

Marteilia refringens and Crassostrea gigas

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Introduction

In the coastal waters of Brittany there are two commercially significant species of oysters, the European flat oyster, *Ostrea edulis*, and the Japanese oyster, *Crassostrea gigas*. Aber disease, caused by *Marteilia refringens* and considered in detail in this symposium, appeared until very recently to be completely absent from *C. gigas*. Now there is some doubt.

While significant mortalities due to the increase of Aber disease in *O. edulis* parks of certain Breton regions was occurring, substitute plantings of *C. gigas* were initiated by oyster growers. During the past several months we have been examining oysters from those plantings for the presence of parasites. In May 1977 standard histological tests clearly showed that parasitic cells were present in the epithelium of the digestive tract of *C. gigas*. Those cells are the subject of this presentation.

Materials and Methods

For light microscope studies of the parasite, fixations were accomplished in formaldehyde with post fixation in Bouin's fixative. Tissues were embedded in paraffin, sectioned at 7 μm , and stained in hematoxylin and eosin.

Samples of 10 oysters each were obtained for histological study from the following locations in France: Morlaix

ABSTRACT—In May 1977 parasitic cells were found in the digestive system of *Crassostrea gigas* collected in Brittany, France. The parasites are restricted to the epithelial cells of the oyster's stomach and are similar to the primary cells of *Marteilia refringens* in the early stages of infection of *Ostrea edulis*.

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Bay (Carantec, Plouezoch), Aber Benoît, Brest Harbour (Tinduff), Morbihan Gulf, and Arcachon.

Results

Parasites were first found in the digestive system of *Crassostrea gigas* from Carantec. The cells are localized in epithelium of the stomach, being situated in the apical part of host cells (Fig. 1, 2) or on their surface in the stomach lumen. These parasitic forms are 5-15 μm and contain only a few nuclei around each of which there is a clear halo. The number of parasites in a histological cross section is low. We have counted up to a hundred parasitic forms in a single histological section; however, most often this number is below 50.

Tissues of the oysters containing parasites are sometimes more or less necrotized and occasionally there may be a leucocytic reaction. However, this is not a general rule, and most often the tissues are in good condition, not appearing to suffer from the presence of the parasite.

The percent of oysters containing the parasite is not very high. Only 13 oysters out of the 310 (7 percent), which have been tested since May 1977, have shown parasitic cells. They all came from Carantec (Table 1).

All histological examinations of those coming from Plouezoch, Aber Benoît, Brest, Morbihan, and Arcachon have yielded negative results thus far.

Discussion

The parasitic cells observed in the digestive system of *C. gigas*, are similar to the primary cells of *M. refringens* (Fig. 3) as observed in the early stages of infection of *O. edulis* (Perkins, 1976). Localization in apical part of the epithelial cells of the stomach is the same as with *M. refringens*. The cellular structure is comparable as is the cell size of 5-15 μm . In addition the two to four nuclei are surrounded by a clear halo in both forms. Several speculations can be expressed concerning this *M. refringens*-like organism.

1) The parasitic forms discovered in *C. gigas* may be considered as transient, whose stay does not induce pathogenicity. There appear to be no ill-effects at either the organismic or tissue levels.

Similar observations have been made concerning *M. refringens*-like parasites in *Cardium edule* and *Mytilus edulis* (Comps et al., 1975). In those cases, the infection levels are low (4 percent); however, the parasites showed "old" or sporulating stages along with wider distribution in the digestive tract.

2) It may be assumed that the presence of these parasitic forms shows infection of *Crassostrea gigas* by *Marteilia refringens*. These infections may represent a de novo infection of *C.*

Table 1.—Percentages of infected oysters in samples of 10 *Crassostrea gigas* oysters in different oyster parks of Carantec, 1977.

Sampling areas	Sampling dates				
	4/5	17/5	26/5	29/6	2/8
Parc Sinquin	0	0	20	20	0
Parc Relaz	0	0	20	0	
Parc Georges	10	0	0	0	0
Parc Herry	0	0	0-0	0-0	
Grand Parc	20	0	10	0-0	10
Parc Bas Coat	10	10	0	0	

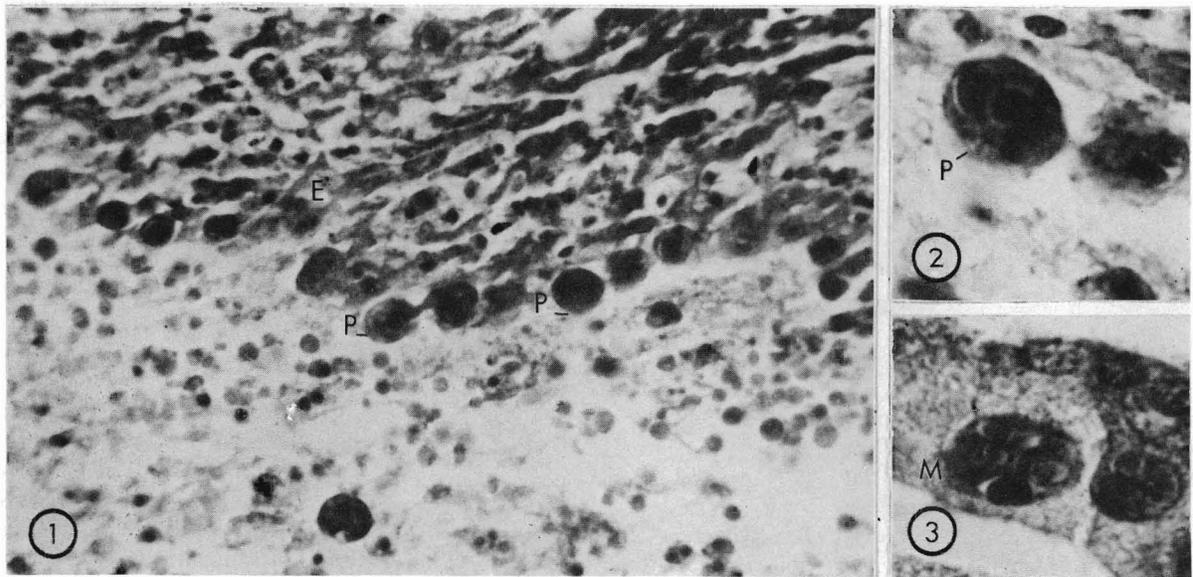


Figure 1.—“Young” parasites (P) in the stomach epithelium (E) of *Crassostrea gigas*. 400×. Figure 2.—“Young” parasites (P) in *C. gigas*. 1,000×. Figure 3.—“Young” forms of *Marteilia refringens* (M) in the stomach epithelium of *Ostrea edulis*. 1,000×.

gigas, because we have never observed advanced (sporulating) stages in the Japanese oyster. The cellular appearance of the *C. gigas* parasite is similar to the parasite of *O. edulis* when the latter is placed in an infested area at the same time of the year. In flat oysters, such cells indicate the outset of an infection. The parasites are “young” or primary cells as described by Grizel et al. (1974) and are situated in the cells of the stomach epithelium and its branches in as many as 45 percent of the oysters. At the beginning of an epizootic, only a low percentage of *O. edulis* contain *M. refringens*.

3) It may be suggested that the parasite of *C. gigas* is also similar to *M. sydneyi*, which is a parasite of the Australian oyster, *C. commercialis*, since the latter parasite described by Wolf (1972) and Perkins and Wolf (1976) is closely related to *M. refringens*. We are not, at present, in a position to critically evaluate such proposed affinities.

It will be essential in the near future to proceed in four areas of study in order to determine the role and identity of the Japanese oyster parasite. Ultra-

structural information is required concerning the substructure of the “young” cells, but is proving to be difficult to obtain because of the paucity of cells. We also need to determine if transmission of infections can be accomplished from oyster to oyster, whether it be from *C. gigas* to *O. edulis* or vice versa. Attempts have been made in our laboratory since July 1977 without success. Infected oysters were held with uninfected ones in aquaria. In natural waters we have been conducting an experiment since June 1977 where *C. gigas* was introduced into a *M. refringens*-endemic area. After 3 months no transmission of infections has been observed. Further epizootiological data will be acquired concerning populations of *C. gigas* in which *M. refringens* has already been observed. It will be important to note whether the incidence of the parasite increases and whether pathogenicity is expressed. Whatever occurs will undoubtedly be linked to the oyster’s resistance, the potential pathogenicity of the parasite, and physical conditions of the environment.

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