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Selecting flat oysters, *Ostrea edulis*, for survival against the parasite *Bonamia ostreae*: assessment of the resistance of a first selected generation

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Abstract

In 1989, a selected strain of flat oyster, *Ostrea edulis*, was produced from breeders that survived *Bonamia ostreae* and that were overselected by injection of purified parasites. This generation (G1) was compared to native oysters which had settled during the same year. After a growing period of 21 months, the oysters were tested according to three procedures: exposure to natural infection in an intertidal area, maintenance at the laboratory in tanks near heavily infested oysters, injection of parasites. The test lasted 7 months. In each procedure, oysters of the first generation (G1) showed a better survival rate than control oysters (72 to 94% versus 48 to 66%). On the other hand, the highest mortalities were observed among injected oysters (G1 and controls). In the laboratory tests, however, infestation rates of surviving oysters were equal or even higher in G1 than in the controls. This reduces the gain of resistance, regarding the number of non infested survivors. Nevertheless, the difference remains significant between oysters overselected by inoculation and their controls (49% versus 32%). This confirms that the injection technique may provide faster assessment of disease resistance and also may be used for overselection. Survivors of the overselected first generation have been kept to produce a second generation (G2).

KEYWORDS: Parasite resistance, *Bonamia ostreae*, *Ostrea edulis*, Genetics.

Résumé

En 1989, une production d'huîtres plates (*Ostrea edulis*) a été réalisée en écloserie à partir de géniteurs naturels ayant survécu en zone infestée par le parasite *Bonamia ostreae* et dont la sélection a été forcée par inoculation de ce parasite. Cette génération (G1) a été comparée à du naissain de captage naturel de la même année. Après 21 mois de prégrossissement, ces huîtres ont été soumises à trois types de tests de qualification de la résistance: mise en élevage en milieu

naturel dans un secteur d'élevage favorable au développement de *Bonamia*, maintien des huîtres en proximité d'huîtres fortement parasitées, inoculation de parasite. Quel que soit le test, les huîtres de la génération G1 ont montré une meilleure survie que les huîtres témoins (72 à 94% contre 48 à 66%). Par ailleurs, les mortalités les plus élevées ont été observées sur les huîtres soumises à l'inoculation de parasites (G1 et témoins). Cependant, dans les tests en laboratoire, les taux d'infestation observés sur les huîtres G1 survivantes étaient égaux ou même supérieurs à ceux des témoins. Si l'on évalue le gain de résistance à partir du nombre de survivantes saines, la différence entre les huîtres sursélectionnées par inoculation et leurs témoins se réduit mais demeure significative (49% contre 32 %). La technique d'inoculation peut donc être utilisée à la fois pour accélérer l'estimation du degré de résistance et pour augmenter la pression de sélection, les huîtres survivantes (G1) devant être utilisées comme géniteurs d'une génération de deuxième niveau (G2).

Introduction

In the past 24 years, two protozoans *Marteilia refringens* and *Bonamia ostreae* (Pichot et al., 1980) induced a sharp decline in the production of flat oyster *Ostrea edulis* in French oyster farming sites (Meuriot and Grizel, 1984).

Several prophylactic measures permitted to maintain the rearing activity in subtidal areas but the remaining occurrence of the two parasites led to start genetic selection programmes. A first generation of oysters was produced in 1985 from breeders that survived *Bonamia ostreae*. This generation showed slower infestation by this parasite and better survival when compared to native oysters of the same age (unpubl.). A new G1 generation was then produced in 1989 in order to confirm these results. Several methods of testing resistance were also compared. This paper describes the three procedures used for testing resistance and gives the results of the comparison.

Materials and methods

INOCULATION

The inoculation of the parasite *Bonamia* has been developed since 1983 (Bachère et al., 1984) and was improved during the next years. The improvements concerned purification of *Bonamia* (Mialhe et al., 1988), and injection technique (Hervio et al., in press). During the first experiments, the oyster shell was truncated at the edge and injection was practised with a micro-needle inside the digestive gland. In further experiments, the use of a solution of $MgCl_2$ (6g/100ml) to anaesthethize oysters allowed to inject directly inside the heart with an attenuated stress. Recovery of the activity was almost immediate when oysters were returned in filtered seawater. In the present experiment, the dose was adjusted to 50 000 cells per oyster, according to their mean size.

PRODUCTION AND OYSTERS BREEDING

Spat was produced in June 1989 at the IFREMER hatchery of La Tremblade. It was nursed at the IFREMER facilities of Bouin before being transferred to South Brittany in December (Fig. 1).

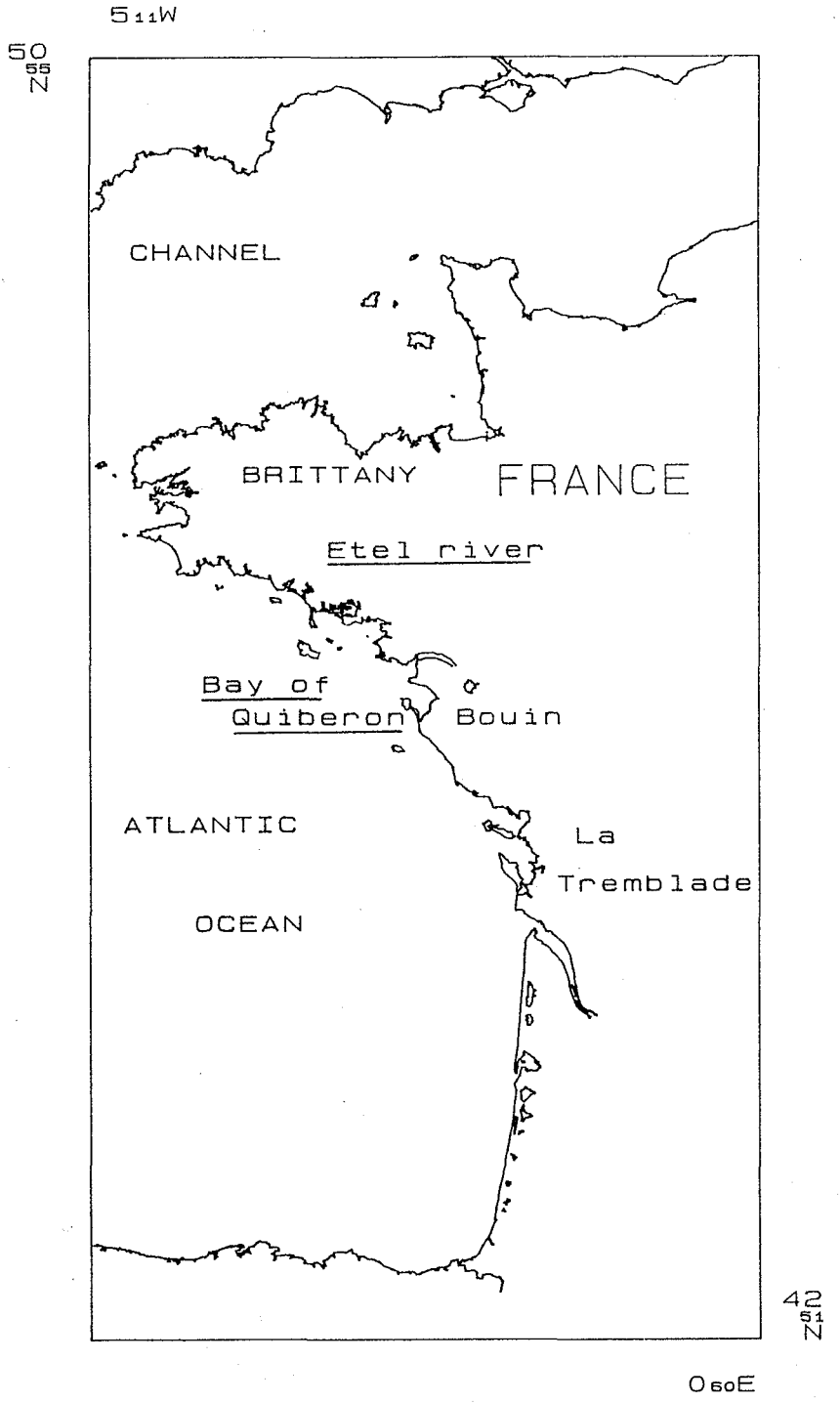

Fig. 1. Location of hatchery, nursery, and growing sites.

Two thousand juveniles were placed in 0.4m^2 plastic bags in the Bay of Quiberon, where *Bonamia* is present. The mesh was progressively adapted to the oyster size. Density was kept to 500 per bag. Bags were secured to metallic tables, 0.5m above the ground in the subtidal area (-5m). Controls were performed with the same quantities of natural spat placed in identical conditions, in May 1990.

The 21-months-old oysters were then placed in test conditions for about 7 months, from March to October 1991. Three procedures were tested: natural infestation in the intertidal area, maintenance at the laboratory in tanks near heavily infested oysters, and direct inoculation of parasites.

SELECTION TESTS

Since intertidal areas are more susceptible to development of *Bonamia* disease than subtidal areas, natural infestation was boosted by exposing oysters in the intertidal area of the Etel river. There were kept in plastic bags at the rate of 150 oysters per bag and three to five bags per lot (control and G1). Laboratory tests concerned batches of 250 oysters disposed in raceways (Fig. 2). For proximity contamination, 150 oysters were taken from a heavily parasited lot (40 to 50%) and placed in the raceways. Inoculation was practised by the method explained previously (50 000 cells of *Bonamia* injected per oyster after opening molluscs with $MgCl_2$).

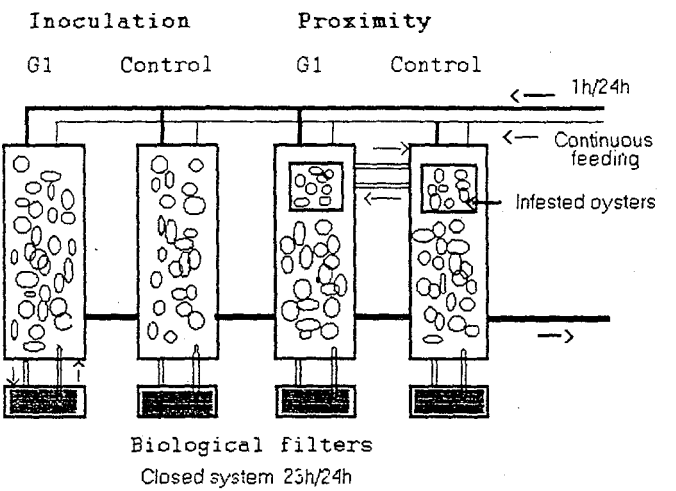

Fig. 2. Sheme of the experimental device used in selection and resistance testing (upper view).

For the laboratory tests, oysters were maintained at a temperature of 15-18°C. They were continuously fed with a suspension of 4.10^9 cells/h/100 l tank. These tanks were used in a closed system with a biological filter. Water was renewed by continuous flowing during 1h every 24h. Inoculated oysters were placed in isolated raceways while a crossed circulation was installed between batches of G1 and control of the proximity test. The

mortality was recorded daily and the parasites prevalence was estimated on 50 ind./batch at the end of the test.

PARASITES EXAMINATIONS

Parasites examinations were realized by photonic microscopy, on smears of heart and digestive gland for detection of *Bonamia ostreae* or *Marteilia refringens*. Smears coloration was obtained with "kit hemacolor".

OTHER PARAMETER

Weight was measured at the beginning and at the end of the experiments on a sample of 50 to 100 oysters.

STATISTICAL ANALYSIS

Differences between treatments were tested using the T test for comparison of means and proportions (ITCF and Statgraphics computer programmes). In the last test, variances were calculated according to the method described in Cochran (1977) concerning the double sampling with stratification.

Results

MORTALITY

The highest mortality was observed in inoculated oysters compared to proximity-test oysters and in control oysters compared to G1 (Fig. 3). Statistical tests performed on survival rates (Table I) revealed that the differences were highly significant between G1 and controls regardless the way of selection. The natural infestation test was the less efficient, with relatively low mortality.

Table I. Numbers and percentages of surviving oysters among G1 and controls in the different tests. "Sign." gives the probability level of the difference between treatments

Treatment	Inoculation	Sign.	Proxim.	Sign.	Natural site
G1	180/250 72%	*	226/250 90.4%	ns	704/755 93.2%
Sign.	*		*		*
Control	119/250 47.6%	*	174/250 69.6%	ns	296/450 65.8%

* $P<0.01$; ns = not significant at the 5% level.

The mortality of inoculated oysters increased rapidly between the 9th and the 24th week of the experiment, and then became stable. In the proximity test, the mortality developed more slowly and was not yet stabilized at the end of the experiment.

INFESTATION RATES

Bonamia observations, practised on surviving oysters, were not as demonstrative as mortalities (Table II). The difference was significant only for natural infestation that was higher for control than for G1. In other cases, infestation rates were equal or even higher for G1 than for controls. Resistance levels were also reported by the rate of surviving oysters that were not infested (Table III). In this case, the difference between treatments decreased but still remained significant for two of the three tests.

Table II. Numbers and percentages of *Bonamia* non infested oysters among surviving oysters in the different tests

Treatment	Inoculation	Sign.	Proxim.	Sign.	Natural site
G1	34/50 68%	ns	31/50 62%	*	49/50 98%
Sign.	ns				*
Control	33/49 67.3%	ns	36/50 72%	ns	40/50 80%

* $P<0.01$; ns = not significant at the 5% level.

Table III. Percentages of surviving oysters non infested with *Bonamia ostreae* at the end of the tests

Treatment	Inoculation	Sign.	Proxim.	Sign.	Natural site
G1	49%	ns	56%	*	91.3%
Sign.	*		ns		*
Control	32%	*	50.1%	ns	52.6%

* $P<0.05$; ns = not significant at the 5% level.

GROWTH RATE

The mean weight of the oysters reached 18 to 22g at the beginning of the experiment with a high dispersion of individual values. Seven months later the mean weight ranked

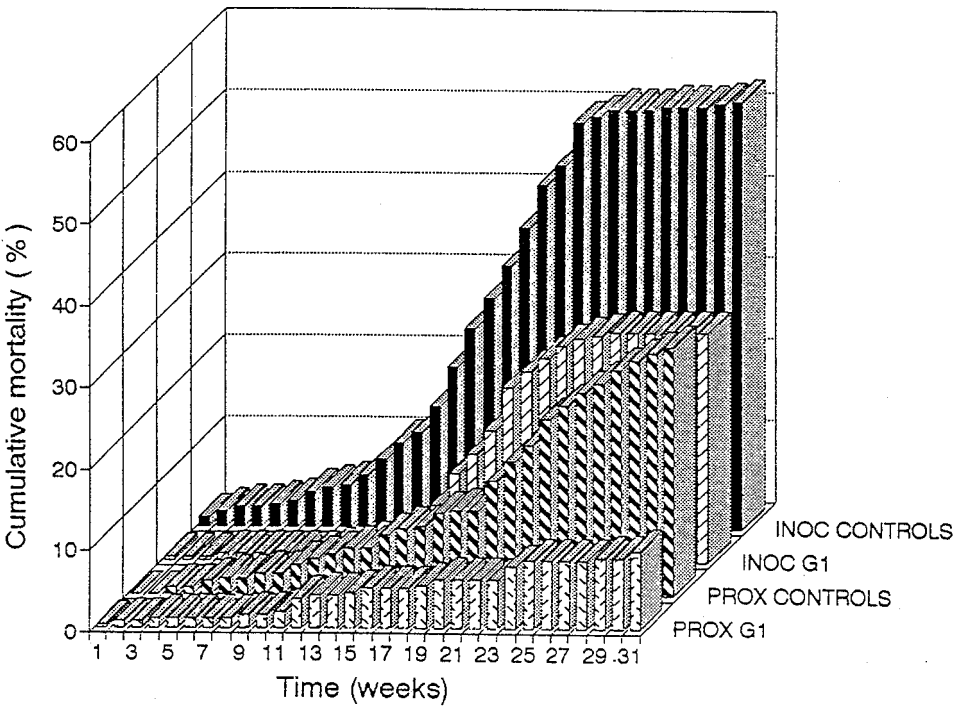

Fig. 3. Evolution of the mortality of G1 and controls during the laboratory tests (inoculation and proximity).

from 23 to 38g (Fig. 4). Differences could not be related to lineage, the deviation between G1 and the controls being reverse from one test to another. G1 had a better growth in the natural site than the controls (significant difference at 5% level) and inversely in laboratory (no statistical test, mean weight of two control batches estimated from their whole weight).

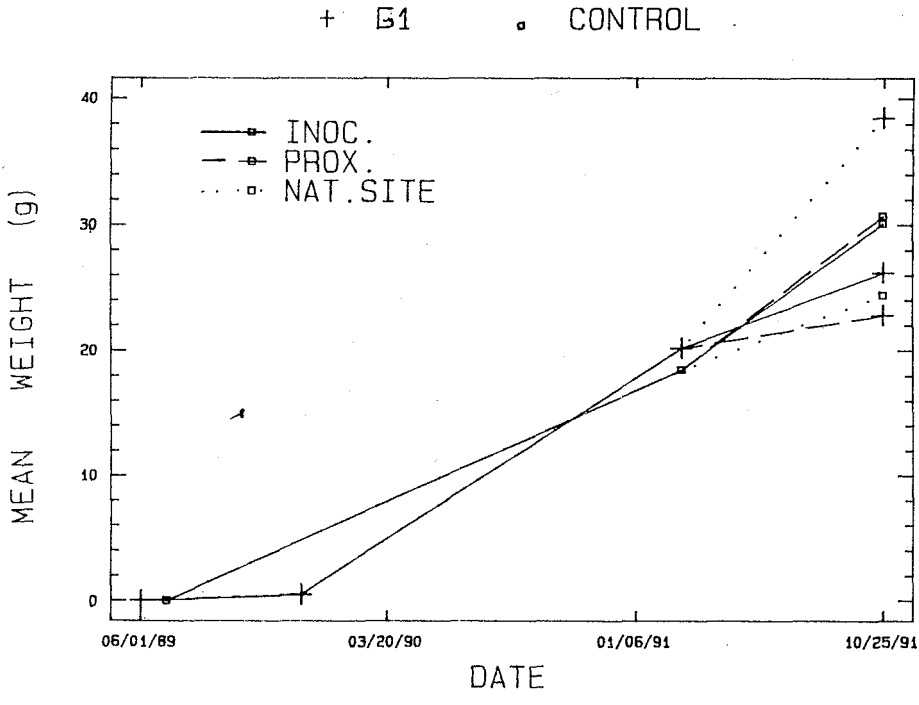

Fig. 4. Growth of the different batches of oysters during the experiments.

Discussion

MORTALITY

During these experiments, no parasite examinations were practised on freshly dead oysters. Mortality was then considered as being related to *Bonamia*. Previous tests have shown that the mortality due to the inoculation method was weak. In this experiment, the mortality during the first 6 weeks did not exceed 0.8% for inoculated F1 and 3.2% for inoculated controls. Initial infestation rates of these batches were respectively 0% (0/98) and 8% (8/99) before inoculation. The last result may explain the first mortalities in the second batch. That could allow to consider that the stress related to the inoculation was negligible.

INFESTATION RATES

The fact that infestation rates of surviving inoculated oysters reached 32% after mortality stabilization may be explained by a second phase of contamination induced by dying individuals and affecting gradually the less resistant oysters. This phase would probably have led to mortality if the test had been pursued.

In the proximity and intertidal tests, the evolution of the controls mortality was similar to usual observations concerning 2-year-old oysters. Nevertheless a significant difference is noticeable compared to the G1. On the other hand, the prevalence of surviving oysters is higher in the proximity test than in the intertidal test. In the first case, the process of infestation-mortality was already in an acceleration phase while, in the second case, it progressed slower as generally occurring in natural conditions where mortality accelerates during the 3rd year.

Inoculation may be considered as a very fast way of selection and of resistance testing. It is, however, difficult to quantify the selection pressure thrusted to oysters compared to natural selection. It would be interesting to know how many months of natural rearing would have been necessary to reach the same mortality rate. The first purpose of this research is, indeed, to permit oyster farmers to conduct flat oyster breeding to a commercial size with a final survival not lower than 25 to 30%.

Anyway, results allow to conclude that flat oysters first generation issued from overselected breeders is more resistant to the parasite *Bonamia ostreae* than natural oysters of the same age. Comparison would have been, however, more rigorous with hatchery produced controls from non selected parents. Natural spat does not present the same history as G1 from settlement to placing in plastic bags. Selection pressure during the first stage of life was not identical and initial natural mortality cannot be quantified. So we are not allowed to certify the heritability of the resistance character of G1 generation. Further experiments will be realized with hatchery products exclusively.

Conclusion

The improvement of resistance to the parasite *Bonamia ostreae* was significant for the first overselected generation of flat oysters when compared to natural spat. The inoculation technique appeared to be rapid and efficient. This technique would therefore allow to accelerate the selection process. Only 2 years should be necessary to produce and test a new generation. Overselected surviving oysters will be used to produce a new generation (G2).

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