Life Cycle and Ecology of *Marteilia sydneyi* in the Australian Oyster, *Crassostrea commercialis*

PETER H. WOLF

Peter H Wolf is with the Scientific Section, New South Wales State Fisheries, 211 Kent Street, Sydney, N.S.W 2000, Australia.

ABSTRACT–Moribund oysters, Crassostrea commercialis, *from Moreton Bay, Queensland, and Evans River, New South Wales, Australia, revealed the presence of a haplosporidian parasite,* Marteilia sydneyi, *similar to* Marteilia refringens *found in French oysters. The life cycle and structure of the Australian pathogen is considered herein and observations of the effects of oyster age, salinity, and temperature are presented. Observations indicate that elevating oysters within the tidal range may lessen the impact of the disease in those populations.*

Introduction

After a particularly heavy outbreak of oyster mortalities in Moreton Bay, Queensland, in 1969-70, moribund specimens of *Crassostrea commercialis* were collected for examination in the Sydney laboratories of New South Wales State Fisheries Department. Some of these specimens were found to be heavily invaded by a microparasite, believed to be a haplosporidian, and at the time named QX (Wolf, 1972). Later it was described by Perkins and Wolf (1976) as *Marteilia sydneyi*. Earlier, vague and unconfirmed reports from estuaries in New South Wales indicated that oyster mortalities had occurred previously, without being brought to the attention of the Fisheries Department at that time.

Since Moreton Bay is only about 60 km from the northernmost New South Wales estuary of the Tweed River (Fig. 1), it was suspected that the mortalities in the latter State could well have been caused by the same organism. This assumption was confirmed in 1973 by an examination of a large number of infected and moribund oysters from the Evans River, about 150 km south of the Queensland border, following an epidemic outbreak of oyster mortalities in that locality which resulted in 80 percent losses of the crop. These latter

Figure 1.—Map of the study area.

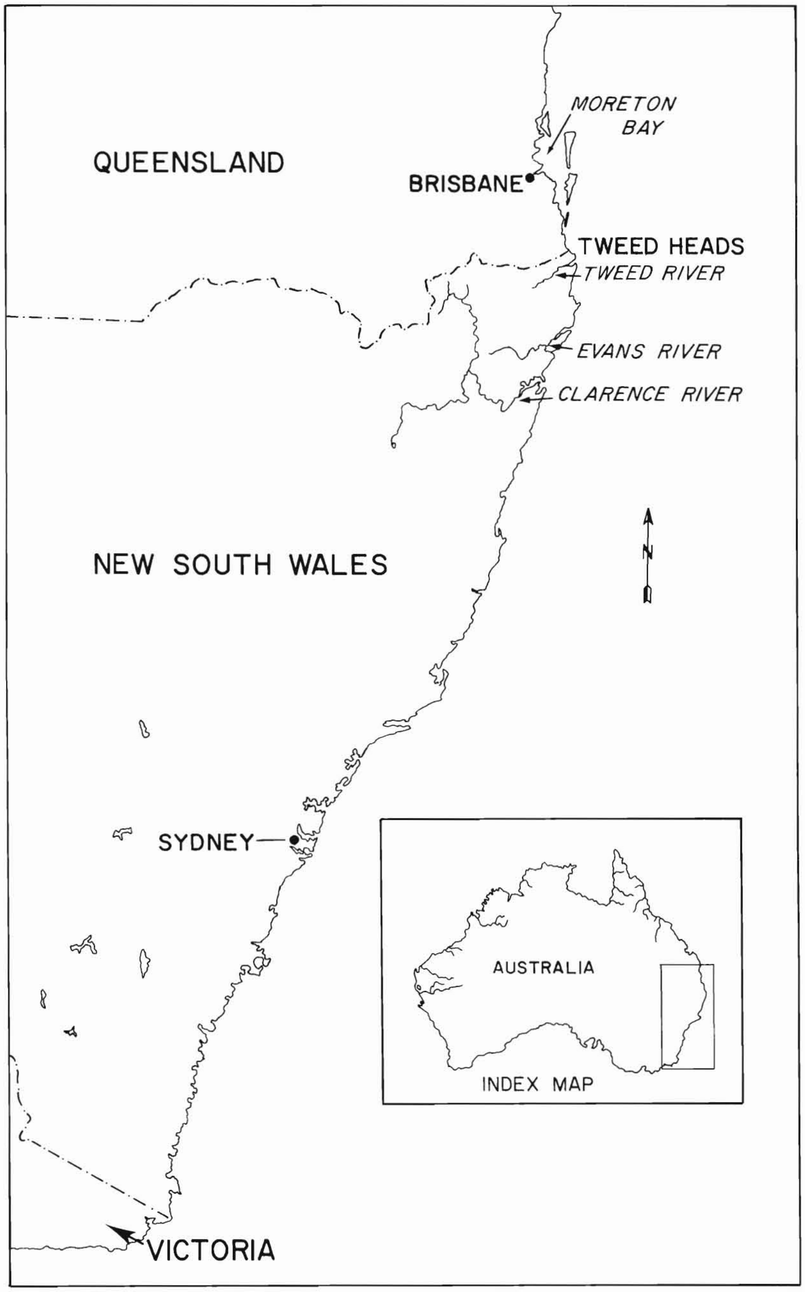

Figure 2.—*Marteilia sydneyi*. Interference contrast micrograph taken from live smear. Unlabeled arrows in Figures 2 and 3 indicate sporangia. Developing spore(s). 1,000 ×.

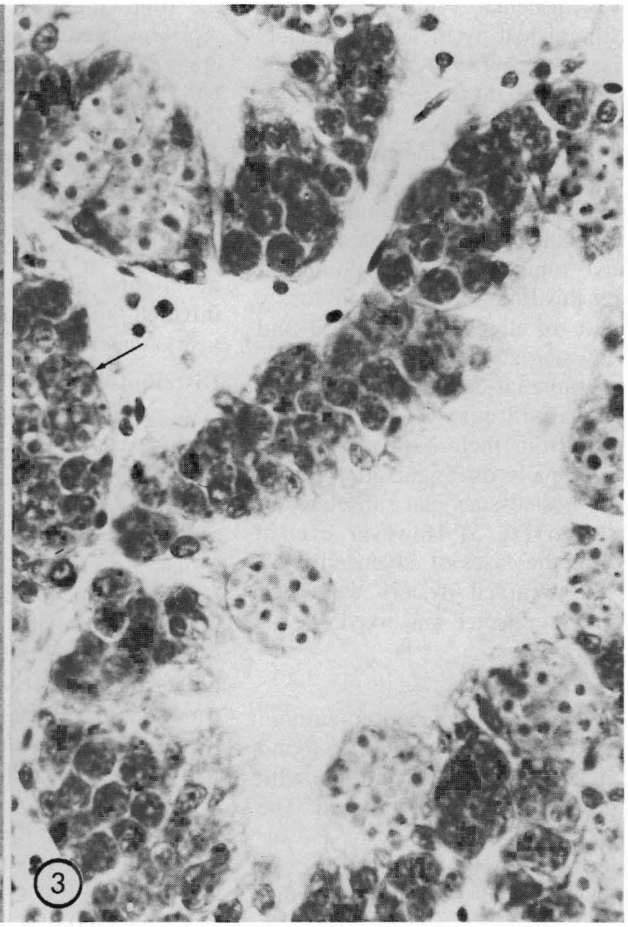

Figure 3.—*Marteilia sydneyi*. Extensive invasion of epithelial cells of the digestive glands by the parasite. 150 ×.

observations have now added considerably to the knowledge of *M. sydneyi,* the available details of which are here recorded.

Materials and Methods

In addition to histological sections obtained after routine fixation in Davidson's fluid, transverse cuts through the digestive diverticulae of freshly opened oysters, followed by rubbing the cut surface against a glass slide, provided living cells of the parasite for interference light microscope observations (Fig. 2). This enabled an efficient and fast method of diagnosis, replacing the time-consuming conventional histological approach. During an epidemic in the Tweed River in 1975, live material was fixed in glutaraldehyde for electron microscope studies and flown to the Virginia Institute of Marine Science where Frank Perkins was able to describe and taxonomically define the newly found species of *Marteilia*.

Results and Discussion

Morphology

Sprague[1] had already indicated that the microparasite was similar to or identical with a species recorded from France (Comps, 1970) by the possession of two spherical spores per membrane-limited body. Comparisons with sections of the French parasite showed that, at least in histological preparations, the form of the bodies and refringent inclusions were dissimilar to our material.

Since then, light microscope observations have revealed more about the life cycle of the oyster parasite. Its mode of entering the host is still uncertain. It could well be that it enters by way of the gills, but this is by no means fully established. The cells develop around or in the digestive gland epithelia and appear to divide into 2, 4, 8, 16, or possibly more, spores (Fig. 3). At this stage the spore packets stain

[1]Sprague, Victor. University of Maryland Center for Environmental and Estuarine Studies, Chesapeake Biological Laboratory, Solomons, MD 20688. Pers. commun.

darkly with hematoxylin and can only be distinguished by their form. Later, the first refringent bodies become visible, staining bright red in eosin. Lastly, each membrane-limited body reveals two spherical spores and many (up to 20) circular refringent bodies. The membrane appears to open and the contents are shed into the lumen of the digestive tubules and surrounding tissue. By this time the host is obviously incapable of digesting any food and death by starvation seems inevitable.

The epithelial cells of the digestive tubules disintegrate after being loosened from their basal membrane and a complete disorganization of the infected host tissues can subsequently be observed (Fig. 3). However, even in these extreme cases of advanced invasion, the sampled oysters were still alive when collected and fixed.

Condition of Affected Oysters

With one notable exception (autumn 1977), all infected oysters were found to be in extremely poor condition with their gonads completely resorbed. The body of such a diseased oyster is usually shrunken to about one-third, or in extreme cases two-thirds, of its normal size. The adductor muscle, however, remains fully functional so that the shell is very difficult to open. Since the gonads are resorbed, the underlying digestive diverticulae can be seen clearly, giving the animal a translucent appearance. Blockage of the digestive glands occurs and the animals can be expected to slowly starve to death.

Material collected in 1977, on the other hand, revealed clearly visible gonads even though in a state of regression. It seems that on this occasion, when the collected specimens showed an overall infection of 79.8 percent, and the mortality was similarly at 80 percent, the invasion by the parasite must have been massive and rapid.

Incubation Period

Following regular samplings of material during 1977, it can now be said that the development period of the parasite is less than 60 days from invasion to death of the host. While samples in mid-February did not show the presence of *M. sydneyi* in about 80 oysters, the following lots, collected in mid-April revealed infection levels at an average of 79.8 percent, with some oysters from particular areas being 100 percent infected.

Age of Affected Oysters

No preference by the parasite for older or younger oysters could be observed. Small (1-year-old) oysters with developing gonads were found to be infected just as much as older (2-3 years) specimens.

Distribution of *M. sydneyi*

Judging from its type locality (Keppel Bay, lat. 23°S), *M. sydneyi* could well be restricted to tropical and subtropical regions. Despite the transportation of commercial oyster stocks from estuary to estuary, as a result of our cultivation methods, the haplosporidian has not yet been reported or observed south of the Evans River (approx. lat. 29°S). Its hitherto known distribution makes it feasible to assume that it could invade, apart from *Crassostrea commercialis,* also our tropical species *C. echinata*. However, in view of the fact that this latter species is hardly cultivated, and that regular sampling in Queensland is difficult, epidemic mortalities of the tropical oyster caused by the microparasite would be hard to discover. Cells resembling *M. sydneyi* have been found in only one specimen of *C. echinata*.

Temperature and Salinity

There seems to be no one season in which the parasite is more likely to express itself. During the past 7 years the northern estuaries in New South Wales experienced epidemics in mid-winter (July-August 1973), in spring (October 1974), in late summer (February-March 1975), and most recently in autumn (April-May 1977). The lowest water temperatures in these estuaries are about 14°C in mid-winter, and the highest around 30°C in mid-summer. On some occasions, water temperatures of up to 32°C have been recorded, and the assessed annual mean temperatures are 22.2°C. Temperature, as well as salinity values in all rivers, has been recorded since 1964-66, and the average readings per month are about 25, or 300 readings per year.

Possible Solutions to the Disease Problem

The Australian oyster industry, unlike its intertidal-subtidal counterpart in the United States, is an entirely intertidal and off-bottom culture with oysters growing on sticks or trays placed on racks at about mid-tide level. In the latitudes of New South Wales the tidal amplitude is around 1.8 m (6 feet). The growing height is uniform in each estuary and the oysters are regularly exposed at low tide for several hours per day.

A number of observations seem to indicate that oysters which are placed higher in the intertidal zone are less likely to become infected. We intend to test this hypothesis by obtaining quantitative information from controlled experiments with racks of variable height.

It also has been ascertained that wild stock, collected on mangroves and boat ramps, is just as vulnerable to invasion by the parasite as cultivated stocks. Unlike other conditions detrimental to oysters, *M. sydneyi* displays no preference for cultivated stocks.

Since 20 percent of all oysters, examined after occurrence of the massive epidemic caused by the haplosporidian in April 1977, showed no signs of infections, it can be assumed that some oysters possibly are resistant. Although it is not yet clear whether such a possible resistance is acquired or genetically based, it is felt that selective breeding for resistance might be a solution to this problem and should be attempted.

[Note added in proof. In April 1978, *Marteilia sydneyi* was found associated with significant mortalities of oysters in the Clarence River (ca. lat. 28°30'S) which is south of the limit previously recorded.]

Literature Cited

Comps, M. 1970. Observations sur les causes d'une mortalité anormale des huitres plates dans le Bassin de Marennes. Rev. Trav. Inst. Pêches. Marit. 34:317-326.

Perkins, F. O., and P. H. Wolf. 1976. Fine structure of *Marteilia sydneyi* sp. n.—haplosporidan pathogen of Australian oysters. J. Parasitol. 62:528-538.

Wolf, P. H. 1972. Occurrence of a haplosporidan in Sydney rock oysters (*Crassostrea commercialis*) from Moreton Bay, Queensland, Australia. J. Invertebr. Pathol. 19:416-417.