Marteilia refringens—Considerations of the Life Cycle and Development of Abers Disease in *Ostrea edulis*

GEORGES BALOUET

ABSTRACT–The parasite of Ostrea edulis, Marteilia refringens, *causes a digestive gland disease which results from a conflict between the parasite and its host with environmental factors collectively regulating development of epidemics. The pathogen has a complex morphology. In its "old" plasmodia, corresponding to sporulation stages, characteristic refringent inclusion bodies can be found. All experiments, conducted to produce infections in the laboratory, have been thus far unsuccessful. In open waters, histologically detectable infections start in July-August, independent of previous exposure time. During the last 3 years of development of the epidemic in Brittany, we have been able to identify high-risk areas, moderate infection areas in equilibrium, and free zones.*

Introduction

Since initiation of epizootic disease of *Ostrea edulis* in Aber Wrach in Brittany (1967) and detection of the causative parasite, *M. refringens* (Grizel, Comps, Bonami, Cousserans, Duthoit, and Le Pennec, 1974), many studies have been conducted, morphological and experimental, to define the taxonomic position of the parasite and its life cycle. In Brest, the Laboratory of Pathology of the Faculty of Medicine has a special interest in epidemiological problems and has carried out since 1974 various experiments in the laboratory and in estuaries and coastal areas to answer questions about *M. refringens*.

Materials and Methods

Since 1974 about 8,000 oysters have been sectioned using standard techniques as a means of detecting the parasite. Six percent neutral formaldehyde, buffered with $CaCO_3$ and made isotonic with NaCl, was used as the fixative. Embeddings were made in paraffin, then sections were cut to 7 μm and stained in hematoxylin and eosin.

At first, an experimental series was conducted in an attempt to produce infections in tanks by injection of minced, infected oyster tissues, ingestion from seawater suspensions of similar material, and contact with infected oysters. All attempts were unsuccessful. Therefore, we directed our attention to epidemiological studies of natural waters.

Results

Morphology of *M. refringens*

Our own investigations have confirmed the results of Grizel, Bonami, Cousserans, Duthoit, and Le Pennec (1974) and Perkins (1976). We agree with the identification of primary, seconary, and tertiary cells described by Grizel, Comps, Cousserans, Bonami, and Vago (1974) and agree that they are sporulation stages, judgments which were emphasized in this symposium held at the Virginia Institute of Marine Science.

From a practical viewpoint, we think that one can distinguish young and old plasmodia in histological slides after routine formaldehyde fixation, embedding, and hematoxylin-eosin staining (Fig. 1). Young plasmodia are from 7 to 15 μm (Fig. 2) and old plasmodia are about 15 to 30 μm. The latter contain characteristic refringent inclusion bodies which are strongly eosinophilic. These mature plasmodia correspond to the sporulation stages (Fig. 3).

In an experiment conducted in Landevennec near Brest in 1976, we observed that old plasmodia appeared 1 month after young stages, and that their number through the summer was lower than those of young stages. In winter old plamodia decrease in numbers and, in most cases, are absent from histological sections.

Epidemiology

1) *Marteilia refringens* has, like many other parasites, an annual cycle which we confirmed in studies at Roscanvel near Brest. In 1975 regular samples showed that the histologically observed infection rate was from 0 to 23 percent from February to June, and 43 to 50 percent from June to September.

2) Oyster transfers made by oyster growers cannot be used generally for scientific interpretation. We were able, nevertheless, to supervise two transplantations: In 1975, from Binic, a noninfected area, to Tinduff, a heavily infected area. The infection level was 0 percent in February and May and reached 96 percent 1 year later in February 1976. In 1975 and 1976, 26 samples were removed from various areas to Binic. In 13 samples, free of infection at the time of movement, no parasites were found after 1 year of supervision. In four samples that were moderately infected (10 to 30 percent), the infection rate decreased after 1 year and became zero for two samples. Nine samples were heavily infected initially (>40 percent) and showed increased infections after 6 to 9 months.

3) In 1976, a test was carried out in Landevennec, a heavily infected area. Disease-free oysters from Binic were immersed in January, and in subsequent months, and regularly monitored. Infections appeared between 20 July and 20 August, independently of when oysters were exposed, including those imported in June which had only 1-month exposure. Other oysters immersed in September 1976 were not infected in July 1977 (Fig. 4). Prevalence rates in infected oysters remained stable from August to December 1976 (86 to 100 percent), then decreased as had been observed in Roscanvel.

This experiment elucidates disease

Georges Balouet is with the Laboratoire d'Anatomie Pathologique, Faculté de Medecine de Brest 29200, Brest, France.

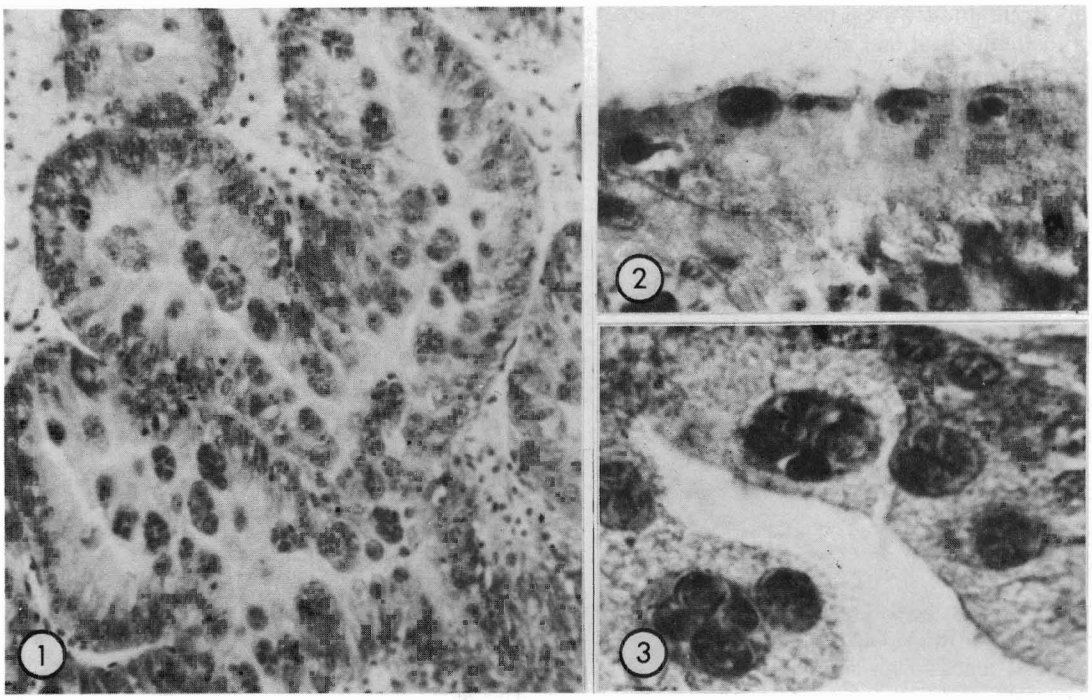
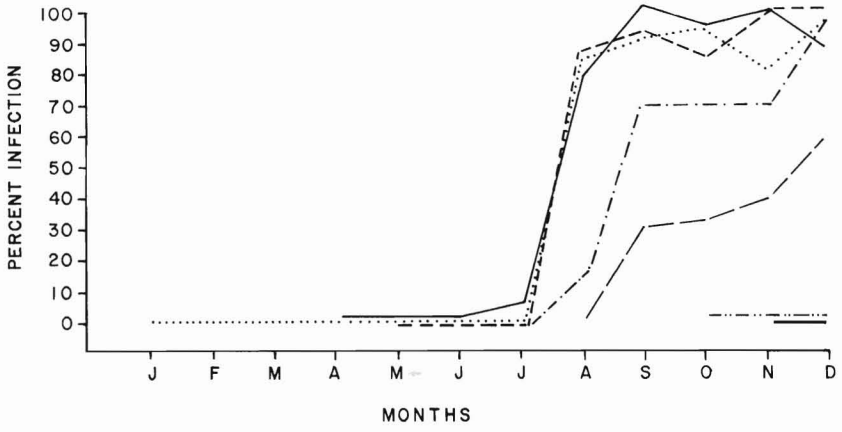


Figure 1.—Numerous stages of *Marteilia refringens* in digestive diverticula of *Ostrea edulis*. Hematoxylin and eosin (H.E.) staining 100×. Figure 2.—Young plasmodia in the stomach wall of *Ostrea edulis*. H.E. staining 250×. Figure 3.—Old plasmodia with refringent inclusion bodies in digestive diverticulum. H.E. staining. 400×.

activities found in commercial transplantings. Oysters can remain free of infections for long periods even in heavily infected areas, an observation which is of practical interest for oyster culture. Since early 1977 we have been conducting experiments in eight different Breton areas to see if annual and geographical variations occur.

Ten years after the outbreak of Aber disease and 3 years after the beginning of our research, collection of samples from various areas has revealed two periods of disease development: 1) From 1967 to 1974 there was an extension of the disease from the Abers. It spread out in 1973 to the Penze River, then to Morlaix-Carantec Bay and, in 1974, to Brest and the Gulf of Morbihan. 2) Since 1974 the epidemiological status seems to have become stable. Infection rates vary among growing areas and we distinguish three groups of oyster culture areas in Brittany (Fig. 5). There are very heavily infected areas (high-risk areas) as, for example, the Bay of Morlaix-Carantec and the eastern part of the Bay of Brest, where infection rates were always above 40 percent and mortalities reached 90 to 100 percent. Secondly, the moderately infected areas, where infection rates remained between 10 and 30 percent for over 3 years. For example, the western part of the Bay of Brest, the Bay of Paimpol, and the Morbihan Gulf are at

Figure 4.—Graph of Landevennec experiment (1976) showing mass infections of oysters in high-risk area during July-August. Different lines are used to distinguish lots of oysters imported at various times.



present in this group. In such areas mortality is at a tolerable level and allows some commercial exploitation. The third type of area is represented by Quiberon in the south of Brittany and Binic-St. Brieuc in the north, where no infection has been found in resident oysters in any type of analysis. In these presently nonendemic areas, some imported, infected oysters seemed able to recover.

Conclusions

We think that all pathological and epidemiological findings can be summarized as the result of a conflict between two organisms, the pathogen and the host.

The Pathogen

The parasite, *M. refringens,* probably has its own unique form of pathogenicity. Existence of an annual cycle is a normal characteristic of parasitic life, but we do not know at present many details about this cycle nor do we know much about the mechanisms of pathogenicity. Development of Aber disease probably occurs over a long time period before starvation and death of the host occurs.

In distinguishing between young and old plasmodia, recent results suggest that the first can correspond to chronic oyster infection all year long, the second to seasonal stages, perhaps responsible for spreading the disease via seawater.

The Host

Ostrea edulis had been, since 1967, the only species of oyster known to become infected by *M. refringens*. However, we have known for several months that the parasite can be found in *Crassostrea gigas* (Cahour, 1979). Susceptibility or resistance of oysters is a truly variable trait to be watched carefully.

Interaction Between Host, Parasite, and Environment

The necessity for an intermediary host, which has been suspected for other haplosporidian parasites, has not been proved for *M. refringens*. The demonstration in *Orchestia gammarellus* Pallas (Ginsburger-Vogel et al., 1976) of cellular stages, morphologically close to *M. refringens,* must not lead one to conclude that the crustacean is an alternate host until transmission of infections to *O. edulis* can be demonstrated.

Among physical parameters, variations in temperature, salinity, and immersion depth seem of little importance in Brittany. The final effect of all factors is only known when their change provokes disasters. In the future we shall have to take them into account and perhaps consider that Aber disease, as with other shellfish diseases, is widespread and is not so much a microbial disease as one arising from unfavorable physiochemical factors in seawater.

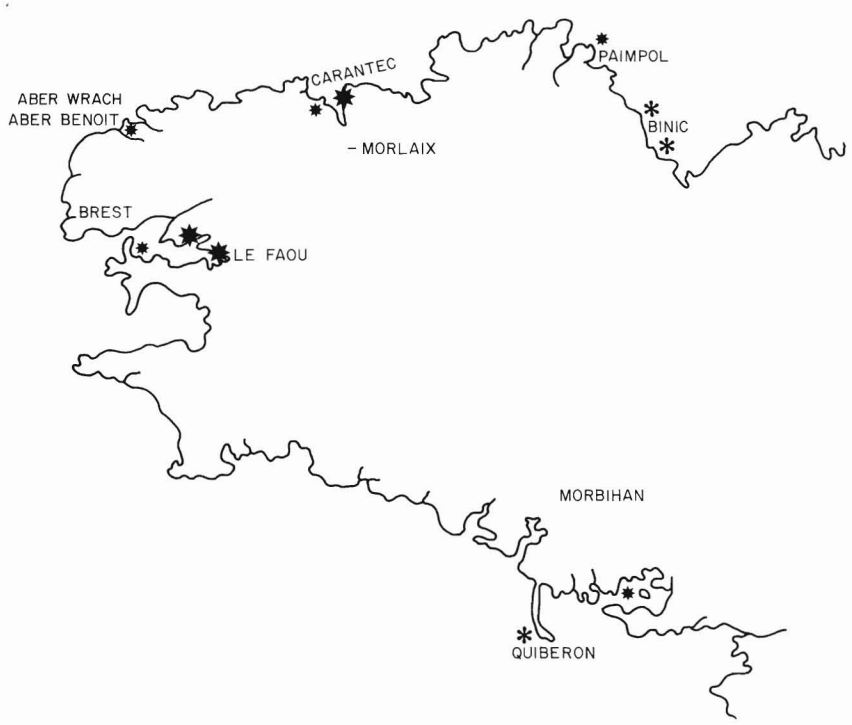


Figure 5.—Distribution of Aber disease in Brittany showing distribution of free and infected areas 3 years after disease began spreading in 1974. Asterisks indicate areas where the disease does not develop, small starts indicate areas where infections are moderate, and large stars show heavily infected (high-risk) areas.

Acknowledgments

This research was supported by a grant from Université de Bretagne Occidentale (Brest) and in part by a grant from CNEXO (77/1690). The author thanks Annie Cahour for her helpful collaboration, Guy Maheo and his associates for professional contributions, and Andrea Pasquale, Renée Begot, Anne Marie Arzel, and Myriam Gouzien for their able technical assistance.

Literature Cited

Cahour, A. 1979. *Marteilia refringens* and *Crassostrea gigas*. Mar. Fish. Rev. 41(1-2):19-20.

Ginsburger-Vogel, T., I. Desportes, and C. Zerbid 1976. Présence chez l'Amphipode *Orchestia gammarellus* (Pallas) d'un Protiste Parasite; ses affinités avec *Marteilia refringens,* agent de l'epizootie de l'Huître plate. C. R. Acad. Sci., Paris 283:939-942.

Grizel, H., M. Comps, J. R. Bonami, F. Cousserans, J. L. Duthoit, and M. A. Le Pennec. 1974. Recherche sur l'agent de la maladie de la glande digestive de *Ostrea edulis* Linné. Sci. Pêche 240:7-30.

_____, _____, F. Cousserans, J. R. Bonami, and C. Vago. 1974. Etude d'un parasite de la glande digestive observé au cours de l'épizootie actuelle de l'Huître plate. C. R. Acad. Sci., Paris 279:783-785.

Perkins, F. O. 1976. Ultrastructure of sporulation in the European flat oyster pathogen, *Marteilia refringens* - taxonomic implications. J. Protozool. 23:67-74.