it is considered that many of these deaths are only indirectly attributable to the effects of the parasite.

A haplosporidian hyperparasite (*Urosporidium constantae* n.sp.) caused total mortality of the embryonic cercariae in the sporocysts of the few infected oysters obtained from north of Tasman Bay. Ecological factors, and the difficulty of collecting large numbers of the hyperparasite from this locality, may preclude its introduction to the Foveaux Strait oyster beds as an effective biological control for *B. longicornutus*.

INTRODUCTION

THE New Zealand mud-oyster, *Ostrea lutaria* Hutton, 1873, forms the basis of a commercial fishery in Foveaux Strait. Although the annual harvest of oysters from the extensive beds in this region has remained more or less constant over recent years (Marine Department Annual Reports, 1951-1964), there has been a marked tendency for the catch per unit effort to decline by more than half over the same period according to unpublished data given to me by the Marine Department. This serious decline has become most noticeable since the 1958 oyster season, and the figure has remained essentially static over the last four years at approximately six sacks/hour as against maximum yields of 14 sacks/hour prior to 1958. In 1963, when Dr R. H. Millar of the Marine Station, Millport, reported the presence of a bucephalid parasite in the gonad of a sample of *O. lutaria*, a possible explanation for this decline in catch per effort became apparent, and studies on the parasite were therefore initiated.

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The main features of the life history of the parasite, *Bucephalus longicornutus*, which, at its sporocyst stage, infects the gonad of the mud-oyster, have been described elsewhere (Howell, 1966). The information contained herein was obtained concurrently with this life history study, and is divided into the following three sections:

1. Geographical distribution and incidence of infection of the sporocysts in the mud-oyster.

2. Effects of the sporocysts on the mud-oyster, prefaced by an historical account of previous observations of the effects of bucephalid parasites on their bivalve hosts.

3. Hyperparasitism of the embryonic cercariae within the sporocysts.

Materials and Methods

Oysters used in this study were obtained from Foveaux Strait between June and October 1963, and March and June 1964, and on one occasion only from each of the localities indicated in Table I. Those from the latter localities were opened and their condition recorded. The majority of Foveaux Strait samples were set up in finger bowls as detailed elsewhere (Howell, 1966), which allowed the incidence of infection in a given sample to be determined within a week. Some samples, however, were opened immediately to determine both the incidence and state of the infection. This was followed by sectioning and staining of portions of the visceral mass of both infected and uninfected specimens using standard techniques to determine the histological effects of the parasite on the oyster.

Four hyperparasitised oysters from Tasman Bay were frozen, then thawed before being received by the author. The visceral mass of two specimens was sectioned and stained using standard techniques. Portions of infected sporocysts were teased out of the visceral mass and mounted in glycerine for examination of the spores of the hyperparasite.

Live oysters from Foveaux Strait were used for three separate mortality experiments. The oysters were set up separately in 7in diameter finger bowls two-thirds filled with sea-water, and the water was changed once or, when time permitted, twice daily. Oysters that died during the experiment, and those that remained alive after the experiment was terminated, were opened and their condition recorded.

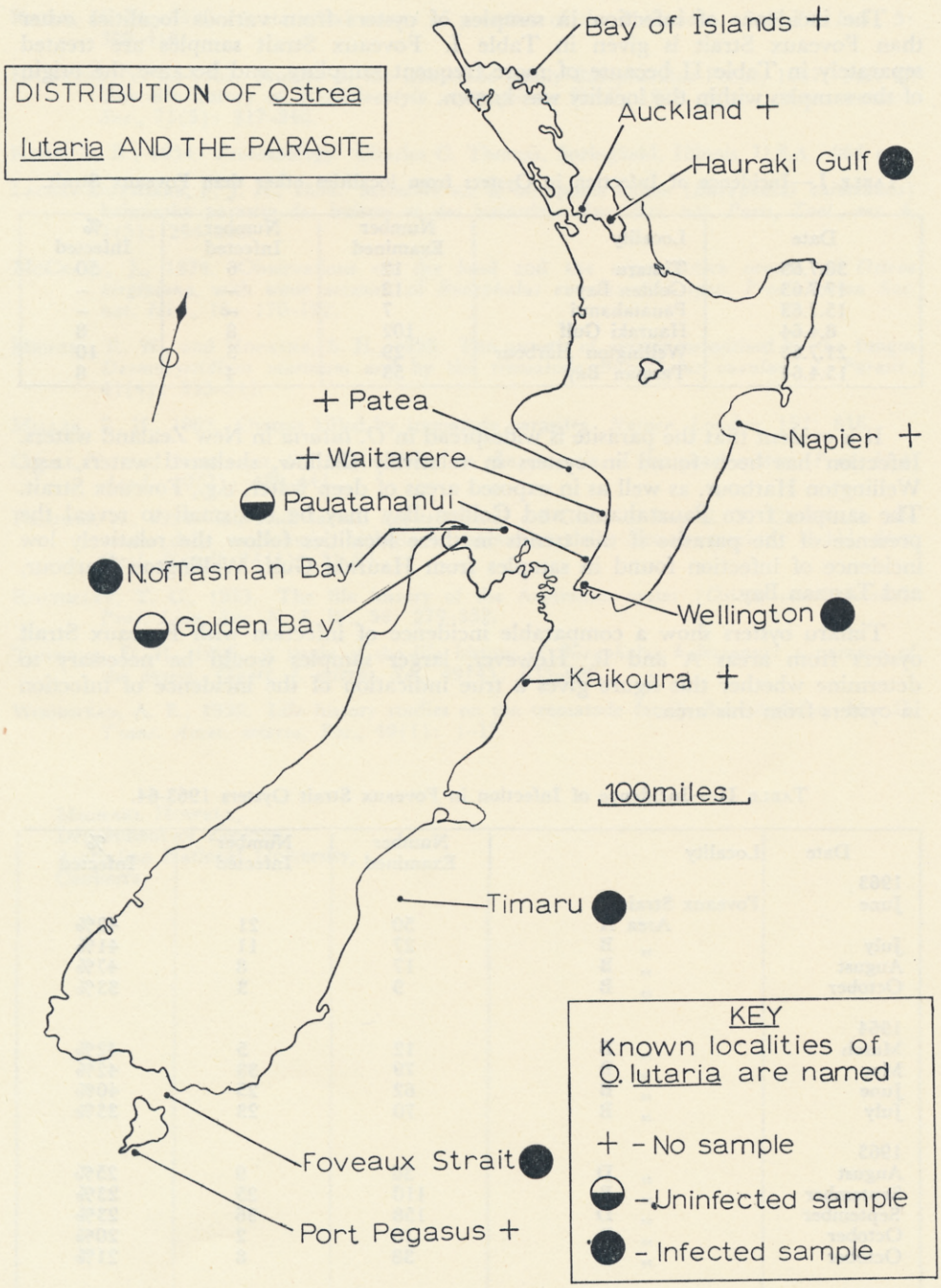
1. Geographical Distribution and Incidence of Infection in *Ostrea lutaria*

Distribution:

Known localities of *Ostrea lutaria* in New Zealand (Hollis, 1963), localities from which samples were received and the condition of these samples are indicated in Text-fig. 1.

The Foveaux Strait region is the major locality in which extensive oyster beds are known to occur. It is also the only region, disregarding minor operations in Golden Bay, where oysters are dredged on a commercial scale. Commercial operations have made possible more or less regular sampling from the Foveaux Strait region in contrast with only occasional sampling from the other known localities.

Hollis did not report *O. lutaria* from north of Tasman Bay. However, as shown in Text-fig. 1, oysters were recovered from the trawl net of the "Constanta", a Rumanian stern trawler, from approximately 173° 30′ E, 40° 10′ S during fishing trials in this area. The fact that only 53 oysters were taken suggests that oysters are sparse in this locality and not present in commercial quantities.



TEXT-FIG. 1.—Distribution of *Ostrea lutaria* and its bucephalid parasite in New Zealand.

Incidence of Infection:

The incidence of infection in samples of oysters from various localities other than Foveaux Strait is given in Table I. Foveaux Strait samples are treated separately in Table II because of more frequent sampling, and because the origin of the samples within the locality was known.

Table I.—Incidence of Infection in Oysters from localities other than Foveaux Strait.

Date	Locality	Number Examined	Number Infected	% Infected
30.7.63	Timaru	12	6	50
17.7.63	Golden Bay	12	–	–
15.7.63	Pauatahanui	7	–	–
8.4.64	Hauraki Gulf	102	8	8
21.7.63	Wellington Harbour	29	3	10
15.4.64	Tasman Bay	53	4	8

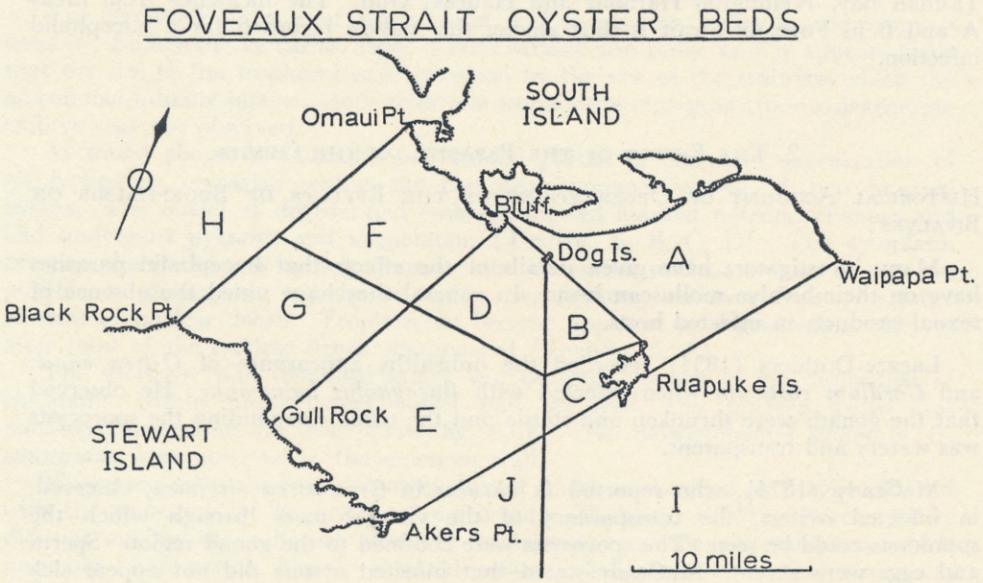
It is evident that the parasite is widespread in *O. lutaria* in New Zealand waters. Infection has been found in oysters in relatively shallow, sheltered waters, e.g.. Wellington Harbour, as well as in exposed areas of deep water, e.g., Foveaux Strait. The samples from Pauatahanui and Golden Bay may be too small to reveal the presence of the parasite if the trends in these localities follow the relatively low incidence of infection found in samples from Hauraki Gulf, Wellington Harbour, and Tasman Bay.

Timaru oysters show a comparable incidence of infection with Foveaux Strait oysters from areas A and B. However, larger samples would be necessary to determine whether this figure gives a true indication of the incidence of infection in oysters from this area.

Table II.—Incidence of Infection in Foveaux Strait Oysters 1963-64.

Date	Locality	Number Examined	Number Infected	% Infected
1963				
June	Foveaux Strait Area A	50	21	42%
July	„ B	27	11	41%
August	„ B	17	8	47%
October	„ B	9	3	33%
1964				
March	„ B	12	5	42%
May	„ B	79	33	42%
June	„ B	62	25	40%
July	„ B	70	23	33%
1963				
August	„ D	36	9	25%
September	„ E	110	25	23%
September	„ D	158	36	23%
October	„ E	10	2	20%
October	„ D	38	8	21%
1964				
April	„ D	24	5	21%
April	„ G	36	7	19%
May	„ E	15	3	20%
July	„ E	100	18	18%

The Marine Department has divided the Foveaux Strait oyster beds into a number of areas as shown in Text-fig. 2. The incidence of infection in every area was not determined because the samples were obtained from oyster boats which confined their dredging mainly to areas B, D and E over the study period. One sample was received from area A in June 1963, but this area was closed to commercial operations during 1964. One sample was received from area G in April 1964.



TEXT-FIG. 2.—Marine Department subdivisions of Foveaux Strait oyster beds.

ABBREVIATIONS FOR ALL FIGURES

a., amoebula; b.v., haemolymph vessel; c., cyst wall; c.m., cell membrane; c.t., connective tissue; cu., cuticle; cy., cytoplasm; d., densely stained region; d.c., degenerating connective tissue; d.e., degenerating embryos; e., distorted epithelium; e.c., degenerating cuticle of cercarial embryo; g., alimentary canal; g.f., gill filament; h.f., hermaphrodite follicle; i.l., interrib; i.m., ruptured investing membrane; l., gill lamella; m., membraneous portion of spore capsule; n., condensed chromatin; n'., necrotic nucleus; n.c., trophozoite nucleus; n.d., nuclear debris of cercarial embryo; ne., pyknotic nucleus; n.i., daughter nuclei of trophozoite nucleus; nu., normal nucleus; o., operculum; p.c., pyloric caeca; ph., phagocyte; s., spores; sp., sporocysts; spo., stages in sporogony; s.w., sporocyst wall; t., trophozoite; v., vacuole containing an irregularly shaped mass of chromatin.

The areas can be grouped into two divisions according to the incidence of infection. Areas D and E maintained a consistent incidence over 1963-64 (22% average) and together with area G (25% incidence in April 1964) constitute one division. Area B over 1963-64 along with area A in June 1963, exhibited almost double this incidence (41% average), thus constituting the other division.

These divisions, based on the incidence of infection, can be correlated with the type of sub-strate found in the different areas. Stead (pers. comm.) stated that areas E, G and D have a predominantly sandy substrate which is in part both compact and loose, whereas areas A and B tend to have a substrate of small pebbles with smaller amounts of sand.

As the presence of the parasite in *O. lutaria,* in particular from Foveaux Strait, was not detected until 1963 by Millar, no comparative figures from previous years are available.

The incidence of bucephalid infections previously reported from bivalve molluscs ranges between 0.1% for *Bucephalus mytili* infecting *Mytilus edulis* from North Wales (Cole, 1935), and greater than 50% for *B. haimeanus* infecting *Tapes aureus* from the Mediterranean (Palombi, 1934). The majority of authors have recorded a 10% incidence or less which is comparable with the incidence in oysters from Tasman Bay, Wellington Harbour and Hauraki Gulf. The incidence from areas A and B in Foveaux Strait is thus among the highest recorded for a bucephalid infection.

2. THE EFFECT OF THE PARASITE ON THE OYSTER

HISTORICAL ACCOUNT OF OBSERVATIONS OF THE EFFECTS OF BUCEPHALIDS ON BIVALVES:

Many investigators have given details of the effects that bucephalid parasites have on their bivalve molluscan hosts. In general they have noted the absence of sexual products in infected hosts.

Lacaze-Duthiers (1854) recorded the unhealthy appearance of *Ostrea edulis* and *Cardium rusticum* when infected with *Bucephalus haimeanus.* He observed that the gonads were shrunken and sterile and the tissue surrounding the sporocysts was watery and transparent.

McCrady (1874), who reported *B. cuculus* in *Crassostrea virginica,* observed, in infected oysters, the transparency of the visceral mass through which the sporocysts could be seen. The sporocysts were confined to the gonad region. Sperm and eggs were absent. McCrady stated that infected oysters did not appear sick or weak and that an infected oyster was probably freed from the parasite with the onset of winter.

Huet (1889) reported *B. haimeanus* in *Cardium edule.* He observed the unhealthy appearance of the visceral mass of infected specimens, and concluded that the parasites probably caused the death of the hosts and then escaped into the water.

Kelly (1899) described gonad destruction in freshwater clams infected with sporocysts of *B. polymorphus.* He also noted changes in shell form, and damage to the kidney in heavily infected specimens. His general conclusion was that bucephalid infections caused parasitic castration.

Cary (1907) noted that the appearance of the visceral mass in an infected oyster from the Louisiana oyster beds is similar in appearance to that of an oyster laden with well-developed sexual products. However, he pointed out that the milky appearance was due to the sporocyst ramifying throughout the visceral mass and not to sexual products.

Tennent (1906) made observations on experimental oyster beds in Newport River, North Carolina, U.S.A. A high mortality of oysters occurred following a summer of freshets in the river and Tennent concluded (p. 682), "... that the presence of the cercaria seems to render the oyster less capable of withstanding adverse conditions. While conditions conducive to the well-being of the oyster prevail, the presence of the cercaria does not seem to cause any great mortality. ... Even during the best of conditions the parasite must be considered as injurious, since it prevents the formation of sexual products."

Woodhead (1930) noted that in light infections of *B. elegans* in *Eurynia iris* the gonad continued to produce eggs over 75% of its area, but in heavy infections the gonad was entirely replaced. He also considered that *E. iris* could maintain the infection for at least two winters.

Roughley (1933) found either no sexual products or only a few degenerating eggs in five *O. commercialis* and one *O. angasi* infected by a bucephalid which he did not describe.

Ozaki and Ishibashi (1934) described the cercaria *B. margaritae* liberated from *Pinctada martensi,* the Japanese pearl oyster. They stated of the sporocyst (p. 440) that " . . . this parasite is a terrible parasite of the pearl oyster and was having serious effects on the formation of the pearl." The parasite first affected gonad and liver, then spread to mantle, gills, palps and adductor muscle.

Andreu (1949) reported *Bucephalopsis haimeana* in the ovary of *Tapes aureus* and noted the destruction of the gonads. He stated the parasite occurred in May, but not in March or June.

Kniskern (1952) found the gonad of *Lampsilis siliquoidea* completely invaded and destroyed by the sporocysts of *Rhipidocotyle septpapillata* in " old and heavy infections ", but he stated (p. 322) that " In light or moderately heavy infection areas of normal gonadial tissue remained to actively produce ova and sperm."

Menzel and Hopkins (1955) gave details of the growth of one specimen of *Crassostrea virginica* (under natural conditions) parasitised by *B. cuculus.* They found that the oyster increased in weight from 59.0 to 227.0 grams over the 16 month experimental period, but failed to state whether the oyster became infected before or after the experiment commenced. The oyster was less than two years old when it became infected. The authors stated (p. 341) " The effects of *Bucephalus cuculus* infection are not so well known. From personal observations on many bucephalus-infected oysters we know that the sporocysts are confined to the gonad in the early stages of the infection. The gonad tissues are eventually destroyed so that oysters with well-developed infections never produce eggs or sperm." They point out that, initially, bucephalid infections may cause increased growth, but after completely replacing the gonad tissues, the sporocysts spread to mantle, gills, digestive glands and the adductor muscle and impair normal functions.

Millar (1963) carried out mortality experiments on a sample of *O. lutaria* from New Zealand. He found that of the oysters that died, 67.3% were infected with sporocysts of *B. longicornutus,* and only 15% of those that remained alive were infected.

Cheng and Burton (1965) found that the initial site of infection of young sporocysts of " *Bucephalus* sp." in *Crassostrea virginica* from Rhode Island (USA) was the pyloric caeca (digestive gland) rather than the gonads. This contrasted with the condition reported in *C. virginica* parasitised by *B. cuculus* further south along the Atlantic coast, and was the authors' reason for maintaining that the species they were dealing with was distinct from *B. cuculus.* (It should be noted that use of the generic name *Bucephalus* for these species may not be correct, *vide* Howell, 1966.) They also found that there is no increase in the number of amoebocytes in parasitised gonads or pyloric caeca.

DESCRIPTION OF THE EFFECTS OF *Bucephalus longicornutus*

Oysters from Foveaux Strait were obtained between June and October 1963, and March and July 1964. Wherever possible infected oysters were compared with uninfected oysters of the same size and from the same locality.

The appearance of the visceral mass of uninfected *O. lutaria* is dependent on the state of the gonad. When the gonad is well-developed and sexual products are differentiated, it is milky white. After spawning, as stated by Hollis (1963, p. 12), the gonad is "flabby, watery and translucent . . . which enables the brownish diverticular tubules to be observed." This latter condition persists until the next sexual phase commences. The condition of the gonads during the time the visceral mass appears milky white varies considerably. Hollis recognised five types of sexual phases in *O. lutaria*. The only one of these phases that was recognised in infected oysters by the author was "hermaphrodite individuals containing ripe sperm and ova." (Text-fig. 3, B). For comparative purposes a brief description of an uninfected hermaphrodite individual is given. The visceral mass is covered by a thin epidermis. Immediately internal to this are the hermaphrodite follicles containing sperm and ova. In section these may be ovoid, circular or sausage-shaped, and vary between 100 and 750μ long and between 100 and 350μ wide. Pyloric caeca (diverticular tubules) are located deeper in the visceral mass. These are generally circular, ovoid or cross-shaped in section. They are smaller than the hermaphrodite follicles, being between 50 and 300μ long by 60 to 150μ wide. Portions of alimentary tract are found in the central regions of the visceral mass. The connective tissue supporting all the above structures (Text-fig. 3, D) consists of polygonal cells each with a large, clear vacuole and only a small amount of cytoplasm, often mostly peripheral in position. The nucleus of these cells is spherical, approximately 4 to 6μ in diameter, usually containing one, but occasionally two nucleoli and several smaller, randomly dispersed granules of chromatin.

One infected oyster examined in each of the months of June, July and September 1963, and five examined in March, four each in April and May and two in July 1964, were comparatively lightly infected, judged by the small number (10 to 20) of sporocyst tubules visible through the epidermis of the visceral mass. The visceral mass appeared milky white due to the presence of both follicles and sporocysts. The oysters were plump and appeared to be in good condition. Sections showed that hermaphrodite follicles were present in all specimens and these contained mature eggs and sperm; but tubules of sporocysts were five to 10 times as common. Sporocyst tubules were more common near the dorsal region of the visceral mass. Connective tissue in the immediate vicinity of a sporocyst showed signs of necrosis with deformation of cells and some condensation of chromatin into small, irregularly shaped masses within the nuclei. Hermaphrodite follicles adjacent to sporocyst tubules were partially or completely collapsed, and contained either a few degenerating eggs and sperm or none.

Very large numbers of sporocysts were visible in the visceral mass of all but three of 123 infected oysters examined between June and October 1963, and in all but 15 of 119 examined between March and July 1964 (Text-fig. 3, A; Plate 1 A). These oysters were classified into two groups depending on the macroscopic appearance of the visceral mass. Those in which the visceral mass appeared milky white due to the large number of sporocyst tubules visible through the epidermis were classified as moderately heavily infected. In many of these the connective tissue, near the dorsal region of the visceral mass in particular, appeared gelatinous and translucent. Collapsed or degenerating genital follicles were only rarely encountered in sections of oysters in this group. Moderately-heavily infected oysters formed the predominant group of infected oysters examined between April and June 1964.

The remainder, classified as heavily infected, were characterised by a yellowish tinge and by reduced size and gelatinous appearance of the visceral mass. The sporocysts, which were closely interwoven, conspicuous and retained their milky white appearance when teased out of the visceral mass, were found to have completely replaced the genital follicles (Text-fig. 3, A). Oysters of this group

comprised the majority of infected oysters examined between June and October 1963 and during July 1964.

The adductor muscle of heavily infected oysters showed signs of weakening. Individuals held in finger bowls gave a slow response in closing the valves when disturbed, were relatively easy to open, and the valves could be readily moved against each other. By comparison, the valves are generally immovable in uninfected, lightly infected and moderately infected oysters.

On three occasions small fish (*Tripterygion* sp.), about 3.5cms total length, were found dead and decaying within the mantle chamber of heavily infected oysters which died in the aquaria. Presumably, the slow reaction of the adductor muscle contraction enabled the fish to gain entry. Furthermore, on several occasions, small shrimps (*Palaemon affinis* Milne Edwards) were observed probing between the two valves of living, but gaping, oysters which proved to be heavily infected on subsequent opening.

In moderately-heavily and heavily infected oysters, sporocysts were found growing over the pericardium and among the pyloric caeca. The appearance of the connective tissue of the visceral mass was similar in sectioned material from both these groups (Text-fig. 3, C). Cells were grossly deformed. In place of readily recognisable large, individual, vacuolated cells, clearly set off from one another, the appearance was that of a syncytium with continuous cytoplasm and scattered nuclei, some of the latter showing marked necrotic changes in the condensation of chromatin. There were also scattered particles of chromatin in the cytoplasm. Phagocytes were common amongst the degenerating tissue and they contained chromatin inclusions, presumably debris of connective tissue nuclei, in their cytoplasm.

In sectioned material, no obvious signs of degeneration of the pyloric caeca were observed. However, the yellowish appearance of the visceral mass in heavily infected oysters may result from liberation of pigment from pyloric caeca which are in a state of degeneration or have degenerated.

The state of infection, determined as outlined above, in all infected oysters examined during the course of the study, is represented graphically in Text-fig. 4. The most noticeable feature is the relative abundance of heavily infected oysters between June and October 1963, their absence from March and April samples 1964, and their reappearance in May 1964, with subsequent increase in abundance in June and July 1964. Lightly infected oysters gradually decrease in abundance from March onwards 1964 as moderately-heavily infected, followed by heavily infected oysters become more abundant. It would have been advantageous to have examined samples between October 1963 and February 1964, in addition to having access to larger samples in some instances. However, despite this, seasonality of the parasite is evident with the majority of infected oysters becoming heavily infected by the later months of the year, following the greater abundance of first lightly infected, then moderately-heavily infected oysters in the earlier months of the year.

In all infected oysters examined during the course of this study, three to 10 interribs of a demibranch of one or more of the gills adjacent to the visceral mass exhibited a characteristic swollen appearance for approximately two-thirds of their proximal length (Plate 1, B). Sporocysts of approximately uniform diameter were located in clusters of three to 100, stretching the epithelium on the water canal side of the demibranch (Text-fig. 3, E). In many instances, blind portions of the sporocysts grew away from, and almost perpendicular to, the gill interrib axis and stretched the gill epithelium so that the gill surface in the infected region had a nodular appearance. These sporocysts became interwoven with and indistinguishable from those of the visceral mass at the proximal ends of the gill. Cercariae in

advanced stages of development were more common than germ balls or germinal masses in the lumen of these sporocysts. The connective tissue between the sporocysts was necrotic, and resembled the connective tissue of the visceral mass of moderately-heavily and heavily infected oysters apart from being more sparse and containing fewer phagocytes. Epithelium of the interribs was distorted through stretching, but remained intact, and gill filaments retained their normal appearance.

No specific deterioration of shell in infected oysters was observed. Brittleness of the shell in the visceral mass region of *O. lutaria* was found to be common in both infected and uninfected specimens.

The smallest infected oysters examined during this study measured approximately 5cms in length. This measurement was made on the flat valve being the farthest distance from the hinge to the end of the valve.

The results of mortality experiments are summarised in Table III. For the purposes of statistical analysis, the results have been pooled (similar trends are evident in each experiment). The % of oysters that died that were infected has been compared with the % of oysters that remained alive that were infected using the comparison of two observed %'s test (Dowdeswell, 1959). The difference between these %'s is highly significant. (Combined S.E. = 8.1).

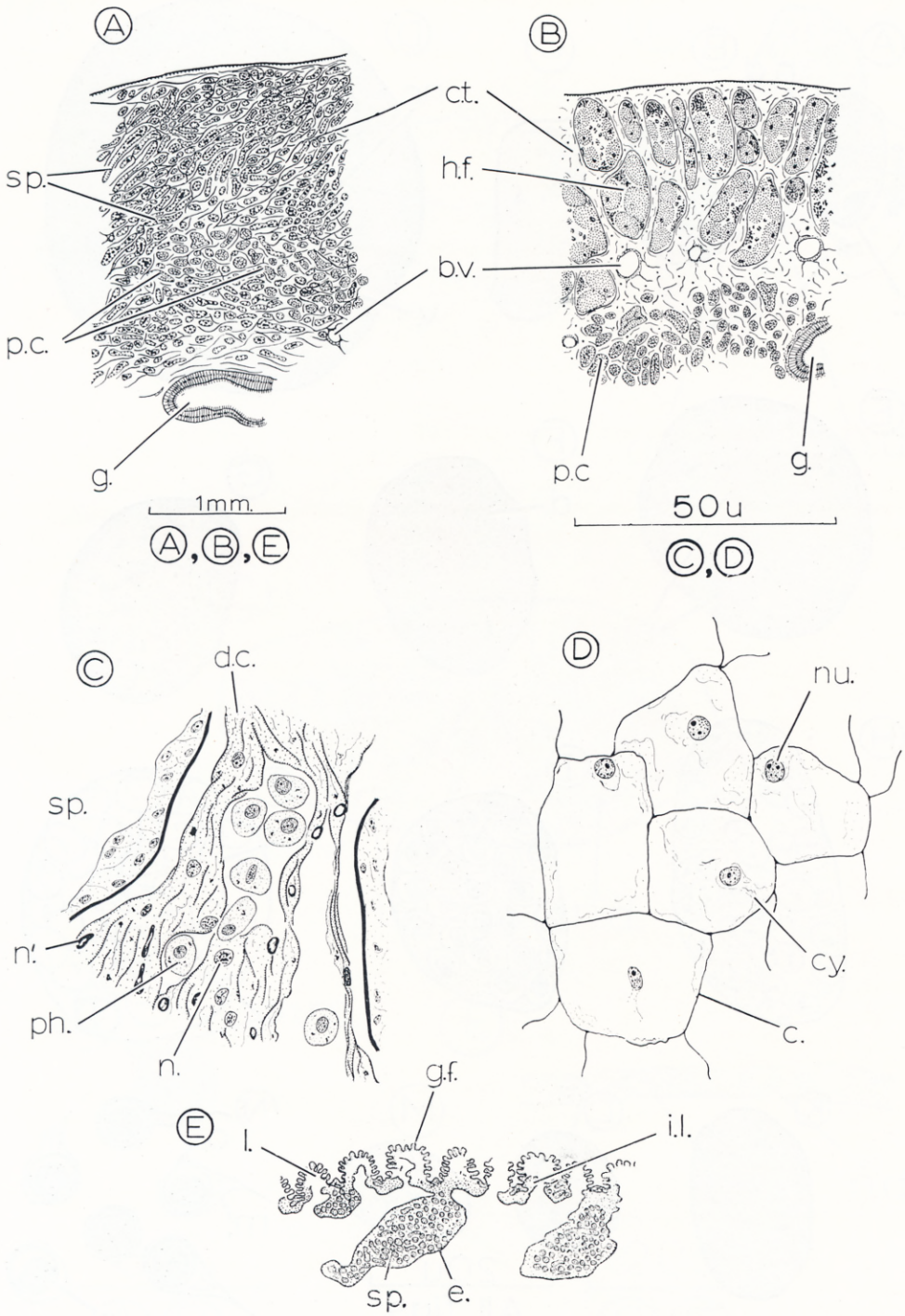
Table III.—Mortality Experiments.

Experiment and No. of specimens	Number that died		Number that remained alive		Length of time in laboratory
	infec.	uninfec.	infec.	uninfec.	
1–46	13	7	5	21	4 months
2–50	12	6	5	27	4 months
3–24	7	5	1	11	4 months
120	32 (64%)	18	11 (16%)	59	

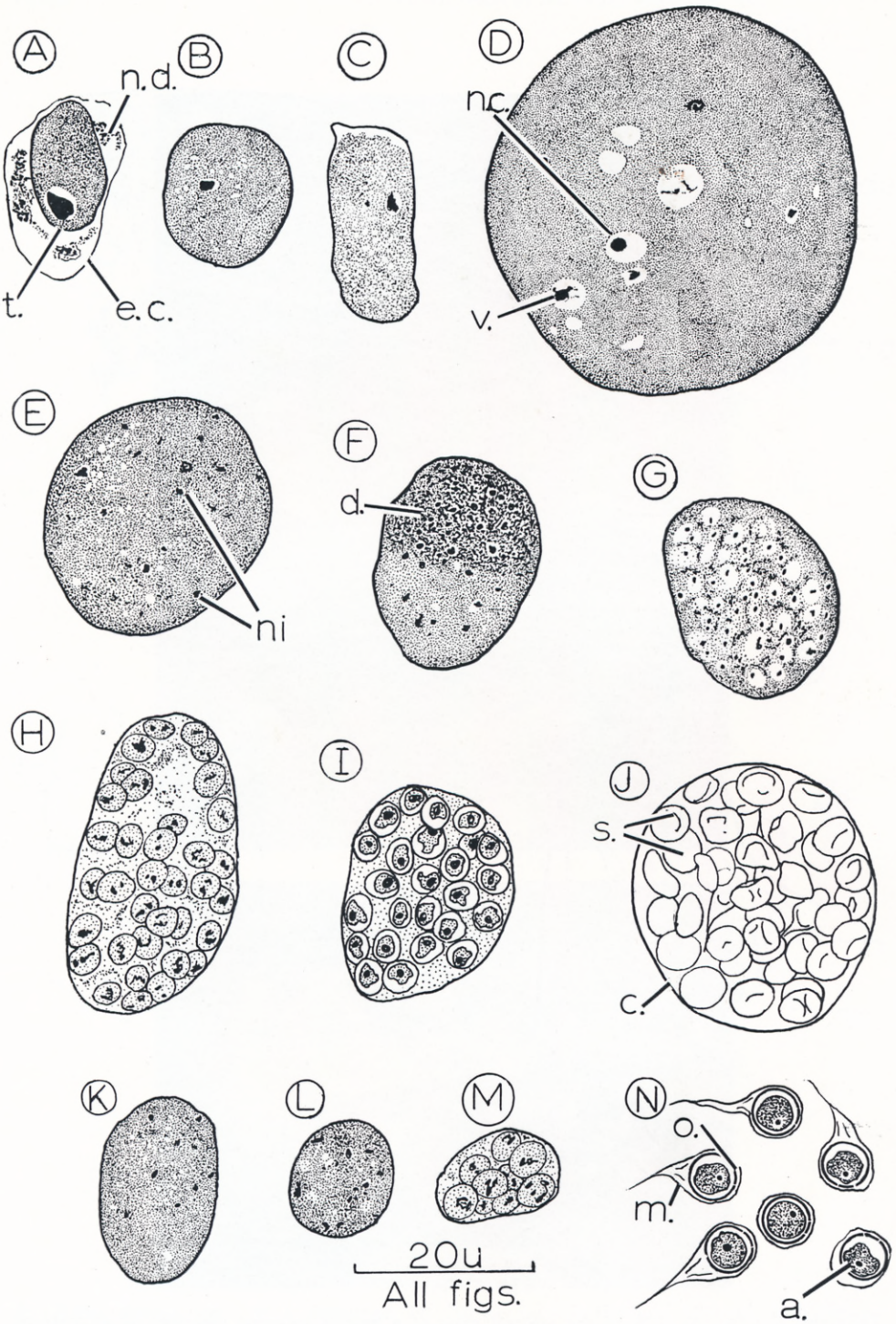
Discussion:

An analysis of the observations of Lacaze-Duthiers (1854), McCrady (1874), Huet (1889), Tennent (1906), Cary (1907), Woodhead (1930), Roughley (1933), Andreu (1949), Kniskern (1952), and Menzel and Hopkins (1955), suggests that a general rule is applicable to the course that bucephalid infections of bivalve molluscs take. The gonad is gradually replaced by sporocysts and, after parasitic castration has occurred, the infection may spread to other organs. This latter phenomenon is especially true of *Bucephalus margaritae* described by Ozaki and Ishibashi (1934), *B. cuculus* described by Tennent (1906), and Menzel and Hopkins (1955). However, an exception to this rule is provided by the observations of Cheng and Burton (1965), who found that the initial site of infection of *Crassostrea virginica* by "*Bucephalus sp.*" was the pyloric caeca, and spread to the gonad occurred later.

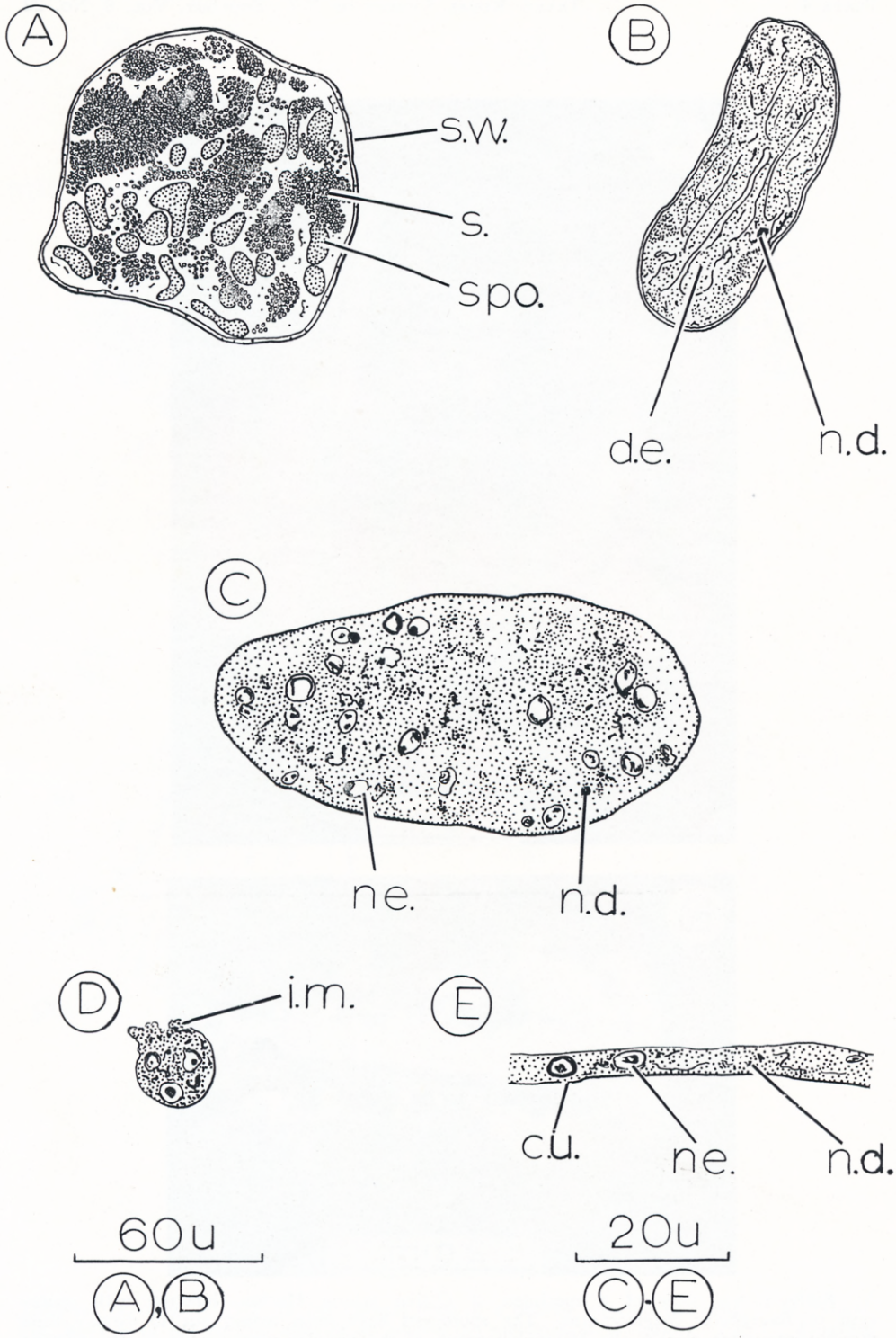
The infection of *O. lutaria* follows a similar course to that outlined above, with gradual replacement of the gonad starting from the dorsal region of the visceral mass, and later spreading at least to the pyloric caeca and pericardium. Sampling between November and February would probably give more details concerning further spread of the infection to other organs. Contrary to the observations of Cheng and Burton (1965), infection of *O. lutaria* by *B. longicornutus* is accompanied by an increase in the number of phagocytes in damaged tissues.



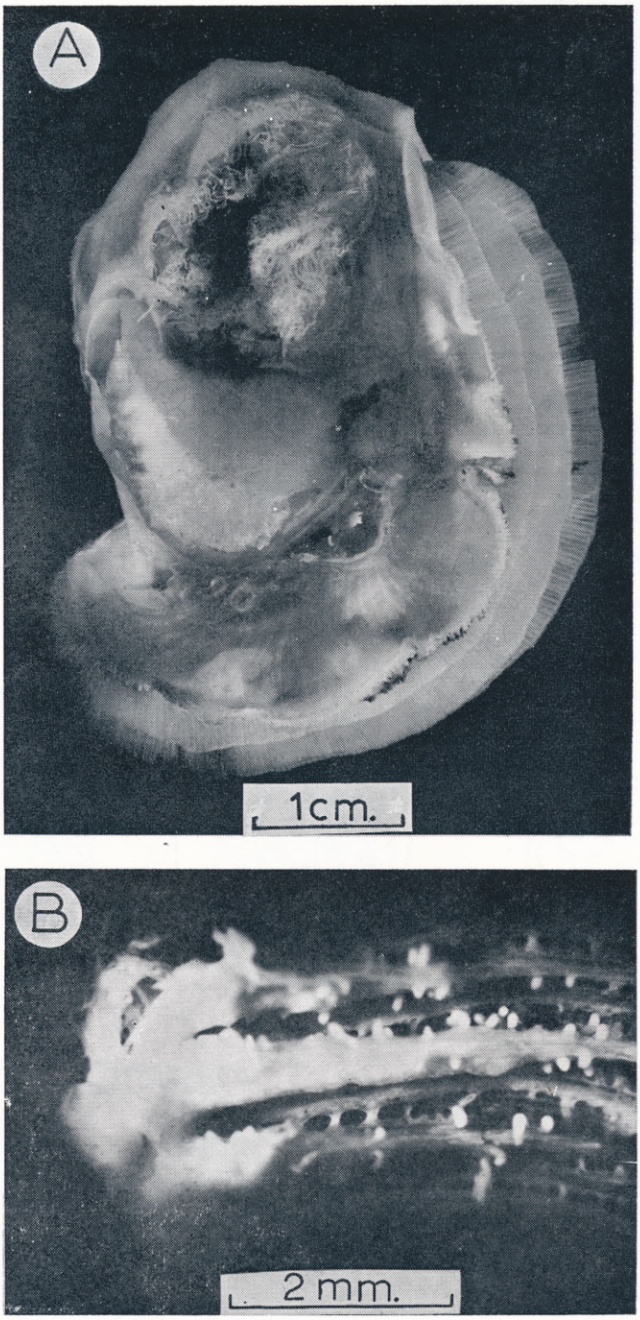
TEXT-FIG. 3.—Fig. A: T.S. of the visceral mass of a heavily infected specimen of *O. lutaria*. Fig. B: T.S. of the visceral mass of an uninfected specimen of *O. lutaria*. Fig. C: Enlarged view of A (above) to show the degenerating connective tissue between the sporocysts. Fig. D: Enlarged view of B (above) to show normal connective tissue. Fig. E: T.S. of a demibranch of gill of an infected specimen of *O. lutaria* showing the clusters of sporocysts swelling the epithelium of the gill interribs.



TEXT-FIG. 5.—*Urosporidium constantae* n.s.p. Fig. A: Trophozoite within a degenerating cercarial embryo. Figs. B and C: Free trophozoites in the sporocyst lumen. Fig. D: Large trophozoite. Fig. E: Multinucleated plasmodium. Fig. F: Plasmodium showing a densely stained region. Fig. G: Pairing of nuclei. Figs. H to J: Stages in sporogony. Figs. K and L: Variably sized plasmodia. Fig. M: Very small cyst containing early stages in sporogony. Fig. N· Free spores.



TEXT-FIG. 6.—*Urosporidium constantae* n.sp. Fig. A: T.S. of an infected sporocyst showing spores and stages in sporogony. Note the absence of embryonic cercariae. Fig. B: T.S. of another region of a sporocyst showing a degenerating mass of embryonic cercariae. Fig. C: T.S. of a degenerating embryo. Fig. D: Degenerating germ ball. Fig. E: T.S. of the wall of an infected sporocyst.



A: Right lateral view of a specimen of *Ostrea lutaria* Hutton, infected with sporocysts of *Bucephalus longicornutus*. The sporocysts have been teased out to display their ramifications more clearly. B: Microphotograph of the nodular areas visible on the efferent water canal surface of the gill of *O. lutaria*.

Right lateral view of a hyperparasitised specimen of *Ostrea lutaria* Hutton, from north of Tasman Bay. The dark sporocyst tubules are those heavily laden with the spores of *Urosporidium constantae* n.sp.

The significance of the sporocysts on the gill interribs is not clear. Their appearance in this region in lightly infected oysters demonstrates that their presence is not necessarily indicative of an advanced state of infection, as has been noted for other bucephalid infections by several authors. The reasons for regarding these sporocysts on the gills as the terminal portions or growing points are given elsewhere (Howell, 1966).

More sampling will be necessary to determine whether lightly infected oysters are capable of spawning. Kniskern (1952) has noted the simultaneous shedding of cercariae and glochidia in *Lampsilis siliquoidea,* infected with sporocysts of *Rhipidocotyle septpapillata.* The presence of mature sperm and eggs in the follicles of lightly infected oysters suggests that spawning may possibly take place before the infection becomes heavy.

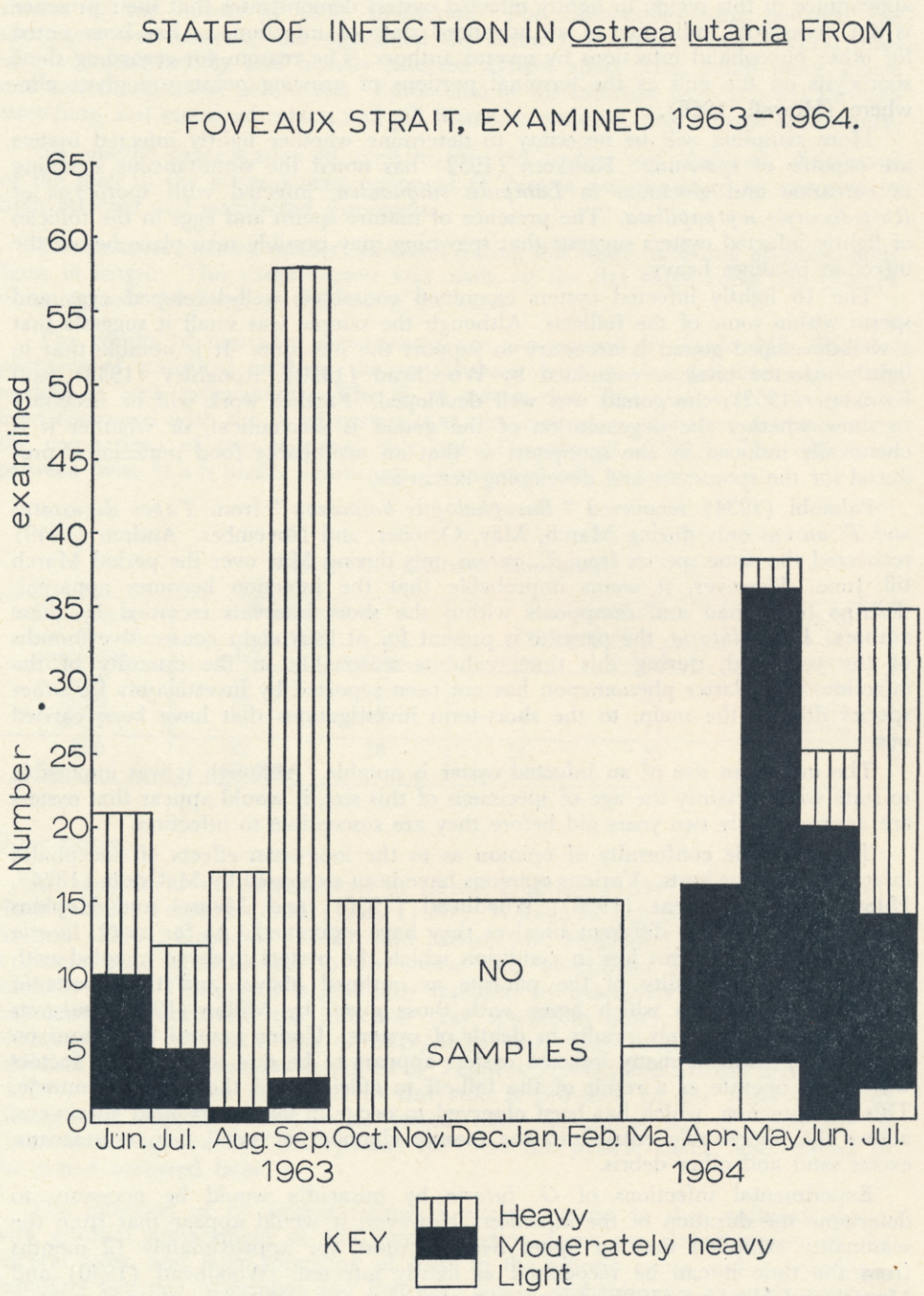
The 18 lightly infected oysters examined contained well-developed eggs and sperm within some of the follicles. Although the sample was small it suggests that a well-developed gonad is necessary to support the infection. It is notable that in lightly infected bivalves examined by Woodhead (1930), Roughley (1933), and Kniskern (1952), the gonad was well developed. Further work will be necessary to show whether the degeneration of the gonad is mechanical, or whether it is chemically induced by the sporocysts so that an assimilable food material is produced for the sporocysts and developing cercariae.

Palombi (1934) recovered "*Bucephalopsis haimeana*" from *Tapes decussatus* and *T. aureus* only during March, May, October, and November. Andreu (1949) recovered the same species from *T. aureus* only during May over the period March till June. However, it seems improbable that the infection becomes apparent, destroys the gonad and disappears within the short intervals recorded by these authors. In *O. lutaria,* the parasite is present for at least eight consecutive months of the year and, during this time, exhibits seasonality in the intensity of the infection. This latter phenomenon has not been reported by investigators for other species due, in the main, to the short-term investigations that have been carried out.

The minimum size of an infected oyster is notable. Although it was impossible to state with certainty the age of specimens of this size, it would appear that oysters are approximately two years old before they are susceptible to infection.

There is little conformity of opinion as to the long-term effects of bucephalid infections on their hosts. Various opinions have been expressed by McCrady (1874), Huet (1889), Tennent (1906), Woodhead (1930), and Menzel and Hopkins (1955), based on the different bivalves they have examined. As far as *O. lutaria* is concerned, the serious loss in condition which the oysters undergo coupled with the apparent seasonality of the parasite as outlined above, and the results of mortality experiments which agree with those given by Millar (1963), suggests that infection ultimately results in death of oysters. Under natural conditions on oyster beds, death of many infected oysters appears to be due to secondary factors which can operate as a result of the fall-off in efficiency of the adductor muscle. This phenomenon, which has been observed to occur in laboratory held specimens, allows the undesirable entry into the mantle chamber of small fish, crustaceans, excess sand and other debris.

Experimental infections of *O. lutaria* by miracidia would be necessary to determine the duration of the infection. However, it would appear that from the seasonality exhibited that an oyster lives, at most, for approximately 12 months from the time it can be recognised as lightly infected. Woodhead (1930) and Menzel and Hopkins (1955) have contended that the infected bivalves they have examined harbour infections for considerably longer periods of time than that suggested above for the present species. However, they have not supplied any statistical data to support their views.



TEXT-FIG. 4.—State of infection in *Ostrea lutaria* from Foveaux Strait, 1963-64.

The possibility of an oyster recovering from an infection was considered, but abandoned for several reasons. First, there is no significant appearance of lightly or moderately-heavily infected oysters between August and September 1963 following the predominance of heavily infected oysters in June and July 1963. Secondly, the predominant group of moderately-heavily infected oysters between April and June 1964 gives way to a predominance of heavily infected oysters by July 1964, and not to lightly infected oysters. The latter would be expected if recovery was occurring. Thirdly, no infected specimen of *O. lutaria* examined had sporocysts which were entirely empty and which appeared to be in the process of being resorbed or shed.

The above observations on the effects of the parasite lend support to the view that the decline in catch/effort, which has become particularly noticeable in the Foveaux Strait oyster beds over the last few years (see p. 2), is largely due to the high incidence of *B. longicornutus.* However, in the absence of data concerning the oyster population in Foveaux Strait, and many details of the biology of *O. lutaria,* a more critical examination of this aspect cannot be made at the present time.

HYPERPARASITISM OF THE EMBRYONIC CERCARIAE

The embryonic cercariae in the sporocysts of four infected oysters recovered from north Tasman Bay in April 1964, were hyperparasitised by a haplosporidian. Many of the sporocysts were rich brown in colour and dilated to approximately twice their normal diameter (Plate 2). Sections of the whole visceral mass of two of these oysters showed that all embryonic cercariae within the sporocysts lumen were degenerating or had been entirely replaced by spores or stages in sporogony of the hyperparasite.

Class: SPOROZOA Leuckart, 1879.

Sub class: AGNIDOSPORIDIA Cepede, 1906.

Order: HAPLOSPORIDIA Caullery and Mesnil, 1905.

This order comprises sporozoans which produce simple spores without polar filaments. The Haplosporidia are cytozoic, histozoic or coelozoic parasites of invertebrates and lower vertebrates. The terminology used follows that of Kudo (1954).

Genus: UROSPORIDIUM Caullery and Mesnil, 1905.

Haplosporidia in which the spores are spherical and operculate with a single long projection.

UROSPORIDIUM CONSTANTAE n.sp.

(Text-fig. 5, A-N; 6, A-E)

TYPE HOST: *Bucephalus longicornutus* (Manter, 1954) sporocysts.

TYPE LOCALITY: North of Tasman Bay, 173° 30′E 40° 10′S, ca., 100 fathoms.

HOLOTYPE AND PARATYPES: Dominion Museum, Wellington, New Zealand, numbers Prot. 1 and Prot. 2 respectively.

Trophozoites ovoid to spherical, ranging from 10 to 45μ long by 9 to 43μ wide; each with a conspicuous ovoid nucleus ranging from 2 to 6μ long by 1 to 4μ wide and containing generally one irregularly shaped mass of chromatin. Cytoplasm densely granular, generally vacuolated, with some affinity for haematoxylin. Vacuoles variable in size, many containing small irregularly shaped masses of chromatin. Rarely, cytoplasm differentiated into hyaline

ectoplasm with pseudopodia apparent. Spores (Text-fig. 5, N) drop-shaped, consisting of spherical operculate portion, 4 to 5μ in diameter, and containing an irregularly shaped amoebula, surrounded by a thin membranous envelope which is drawn out as a thin filament 10 to 12μ long, directly opposite operculum. Filament curved, straight or irregular. Nucleus of amoebula ovoid to spherical, 1μ in diameter, containing one spherical mass of chromatin which occupies one-third nucleus volume. Cytoplasm of amoebula densely granular.

DISCUSSION:

Three other species of *Urosporidium* have been described: *U. pelseneeri* (Caullery and Chapellier, 1906), *U. fuliginosum* Caullery and Mesnil, 1905, and *U.* sp. Guyenot, 1943. *U. constantae* differs from *U. fuliginosum* in that the spore filament is shorter by 3 to 5μ and the membranous envelope has no ridge extending back from the filament over the spherical portion of the spore; from *U. pelseneeri* and *U.* sp. in that the spore filament is longer by 3 to 5μ.

The specific name *constantae* is the genitival derivative of *Constanta,* the Rumanian fishing vessel which collected the material.

Trophozoites of *Urosporidium constantae* undergo growth inside embryonic cercariae causing complete degeneration, and are eventually liberated into the lumen of the sporocyst (Text-fig. 5, B, C, D). Several divisions of the trophozoite nucleus occur so that a multinucleated plasmodium is formed (Text-fig. 5, E). In general, there is no significant change in cytoplasmic detail at this stage. However, in some plasmodia, parts of the cytoplasm are intensely stained by haematoxylin (Text-fig. 5, F). It appears that division of the trophozoite nucleus leads directly to gametogony and sporogony; schizogony was not clearly recognised in the material examined. Each daughter nucleus within a plasmodium becomes surrounded by a clear, unstained area which eventually is occupied by a less dense cytoplasm than in the remainder of the plasmodium (Text-fig. 5, G). The nuclei pair and a definite membrane encloses each pair of nuclei and their surrounding cytoplasm (Text-fig. 5, H). At this stage, a small amount of residual cytoplasm remains dispersed between these bodies. The binucleated bodies thus formed are generally spherical, approximately 4 to 5μ in diameter, and confined within the cell membrane of the parent plasmodium. The paired nuclei fuse and the cytoplasm retracts from the thin membrane within which it is enclosed (Text-fig. 5, I). The nucleated individuals become the amoebula and the enclosing membrane differentiates into the operculate spore capsule and membranous envelope. In section, spores appear most often to be spherical in shape and the membranous portion is inconspicuous (Text-fig. 5, J). The cyst wall enclosing the spores is the cell membrane of the parent plasmodium. In the material examined, it had generally ruptured and liberated the spores into the lumen of the sporocyst. Cysts vary considerably in size, falling within the same size ranges given for the trophozoites.

Caullery and Mesnil (1905) noted that in *Urosporidium fuliginosum,* the amoebulae grow in the coelom of the host, *Syllis gracilis,* and undergo repeated nuclear division during growth so that the amoebulae omit the trophozoite stage and proceed directly to plasmodia. They also found that cysts were constant in size and spores were arranged radially within the cysts. They did not describe pairing of the plasmodium nuclei but, however, they did recognise a division of the amoebula nucleus prior to the formation of the spore capsule. This division was also observed by Cepede (1911) in *U. pelseneeri.* A similar division was not recognised in *U. constantae,* but the fact that the pairing of the two nuclei results in a relatively large nucleus which decreases to about half its size in a mature spore suggests that a division may take place.

The number of embryonic cercariae within the complete sporocyst system of an infected oyster is enormous, and total infection of these by the hyperparasite raises a number of points which will need clarification by reference to further

material which is, preferably, not as heavily infected with the hyperparasite as that examined by the author. Whether large numbers of spores are taken in initially, or gradually over a period, whether spores are capable of germination in the lumen in which they are formed and thereby infect remaining embryos, or whether schizogony does occur are some of these points.

Schizogony has been recognised in *U. pelseneeri* by Cepede (1911) and may possibly occur in *U. fuliginosum* according to Caullery and Mesnil (1905). In the present species, schizogony would be the most probable method of producing sufficient amoebulae for the infection of all cercarial embryos.

The variations in size of trophozoites and succeeding stages in sporogony is notable. Embryonic cercariae cover a considerable size range and it appears likely that the size of the trophozoites is governed by the size of the embryos which the amoebulae initially infect. More than one trophozoite emerging from a degenerate embryo was not observed.

As stated above, the parasite had caused partial or complete degeneration of all embryonic cercariae within the complete sporocyst system of two infected oysters. The nuclei of degenerated embryos showed marked necrotic changes and had undergone pyknosis and vacuolation (Text-fig. 6, B, C, D). The cytoplasm between the nuclei was densely granular and contained nuclear debris. It was not possible to distinguish amoebulae within degenerating embryos from the large amount of nuclear debris. Trophozoites became apparent in degenerating embryos after most of the nuclear debris disappeared (Text-fig. 5, A).

The sporocyst wall remained intact, but necrotic changes in the disposition of chromatin within mesenchymal nuclei had occurred, and nuclear debris was scattered throughout the wall (Text-fig. 6, E). No developmental stages of *U. constantae* were observed in the sporocyst wall.

Macroscopically, the four oysters examined could be classified as heavily infected with the sporocysts of *Bucephalus longicornutus* (Manter, 1954). However, they did not exhibit the yellowish tinge or translucency of the visceral mass which normally characterises heavily infected oysters. Instead, the visceral mass was milky-white apart from the brownish streaks of sporocysts filled with spores (Plate 2).

The connective tissue of the visceral mass had a syncytial appearance and no genital follicles were observed. No obvious signs of repair or regeneration of tissue were apparent.

The complete destruction by *U. constantae* of the embryonic cercariae may afford a method of biologically controlling the bucephalid infection of *Ostrea lutaria* in the commercially fished oyster beds at Foveaux Strait since total mortality of cercarial embryos would be reflected in fewer definitive hosts becoming infected. This, in turn, would be reflected in fewer oysters becoming infected with sporocysts. *U. constantae* has not been recorded from the 242 infected oysters examined from Foveaux Strait and its absence from this locality would appear to be likely. Thus, the introduction of the hyperparasite into the Foveaux Strait region may present ecological difficulties. Furthermore, the collection of a sufficient number of hyperparasitised specimens from Tasman Bay, to attempt such an introduction, may also prove difficult since oysters do not occur in larger numbers in the locality and the incidence of bucephalid infection was low (8%) in the sample of 54 oysters examined.

SUMMARY

1. The incidence of infection of *Bucephalus longicornutus* sporocysts in *Ostrea lutaria,* from seven localities in New Zealand, is given. The incidence within areas A and B, Foveaux Strait, is approximately 40% while in other areas it is approximately 20%. These different incidences in Foveaux Strait can be correlated with the type of substrate in the respective areas.

2. The effects of the parasite of *O. lutaria* are described and compared with what is known regarding bucephalid infections in other species of bivalve molluscs. Infected oysters can be divided into three categories depending on the macroscopic appearance of the visceral mass: in lightly infected specimens it is milky-white due to the presence of both hermaphrodite follicles and a few sporocysts; in moderately-heavily infected specimens it is milky-white (but occasionally gelatinous near the dorsal region) due entirely to sporocysts; and in heavily infected specimens it is yellowish, gelatinous, and reduced in size. The adductor muscle becomes less effective as the infection becomes heavier. Seasonality in the intensity of the infection and the results of mortality experiments show that death of a majority of infected oysters occurs although it is considered that death may only be indirectly caused by the effects of *B. longicornutus* in many instances. It is suggested that *B. longicornutus* may be responsible for the decline in catch/effort for the Foveaux Strait oyster beds.

3. A haplosporidian hyperparasite, *Urosporidium constantae* n.sp., is described from sporocysts of infected oysters obtained from north of Tasman Bay. It is found to cause total mortality of cercarial embryos. The possibilities of *U. constantae* as a biological control for the parasite in Foveaux Strait may present ecological difficulties and collection problems from the type locality.

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