

Growth and mortality of the European oyster *Ostrea edulis* in the Bay of Arcachon (France)

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Abstract

A study of the growth and mortality of the European oyster *Ostrea edulis*, including the effects of *Marteilia refringens* and *Bonamia ostreae*, was carried out in three oceanic areas in the Bay of Arcachon from May 1989 to February 1991. One-year-old spat, free from disease were used for the trials. Six months after beginning the trials, *Marteilia refringens* had severely infested the cultures. Although *Bonamia ostreae* was found during the first year, the rate of infestation was low. In the second year Bonamiasis increased and high mortality (>60%) occurred in *Ostrea edulis* populations. Although the growth of the oysters was good, their condition index was generally poor. Moreover, shell perforations by *Polydora* sp. were observed. In spring 1990, a low glycogen storage was noted which may have been related to the *Marteilia refringens* infestation although no relationship with the carbohydrate content and mortality was detected. The influence of different rearing procedures on *Ostrea edulis* survival and development in four commercial batches of oysters, free of disease, was also studied. The growth of *Ostrea edulis* in single culture was better than in cultures mixed with *Crassostrea gigas*. However, infestation by *Marteilia refringens* and *Bonamia ostreae* in the laid cultures was quite similar to that of the experimental cultures (bags). Due to the persistence of these two parasites, the culture of *Ostrea edulis* on a commercial scale is not advisable in the Bay of Arcachon.

Keywords : *Ostrea edulis*, pathology, mortality, growth, reproduction, gross biochemical composition, bay of Arcachon.

Croissance et mortalité de l'huître plate Ostrea edulis dans le Bassin d'Arcachon (France).

Résumé

Une étude de la croissance, de la mortalité et des taux d'infestation parasitaire, par *Marteilia refringens* et *Bonamia ostreae*, de l'huître plate *Ostrea edulis*, a été réalisée dans le bassin d'Arcachon de mai 1989 à février 1991, à partir de naissain âgé d'un an et indemne de parasite à l'origine. Dans les trois secteurs concernés, une importante infestation par *Marteilia refringens* était notée six mois après la mise en culture. Bien qu'au cours de la première année d'élevage, la présence de *Bonamia ostreae* ait été décelée, son degré d'infestation est resté faible. Au cours de la deuxième année, ce parasite affectait 10 à 15 % de la population. Au-delà de la première année, de fortes mortalités d'*Ostrea edulis*, supérieures à 60 %, ont été enregistrées. Si une bonne croissance a été relevée, les huîtres ont généralement présenté un index de condition médiocre. De plus, dans la zone la plus performante sur le plan de la croissance, le Banc, la qualité de la coquille a été sévèrement affectée par *Polydora* sp. Au printemps 1990, une faible accumulation des réserves de glycogène était observée, celle-ci pouvant être liée à l'infestation des mollusques par *Marteilia refringens*. Par contre, il n'a pas été possible d'associer les mortalités observées à un épuisement préalable de ces réserves. D'autre part, le suivi de quatre élevages professionnels, exempts de parasite à l'origine, a permis de déterminer l'influence de la pratique culturale sur le développement d'*Ostrea edulis*. Comparées à des cultures mixtes (*Crassostrea gigas* + *Ostrea edulis*) de meilleures croissances étaient enregistrées dans les cultures

monospécifiques. Par contre, la pratique culturale semble n'avoir que peu d'effet sur la propagation de ces parasitoses, les taux d'infestation par *Marteilia refringens* et *Bonamia ostreae* dans ce type de culture (au sol) étant aussi importants que dans les cultures expérimentales (poche). En raison de la persistance de ces deux parasites, l'élevage d'*Ostrea edulis*, à une échelle commerciale, n'est pas recommandé dans le bassin d'Arcachon.

Mots-clés : *Ostrea edulis*, pathologie, mortalité, croissance, reproduction, composition biochimique, bassin d'Arcachon.

INTRODUCTION

As a result of epizootic diseases, the annual production of the European oyster, *Ostrea edulis*, in the bay of Arcachon has dropped from 20 000 t in 1900 to 2 tonnes in 1990 (Deltreil pers. com.). The first disease, whose origin is still unknown, appeared in 1920. A dramatic fall in flat oyster production occurred and the stock was exhausted in 1922. Ten years later, its culture was reinitiated in the bay, but competition with the Portuguese oyster (*Crassostrea angulata*) regularly introduced into the bay during the previous ten years, limited its extension. Because of *Crassostrea angulata* spread, only 10% of the appropriate sites were available for *Ostrea edulis* culture and its production never exceeded 1 500 tonnes.

Since 1970, a new disease, caused by *Marteilia refringens*, has affected *Ostrea edulis*. From 1970 to 1976 the disease increased in the bay (His *et al.*, 1976), after which the parasitosis seemed to decline (Tigé *et al.*, 1979). From 1980, the disease reappeared and most of the commercial oyster cultures failed (Deltreil pers. com.).

In 1980, *Bonamia ostreae*, was found in Brittany (Comps *et al.*, 1980), and was noticed in the bay of Arcachon. As a result, the commercial culture of the flat oyster in the bay was stopped.

Nevertheless, since 1987, the number of *Ostrea edulis* larvae in the bay has increased and two wild oyster stocks were discovered in the main channel of the bay. In spite of substitution of this species by the cupped oysters, the French market's demand of flat oysters greatly exceeds supply. In answer to the Arcachon oyster farmers' request, the present study was carried out to assess the potential for the renewal of *Ostrea edulis* culture in the bay.

MATERIALS AND METHODS

Previous studies have shown the importance of local environmental conditions on the growth of the Japanese oyster *Crassostrea gigas* (Maurer, pers. com.) and on the Manila clam *Ruditapes philippinarum* in the bay of Arcachon (Robert *et al.*, 1991). Furthermore, high salinities have negative effects on

Marteilia refringens development (Grizel, 1985). Consequently, to locate the most suitable area for the cultivation of the European oyster in the bay, one-year-old *Ostrea edulis* spat (30 mm shell length approximately 3 g), free of disease, purchased from Morbihan (Brittany-France) were reared in bags, in three oceanic areas of the bay of Arcachon, from May 1989 to February 1991. These cultures will be called experimental cultures. To determine the influence of the rearing procedure on *Ostrea edulis* survival and development, one-year-old 3 g spat (30 mm), also purchased from Morbihan, were laid on sites located in an oceanic area of the bay of Arcachon. They were reared in single or mixed cultures with *Crassostrea gigas*, at different densities, from May 1989 to December 1990. These cultures will be called commercial cultures.

Experimental cultures

Mortality, growth and condition index

Experimental cultures were placed at the density of 200 to 500 individuals.m⁻², in oyster bags held on 30 cm high trestles, in the intertidal zone, at three oceanic areas of the bay of Arcachon, "Ferret", "Banc" and "Courbey" (fig. 1). At each site, two kinds of surveys were carried out over 21 months. In the first, mortality, shell length and total weight increment were determined monthly from whole populations of 100 oysters per bag (200 individuals.m⁻²). In the second survey, samples of 30 individuals were taken each month from other populations placed in 6 oyster bags per site at a density of 500 individuals.m⁻² (simple random sampling without replacement). Length, total weight, dry meat weight, dry shell weight and condition index (according to Medcoff and Needler, 1941) were recorded. Differences between stations were assessed using one-way analysis of variance (ANOVA) for the survey 1 and *t*-tests for the survey 2.

Gametogenesis and shell infestation

During the second year, fatness of the molluscs, gametogenic development and degree of infestation of the shells by *Polydora* sp. were also recorded monthly from the second survey. Three arbitrary stages were used to describe the change in fatness: glairy

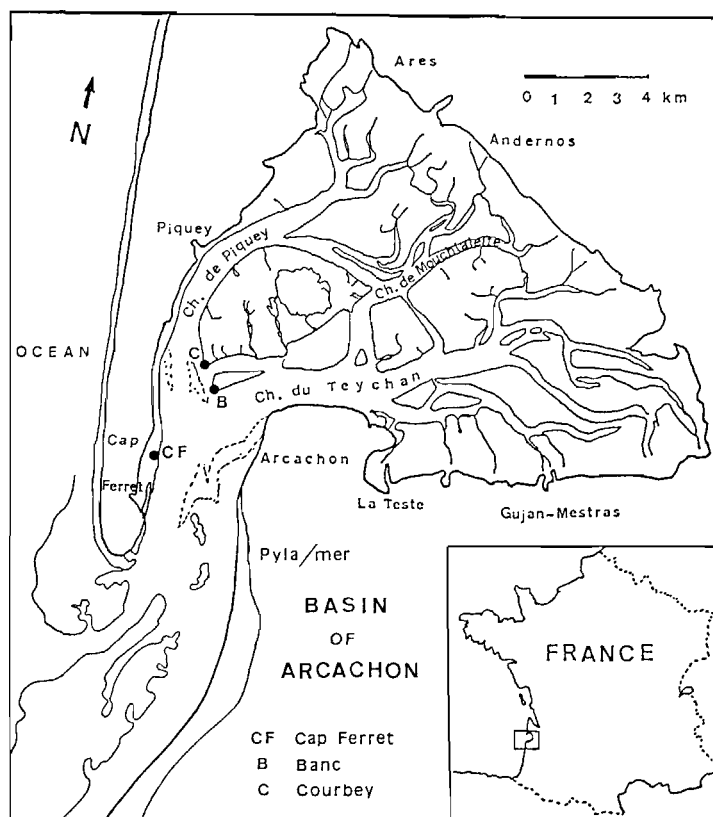


Figure 1. — Situation of the experimental and commercial cultures of *Ostrea edulis* in the bay of Arcachon.

to thin, thin to fatty and fatty to very fatty. The gametogenic development was studied by means of microscopic observations after piercing the gonad. Four stages according to Marteil (1960) were used to describe the reproductive cycle of *Ostrea edulis*: stage 0-1, no or rare traces of sexuality present; stage 2, oocytes or spermatozoa present but few in number; stage 3, numerous and active spermatozoa or free oocytes; stage 4, spawning. Direct observations of the number of flat larvae in the plankton were also made.

Biochemistry

From Banc only, the gross biochemical composition was also recorded monthly using vacuum-dried pooled tissue (30 individuals sampled from the second survey) as recommended by Giese (1966). For each component, 3 to 5 grammes of homogenized tissue were used and all measurements were performed in duplicate. The methods of Lowry *et al.*, (1951), Dubois *et al.*, (1956), Bligh and Dyer (1959) and Marsh and Weinstein (1966) were used to determine the protein, carbohydrate and lipid levels respectively. The biochemical results were expressed as percentage of ash-free dry weight. The proportion of ash was estimated after incinerating tissue homogenates in a muffle furnace at 500°C for 24 hrs.

Histopathology

Infestation by *Marteilia refringens* and *Bonamia ostreae* was determined quarterly from histological observations of 20 to 30 individuals sampled from each area from the second survey. For light microscopy, animals were fixed in Davidson's solution and embedded in paraffin. The sections were stained with Masson's trichromic (Pichot, 1984). *Bonamia ostreae* was also detected from squash preparations made of the heart which were stained with methylene blue-eosine (RAL 555).

Commercial cultures

Four commercial cultures were surveyed over a 19-month period. Two batches were reared at low density of 50 *Ostrea edulis*.m⁻² and two others were mixed with *Crassostrea gigas* at a density of 200 individuals.m⁻² (50 flat oysters.m⁻² and 150 cupped oysters.m⁻²). Samples of 30 animals were randomly taken quarterly, and length, total weight, condition index, prevalence of Martelliasis and Bonamiasis were measured using the techniques described previously.

Hydrobiology

Seawater temperature, salinity and chlorophyll *a* were monitored weekly at midday on water surface at the stations of Banc and Courbey. To minimize advection problems, sampling was made along with the incoming tide, starting 1 hrs. before high or low tide. Temperature and salinity were measured with a portable T-S Meter (YSI model 33). For chlorophyll *a* determination, 100 ml of seawater was filtered on GF/C membranes. Filters were kept in an ice box until fluorometer analysis (Turner model 112), according to Lorenzen (1967).

RESULTS

Experimental cultures

Survey 1

Mortality. — In all areas, high mortality (60 to 80%) was recorded at the end of the trials (*fig. 2a*). Severe mortalities began in February 1990, ten months after the beginning of the trials. In spring and summer 1990, the mean lengths of live and dead oysters were similar, indicating that mortality was not selective but covered a large size range. The highest mortality rates occurred in summer 1990: 15% in the Courbey area, 20% in the Ferret and 23% in the Banc.

Growth. — From July to September 1989, growth rates sharply increased, then declined until the following summer (*fig. 2b*). From May 1989 to February 1991, flat oysters grew from 30 to 50-60 mm. Increases in total weight were steadier in all areas but development was better at the Banc site (*fig. 2c*; table 1). At the end of the trials, the mean total weights ranged from 30 to 40 g and a high dispersion of these values was noted with a coefficient of variation of 52%.

Survey 2

Growth. — As shown by Newman-Keuls tests in the survey 1, Ferret was an intermediate growing area, and consequently, results will only concern Banc and Courbey in this survey 2. On the other hand, to make *figure 3* clearer, data will be represented quarterly. As previously shown, flat oysters grew faster in the Banc area at $p=0.05$ (*fig. 3a*). Compared to total weight, increases in dry shell weight and dry meat weight followed similar patterns (*figs. 3b,c*). In February 1991, mean dry meat weight ranged from 0.45 to 0.65 g and a high dispersion of these values was recorded with a coefficient of variation of 65%.

Gametogenesis. — In 1990, one or two spawning periods occurred in each area in April-May and June-August (*figs. 4a,b*). However, in both stations most of the oysters (>60%) generally exhibited spent gonads characteristic of the stage 0-1. Larvae were

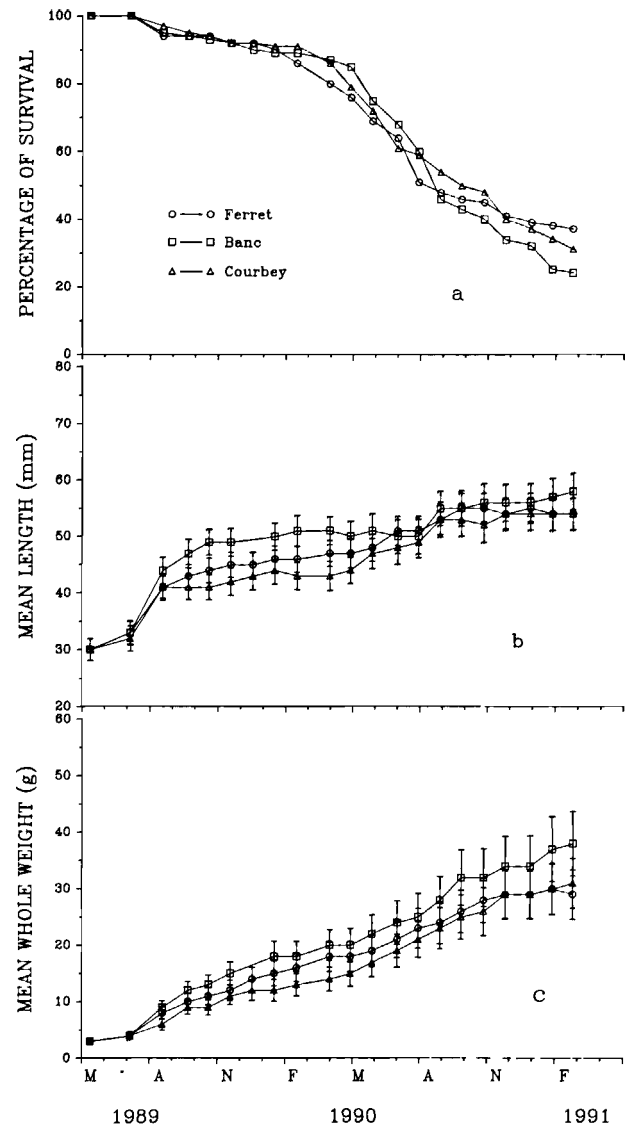


Figure 2. — Percentage of survival (a), shell length (b) and live weight increment (c) of *Ostrea edulis*, in experimental cultures, in Banc, Ferret and Courbey, three oceanic areas of the bay of Arcachon, from May 1989 to February 1991 (monthly means calculated from whole populations).

found in the plankton from June (beginning of plankton sampling) to September (*fig. 4c*).

Fatness, condition index and shell infestation. — In spring and summer 1990, only 40% of oysters were fatty (*fig. 5a*) and condition index was generally poor with values of 40 to 80 (*fig. 5b*). In February 1991, individual measurements of this index showed important dispersion with a coefficient of variation of 48%. In both stations, early infestation by *Polydora* sp. was noted but was higher in Banc (*fig. 5c*).

Proximate biochemical composition. — In Banc, ash content showed little change over the study period

Table 1. — Effect of site on weight of *Ostrea edulis*. One-way analyses of variance were made in May 1990 and in February 1991 ($1-\beta$: power of the test; ** significant at $p=0.001$; * significant at $p=0.05$).

Source of variation	1 year culture					2 years culture				
	Degree of freedom	Sum squared	F (ratio)	p	$1-\beta$ (%)	Degree of freedom	Sum squared	F (ratio)	p	$1-\beta$ (%)
Factor (site)	2	570.18	5.57	**	88	2	1005.27	3.73	*	77
Residual (error)	204	102.32				69	269.56			
Total	206	106.86				71	290.29			

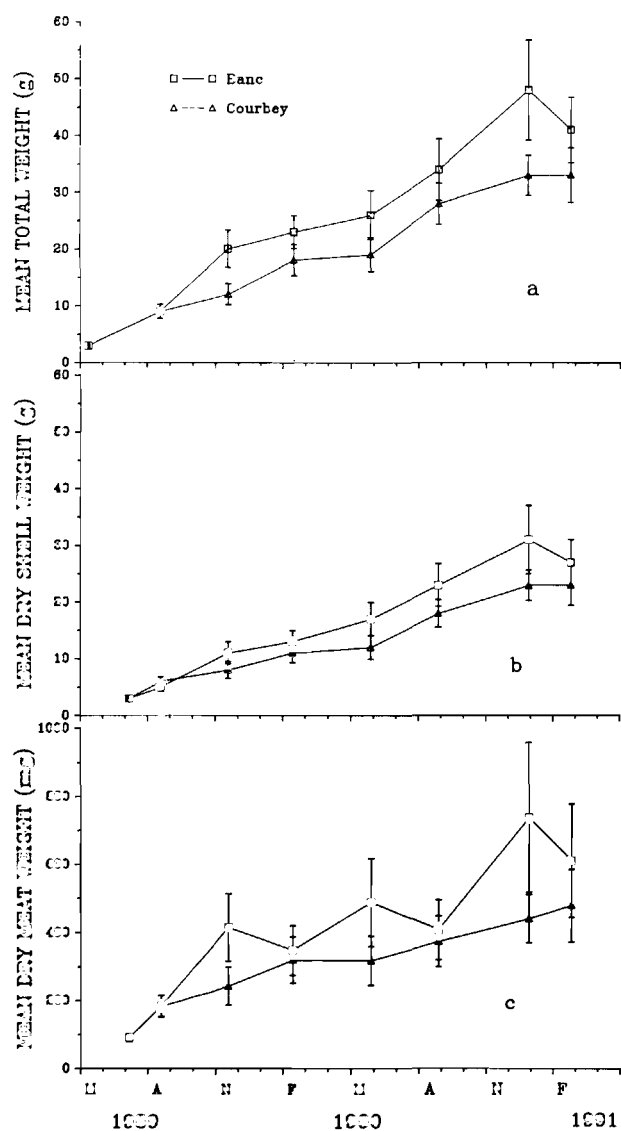


Figure 3. — Increase in live weight, shell weight and dry meat weight of *Ostrea edulis*, in experimental cultures, in Banc and Courbey (bay of Arcachon), from May 1989 to February 1991 (quarterly means calculated from samples of $n=30$).

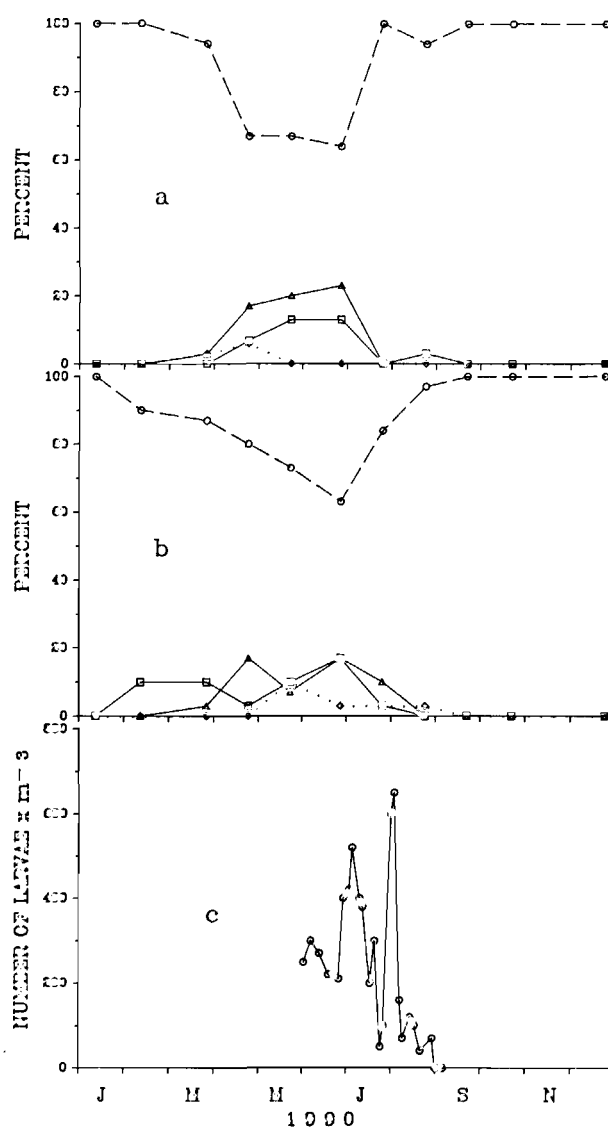


Figure 4. — Gametogenesis in *Ostrea edulis*, in experimental cultures, in Banc (a), and Courbey (b) and number of flat oyster larvae in the channel of Piqueu, West part of the bay of Arcachon, (c) from January 1990 to December 1990.

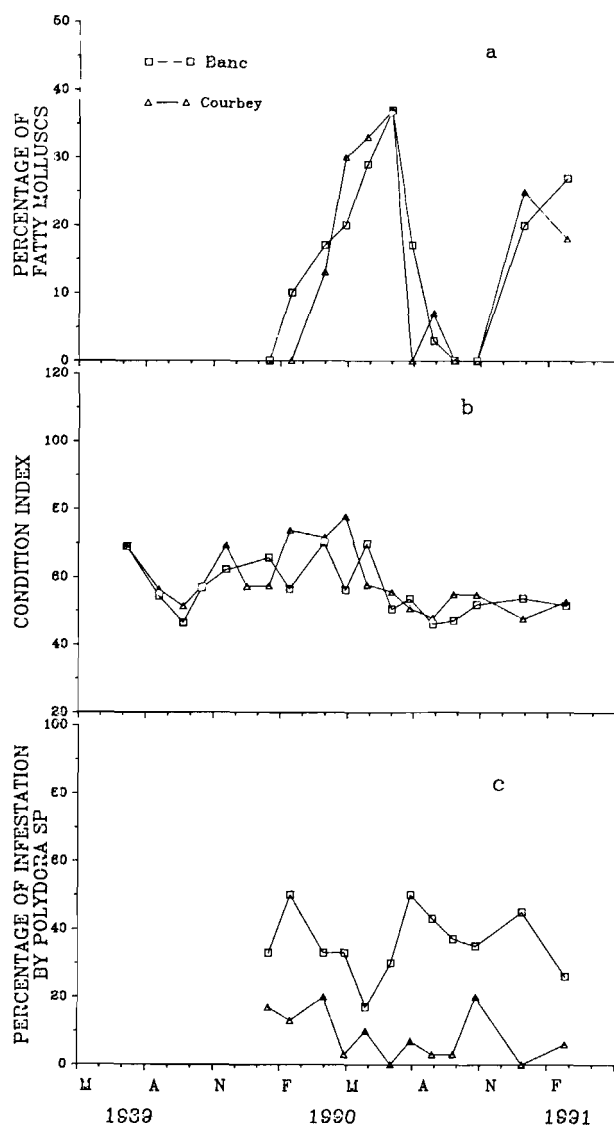


Figure 5. — Index of condition (a), fatness (b) and degree of infestation of the shells by *Polydora* sp. (c) of *Ostrea edulis*, in experimental cultures, in Banc and Courbey (bay of Arcachon), from May 1989 to February 1991.

(fig. 6a) and the average ash content of the dry meat in all samples examined was $19.42 \pm 1.90\%$. Carbohydrate contents increased from 3.2% in July 1989 to 15.7% in November 1989 (fig. 6b). A low glucid storage was noted in spring 1990 with values fluctuating from 11.2 to 16.9%. Glucid contents decreased from 16.6% in May 1990 to 8.8% in July 1990 and then recovered to earlier levels. Glycogen represented at least 85% of total carbohydrate and followed the same pattern (fig. 6b). From July 1989 to February 1991, neither lipid storage nor depletion were observed (fig. 6c). The values ranged from 9.2 to 12.7% and the average lipid content of organic matter in

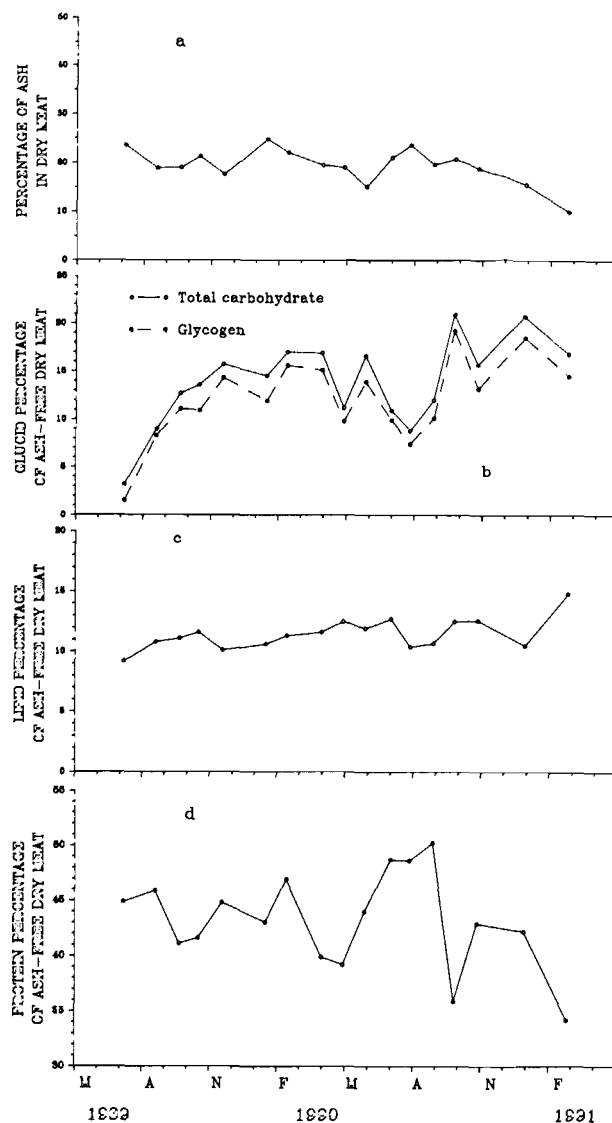


Figure 6. — Diagram of the change of ash in the dry meat (a) and the change of carbohydrate (b), lipid (c) and protein (d) in ash-free dry meat of *Ostrea edulis*, in experimental cultures, in Banc (bay of Arcachon), from May 1989 to February 1991.

all samples examined was $11.30 \pm 0.54\%$. Over the experimental period protein contents fluctuated erratically, ranging from 27.7 to 49% (fig. 6d).

Prevalence of *Marteilia* and *Bonamia*. — In October 1989, six months after the beginning of the trials, infestation by *Marteilia refringens* was high. In both areas, 50 to 60% of oysters were affected, particularly with numerous young plasmodia in the stomach epithelium (fig. 7a). From September 1989 to September 1990, infestation sharply decreased from 60 to 10% in Courbey, but *Marteilia* infestation was still high in Banc.

Infestation by *Bonamia ostreae* was low during the first 16 months (<5%) then increased, with values rising to 10-15% (fig. 7b). The degree of infestation by both protozoans seemed to be related to the loss of flesh, because the change in ratio of dry flesh weight/dry shell weight decreased with time (fig. 7c).

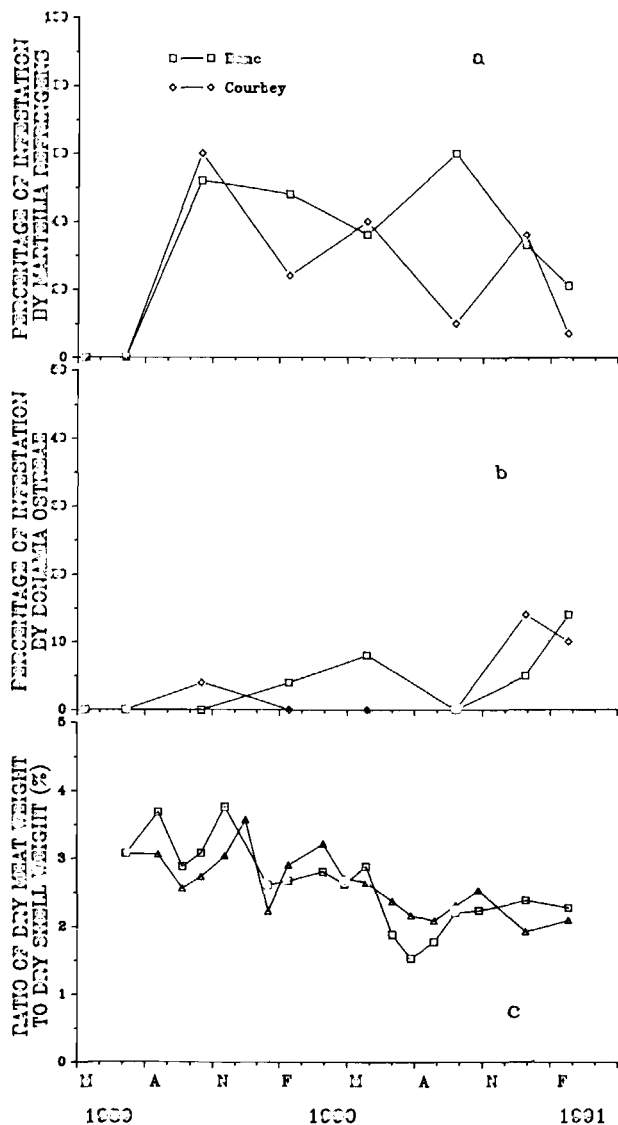


Figure 7. — Degree of infestation by *Marteilia refringens* (a), *Bonamia ostreae* (b) and change in ratio of dry flesh weight/dry shell weight (c) in *Ostrea edulis*, in experimental cultures, in Banc and Courbey (bay of Arcachon), from May 1989 to February 1991.

Commercial cultures

During the first year, *Ostrea edulis* had a significantly higher growth rate when grown alone than when mixed with *Crassostrea gigas* (fig. 8a), then

differences became less apparent. At the end of the survey, the mean lengths of 62 to 65 mm and the mean total weights of 57 to 58 g were recorded in single cultures. Respective values were 55 to 59 mm and 44 to 48 g in mixed cultures. Moreover, during the first year, better condition index were noted when *Ostrea edulis* was grown alone (fig. 8b).

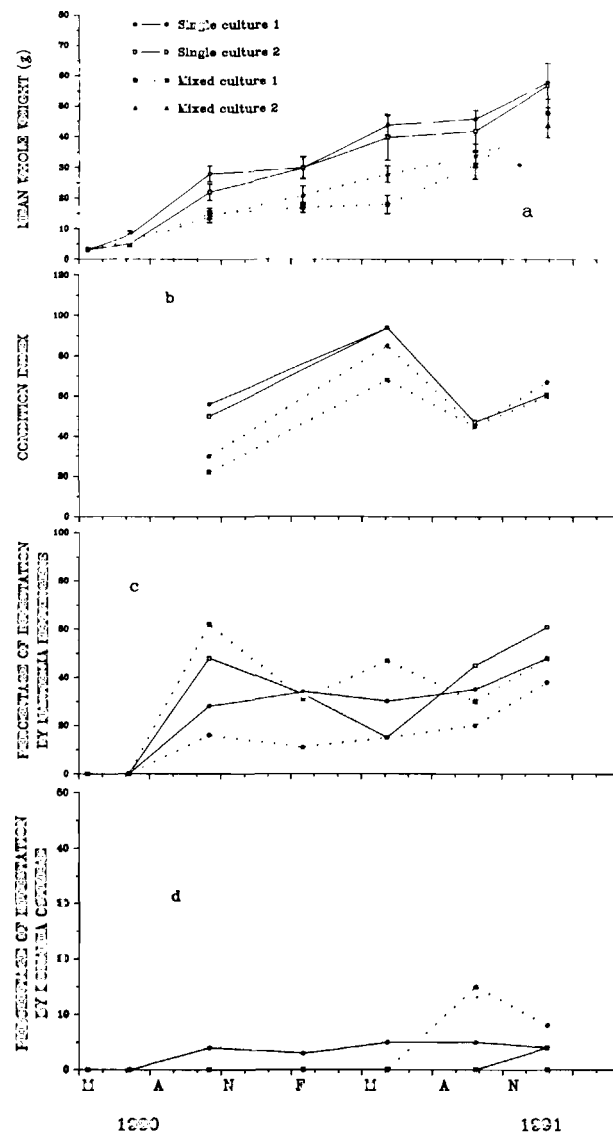


Figure 8. — Increase in live weight (a), change in condition index (b) and degree of infestation by *Marteilia refringens* (c) and *Bonamia ostreae* (d) in 4 commercial cultures, single and mixed, of *Ostrea edulis*, laid on the ground, in Banc area (bay of Arcachon), from May 1989 to February 1991.

Infestation by *Marteilia refringens* was high in one mixed culture, with values higher than 30%, and quite low in the second mixed culture with values generally

lower than 20%. In single cultures, infestation rates ranged from 30 to 60% and followed similar patterns to experimental cultures (fig. 8c). In all cultures, Bonamiasis prevalence was low (fig. 8d).

Hydrobiology

Fluctuations in seawater temperature, salinity and chlorophyll *a* are represented in figure 9 and show little differences between both stations. Winter 1989 was mild with water temperature exceeding 9°C. With salinity values generally greater than 32‰, the year of 1990 was especially dry. In 1989, unusual summer and autumnal phytoplankton blooms of high intensities were recorded. In contrast, blooms of low intensities were noted in spring 1990.

DISCUSSION AND CONCLUSION

In the bay of Arcachon, the failure of *Ostrea edulis* culture is clearly linked with the persistence of Marteiliasis and Bonamiasis.

The effects of *Bonamia ostreae* increased after 16 months on-growing but this could not be clearly shown in this study because the period of testing was not long enough. In contrast, the effects of *Marteilia refringens* on *Ostrea edulis* have been reported. Although the degree of infestation was higher in Arcachon, the pattern of *Marteilia refringens* disease is quite similar to those already described in Brittany (Grizel, 1985). While in Brittany mortality occurred in the autumn of the second year, severe mortalities were recorded in the spring in Arcachon which may result in an earlier and higher increase of seawater temperature. For similarly reared disease-free oysters, natural mortality of 5 to 15% has been recorded in other regions (Walne, 1961; Marteil, 1979; Paquette and Moriceau, 1987), and consequently, more than 50% mortality may be attributed to *Marteilia refringens* infestation. Similar results have been reported in Spain by Figueras (1991).

Amongst the different clinical signs proposed by Grizel and Tigé (1973) a low mollusc fatness was also observed. Because the loss of oyster flesh was related to the degree of infestation, Marteiliasis induces or exacerbates the poor condition of the molluscs.

Infestation by *Marteilia refringens* seemed to have negative effects on the gametogenesis of *Ostrea edulis*. A low number of individuals reached stage 3 and only 25% of oysters were ripe. Nevertheless, the delay between two consecutive observations was too long for a more detailed reproductive cycle study. To counterbalance this inconvenience, the total number of oysters belonging to the subsequent stages 2 and 3 will be taken into account. From this, only 35% of oysters were estimated to be in active stages. In Brittany before Marteiliasis spread, or today in regions free of disease, 60 to 80% of oysters generally reached

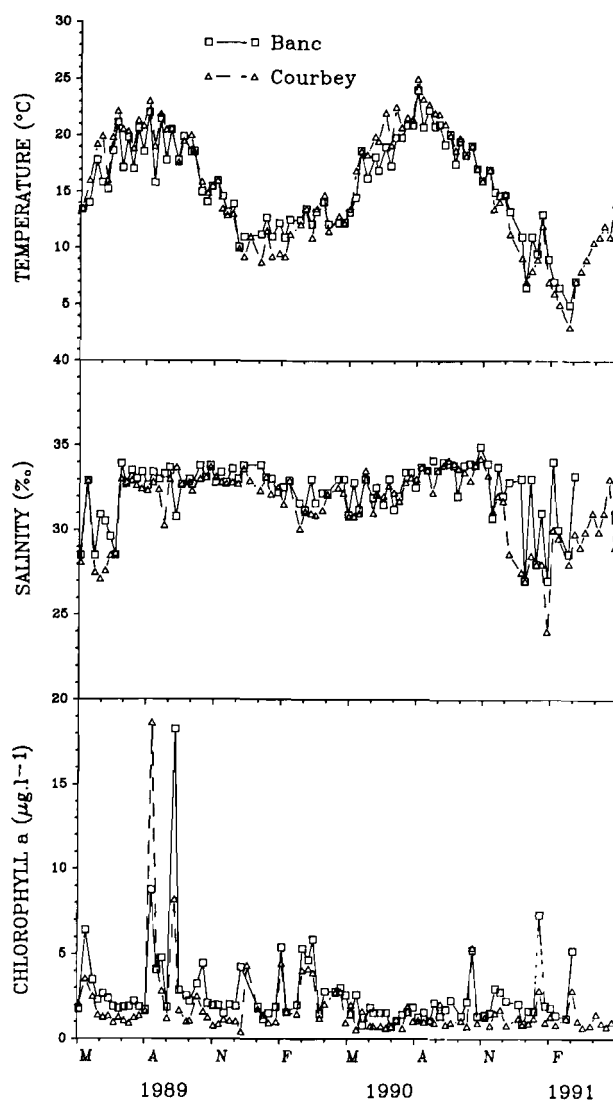


Figure 9. — Fluctuation in seawater temperature (a), salinity (b) and chlorophyll *a* (c) in Banc and Courbey areas (bay of Arcachon), from May 1989 to February 1991.

these gonadal development stages (Marteil, 1960; Wilson and Simons, 1985). Similar observations have been reported with *Bonamia ostreae* (Van Banning, 1990).

The evolution of the proximate biochemical composition confirms a disturbance of *Ostrea edulis* gametogenic development. In normal conditions, the accumulation and storage of carbohydrate in spring precedes gonad development (Eble, 1969; Walne, 1970; Holland and Hannant, 1974, 1976). These authors found low percentages of carbohydrate in winter, with values ranging from 5 to 10% of dry flesh weight, an increase in spring and a maximum rate in June with

values of 20 to 30%. In the bay of Arcachon, the lowest values were also found in winter, ranging from 11 to 13.5% but the highest value recorded in May 1990 was only 14%. This poor carbohydrate storage during spring 1990 (3%) may result from low sexual activity in *Ostrea edulis*. Moreover the lipids showed little variation over the 2 year period.

No relationship was found between previous glycogen depletion and *Ostrea edulis* mortality. Such a correlation has been reported on *Crassostrea gigas* by Maurer *et al.* (1986) and on *Ruditapes philippinarum* by Goulletquer (1989), with glycogen values lower than 1% of dry flesh weight. In the present work 5.6% was the lowest value recorded. Mortalities, which are not coarse but progressive, and sampling of infected oysters mixed with uninfected ones may explain this result.

A decline in infected oyster growth rate has been shown by Grizel and Tigé (1973). In the present study this pattern is not consistent. Mean total weights of 10 to 20 g and 30 to 60 g were recorded at the end of the first and second year, respectively. Such growth rates have been monitored in regions free of disease (Walne, 1958; Walne and Mann, 1975; Askew, 1978). Differences in growth rates have been found between the three stations selected here, with the best growth in Banc. There were little differences in environmental conditions between the stations. The differences in growth recorded here may be related to differences in immersion times. The experimental site located in Banc was at the lowest intertidal level, resulting in a greater immersion time during which oysters fed more. Differences in growth were also observed when oysters were reared in single or mixed cultures. When grown with *Crassostrea gigas*, the development of *Ostrea edulis* is poorer because of differences in total density, as proposed by Le Bec *et al.* (1991) or inter-specific competition, as proposed by Briggs (1978).

The losses caused by *Bonamia ostreae* must not be minimized, however the swift and high infestation by *Marteilia refringens* alone explains the failure of commercial *Ostrea edulis* culture in the bay of Arcachon.

For short-term purposes, two alternatives may be proposed to Arcachon oyster farmers. The first one is to attempt to restore the bay by de-stocking the area by either stopping commercial cultures or by dredging and destroying the infected oyster beds. It is well known that a small infected stock is sufficient to infect newly laid oysters (Tigé *et al.*, 1984; Van Banning, 1987, 1988).

The second solution is to continue such cultures but by minimizing the incidence of Marteiliasis and Bonamiasis in relaid stocks. For such a management, the culture strategy should be to rotate oyster beds and to employ only short-term culture periods (one year). Medium-sized juveniles (30 mm length) originating from regions free of disease must be planted in the oceanic areas of the bay in March-April at low densities of 50 individuals.m⁻² to improve their growth. In the following spring, when oysters exhibit the best condition index, the oysters should be harvested and the unwanted ones destroyed on land away from seawater.

For a medium-term solution the introduction and acclimatization of non-indigenous flat oyster species, resistant to disease, may be another alternative to improve flat oyster production. At present, four non-indigenous species have been tested on French coasts; *Ostrea chilensis* (Grizel *et al.*, 1983), *Ostrea denselamellosa* (Le Borgne and Le Pennec, 1983), *Ostrea angasi* (Bougrier *et al.*, 1986) and *Ostrea puelchana* (Pascual *et al.*, 1991). The sensitivity of these exotic species to *Marteilia* and *Bonamia* or with respect to cold temperature has prevented any success to date.

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