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DOES THE PREVALENCE OF *BONAMIA* AND *MARTEILIA* DISEASES BE REDUCED ON FLAT OYSTERS (*OSTREA EDULIS*) OF ATLANTIC AND MEDITERRANEAN ORIGIN, WHEN THEY ARE REARED TOGETHER WITH THE JAPANESE OYSTER (*CRASSOSTREA GIGAS*) IN TIDAL PONDS ?

by

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ABSTRACT

The flat oyster *Ostrea edulis* was originally reared in several regions of the European waters. The tidal saline ponds on the Atlantic coasts of France were highly productive before being hit by two consecutive epizooties. Parasitic diseases were described on this species, both of them leading to economic losses, as heavy mortalities have usually been recorded before the oysters reach the commercial size. Repeated experiments showed that the two diseases, *Marteilia refringens* and *Bonamia ostreae* are still active in this environment. Recently, some evidences were given that haemocytoblasts of *Crassostrea gigas* had the ability to lyze the Bonamia cells. Therefore, in an experiment of contiguous culture of the two species of oysters, the growth, mortality and prevalence of the two diseases were recorded of two populations of the flat oyster. The prevalence of *Marteilia* was higher then the one of Bonamia (49.8 % against 7.4 %). The oysters originated from the area exhibited a better growth and survival then the ones coming from mediterranean waters. However, the Mediterranean origin was significantly less sensitive then the Atlantic one to *Bonamia* (respective prevalence of 5.6 % and 9.2 %). The rearing in different proportions with *Crassostrea gigas*, in the same oyster bags, resulted in an absence of significant difference between these proportions, thus leading to the conclusion that the mixed rearing of the two species did not reduce significantly the prevalence of *Bonamia* parasites on the flat oyster. The very high level of the prevalence for *Marteilia refringens* after 8 months of cultivation, should be emphasized. For this disease, a significant effect of the mixed rearing was observed which may be due to the pond variability. These results were discussed in terms of culture management.

Key-words : *Bonamia ostreae*, *Marteilia refringens*, *Ostrea edulis, Crassostrea gigas*, pathology, growth, mortalities, molluscs.

INTRODUCTION

The flat oyster industry, formerly flourhising in European countries, has been hit by a parasite, *Bonamia ostreae*, since 1973 (Grizel et al., 1976). This haemocytic parasite (Pichot et al., 1980) spread over the European coasts and then in several parts of the world. While it was signalized once in the Mediterranean sea, these populations of oyster may still be considered as uncontaminated. Along the coasts of Brittanny (France), the economic losses resulting from the impact of the *Bonamia* disease on the local oyster industries have been computed as to equal the damages caused by the wreck of the Amoco Cadiz (Grizel, 1985). However, among the parasites already described on *Ostrea edulis*, another one has also caused significant mortalities, while much reduced. This parasite, *Marteilia refringens* (Comps, 1970) seemed to have lost part of its virulence in this area.

Researches have been conducted on the development of diagnostics tools for the *Bonamia* disease (Grizel et al., 1988 ; Boulo et al., 1989) and on the establishment of cellular lines (Mialhe et al., 1988), in order to propose a model of this epizooty (Mourton et al., 1991). Other efforts are devoted to the genetics, aiming at the obtention of *Bonamia*-resistant strains of flat oysters (Elston et al., 1987). Practical aspects have concerned the technical rules of cultivation, in order to reduce the risks of local propagation and to improve the survival rates and the production. However, it has recently been established that *Bonamia ostreae* had also the ability to infect the haemocytoblasts of the Japanese oyster, *Crassostrea gigas*, with the absence of resulting mortalities for this species (Chagot, 1989 ; Chagot et al., in press).

Furthermore, experimental infections of *O. edulis* by known amounts of *B. ostreae* showed that the resultant prevalence was proportional to the quantity of parasites introduced. Then the presence of *C. gigas* together with *O. edulis* may contribute to reduce the number of *B. ostreae* cells which could infect the flat oysters. This might result in reduced prevalences and better survival of the flat oyster, in field conditions. *M. refringens* may also contaminate *C. gigas*, but until now, it has only been found in the younger oysters (Cahour, 1979). A comparable hypothesis can therefore be proposed, about the infestation of *C. gigas* by *M. refringens* while its mechanism remains unknown. Field experiments on the mixed rearing of *O. edulis* and *C. gigas* were then conducted, in order to test the hypothetic reduction of prevalence by the phagocytosis of *B. ostreae* from haemacytoblasts of *C. gigas*. They were conducted in traditional tidal ponds, near the city of Marennes (Atlantic coasts of France) which was in the past, a well known center for the flat oyster production. Two different populations of *Bonamia* -free flat oysters were tested, one from uncontaminated, natural beds in the Atlantic coasts and the other from the Mediterranean sea. Until recently the last one was protected from any *Bonamia* contamination. Similar experiments have been conducted in an intertidal area, along the coasts of Brittanny (Le Bec et al., 1991), on the impact of *B. Ostreae* on Atlantic *O. edulis*, reared at different proportions and densities with *C. gigas*.

MATERIALS AND METHODS

Facilities and biological materials

The tidal ponds used in this experiment are very similar to those widely in use for the on-growing and the fattening of oysters in this area (Zanette and Garnier, 1981). These shallow (0.4 m) earth ponds have a surface of 300 m^2 (fig. 1). They are irrigated from sea waters every Spring Tide, thus providing uniformous rearing conditions. The flat oysters were dredged on naturel beds, in the Thau lagoon, for the Mediterranean origin and near the

Oleron island for the Atlantic origin. Until now, both of these places are *Bonamia*-free. The oysters were sorted to obtain an average size of 30 g and an initial sample was taken to determine the prevalence of *Bonamia* and *Marteilia* diseases. The initial weight of the japanese oysters used in the experiment was also of 30 g. They were obtained from an oyster farm in the Marennes-Oléron bay.

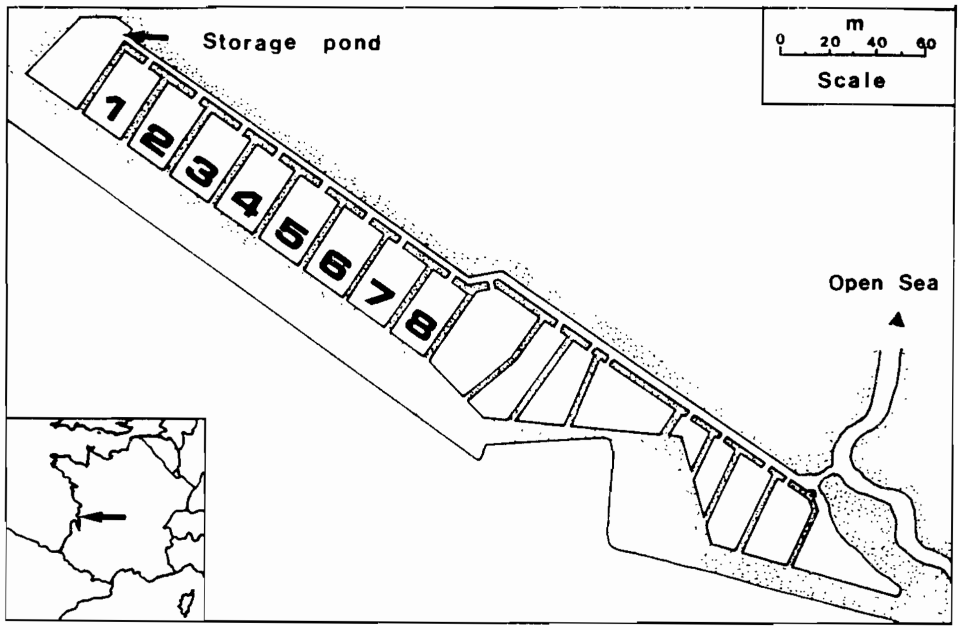

Figure 1 : Location and scheme of the experimental ponds. The last four ponds (5 to 8) were utilized for the experiment.

Experimental design

Four different percentages of mixed culture of *O. edulis* with *C. gigas* were installed : 25 %, 50 %, 75 % and 100 % of *O. edulis*. The flat oysters were chosen in equal quantities from Atlantic and from Mediterranean populations. Adequate weights of each species and origin of oysters were sowed in the earth bottom of the ponds, so as to reach in every ponds a total biomass of 200 g/m^2. Such biomass would not cause any significant impact on the algal populations and consecutively reduce the growth (Zanette and Garnier, 1981). Each of the four ponds used was then given a value of the four percentages. For easier controls, individually labelled oysters (50 of each species and origin per pond) were installed in racks put on the bottom of the ponds, in order to monitor the growth in live weight and mortality for every condition. Growth was assessed by means of the daily instantaneous growth rate (Askew, 1978) which normalize the variances. Oysters were measured every two months from April 1988 to February 1989 while survival rates were counted every month. Results on the growth of the Japanese oyster were not reported here.

Histological examinations

They were performed on the two origins for the flat oysters, in order to determine the prevalences of *Marteilia* and *Bonamia* diseases. Approximately 100 oysters of every modalities (percentage and origine) were dissected, and smears of the digestive gland and of the heart were air-dried. After a fixation with methanol, they were stained with Haemocolor kit (Merck) before light microscopy examination. The data were processed (ANOVA and Chi square tests) with the statistical package STATGRAPHICS.

RESULTS

The two different origins (Atlantic and Mediterranean) and the different proportions were analysed in terms of final weight and growth during the experiment, by means of ANOVA. The first analysis performed on the final weight (table) showed that highly significant differences ($p < 0.001$) were observed, both for the origin of the oysters and for the proportions of flat oysters.

Table 1 : Analysis of variance performed on the final weight (in grammes) of surviving flat oysters from different origins (Atlantic and Mediterranean) ant at different proportions among Japanese oysters, after 10 months of rearing in tidal ponds. d.f. = degrees of freedom.

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. level
MAIN EFFECTS	16 405.0	4	4 101.2	65.18	< 0.001
Origin	5 394.9	1	5 394.9	85.74	< 0.001
Proportion	12 662.7	3	4 220.9	67.08	< 0.001
FACTOR INTERACTIONS	1 331.1	3	443.7	7.05	< 0.001
RESIDUAL VARIATIONS	16 610.2	264	62.9		
TOTAL	34 346.5	271			

A multiple range analysis, performed by means of a test of Newman-Keuls showed that the two lower proportions (50 % and 25 %) did not significantly differed between them, while each other proportion corresponded to an homogenous group. The higher proportions (75 % and 100 %) were then significantly different between them and with the other group. Another analysis was performed on the daily instantaneous growth rate, which was computed over the whole experiment. Such variable may then have integrated the growth conditions encountered during the 10 months of experiment.

Table 2 : Analysis of variance performed on the daily instantaneous growth rate of surviving flat oysters from different origines (Atlantic and Mediterranean) and at different proportion, among Japanese oysters, after 10 months of rearing in tidal ponds. d.f. = degrees of freedom.

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig.level
MAIN EFFECTS	0.0003717	4	$9.293\ 10^{-5}$	105.33	< 0.0010
Origin	0.0001664	1	$1.664\ 10^{-4}$	188.68	< 0.0010
Proportion	0.0002449	3	$8.166\ 10^{-5}$	92.56	< 0.0010
FACTOR INTERACTIONS	0.0000211	3	$7.032\ 10^{-6}$	7.97	< 0.0010
RESIDUALS VARIATIONS	0.0002329	264	$8.822\ 10^{-7}$		
TOTAL	0.0006257	271			

The two factors were acting on the daily instantaneous growth rate, with very high significant levels ($p < 0.001$), thus indicating that both the origin and the proportion of flat oysters had an influence on the growth rate. As for the analysis performed on the final weight, the interaction between the two factors was also very highly significant ($p < 0.001$). The actions of the origin of the oyster and of their respective proportions on the growth rate were then dependant from each other.

The test of Newman Keuls ($p = 0.05$) performed on these data, gave results identical to those found on the final weight : the same groups were identified, the lower proportions (50 % and 25 %) being not differenciated from each other.

A seasonnality in the growth appeared clearly over the ten months of experiment (fig. 2 and 3). At the beginning, from April to July, the growth was fast for both species. Afterwards, growth stopped during Summer and some emaciation may even be seen during Winter, mainly for the Atlantic oysters. The mere difference between the two origins was that the Atlantic oysters (fig. 3) reached in December a much larger weight (49.73 g ± 1.21 g) than the Mediterranean ones (40.42 g ± 1.43 g) (fig. 2).

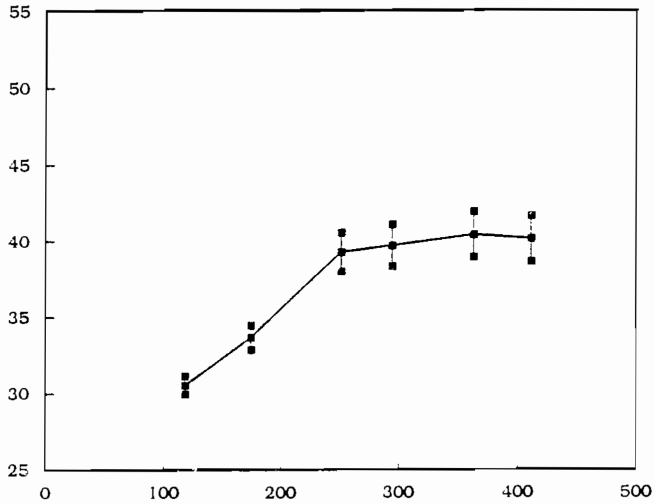

Figure 2 : Average growth in live weight (in grammes) of Mediterranean *O. edulis*, reared in different proportions with *C. gigas* over a period of 10 months in tidal ponds.

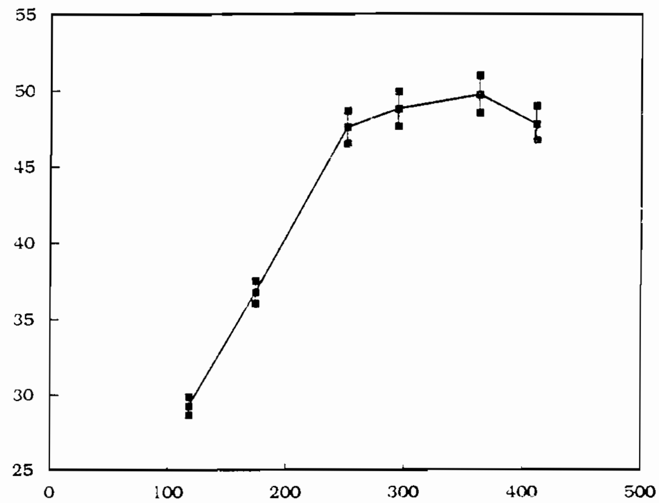

Figure 3 : Average growth in live weight (in grammes) of Atlantic *O. edulis* reared in different proportions with *C. gigas*, over a period of 10 months in tidal ponds.

The statisticals results related to the different proportion of oysters were illustrated clearly in the figure 4. The faster growth was observed for a proportion of 100 % of flat oysters, and then for a proportion of 75 %, while the two other curves were not merely distinguished. An emaciation during Winter was observed in every conditions.

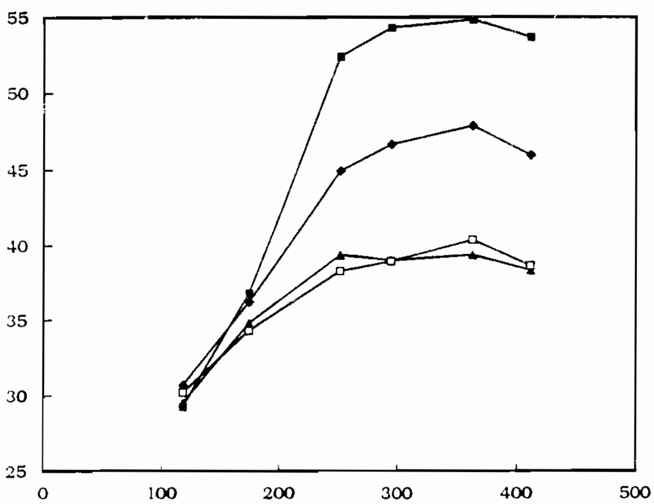

Figure 4 : Average growth of *O. edulis* of different origin (in grammes), reared in the following proportions with *C. gigas* in tidal ponds. Full squares : 100 % of O. edulis. Diamonds : 75 % of *O. edulis*. Open squares : 50 % of *O. edulis*. Triangles : 25 % of *O. edulis*.

The survival rates, expressed in percentages (table 3) were highly different between the two origins of *O. edulis*. Individuals originated from the Atlantic coasts were much more resistant, since the average survival was of 91 % during the experiment, against 61 % for the oysters from the Mediterranean sea. Such better survival was observed at every proportion, since the worst survival rate of Atlantic oysters is even higher than the best result of Mediterranean oysters. A period of high mortalities was found during June and July for the Mediterranean origin, while nothing comparable could be detected for the Atlantic one.

Table 3 : Cumulated survival rates of the flat oysters, computed from monthly mortality counts. Results in each column correspond initially to 50 individually labelled oysters (27th April 1988). 100 %, 75 %, 50 % and 25 % correspond to the proportion of flat oyster reared together with Japanese oysters.

Date	Atlantic origin				Mediterranean origin			
27/04/88	100	100	100	100	100	100	100	100
26/05/88	98	98	100	100	86	92	74	92
22/06/88	94	98	98	92	86	92	72	76
20/07/88	94	98	98	92	84	74	62	68
07/09/88	92	98	96	88	80	74	60	60
21/09/88	92	98	96	88	80	74	60	58
20/10/88	92	98	96	88	74	74	56	56
22/11/88	90	98	96	88	74	74	48	56
28/12/88	88	96	94	86	72	72	48	52

The different proportions of mixed rearing were characterised by homogenous survival rates both for Atlantic origins (chi2 = 0.00725, df = 2, $p > 0.05$) and for the Mediterranean one (Chi2 = 0.132, df = 2, $p > 0.05$). However, the mortalities were surprisingly higher at the lowest proportions of Mediterranean *O. edulis*. No clear differences could be detected between the different proportion for Atlantic *O. edulis*.

Prevalences of *Marteilia* and *Bonamia* were examined separately. The prevalence of *Marteilia* disease was very high, since 49.8 % of the flat oysters were contamined after 10 months of experiments (table 4).

Table 4 : Prevalence in percentages of flat oysters *Ostrea edulis* contaminated by *Marteilia refringens* after 8 months of on-growing in tidal ponds. 25, 50, 75 and 100 % are percentage of flat oysters reared together with Japanese oysters.

Origin	100 %	75 %	50 %	25 %	Average
Mediterranean	43.7	38.0	62.0	39.1	45.6
Atlantic	55.5	31.4	74.0	55.2	54.0

Between the two populations, no significant difference was observed in the final prevalence of Marteilia (Chi 2 = 2.779, dl = 3, p = 0.095). However, significant differences were observed for the prevalence measured at the different proportions, both for the Mediterranean population : Chi2 = 12.296, dl = 3 and p = 0.0064, and for the Atlantic one : Chi2 = 16.886, dl = 3 and p = 0.00075. Independance between the prevalence at different proportions and origins of the flat oysters was tested from the contingency table and no significant dependance was observed : Chi 2 = 3.1, dl = 7, which is lower then the theoretical distribution of prevalence (Chi2 = 14.067, dl = 7, for p = 0.05).

The average prevalence of *B. ostreae* was much reduced, since 7.4 % of the oysters were contaminated by this parasite at the end of the experiment (table 5).

Table 5 : Prevalence (in percentage) of flat oysters contaminated by *B. ostreae*, after 8 months of on-growing in tidal ponds. 25, 50, 75 and 100 % are percentages of flat oysters reared together with Japanese oysters.

Origin	100 %	75 %	50 %	25 %	Average
Mediterranean	10.3	7.2	0	5.2	5.7
Atlantic	13.3	8.3	6.6	8.6	9.2

A significant difference was observed in the prevalence of *Bonamia*, between the two origins (Chi 2 = 0.0144, dl = 7). The flat oysters from naturel beds of the Atlantic coasts were then apparently more sensitive to *B. ostreae* ofthe Mediterranean ones. For the Mediterranean origin, the mixed culture resulted in prevalences significantly related to the proportions of flat oysters (Chi 2 = 8.094, dl = 3, p = 0.044 < 0.05). This was not observed for the Atlantic origine (Chi 2 = 3.41, dl = 3, p = 0.333). The last test on the dependance between prevalences observed at different proportions and the origin of the oyster, showed that no dependance was detected : Chi2 observed = 3.158, dl = 7, which is less then the theoretical distribution of the prevalence (Chi2 = 14.067, dl = 7 for p = 0.05).

DISCUSSION

The growth and mortalities of Bivalves Molluscs are known to be dependant on the ecological environment, the nutritional availability, as well as the genetic characters and the pathological status of the populations.

In this experiment, environmental conditions may be considered as homogenous, as previous works have established that no difference was observed in the growth and mortality of *C. gigas* among these different ponds (Bougrier and Bodoy, unpubl. results). The tidal regime of these salt marshes make them quite uniformous, as they are regularly submerged every Spring tides. Any unforeseen difference within the contiguous ponds could have act on the results concerning the proportions, but not on these related to the origin of the oysters, since the two populations were represented in each pond.

The genetic influence was clearly demonstrated for these geographically separated populations. The Mediterranean oysters appeared not to be adapted to such environment. Their growth was slower than for the Atlantic oysters and their mortalities higher, as is often the case (Pacquotte and Moriceau, 1987). This may be related with the smaller hydrological fluctuations encountered in the Mediterranean sea, if compared with shallow, coastal areas submitted to tidal regime on the Atlantic coast.

The rearing at different proportions of flat oysters did not result in positive effects for the growth and mortalities. Since the higher proportion of *O. edulis* had a significantly better growth, a trophic competition may have occurred with *C. gigas* at the lower proportions. The latter species is known to have a more active metabolism (Héral and Deslous-Paoli, 1991) which in turns may have deprived the flat oysters from sufficient amounts of food. No clear trend could be detected for the mortalities, excepted a negative effects on the Mediterranean oysters. These have surprisingly suffered higher mortalities at the lower proportion. This fact, related to their slower growth, indicates that the main effect on these two parametres was probably due to a trophic competition with *C. gigas*, rather than an hypothetical decrease of *Bonamia* related mortalities with the contact of the Japanese species.

The low levels of prevalence observed for *B. ostreae* on surviving oysters, are in accordance with the previous observations. The oysters of the two origins were, at the beginning, *Bonamia*-free (no prevalence or under the detection threshold) and their final contamination for this parasite was not correlated with the observed growth and mortalities. Therefore, it seemed that these field results did not allow to confirm that *Bonamia* cells are densely phagocyted by the haemocytes of *C. gigas* under rearing conditions, in field environments.

In a similar experiment, it has been advocated by Lebec et al. (1991), that another factor may have interact with proportions and densities of *O. edulis* reared with *C. gigas*. As already mentionned, the oysters were identified as Bonamia-free at the beginning of the experiment for the two populations, and no other flat oysters were reared in the neighbouring. The main input of *Bonamia* should then have come from floods of sea water at Spring Tide. The prevalence of *B. ostreae* in *C. gigas* was very low, and the ponds were allowed to dry for two months before the experiment, thus killing any remaining Bivalve which may act an host. A lag time for the infestation of flat oyster by *B. ostreae* has already been described by Montes (1991). All these facts indicates that probably the *Bonamia* cells were evenly available for the infestation of flat oysters. Furthermore, the prevalences observed for *B. ostreae* in our experiment, after 10 months, were much lower than the ones measured by Le Bec et al. (1991) after 15 months in an open environment. This implies that the infestation by *B. ostreae* may not be evoked as the major cause for the mortalities observed in the two origins for this experiment. The lower prevalences observed for the Mediterranean oysters, if significant, received no satisfactory explanation.

High prevalences of *M. refringens*, around 50 %, were observed for the two populations. The life cycle of this parasite received a noticeable attention (Balouet, 1979). The contamination of oysters may occurr during the early period of life. But it has been demonstrated that the infestation rate is under strict dependance of the water temperature, which should be higher then 17°C. However the parasite development may occurr at lower temperatures, down to 12°C, once the oyster has been infected. In the experimental ponds, the water temperature was higher then 20°C, from the beginning of the experiment until late September, thus giving the opportunity for a large infestation of the oysters. Furthermore, *M. refringens* preferentially develops in rather confined environments, such semi closed bays, rias and abers, or rearing ponds (Grizel, comm. pers.). Adequate conditions were then encountered for high prevalences of this disease. The epidemiological results, along the Atlantic coasts of France during the warm Summers of 1989 and 1990, have confirmed these high prevalences observed in a confined site.

No difference was observed in the prevalence of *M. refringens* between the two populations. However, this disease may have been involved in part of the observed mortalities. The oysters which were examined for the determination of the prevalence showed noticeable alterations of the digestive system. They were also emaciated, and obvious histological lesions were found on contaminated oysters.

The mixed rearing of oysters did not give any clear advantage in terms of growth, mortality and reduction of the prevalence of *Bonamia*. However, because of the virulence of *M. refringens*, it appeared that any significant production of the flat oyster in these ponds could only been obtained with very short cycles of production, so as to reach a commercial size, before the contamination by *M. refringens* had caused heavy mortalities.

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