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Testing of the Argentina oyster, *Ostrea puelchana*, in several French oyster farming sites.

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ABSTRACT

Because of diseases affecting flat oyster *Ostrea edulis*, acclimatization of the Argentina oyster *Ostrea puelchana* has been tested in 1989-90 in five French oyster farming sites.

Following ICES recommendations, hatchery produced juveniles, from parents imported from Argentina, were transferred to experimental sites in summer 1989. Very high mortalities occurred, reaching 46 to 98% in March-April 1990. They continued later on, resulting in the end of experiments in March 1991. The growth of surviving oysters was relatively poor in comparison of the local species.

Parasites *Marteilia refringens* and *Bonamia ostreae* have been detected but at rates inadequate to explain the high mortalities.

All these results lead not to retain this species for breeding on French coasts.

RESUME

En raison des parasitoses sévissant actuellement sur l'huître plate *Ostrea edulis*, des essais d'acclimatation de l'huître argentine *Ostrea puelchana* ont été réalisés en 1989-90 dans cinq secteurs ostréicoles français.

Après production en écloserie à partir de géniteurs importés d'Argentine, dans le respect des recommandations du C.I.E.M., les juvéniles ont été répartis sur site en été 1989. De très fortes mortalités se sont déclarées, atteignant 46 à 98 % dès mars-avril 1990. Elles se sont poursuivies par la suite entraînant l'arrêt des essais en mars 1991. La croissance des survivantes s'est révélée relativement médiocre en comparaison de l'espèce locale.

Les parasites *Marteilia refringens* et *Bonamia ostreae* ont été décelés à plusieurs reprises mais à des taux insuffisants pour expliquer les fortes mortalités.

L'ensemble de ces résultats conduit à ne pas retenir cette espèce pour élevage sur les côtes françaises.

INTRODUCTION

In order to reduce the impact of the two protozoans diseases which have induced a high drop of the production of the flat oyster *Ostrea edulis* (Meuriot and Grizel, 1984), the French government built a prophylactic plan including the introduction of new species.

Regarding its biological requirements, the flat oyster *Ostrea puelchana* D'Orbigny, 1841, distributed from Southern Brazil to Northern Patagonia, Argentina (Castellanos, 1957) is one of the candidates. The common interest of one team of the Instituto de Biologia Marina y Pesquera, which desires to develop the oyster culture in Argentina, and of IFREMER, interested in testing the resistance of *O. puelchana*, has permitted to plan an experimental introduction, following the rules proposed by the ICES Working Group "Introduction and Transfer of Marine Organisms".

This paper reports the results of the transport and maturation of a broodstock of Argentinian oysters, the larval rearing and nursery growing of juveniles at the IFREMER hatchery of La Tremblade and at a private nursery, the implantation and the results of two years rearing of the species in oyster parks, in different sites of the French coast.

I.- SPAT PRODUCTION

I.1.- Parent stock : collection, transport, maturation and spawning

As all species of the genus *Ostrea*, *O.puelchana* is a protandric hermaphrodite, with consecutive rhythmic sexuality (Coe, 1942). While fitting this general pattern, this species shows a unique breeding system (Calvo and Morriconi, 1978). During the reproductive season, from mid November to mid

March, oysters smaller than 55 mm in height are predominantly males while large oysters are predominantly females. These large oysters often carry small individuals attached to a flat platform originating from the anterior edge of the concave shell. These small epibiotic individuals mature as males at about 2 mm of shell diameter (Morriconi and Calvo, 1978). Recent experimental research on the subject demonstrated that "carrier" females have a strong influence in retarding the growth rate of small epibionts which are able to live in this "dwarf" condition during a long period of their life (Pascual *et al.*,1989). This alternative mating system provides a greater success in fertilization which takes place, in the mantle cavity, where larvae are incubated.

In autumn 1988, oysters were randomly collected by diving at Banco Reparo and Las Grutas, the two main beds of San Matias Gulf (Fig.1)(Pascual, Thesis in progress). No abnormal mortality or disease has been recognized in these natural populations and no parasite was found on the analysed lot. After transport in isotherm boxes, the batch arrived to the IFREMER hatchery on October 26th 1988. There, it was placed in a quarantine system at water temperature of 13-15°C and fed daily with a mixed diet of *Isochrysis galbana, Chaetoceros calcitrans* and *Tetraselmis suecica*. The broodstock consisted of 500 free living oysters (size ranging from 23 to 107 mm of total height), 50 carrier females (size ranging from 60 to 90 mm) and 64 small epibiotic males (size ranging from 10 to 25 mm) detached from sacrificed carrier females.

Maturation and spawning took place between November 1988 and April 1989. Non-carrier females joined to free males were treated by lots while non-carrier females with detached epibiotic males and carrier females with attached male were matured by isolated couples.

The descendants of the different kinds of crossings were maintained separated during all subsequent manipulations.

I.2.- Larvae : rearing and settlement

The larval rearing methods were essentially the same for all larval origins.

After filtration through a 150 µm mesh and counting, the larvae were reared in 150 l tanks at a density of 1 to 5 larvae/ml. Water was 1 µm filtered and furtherly U.V. sterilized. It was changed every 48 hours.

The usual daily diet consisted of *I.galbana* but mixed algal diets were also used during the last period of larval life.

At water temperatures ranging from 18 to 20°C, larval life lasted 20 to 24 days, comparable to previous data (Zampatti, unpublished). A significant reduction in larval duration was obtained when temperature was maintained at 23-25°C.

The mean total mortality ranged from 35 to 50% except in one case of rod-bacterium development basifying the culture medium. No difference was shown in mortality rates among larval batches of different origins.

Height growth is approximatively linear during larval period. Figure 2 shows three typical curves, each one representing a different kind of descendance. No striking difference in growth rates was detected in relation to larval origins.

The larvae were transferred to fixation raceways when 50% pediveligers were detected. Seawater temperature was maintained at 24°C and food ration was doubled. Fragments of oyster shell (300-500µm) were used as collectors. Settlement occurred at the size of 260 to 310 µm after 17 to 31 days of larval life.

The fixation rate, post-estimated by spat countings, ranged from 38 to 99%, no mortality being detected during the periodic sampling of the sieves. However the first lot of spat was excluded due to a great mortality from an excess of Nitrogen in water.

I.3.- Spat : nursery treatment

Once settled, the spat was transferred in fixation sieves to the nursery room. Water temperature was maintained to 20°C and a water fall system was set out. Algae were added daily through a continuous drop to drop system.

In May 1989, the total quantity of spat was estimated to 800,000 and two thirds of it were transferred to a private nursery (Y. Boisard, Marennes) in order to improve growth rates and fasten transplantation to oyster parks. During the following month, growth rate was twice higher at Marennes. On the other hand, mortality was more important at this site (27.71% vs 12.06%). The higher water flow at Marennes but also the higher temperature may explain these results.

The highest mortality occurred with non-carriers' descendants (30% at Marennes vs 5% for epibiotic males' descendants). These results suggest that they have a greater susceptibility to extreme conditions.

Transfers were then decided in order to provide better growing conditions to the spat.

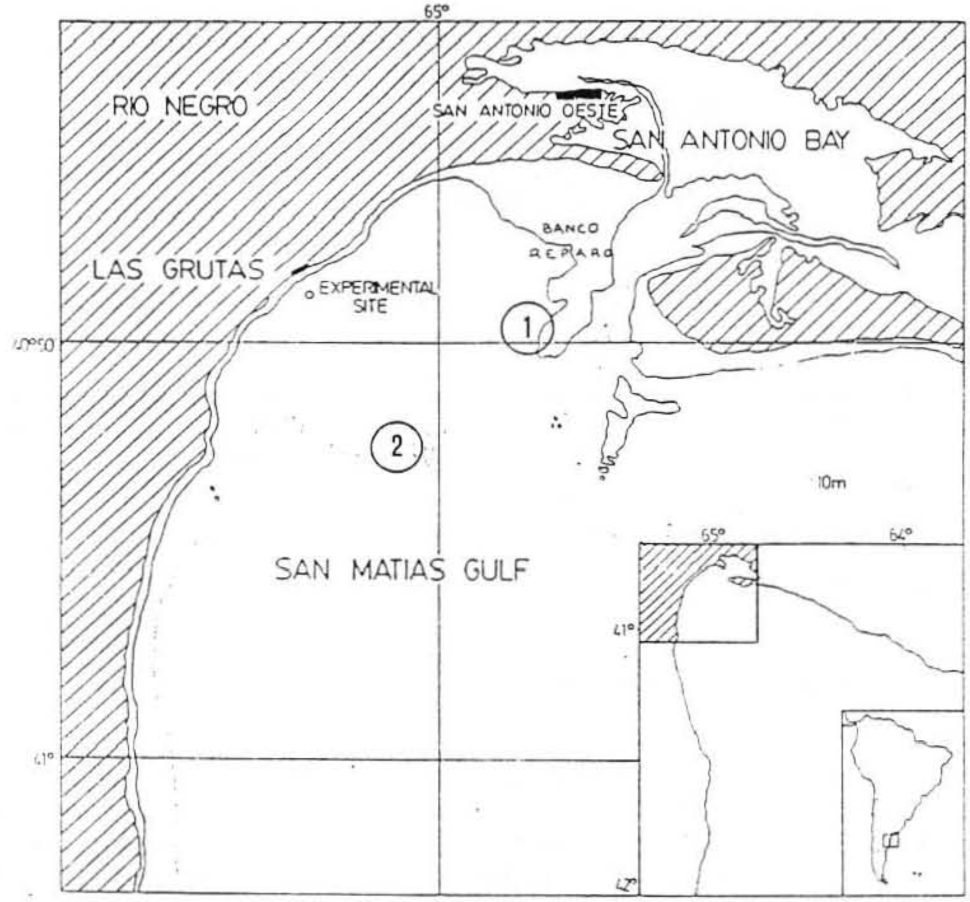


Figure 1.-The natural grounds of *Ostrea puelchana*: geographic location.

1: Banco Reparo
2: Bajo Oliveira

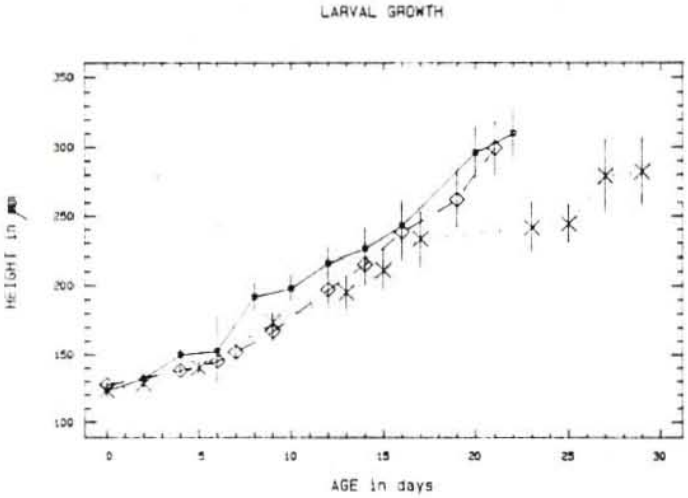


Figure 2.- Type larval growth curves. (mean height ± standard deviation)

- ▪ I: non carriers' descendants
- × II: descendants of non-carriers and epibiotic males
- ◇ III: carriers' descendants

I.4.- TRANSFER TO OYSTER PARKS

Spat was inequally allocated among regions (Fig.3): the greatest quantity was sent to Brittany where *Bonamia ostreae* and *Marteilia refringens* are present.

The first batch of 160,000 juveniles was transferred on the 29th of June 1989 to three sites of Brittany: Bay of Quiberon, St Philibert river and Penzé river.

The second batch (31,000 juveniles) was transferred to the Bay of Arcachon on the 20th of July 1989.

The third one (12,000 juveniles), after spending a few weeks at IFREMER nursery of Bouin, was transferred to Sète, on the Mediterranean coast, on the 22th of August.

II.- GROWING TESTS

II.1.- EXPERIMENTAL PROTOCOL

Repartition of different kinds of descendants in each site is quoted in Table 1. For the first transfer, it may be noticed that about 7% of mortality occurred during transport in lot I (non-carriers' descendants) versus 2% and 0% in lots II and III. Moreover, there is a great variability in mean weights according to staggering of larval productions and duration of nursing.

The juveniles were placed in 0.4 to 0.5 m^2 plastic bags with a mesh adapted to oysters size. Density per bag varied from 320 to 2 200, according to size. The bags were secured to metal trestles, 0.5 m above ground, either in intertidal (St Philibert, Penzé, Arcachon) or in subtidal (Quiberon -5m) areas. In Mediterranean, the bags were inserted into metal structures, following the local experimental culture method in open sea, and immersed at -30 m.

Small quantities of *Ostrea edulis* were produced at the same period by a private hatchery (SATMAR) to be used as controls in Brittany sites. The initial individual mean weight was 0.45 g. In the other sites, natural spat, collected during summer 1988 and reared near by, might serve only as indicator, being about six months older.

Growth, mortality and parasites prevalence were assessed every two to six months depending on sites. Nevertheless, sampling was disturbed by high rates of mortality and, in open sea, by bad weather or unavailability of professional boats.

Country	Site		Group I			Group II		Group III			TOTAL
			Large	Medium	Small	Medium	Small	Large	Medium	Small	
BRITTANY 29/06/89	Saint Philibert	Nb	10 000	23 900	-	20 000	-	-	-	21 400	75 300
		Mean Weight	0,74	0,32	-	0,36	-	-	-	0,21	
	Penzé	Nb	-	12 900	41 600	8 000	7 300	-	8 000	-	77 800
		Mean Weight	-	0,32	0,11	0,36	0,22	-	0,27	-	
	Quiberon	Nb	-	900	1 300	1 000	900	-	2 000		6 100
		Mean Weight	-	0,32	0,11	0,36	0,22	-	0,27		
ARCACHON 20/07/89	Banc	Nb	300	2 700	8 500	1 700	3 000	300	3 600	11 000	31 100
		Mean Weight	0,5	0,05	0,05	0,13	0,05	0,5	0,05	0,03	
MEDITERRANEAN 22/08/89	Sète	Nb	800	6 500	-	1 400	-	800	3 000	-	12 500
		Mean Weight	1,2	0,26	-	1,0	-	1,8	0,6	-	
TOTAL			109 400			43 300		50 100			202 800

Table 1. *O. puelchana* : Repartition of different kinds of descendants in French sites

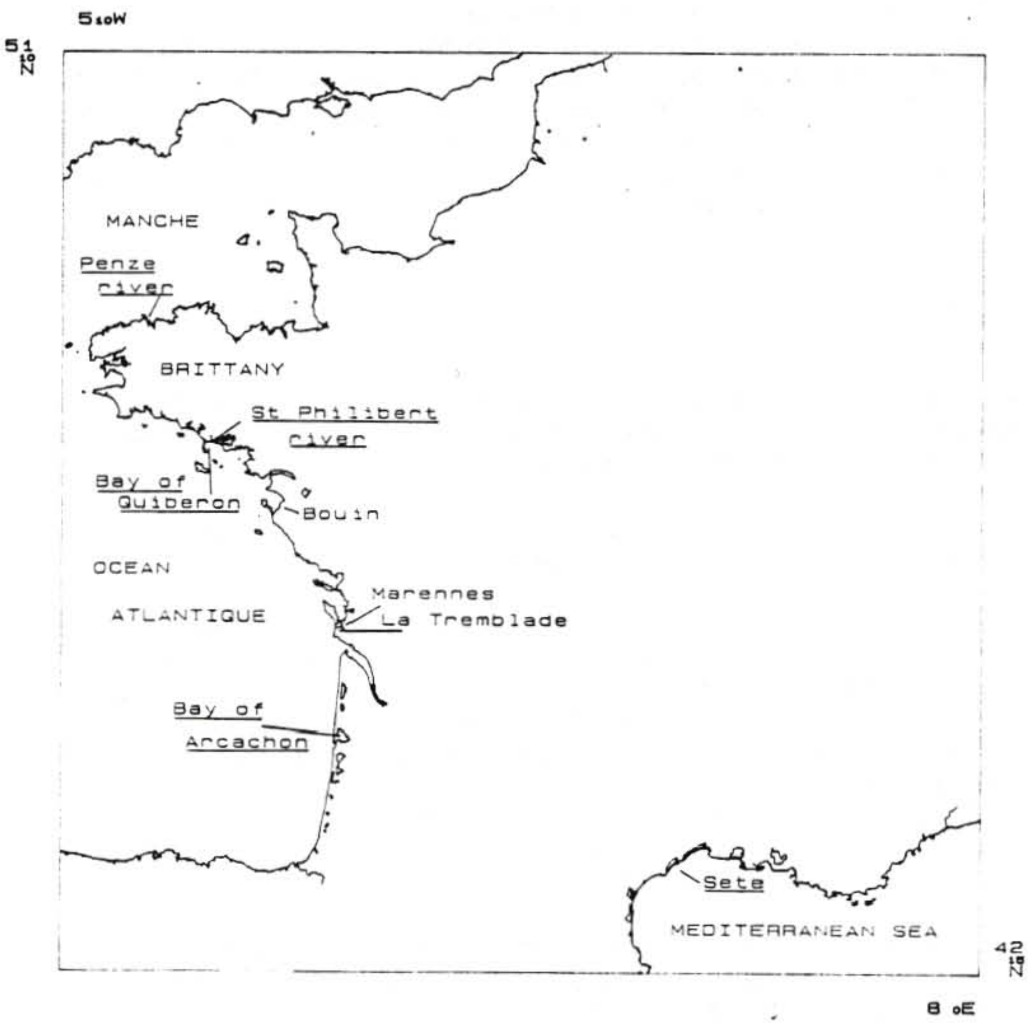


Figure 3.- *O.puelchana* tests on French coasts: location of spat production sites and growing sites.

II.2.- RESULTS

Mortality

Mortality rates are presented in Figures 4. a,b,c,d,e.

In Brittany, they occurred very early, first affecting preferentially non-carriers' descendants, then touching every group, at rates from 80 to 93% three months after planting. They continued during following months, leading to an end of the comparison, with survivals as low as 0.5 to 1.5% versus about 40% for controls.

In intertidal areas, mortality was higher in Penzé than in St Philibert during winter 89-90 (50 to 80% vs 30 to 50%), difference also observed for control oysters (22% vs 9%). This difference fainted later on.

At Arcachon, the same evolution as at St Philibert was observed for both species with *O.puelchana* survival less than 2% after eighteen months (22% for *O.edulis*).

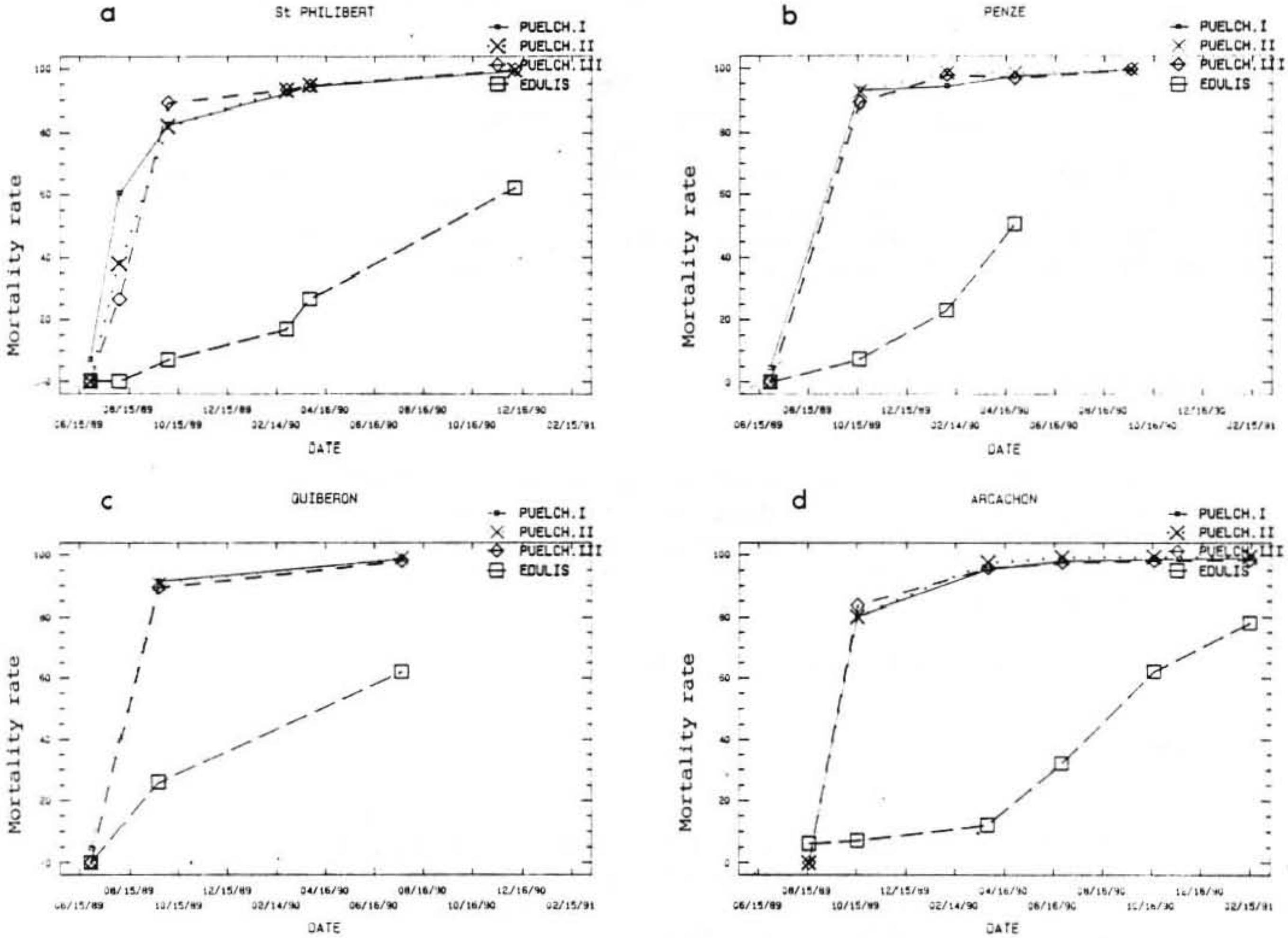
Mortality was slower in the Mediterranean site, with more pronounced differences between groups: better survival was obtained for carriers' descendants, at least for the first months, mortality increasing later to reach 82% at the end of the test (90 to 95% for the other groups). Comparatively, mortality of natural spat amounted to 20-30% from six to thirteen months of age (Paquotte, 1990) and was no more than 10% during the second year.

Growth

Figures 5.a,b,c,d show growth curves in each site, each value representing the mean height (from the umbo to the opposite edge of the shell) or weight of 30 to 100 individuals with confidence interval (95%). There are few differences between the groups of the Argentinian species, and curves are represented only for group III and control.

In Brittany sites, a marked difference between the two species was observed, specially in intertidal areas: the local oyster weight reached three to four times *Ostrea puelchana*'s (24 to 29 g vs 6 to 9.5 g at the end of the test). The growth of the Argentinian oyster is also very poor comparatively to performances recorded in its natural habitat: while Fernandez Castro and Bodoy (1987) mention an annual growth rate of 33 to 38 mm for one year old oysters, the observed annual growth rate in this test reaches only 10 to 20 mm.

Similar observations were made in the Mediterranean site without any possible comparison with local oysters which were selectively graded. However, the relatively poor quality indices recorded on the local oyster (condition index of



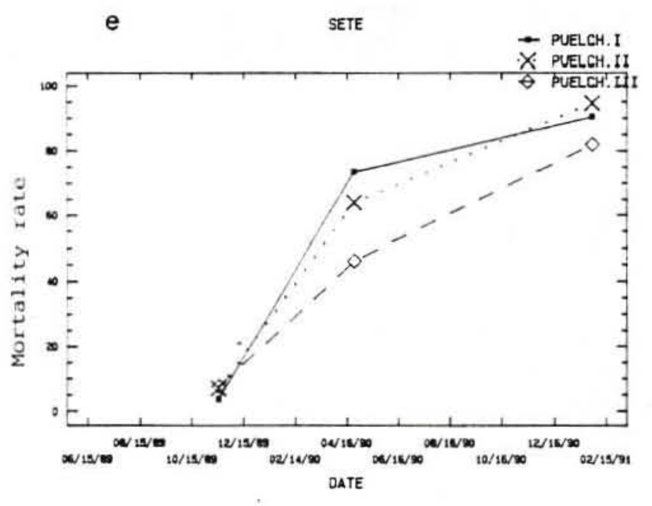


Figure 4. a,b,c,d,e -

Mortality rates of *O.puelchana* and *O.edulis* observed during test on French sites.

a : St Philibert
b : Penzé Brittany sites
c : Quiberon

d : Arcachon

e : Sète

a ST PHILIBERT PENZE QUIBERON

Medcof-Needler < 60) may suggest insufficient availability of food at this depth.

The best growth rate of *Ostrea puelchana* was recorded in Bay of Arcachon, with an annual increment of 23 to 31 mm according to groups and a final weight of 20 to 24 g (two years old oysters). *O.edulis* reared in the same area had reached similar size at the same age (50 mm and 24 g) but six months backwards. This species was also reared differently (ten months on collectors and then inside bags) (ROBERT *et al* in press).

Reproduction behaviour

In South Brittany several oysters with mature gametes or incubating larvae were observed in autumn 1989 (eight to ten months old oysters, size of 25 and 39 mm) and in summer 1990. The first incubations were also observed in autumn 1989 with *Ostrea edulis*.

No controls were made at other sites.

Pathology

Results of parasites observations are detailed in table 2 and 3 and percentages represented in Figure 6.a,b. Those observations were realized by photonic microscopy, either on histological sections or on smears.

Marteilia refringens was observed from October 1989 to March 1990 in intertidal area of St Philibert. *Bonamia ostreae* followed it, affecting sligthly *Ostrea puelchana*, but severely *Ostrea edulis*.

In Penzé, where *Marteilia* has no more been noticed for several years, only *Bonamia* occurred, but at higher rates on *Ostrea edulis* than on *O.puelchana*.

In the Bay of Quiberon, where *Marteilia* has been never found on native *Ostrea edulis*, this parasite was detected on one Argentinian oyster. *Bonamia* was noticed at low rates, only on *O.edulis*.

In the Bay of Arcachon, *Marteilia* affected severely the two species, but the non indigenous species later than the local one. *Bonamia* was also observed but later on and in only 1% of Argentinian oysters compared to 14% of local oysters.

In addition to those two protozoans, the copepod *Mytilicola* was detected inside digestive gland, occasionally in Arcachon, but at higher rates in Brittany (specially in

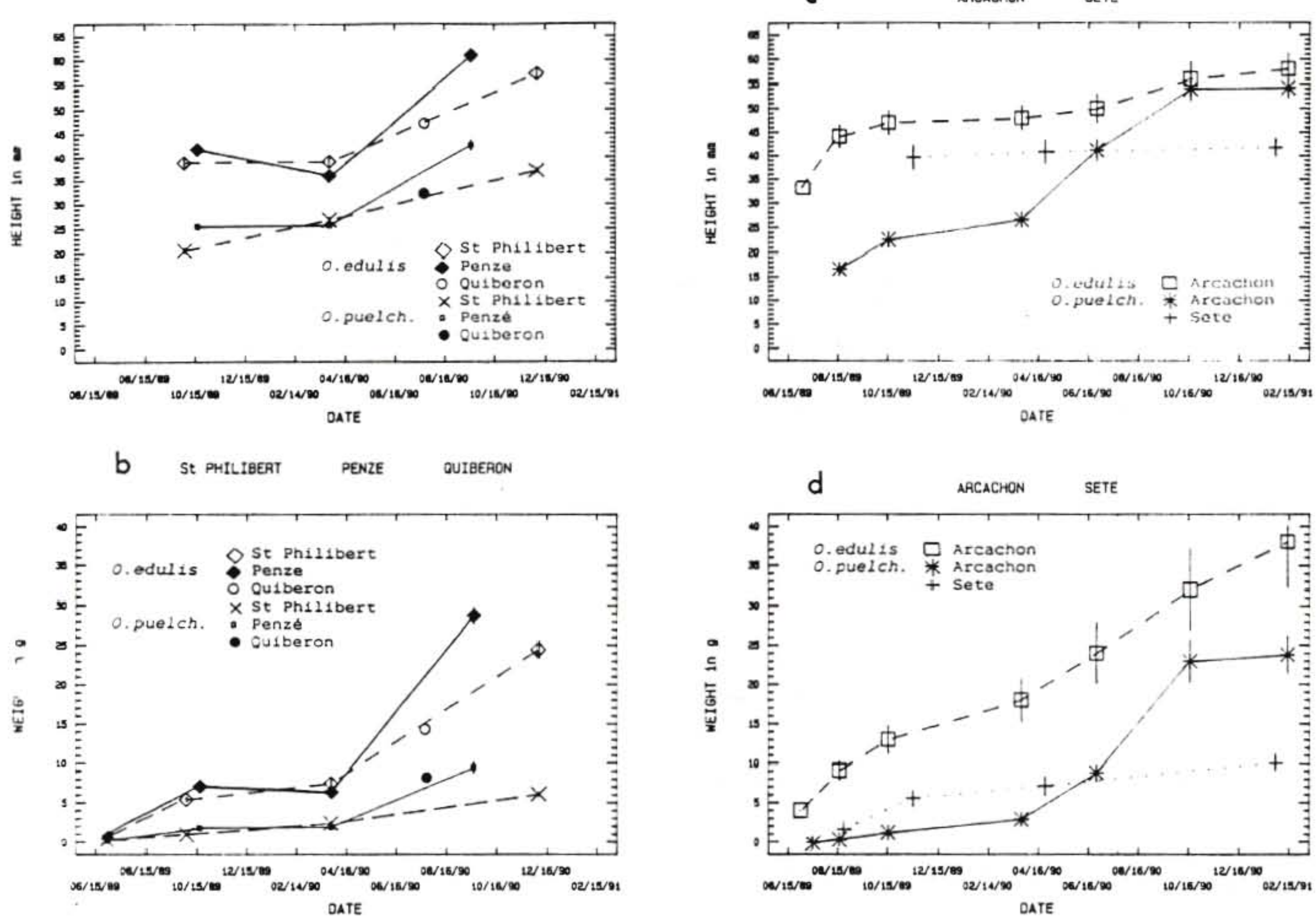


Figure 5. a,b,c,d - Growth curves of *O.puelchana* and *O.edulis* during tests in French sites (mean height + confidence interval 95%).

a and b : height and weight in Brittany sites
c and d : height and weight in Arcachon and Sète

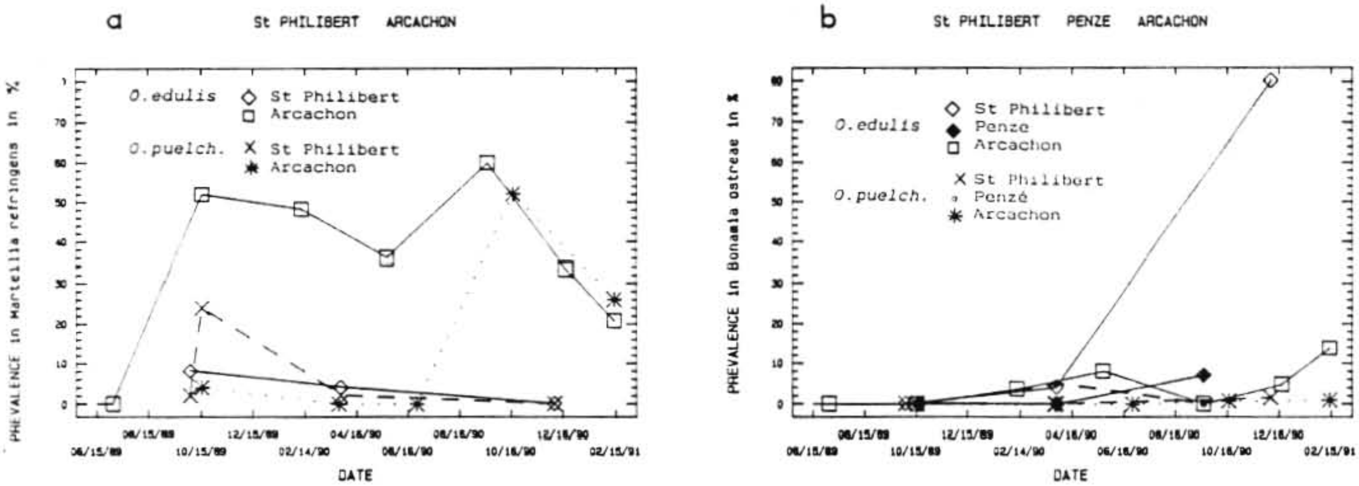


Figure 6.- Evolution of parasites *Marteilia refringens* (a) and *Bonamia ostreae* (b) on *O.puelchana* and *O.edulis* during tests in French sites (in percentages).

Date	St- Philibert		Penzé		Quiberon		Arcachon		Sète	
	Puelch.	*Edulis*	*Puelch.*	*Edulis*	*Puelch.*	*Edulis*	*Puelch.*	*Edulis*	*Puelch.*	*Edulis*
Oct. - Nov. 89	3/150 12/50	4/50 -	0/144	0/50	-	-	2/50	13/25	-	-
Feb. - March - April 90	3/150	2/50	0/200	0/50	-	-	0/117	13/27	0/71	-
May - June - July 90	-	-	-	-	1/77	0/100	0/59	9/25	-	
Sept. - Dec. 90	0/327	0/99	0/227	0/100	-	-	62/119	12/20 7/21	-	0/40
Jan. - Feb. 91	-	-	-	-	-	-	26/99	6/29	0/81	

Table 2. Evolution of parasite *Marteilia refringens* on *O. puelchana* and *O. edulis* during eigthteen months rearing in French sites.

Date	St- Philibert		Penzé		Quiberon		Arcachon		Sète	
	Puelch.	*Edulis*	*Puelch.*	*Edulis*	*Puelch.*	*Edulis*	*Puelch.*	*Edulis*	*Puelch.*	*Edulis*
Oct. - Nov. 89	0/150	0/50	0/144	0/50	-	-	0/50	0/25	-	-
Feb. - March - April 90	0/150	2/50	1/200	0/50	-	-	0/117	1/27	0/71	-
May - June - July 90	-	-	-	-	0/93	4/100	0/59	2/25	-	
Sept. - Dec. 90	5/327	80/99	0/227	7/100	-	-	1/119	0/20 1/21	-	0/40
Jan. - Feb. 91	-	-	-	-	-	-	1/99	4/29	0/81	

Table 3. Evolution of parasite *Bonamia ostreae* on *O. puelchana* and *O. edulis* during eighteen months rearing in French sites.

Quiberon). Moreover, an annelid of genus *Polydora*, strongly invaded the oysters shells of both species since the first months of rearing in intertidal sites.

Marteilia or *Bonamia* were not observed in the Mediterranean site.

II.3.- DISCUSSION

Mortality and parasitism

Most of mortalities observed on Argentinian oysters cannot be imputed to protozoans, these occurring either before infestation (Brittany, Arcachon) or even without infestation (Mediterranean). Moreover, control oysters, finally more affected than tested oysters, presented a better survival for the same period.

Marteilia appeared first, in endemic sectors, during a particularly hot summer, propitious to its development (Grizel, 1985): temperatures over 17°C from the end of May to the end of September 1989, the first days of June excepted. Infestation rates as well as observed stages allow to argue that *Ostrea puelchana* is really susceptible to this parasite. Its presence detected after one year in a non endemic sector may receive two interpretations: previous infestation or higher sensitivity than local oyster.

Bonamia appeared later than *Marteilia* as usually observed: eighteen months old oysters are generally little infested (Grizel, 1985). The difference in infection rates between the two species, particularly obvious in St Philibert, does not prove a different susceptibility. It may be the result of least filtration rates due to smaller sizes of *O.puelchana*.

Moreover, early infection by *Polydora sp* or *Mytilicola* may have contributed to weaken both species, the non indigenous oysters being proportionaly more affected, due to their smaller size.

Mortality and environmental factors.

As parasitism is apparently not the main cause of mortality, at least during the first year, environmental conditions may be put forward. Some values of temperatures and salinity are available at three sites (Fig. 7. a,b,c,d,e).

The extreme temperatures recorded here (5-24°C) are similar to those mentioned in Argentinian sites: 7-23°C at natural oyster beds (Zampatti and Pascual, 1989), 5-18°C at experimental sites (Fernandez Castro, 1986). In the Mediterranean site, the observed amplitude was however lower (10-19°C) than in other sites.

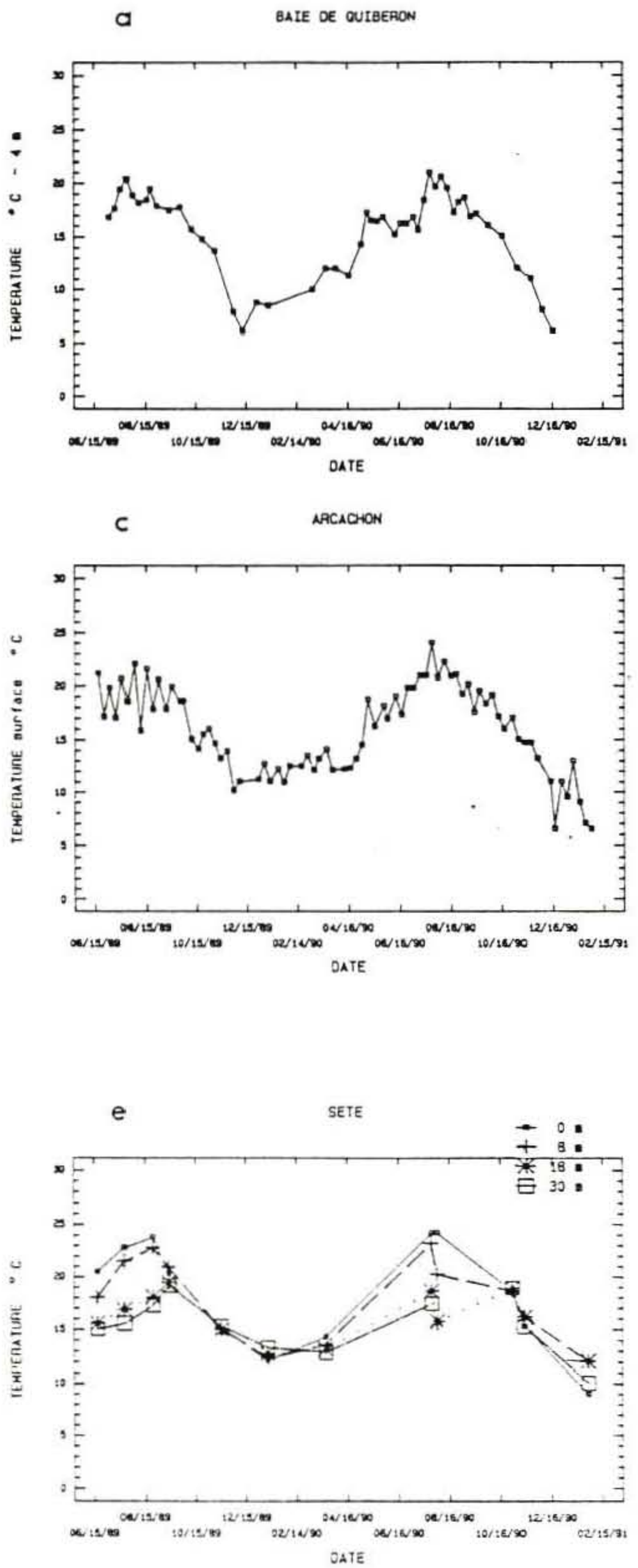


Figure 7.- Evolution of temperature and salinity during tests in French sites.

a: temperature
Baie de Quiberon
b: salinity

c: temperature
Arcachon
d: salinité

e: temperature Sete

Salinity fluctuations (31-37‰ at Quiberon and 27-35‰ at Arcachon) were more important that those noted in Fernandez Castro (33-34‰). In Sète, variations are supposed to be weaker due to location and depth.

In intertidal areas, the oysters were submitted to higher amplitudes of these parameters as well as to emersions, more frequent at St Philibert and Penzé (tidal coefficient 80-85) than at Arcachon (coefficient 95).

The lower temperatures observed at Sète at the beginning of rearing may explain better survivals consecutive to transfer. Better survivals were also recorded at IFREMER hatchery with lower temperature than at private nursery. In the same way, Fernandez Castro (1986) reports that mortality occurred during Argentinian summer (October to February). However, the temperature factor seems no more predominant afterwards.

Subsequent mortality may have been favoured by very important fouling consecutive to hot summer. It was also observed in the Mediterranean site during the second year, because of scarce interventions.

Growth and reproduction behaviour

As spat was produced in winter, immersion on sites occurred at the beginning of summer, leading to early maturation with effective reproduction before one year of age, at least for female individuals.

In Argentina, according to Fernandez Castro (1986), juvenile sexuality of *O.puelchana*, which may affect one third of individuals, is characterized by male gametes non becoming functional, due to concomitant fall of temperatures. So, first functional male maturation appears at one year of age and first female maturation at two years of age.

In Brittany, the first female maturation was effective for ten months old oysters, but early male maturation cannot be certain as fertilization by *Ostrea edulis* males cannot be excluded.

Nevertheless, the energy waste in gametogenesis may explain the poor initial growth. Then this negative effect may have been subsequently amplified by fouling and parasitism: *Marteilia* (St Philibert) *Polydora* (St Philibert,Penzé) and *Mytilicola* (Quiberon).

In Arcachon site, the same hypothesis may be put forward during the first summer. However, growth rates were much higher in 1990 than in other sites. The only known difference concerns temperature, perceptibly higher in Arcachon. Fernandez Castro and Bodoy (1987) showed that this factor could be positively correlated with growth.

CONCLUSION

Several causes may explain the high mortality recorded on *O.puelchana* during tests on French coasts. However none of them is determining. Growth was generally very poor except in one site. Temperature seems to be an important though indirect factor, in relation with physiological and environmental mechanisms. Knowledge concerning environmental factors (food availability and phytoplancton quality) is lacking for further interpretation.

Nevertheless susceptibility of *Ostrea puelchana* to *Marteilia* and probably *Bonamia* as well as bad acclimatization (high mortality and relatively poor growth) are sufficient reasons not to retain this species for breeding on french coasts.

With *Ostrea chilensis* (Grizel *et al*, 1983) and *Ostrea angasi* (Bougrier *et al*, 1986), it is the third non indigenous species of genus *Ostrea* showing infection by the European flat oyster parasites.

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