Epizootiology of *Marteilia refringens* in Europe

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Historical Background

In the late 19th century the European oyster industry was a large and flourishing one, based mainly on the production of the flat oyster, *Ostrea edulis*, from natural beds. In France alone, an elaborate "farming" type of situation had recently been developed. World War I resulted in considerable neglect of the oyster beds and throughout Europe the industry was just becoming reestablished when, in the early 1920's, a large-scale mortality occurred (Orton, 1924). The United Kingdom oyster industry never recovered and even today is only a fraction of its original size.

The French oyster industry did recover and went rapidly from strength to strength, remaining without important problems until the mid-1960's. When problems occurred they were in the sector of the industry utilizing the introduced Portuguese oyster, *Crassostrea angulata*. Mortalities of this oyster in the latter 1960's and early 1970's coincided with early experimental introductions of the Japanese oyster, *C. gigas*, made on a limited scale from 1967 onwards. The mortalities and the availability of an alternative led to the replacement of the Portuguese oyster, *C. angulata*, by the Japanese oyster, *C. gigas*.

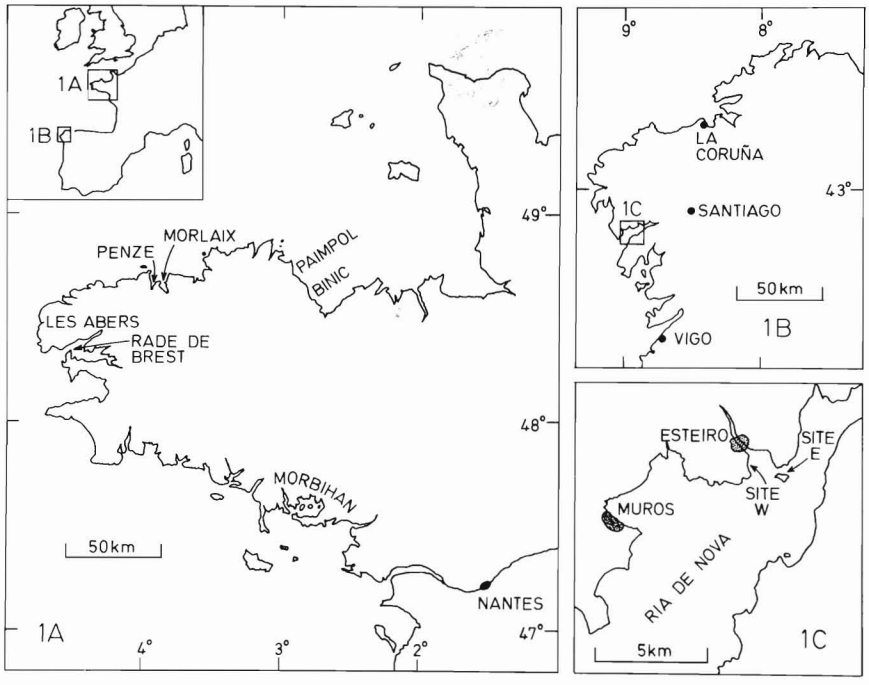
Also in 1967, the first drastic mortality of the flat oyster, *O. edulis*, occurred in a small estuary on the northwestern coast of Brittany, Aber Wrach. Occasional mortalities had been noticed in this estuary, perhaps up to 50 percent in some previous years, but in the summer of 1967 the mortality exceeded 90 percent (Comps, 1970; Herrbach, 1971).

Stages of the parasite which have since been identified as *Marteilia refringens* were recognized immediately in histological examination of oysters from the mortality site (Herrbach, 1971). By the time the parasite was recognized, oysters from the Abers had already been transferred that year to the Rade de Brest, to Marennes, and Galicia in Spain. Samples from these oysters proved to be positive for the presence of the parasite. By 1970, Comps was reporting abnormal mortalities in the Marennes basin associated with the parasite's presence. He also reported it from the Rade de Brest, in Aber Benoit, in Spain, and at Arcachon. The mortalities at Marennes were in stocks introduced from Aradon, Paimpol, and also from Arcachon. Thus it is clear that although severe oyster mortalities at that time were occurring only in relatively limited areas, the presence of the parasite in the major oyster-producing areas of France and Spain had already been established.

The next few years saw a slow increase in the frequency of mortalities and by 1973 the estuary at Penze (Fig. 1A) had become infected. By 1974 the Morlaix estuary was affected and by 1975 it was clear that the disease was present in Paimpol, Binic, and in the Morbihan. Since Morbihan, together with the Rade de Brest, was the major source of young *O. edulis* for transplanting and growing, this was disastrous in the extreme.

Meanwhile, oysters from diseased stocks had continued to be exported to

Figure 1.—Location maps of areas described in text.



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the Netherlands and to Spain. At first, neither country appeared to be having difficulties with introduced stocks. However, by 1975, it was clear that some areas of Spain at least were subject to severe mortalities from Aber disease. In contrast, no significant losses had been noted in the Netherlands.

Table 1 illustrates the effects of the various diseases on oyster production in France. There was a severe decline in production of *C. angulata* from 65,900 tons in 1960 to below 20,000 tons in 1971 when this species was already being replaced by *C. gigas*. This production has now reached in excess of 85,000 tons. In the case of *O. edulis*, the figures show a slow but remorseless and accelerating decline as a direct result of the spread of Aber disease from 1967 onward. There was little significant reduction between 1960 and 1970 when production was in the region of 20,000 tons per annum. However, by 1971 there were the first indications that Aber disease was beginning to become significant and by 1973 production was cut by half to 10,000 tons. Resurgence in 1974 to 14,000 tons almost certainly represents the sale of young stock to realize an asset before it could be lost. The more indicative figure is seen in 1975 when total flat oyster production in France was down to 8,400 tons.

Table 1.—Total oyster production in France in metric tons.

Year	*Ostrea*	*Crassostrea*
1960	21,600	65,900
1969	18,000	43,200
1970	19,100	40,300
1971	14,100	20,100
1972	14,900	53,900
1973	10,300	61,700
1974	14,100	59,500
1975	8,400	85,000

Table 2.—Oyster production of Les Abers and Rade de Brest in metric tons.

	Aber Wrach and Aber Benoit		Rade de Brest	
Year	*Ostrea*	*Crassostrea*	*Ostrea*	*Crassostrea*
1971	1,300	0	6,366	50
1972	1,275	0	5,572	50
1973	1,125	25	5,888	125
1974	—	—	3,654	91
1975	666	376	1,341	441

In the Abers, production of *O. edulis* fell from 1,300 tons to 666 tons between 1971 and 1975 (Table 2) while production of *Crassostrea,* which was zero in 1971, had reached 370 tons by 1975. In the Rade de Brest a similar picture is found. *Ostrea* production fell from 6,366 tons in 1971 to 1,340 tons in 1975, and again production of *Crassostrea* has risen from 50 tons to 441 tons. In neither case has the production of *Crassostrea* risen to equal the level of *Ostrea* production which has been lost. For this, economic forces are largely to blame.

Life Cycle Considerations

Life cycle problems are discussed elsewhere in this symposium (Balouet, 1979; Cahour, 1979; Grizel, 1979). In samples received bimonthly from Carantec in the Morlaix River during 1975 an apparent sequence of parasite stages was visible. During the winter period (from December to February), less than 10 percent of specimens showed the presence of *M. refringens*. The stages present were mainly those with mature spores and refringent inclusion bodies and were located in small areas of the infected digestive gland. During the early spring, stages which Perkins (1976) has referred to as plasmodial stages became apparent in the stomach epithelia both of animals with visible "old" infections and those without. Since the winter infections seemed to consist of small, limited foci in one or two branches of digestive gland, the problem of establishing true levels of infection is great. Although the apparent level of infection may be only 10 percent, it is impractical to expect to find every focus of infection because of the limited number of sections which can be made and examined from a single animal. Therefore, many apparently new infections may simply represent further infection of an already locally infected animal.

The number of mature and maturing parasite cells in the digestive epithelia also began to increase in the spring and infection levels began to climb, eventually reaching 80 percent by July. Levels remained high until October but there were relatively few stomach epithelial infections during the peak of the summer. An increased level of these infections was measured again in the autumn (September to October) period. After October, general levels of apparent infection began to fall, reaching 10 percent in January.

The occurrence of refringent inclusion bodies in the gut presumably indicates sporangial breakdown and spore release. Refringent bodies were present in the gut throughout most of the summer season, but the most impressive were the occasional animals which appeared to have been subject to massive and simultaneous spore release. These were the only specimens with really significant tissue damage. Variable amounts of hemocyte reaction may be observed at different stages of the disease, but the massive spore release is accompanied by breakdown of the oyster tissues. It seems necessary that some physical initiator for such a mass spore release must exist. The tissue damage which accompanies this type of spore release contrasts strongly with the apparent lack of severe reaction to the presence of enormous numbers of mature parasites, and may account for the catastrophic localized mortalities which characterize the progress of Aber disease.

An interesting example of the epizootiological problems posed by Aber disease is seen in Galicia, Spain (Fig. 1B). As has been mentioned previously, oysters infected with *M. refringens* had been imported into Spain as early as 1969. There is no information as to when the disease became naturalized in Spanish waters, but during the autumn of 1975 samples from the Rio de Muros showed high levels of *M. refringens*. Culture of the flat oyster, *O. edulis,* in Spain is limited to the northwest of Galicia and bottom culture is practiced only at Ribadeo. Elsewhere, oysters are suspended either in trays or attached to ropes from rafts, such as are used in the same area for mussel culture. The two sites (W and E) marked on Fig. 1C are operated by two separate companies. The company operating the western (W) site also has an oyster hatchery built to produce local spat, but both companies have imported

large numbers of oysters for ongrowing from France.

In the autumn of 1975, a number of samples of oysters from five groups of animals which had originally been imported from France were examined from the western (W) site. All of these samples showed levels of *M. refringens* infection between 80 and 100 percent. The sixth sample of oysters which consisted of hatchery spat produced in the winter of 1975 at the nearby hatchery also showed 95 percent infection. Clearly, *M. refringens* is able to spread to local stocks at least within the limits of the western site raft system. The seventh sample examined at the same time came from the eastern (E) site set of rafts and showed zero levels of *M. refringens* infection. Samples taken from this site in the following summer, 1976, again consistently showed no *M. refringens*.

There is, however, a complicating factor. Although the eastern site showed no levels of Aber disease either in the imported French oysters or in various other stocks, mortalities still occurred, particularly in the French stocks. The oysters in question were originally imported into Ribadeo and laid there for some months before being removed to the Muros rafts. At about 120 days after arrival at Muros, mortalities commenced and ran at approximately 10 percent per week throughout the summer. The oysters had been examined and found to be free of *M. refringens* before they left France and they remained free of *M. refringens* during the period of examination at Muros. While no indications of *M. refringens* were observed, some 30 percent of the animals examined showed evidence of a hematopoietic neoplasm. The cell type of this neoplasm appears to be equivalent to that noted by Brown et al. (1976) in *Mya arenaria* and designated Type II. It is currently assumed that the mortality and the neoplasm at Muros are interrelated (Alderman et al., 1977). A similar neoplasm was observed in *O. edulis* imported from the Adriatic Sea and examined from Ribadeo where no mortalities were reported. Subsequent reexamination of the material from the western site 1975 investigations showed a number of specimens with Type II neoplastic cells to be present. There is no positive evidence at present that this neoplasm is infectious and occasional cases (van Banning[1]) have been noted in oysters from Le Pô in the Morbihan.

The two Spanish sites at Muros are separated by approximately 2 km of open water in a wholly marine estuary with little input of fresh water and a reasonable tidal circulation. The situation is thus that *M. refringens* is able to become established in the western site and infects local oysters to very high levels within the raft systems and produces very severe mortalities. However, the parasite appears unable to cross the distance of 2 km of open sea and infect oysters on the eastern site rafts. The complicating factor of the neoplasm, which is apparently related to significant mortalities on the eastern site and is also present on the western site, makes it difficult to be certain of the relative importance of *Marteilia* infection in terms of the western site mortalities.

The inability of *Marteilia* to cross the 2 km gap in Spain is indicative of some of the puzzling problems that this disease is presenting. To provide a better understanding of the epizootiology of this disease organism, controlled tray experiments of a type carried out over many years by Andrews (1965, 1966, 1967) are necessary.

[1]van Banning, Paul. The Netherlands Institute for Fishery Investigations, Haringkade 1, Ijmuiden-1620, The Netherlands. Pers. commun.

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Literature Cited

Alderman, D. J., P van Banning, and A. Perez-Colomer 1977 Two European oyster (*Ostrea edulis*) mortalities associated with an abnormal haemocytic condition Aquaculture 10:335-340.

Andrews, J. D. 1965. Infection experiments in nature with *Dermocystidium marinum* in Chesapeake Bay. Chesapeake Sci. 6:60-67

———. 1966. Oyster mortality studies in Virginia. V Epizootiology of MSX, a protistan pathogen of oysters. Ecology 47:19-31.

———. 1967. Interaction of two diseases of oysters in natural waters. Proc. Natl. Shellfish. Assoc. 57:38-49.

Balouet, G. 1979. *Marteilia refringens*—considerations of the life cycle and development of Abers disease in *Ostrea edulis*. *In* F O. Perkins (editor), Haplosporidian and haplosporidian-like diseases of shellfish. Mar. Fish. Rev. 41(1-2):64-66.

Brown, R. S., R. E. Wolke, and S. B. Saila. 1976. A preliminary report on neoplasia in feral populations of the soft shell clam, *Mya arenaria*: prevalence, histopathology and diagnosis. *In* Proceedings of the IX Conference, Society for Invertebrate Pathology, p. 151-158. Printing Dep., Queens University, Kingston, Ont.

Cahour, A. 1979. *Marteilia refringens* and *Crassostrea gigas*. *In* F. O. Perkins (editor), Haplosporidian and haplosporidian-like diseases of shellfish. Mar. Fish. Rev. 41(1-2):19-20.

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

Grizel, H. 1979. *Marteilia refringens* and oyster disease—recent observations. *In* F. O. Perkins (editor), Haplosporidian and haplosporidian-like diseases of shellfish. Mar. Fish. Rev. 41(1-2):38-40.

Herrbach, B. 1971. Sur une affection parasitaire de la glande digestive de l'huitre plate, *Ostrea edulis* Linne. Rev. Trav. Inst. Pêches Marit. 35:79-87.

Orton, J. H. 1924. An account of investigations into the cause or causes of the unusual mortality among oysters in English oyster beds during 1920 and 1921 Fish. Invest. Ser. II. Mar. Fish. G. B. Minist. Agric. Fish. Food 6(3):1-199.

Perkins, F. O. 1976. Ultrastructure of sporulation in the European flat oyster pathogen, *Marteilia refringens* — taxonomic implications. J. Protozool. 23:64-74.