

## *Marteilia refringens* and Oyster Disease—Recent Observations

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### Introduction

The parasite responsible for the disease of the flat oyster, *Ostrea edulis*, has been the object of numerous studies (Comps, 1970; Tigé and Morel, 1974; Perkins, 1976) in which most considered the internal cycle of development of the parasite in the oyster, and the parasite's structure, ultrastructure, and systematic position, of which the latter remains debatable.

In this paper I mention observations made at the time of diagnosis for the presence of infections and information gained through studies of oysters on the bottoms of estuaries and coastal waters. The research is designed to provide practical solutions to problems facing oyster culture.

### Methods

Since there are no specific clinical signs (Grizel et al., 1974), the disease can best be detected by histological techniques. Each month, oysters of all ages, coming from a diversity of Breton coastal regions, are examined in our laboratory.

From September 1976 to September 1977, histological sections from 14,000 oysters were observed. This operation provided oyster growers with a personal knowledge of the condition of their oysters through the monthly distribution of a bulletin informing them of the general epidemiological picture.

The areas for flat oyster rearing can be classified into three zones (Fig. 1): 1) High activity of the parasite where rearing is not advised at present; 2) low activity areas such as Saint Philibert River and the harbor of Brest (Roscanvel); and 3) disease-free areas with three important centers being Cancale, Binic, and Quiberon of which Quiberon is the only important center for recovery of planted, flat oysters. It should be noted that there are several unaffected

river estuaries of less importance such as the Rance River.

### Results

#### In Situ Studies of Infections

Preliminary information, arising from part of a 3-year study, was obtained in 1976 from four stations in the rivers of Auray, Crach, Morlaix, and Penzé. Spat collected from the Pô in

1975, free of the parasite, were transferred in March 1976 to those rivers, and a control lot was kept in the Pô. Monthly observations are presented in Table 1. Analysis of these results show that the period of infection occurs, de-

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Figure 1.—The areas of Brittany, France, described in text.

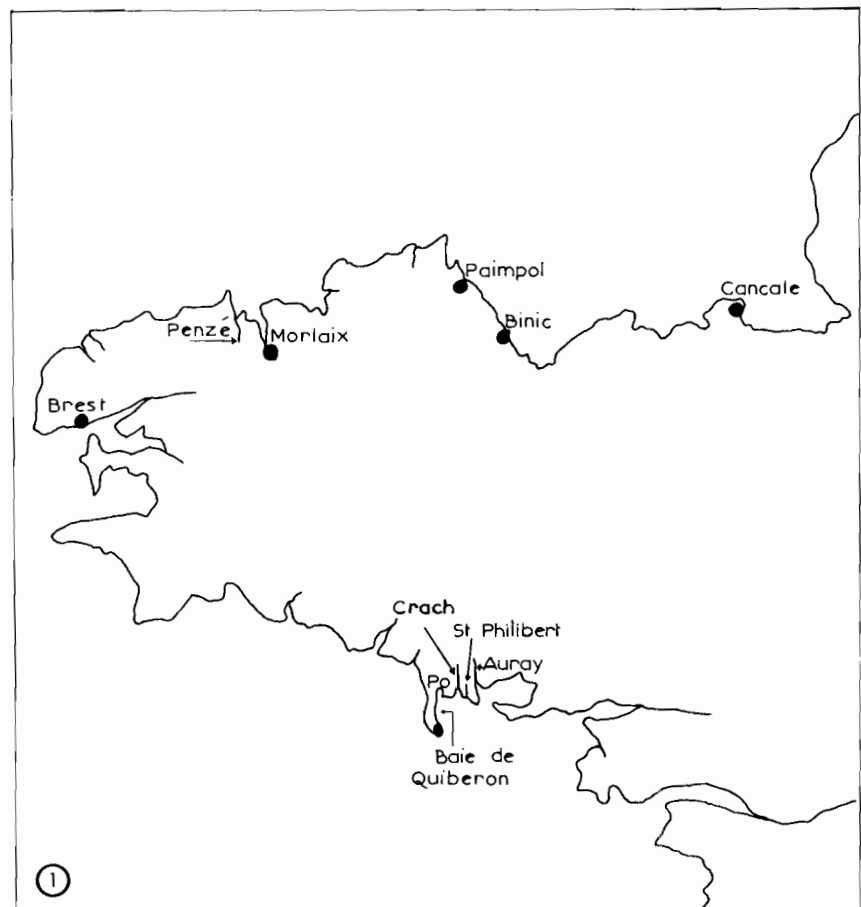


Table 1.—Incidence of infection by *Marteilia refringens* in young oysters from selected localities (no. infected over no. in sample) by year and month.

Stations	1976								1977									
	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S
Auray River	0	1	4	3	8	18	17	17	18	11	3	0	0	2	6	6	—	6
	50	20	20	20	20	19	20	19	20	20	20	20	17	20	20	18	—	20
Crach River	0	0	0	1	1	15	22	17	20	12	0	0	0	2	3	2	—	6
	50	20	20	20	18	20	25	19	22	20	20	20	17	23	20	20	—	20
Penzé	0	0	0	0	1	10	15	15	18	—	11	8	6	6	—	14	—	—
	50	20	20	20	20	20	20	20	18	—	20	20	20	18	—	15	—	—
Morlaix River	0	0	0	0	0	1	0	4	7	—	1	5	2	9	7	9	—	—
	50	20	20	20	20	20	20	20	20	—	20	20	15	20	20	20	—	—
Pô (Control)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	—	0
	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	—	20

pending upon the river, between the first of May and the first of September. The results of this year confirm that fact, but it appears that the months of July and August are the most favorable.

The parasite follows a developmental cycle in which young stages appear first in the oyster's stomach, followed rapidly by an invasion of the digestive diverticula by further developed cell types containing refringent granules. At the end of winter and the beginning of spring, it appears that the known forms of the parasite are eliminated from the oyster, especially along the center of the southern coast of Brittany where the percentage of parasitized oysters becomes zero.

### Studies of the Disease Resistance of Flat Oysters

During the time of range extension of the parasite, therapeutic agents were tried in an effort to find a chemical

Table 2.—Incidences of *Marteilia refringens* infections (no. infected over total in sample) in *Ostrea edulis* of different origins, ages, and time in an endemic area (Crach River).

Origin	Mar. 1976	May 1976	Aug. 1976	Sept. 1976	Nov. 1976	Dec. 1976	Jan. 1977
Adults from Ireland	0	—	4	5	17	17	12
	20	—	13	12	20	20	20
Spat from hatchery	—	0	—	14	10	15	6
	—	20	—	23	17	20	20
"Pied cheval" spat from hatchery	—	0	—	3	16	6	5
	—	20	—	4	19	12	20
Adults from the Mediterranean coasts of France & Greece	—	0	—	18	11	—	—
	—	30	—	19	11	—	—

which could be prescribed for treatment of the disease. In the laboratory, different chemical products commonly utilized in aquaculture (methylene blue, malachite green, and Furanace<sup>1</sup>) have been tested on parasitized oysters. The chemicals were ineffective against *Marteilia refringens*.

On the bottom of the Crach River, native oysters (2-3 years old, from Ireland), spat from the Houat hatchery, spat from the SATMAR hatchery ("pied de cheval" race of oysters), and oysters of diverse ages from the Mediterranean coasts of France and Greece were placed in rearing conditions in bags placed on racks. All of the oysters were known to have disease-free origins. Subsequent examinations of the oysters during the months of August and September revealed that all lots were infected with *M. refringens* (Table 2). The experiment demonstrated for the first time that the age of oysters is not a factor in the initiation of infections. Only the time of submergence in the water plays a role. In addition, origin of the oysters, contrary to previously expressed ideas, does not influence development of the parasite. All of the oysters are parasitized in the same way.

In effect, initiation of infections and subsequent development of the disease follows a set pattern. Heavy mortalities

<sup>1</sup>Mention of trade names or commercial products does not imply endorsement by the National Marine Fisheries Service, NOAA.

strike native oysters from the Mediterranean and the hatchery oysters by the first winter. The remaining ones are all dead by that time.

### Discussion

The epizootic which has ravaged France's flat oyster populations since 1969 has involved serious repercussions for oyster culture. The different stages of development of the organism believed to be responsible for the disease are well known. However, results of recent experiments and the failures experienced in attempting to transmit infections in the laboratory, led us to assume the presence of an additional cell stage not yet described. The unusual ultrastructure and developmental cycle of the parasite still pose, in spite of its being allied by Perkins (1976) with the haplosporidians, problems with its classification. Opinions are divided on its systematic position.

Studies of the life history have permitted us to determine the infection period. This has been identified, independent of the age of the oyster, as being during the first month of summer and can vary several weeks depending upon the region. Research on the life cycle is being pursued actively, because knowledge of the cycle may permit reestablishment of flat oyster culture.

Finally, it is desirable to have a stock of flat oysters resistant to the disease. We plan to determine if such oysters can be obtained by looking for adults which survive in the highly endemic rivers we have already identified in our previous studies.

### Literature Cited

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