Genet. Res., Camb. (2000), **76**, *pp.* 159–168. *With* 3 *figures.* Printed in the United Kingdom © 2000 Cambridge University Press

Influence of parentage upon growth in *Ostrea edulis*: evidence for inbreeding depression

YAMAMA NACIRI-GRAVEN[1]*, SOPHIE LAUNEY[1]†, NICOLAS LEBAYON[2], ANDRE GERARD[1] AND JEAN-PIERRE BAUD[2]

[1] *IFREMER, Laboratoire Génétique et Pathologie (LGP), BP 133 Ronce les Bains, 17390 La Tremblade, France*
[2] *IFREMER, Laboratoire Conchylicole des Pays de la Loire (LCPL), Polder des Champs, 85230 Bouin, France*

(*Received 12 May 1999 and in revised form 1 November 1999 and 25 February 2000*)

Summary

Genetic variability for growth was analysed in three populations of *Ostrea edulis*, selected for resistance to the protozoan parasite *Bonamia ostreae*. This study was undertaken first to determine the potential for selection for growth in populations that have never been selected for this character, and second to estimate heterosis versus inbreeding depression. Growth was monitored in culture for 10 months. The selected populations (namely S85-G3, S89I-G2 and S89W-G2), their crossbred population and a control population were composed of full-sib families whose parents were already genotyped using five microsatellite markers. This genotyping allowed the estimation of genetic relatedness among pairs of parents. The parents' relatedness was then correlated with the growth performance of their offspring within each of the three populations, and inbreeding depression was estimated. The population effect for growth was highly significant, with the crossbred population having the highest growth rate, followed by S89I-G2 and S89W-G2, S85-G3 and the control population. The within-populations family effect was also highly significant, indicating, as well as the high value for heritability at the family level (between 0·57 and 0·92), that a potential for a further selection for growth still exists within the three populations. Estimates of inbreeding depression (relative to the mean, for complete inbreeding) were high (1 for S891-G2, 0·44 for S89W-G2 and between 0·02 and 0·43 for S85-G3), which correlates with the apparent heterosis for growth observed in the crossbred population. These results are discussed in the context of the future management of the selected populations.

1. Introduction

In small and isolated populations, increased homozygosity may occur due to genetic drift or to chance mating between relatives and may result in a reduction of the mean phenotypic value of traits associated with fitness, i.e. inbreeding depression (Charlesworth & Charlesworth, 1987; Falconer & Mackay, 1996). There are two explanations for this: first, increasing homozygosity increases the chance that deleterious alleles will be expressed, and secondly, homozygotes may have a reduced fitness value for traits which are controlled by directionally dominant alleles (Lynch & Walsh, 1998). Estimation of inbreeding levels is particularly relevant in closed cultivated populations since they are usually small and since inbreeding may alter the response to artificial selection pressure and endanger the future of a selection programme. Moreover, inbreeding depression seems to be important in some bivalves (Beattie *et al.*, 1987; Wada & Komaru, 1994; Ibarra *et al.*, 1995; McGoldrick & Hedgecock, 1997), among them *Ostrea edulis* (Bierne *et al.*, 1998).

In this study we investigated the effect of inbreeding on growth in closed populations of the European flat oyster *Ostrea edulis* selected by IFREMER for resistance to *Bonamia ostreae*, a widespread haemolymph protozoan parasite that has been

* Corresponding author. Conservatoire et Jardin botaniques, 1 chemin de l'Impératrice, Case Postale 60, CH-1292 Chambésy, Genève, Switzerland. Tel: +41 22 418 5165/5218. Fax: +41 22 418 5101. email Yamama.Naciri-Graven@cjb.ville-ge.ch
† Current address: Laboratoire de Génétique des Poissons, INRA, 78352 Jouy-en-Josas, France.

endangering the flat oyster populations since the late 1970s in several parts of the world (Comps *et al*., 1980; Polanco *et al*. 1984; Van Banning, 1985; Elston *et al*., 1986; Grizel *et al*., 1988; Friedman *et al*., 1989; Figueras, 1991; Friedman & Perkins, 1994; Culloty & Mulcahy, 1996). We studied three populations – S85, S89I and S89W – that were initially produced by mass spawning and for which the actual number of real genitors contributing to the variability was unknown (Martin *et al*., 1993; Naciri-Graven *et al*., 1998). Using microsatellite markers (Naciri *et al*., 1995; Launey, 1998), Launey *et al*. (submitted) have recently shown that S89W and S89I were derived from 3 and about 10–12 individuals respectively, whereas S85 was established from about 20 animals. Launey *et al*. (submitted) also showed that S89I, S89W and S85 are highly genetically differentiated. This had already been hypothesized by Baud *et al*. (1997) and Naciri-Graven *et al*. (1999) who monitored the growth of two crosses involving the former populations for two consecutive years. The faster growth of the two resistant crosses were attributed to their better physiological condition in a confined environment compared with the control (Naciri-Graven *et al*., 1999), but also to putative hybrid vigour. A correlated inbreeding depression was therefore expected in the selected populations that were not monitored at that time. The experiment reported here was designed with the aim of studying the growth of full-sib families of S85, S89I and S89W, as well as full-sib families of the cross between the three former populations. We additionally investigated the relationships between family growth and the relatedness or the inbreeding coefficient of their parents.

2. Materials and methods

(i) *Biological material*

Four populations derived from three *Ostrea edulis* populations (S85, S89I and S89W) selected by IFREMER for their resistance to *Bonamia ostreae* (Martin *et al*., 1993; Naciri-Graven *et al*., 1998) and a wild population were used in this study. These were the third generation of population S85 (S85-G3), the second generation of S89I and S89W (S89I-G2 and S89W-G2) and a crossbred population between S85-G2, S89I-G1 and S89W-G1.) The control population was reared from oysters from Quiberon Bay (Brittany, France), which is known to be a *B. ostreae* endemic area. The five populations were produced between March and April 1995 at the IFREMER hatchery of La Tremblade (Charente Maritime, France). For each population, the families were produced from two oysters conditioned in 2 litre beakers at 18–20 °C and spawning was induced by heat shocks (up to 30 °C), thereby improving Newkirk's (1986) protocol. The same parents were used for crosses both within (G2 and G3) and between the selected population (G1G2).

Larvae and spat were cultured according to Walne's (1974) protocol. Spat, cultured to a minimum size of 4 mm, were transferred to the IFREMER facilities at Bouin (Baie de Bourgneuf, France) where they were grown in an upwelling system with *Skeletonema costatum*-enriched seawater (Bacher & Baud, 1992) until the spring of 1996. Oysters at this site are known to be weakly infected by *Bonamia ostreae* (REseau PAthologie MOllusques, REPAMO, IFREMER). Consequently, the aim of this latter experiment was not to test for *B. ostreae* resistance but to monitor growth in such a way as to obtain valid estimates of genetic variability.

(ii) *Experimental design*

Fifty families were monitored for growth. The families were distributed as follows: 17 families for S85-G3, 10 for S89W-G2, 7 for S89I-G2, 11 for the crossbred population and only 5 for the Quiberon control. A similar experimental design to that presented in Naciri-Graven *et al*. (1999) was used. In April, the five oyster populations were transferred to four concrete tanks of 7 m^3 (7·70 m long × 1·90 m wide × 0·55 m deep) considered as four blocks. Each block consisted of the 50 families distributed in seven series of four superposed trays that were distributed at random. Each tray was split into two parts and contained 100 oysters from two different families. For each family in each block, 10 animals were individually labelled and weighed (a total of 40 for each family). Measurements were made from April 1996 (day 109) to January 1997 (day 309), every 15 days during the first 3 months and every month from then on. The oysters were fed with *Skeletonema costatum* produced in subterranean salt-water following previously tested protocols (Baud & Bacher, 1990; Haure & Baud, 1993; Baud *et al*., 1997).

(iii) *Microsatellite analysis, assessment of parentage and/or genetic relatedness*

Microsatellite analyses were carried out on the parents using five different markers (*Oedu*J12, *Oedu*U2, *Oedu*H15, *Oedu*O9 and *Oedu*T5; Launey *et al*., submitted). For S89I and S89W, it was easy to infer the parentage of the different individuals involved in the production of the second generation studied here. S89W-G2 was indeed composed of full-sibs and half-sibs derived from three parents, and S89I-G2 of full-sibs, half-sibs and unrelated individuals from about 10–12 parents (Launey *et al*., submitted).

It was more difficult to infer parentage for S85-G3, as it was composed of a larger number of individuals. Therefore, for this population, we measured the

https://doi.org/10.1017/S0016672300004663 Published online by Cambridge University Press

genetic relatedness, r, between the parents of each cross according to Queller & Goodnight (1989) using the software 'Relatedness' they developed.

(iv) *Statistical analysis*

The within-family variances were compared using an $F_{\max}$ test for each of the four populations (Sokal & Rohlf, 1995). In each case heteroscedasticity of variances was found. As a consequence, the use of variance analyses was kept for variance estimations only, and a bootstrap approach was preferred to test the mean squares. It was impossible, using the bootstrap method, to analyse a mixed model since the data need to be resampled with replacement for the random effects and without replacement for the fixed effects. Consequently, the family and the interaction effects were treated as fixed; the data were then resampled 1000 times without replacement and mean squares were calculated for each resampling. The true mean square was declared significant at the α level when it was higher than $(1-\alpha)$ per cent of the resampled mean squares (Manly, 1997). Calculations were performed using S-Plus software (Statistical Sciences, 1995). The five populations were analysed together using the following fixed model:

$$Y_{ijkl} = \mu + p_i + f_{j/i} + b_k + i_{ik} + E_{ijk}, \quad (1)$$

where Y_{ijkl} is the measurement of the lth individual of the jth family in the ith population of the kth block, μ is the grand mean, p_i is the population effect, $f_{j/i}$ is the within-population family effect, b_k is the block effect, i_{ik} is the interaction between populations and blocks and E_{ijk} is the residual. Each population was then analysed separately using the following two-fixed model:

$$Y_{ijk} = \mu + f_i + b_j + i_{ij} + E_{ijk}, \quad (2)$$

where Y_{ijk} is the measurement of the kth individual of the ith family in the jth block, with similar notation for the different effects. Mean comparisons were performed using the minimum significant differences (MSD_{ij}) between pairs (i,j) of populations based on the Game and Howell method (Sokal & Rohlf, 1995) which is appropriate when the variances are heterogeneous.

Heritability estimates were computed for each of the five populations. The following mixed model of variance analysis was used to obtain the variance estimates (and not to test for the significance effect):

$$Y_{ijk} = \mu + F_i + b_j + I_{ij} + E_{ijk}, \quad (3)$$

with the same expression as for models (1) and (2), and where F_i is the family random effect, I_{ij} the random interaction effect, and σ^2_{F} and σ^2_{I} their respective variance estimates. Heritability at the family means level (h^2_{FM}) and its confidence interval at 95% were estimated within each population following Knapp *et al.* (1985). h^2_{FM} was calculated as $(1-MS_{\mathrm{I}}/MS_{\mathrm{F}})$, where MS_{I} is the interaction mean square and MS_{F} the family means square. Heritability (h^2), calculated as $2\sigma^2_{\mathrm{F}}/(\sigma^2_{\mathrm{F}}+\sigma^2_{\mathrm{I}}+\sigma^2_{\mathrm{E}})$, and its 95% confidence interval were also estimated following Becker (1992) and using (3).

Regression analyses were performed on S85, S89I, S89W and the control population, between heritability (h^2 or h^2_{FM}) as the dependent variable and, as independent variables, both the number of families (nf) and the mean relatedness (r; Launey *et al.*, submitted) in each population.

The relationship between parentage and growth performance in S89I and S89W was tested using the bootstrap randomization method on model (2) with p_i, the parentage effect (full-sib, FS; half-sib, HS; unrelated, UR), instead of f_i, the family effect. Mean comparisons were performed as before, using the Game and Howell method (Sokal & Rohlf, 1995). For S89 (S89I + S89W), a regression analysis was performed between inbreeding coefficients f ($f = 0$ for UR, $f = 0{\cdot}125$ for HS and $f = 0{\cdot}25$ for FS) and family growth rates. For S85, a regression analysis was also performed between genetic relatedness (r) and family growth rates. Using the regression analyses, an estimation of the inbreeding depression was computed as:

$$ID = 1 - Y_1/Y_0,$$

with Y_0 being the phenotypic mean with no inbreeding ($f = 0$) or with a null relatedness ($r = 0$) and Y_{I} the phenotypic mean with inbreeding ($f = 1$) or with a complete relatedness ($r = 1$).

3. Results

Oyster growth was linear during the experimental period (data not shown). Regression analyses between time and weight were performed on each monitored oyster and their R^2 values were on average as high as 98% (minimum value 82%). As a consequence the linear slopes (growth in g day^{-1}) were used as indicators of individual growth performances.

(i) *Differences between and within populations*

The bootstrap analysis using model (1) of variance analysis shows that the population effect for growth was highly significant, as was the within-populations family effect and the block effect ($P < 0{\cdot}001$; Table 1). The interaction between blocks and populations was not significant ($\alpha = 0{\cdot}05$, $P = 0{\cdot}70$), which allowed us to correct the data for the effect of blocks. Fig. 1 illustrates the corrected means and the 95% confidence

https://doi.org/10.1017/S0016672300004663 Published online by Cambridge University Press

Table 1. *Analyses of variance for growth rate* (*in g day*$^{-1}$) *using the bootstrap randomization method for the data as a whole* (*model 1*) *and for each population taken separately* (*model 2*)

	Populations					
Source of variation	Whole[a]	S85[b]	S89W[b]	S89I[b]	Cross[b]	Control[b]
Populations	***[c]	–	–	–	–	–
Families	***	***	***	***	***	**
Blocks	***	***	***	*	***	*
Interactions	NS	NS	NS	NS	NS	NS
Means ± *95% confidence intervals* (*in g day*$^{-1}$)						
	–	0·198c[d] ± 0·004	0·223b ± 0·005	0·219b ± 0·007	0·238a ± 0·006	0·185c ± 0·006

[a] Data set analysed using model 1. In this model, the family effect is nested to the population effect and the interaction effect is between populations and blocks.
[b] Data set analysed using model 2. In this model, the interaction effect is between families and blocks.
[c] NS for $P > 0{\cdot}05$, * for $P < 0{\cdot}05$, ** for $P < 0{\cdot}01$ and *** for $P < 0{\cdot}001$.
[d] Means with different letters are significantly different at the 5% level, using the Game and Howell method.

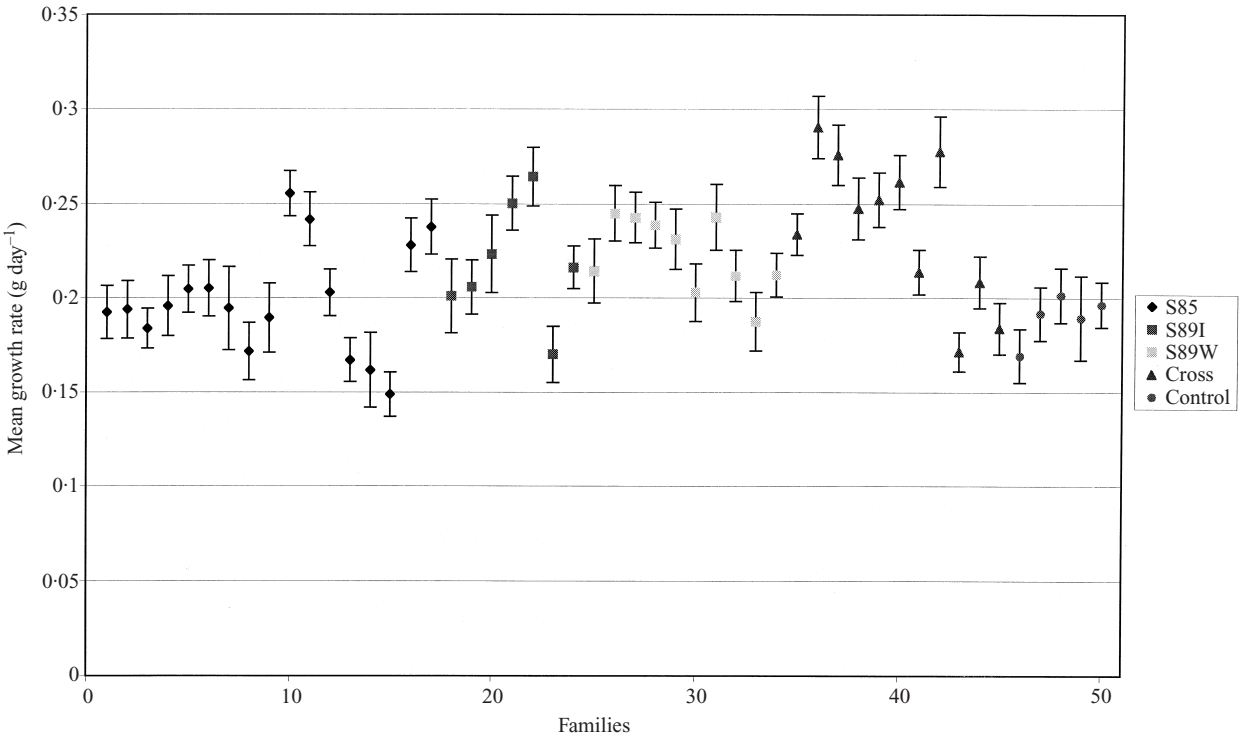

Fig. 1. Mean growth rates and 95% confidence intervals for S85-G3, S89I-G2, S89W-G2, the crossbred population and the control population.

intervals of the families studied. It is clear from this figure that the highest growth rates were obtained for families derived from crosses between populations. The Game and Howell multiple comparison of means at the 5% level indicates that the crossbred families had significantly higher growth rates than either S89W or S89I, which were similar to each other. S85 and the Quiberon control had significantly lower growth rates than all other populations (Table 1).

The bootstrap analysis using model (2) shows that the family effect was always highly significant within each population ($P = 0{\cdot}004$ for the Quiberon Control and $P < 0{\cdot}001$ for the other populations; Table 1). The block effect was highly significant for S85, S89W and the crossbred population ($P < 0{\cdot}001$) but only significant for S89I ($P = 0{\cdot}046$) and for the Quiberon Control ($P = 0{\cdot}040$). None of the interactions between blocks and families was significant ($P > 0{\cdot}05$).

https://doi.org/10.1017/S0016672300004663 Published online by Cambridge University Press

Table 2. *Heritability of growth rate estimated at the family mean level* (h^2_{FM}) *and at the family level* (h^2) *and their 95% confidence interval*

	Populations				
Heritability	S85	S89W	S89I	Cross	Control
h^2_{FM}	0·92 (0·83, 0·97)	0·78 (0·41, 0·94)	0·92 (0·73, 0·98)	0·95 (0·88, 0·99)	0·57 (−0·78, 0·95)
h^2	0·54 (0·25, 0·83)	0·27 (0·01, 0·53)	0·52 (0·10, 0·94)	0·84 (0·42, 1·25)	0·11 (−0·11, 0·34)

Table 3. *Analyses of variance for growth rate* (*in g day*$^{-1}$) *using the bootstrap randomization method for the S89 populations* (*model 2*)

	Populations		
Source of variation	S89W	S89I	S89I + S89W
Parentage	*[a]	***	***
Blocks	***	*	***
Interactions	NS	NS	NS
Means ± 95% confidence intervals (*g day*$^{-1}$)			
Full-sibs	0·214a ± 0·009[b]	0·170c ± 0·015	0·203b ± 0·009
Half-sibs	0·228a ± 0·007	0·208b ± 0·012	0·222a ± 0·006
Unrelated	–	0·236a ± 0·009	0·236a ± 0·009

[a] NS for $P > 0·05$, * for $P < 0·05$, ** for $P < 0·01$ and *** for $P < 0·001$.
[b] Means with different letters are significantly different at the 5% level, using the Game and Howell method.

(ii) *Heritability estimates*

Heritabilities of growth rate at the family mean level (h^2_{FM}) and at the family level (h^2) were estimated for each population with a confidence interval at the 95% level. The highest heritabilities were obtained for the crossbred population followed by S85 and S89I (Table 2). The heritabilities for S89W was lower but still significant. The control had the lowest heritabilities, which were not significantly different from 0. Both heritability estimates were positively correlated with the mean genetic relatedness (r) and the number of families (nf) studied in each population. Relatedness and family number explained 46% of the variation for h^2 and 61% of the variation for h^2_{FM}. The partial correlation coefficients (PC) were, however, not significant at the 5% level, whatever the order of the two variables (r and nf) in the model. With r as a first variable, the partial correlation coefficients were as follows: $PC_{(h^2, r)} = 0·396$, $PC_{(h^2, nf)} = 0·550$, $PC_{(h^2FM, r)} = 0·594$ and $PC_{(h^2FM, nf)} = 0·509$.

(iii) *Influence of kinship in S89I and S89W families*

The effect of parent kinship on growth rate was tested separately on S89I and S89W families (Table 3). The effect was highly significant ($P < 0·001$) for the S89I population. In this population, families derived from unrelated individuals performed significantly better than families derived from half- or full-sibs (Table 3). In S89W population, kinship had only a marginally significant effect on growth rate ($P = 0·041$) and families derived from half-sibs performed slightly but not significantly better than those from full-sibs. The kinship effect on pooled data (S89I + S89W) was highly significant ($P < 0·001$). Families derived from unrelated individuals performed significantly better than families from full-sibs and slightly better than families from half-sibs. In each of the three analyses, the block effect was significant ($P = 0·045$ for S89I and $P < 0·001$ for S89W and pooled S89) but the interaction effect was not ($P > 0·42$).

Within S89 populations, the mean growth rates decreased with increasing inbreeding for both populations (Fig. 2). However, the regression analysis was significant only for S89I ($P = 0·033$; $P = 0·407$ for S89W). The intercepts were highly significant ($P < 0·001$) and similar for the two regression lines (S89I, 0·237; S89W, 0·241). The adjusted R^2 for S89I (Fig. 2) indicates that the model explained about 56% of the observed variation. The slopes for the two populations were not significantly different from each other ($\alpha = 5\%$), which allowed the two populations to be pooled. The regression analysis performed on pooled S89 gave a non-significant result ($Y = -0·129^{NS} X + 0·237^{***}$; $P = 0·065$, adjusted $R^2 = 0·180$).

https://doi.org/10.1017/S0016672300004663 Published online by Cambridge University Press

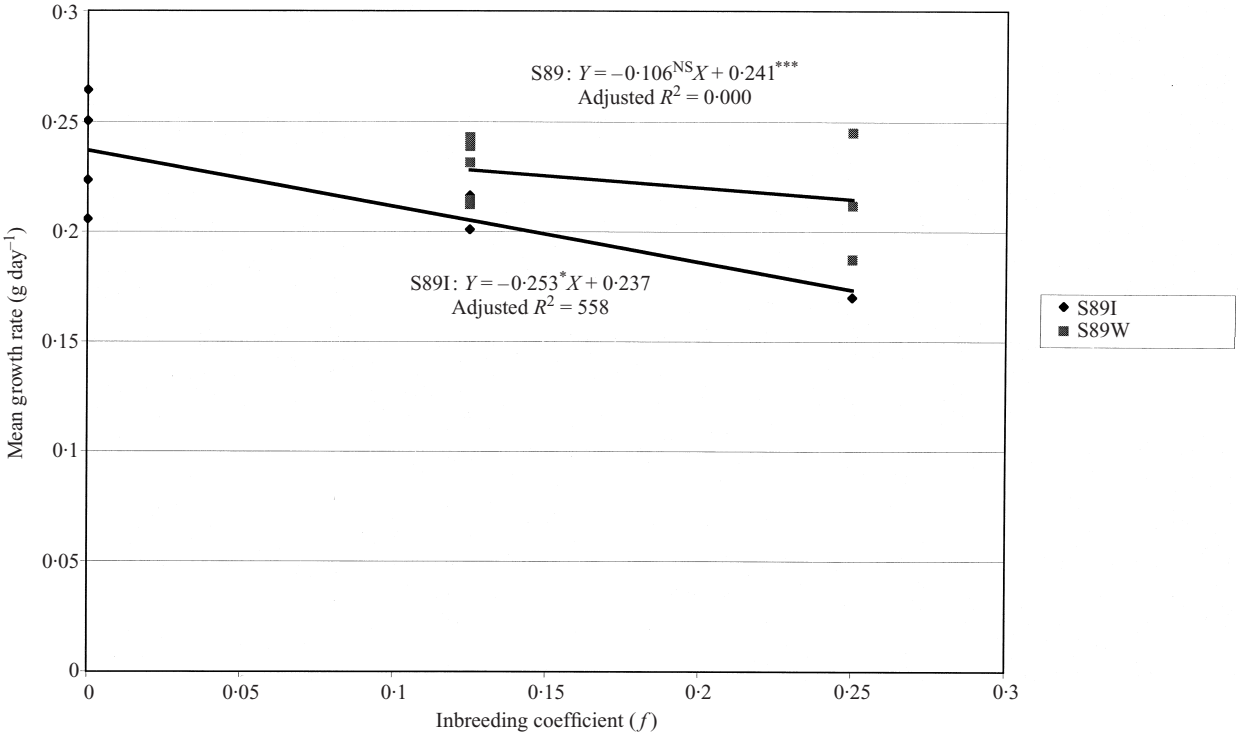

Fig. 2. Changes in mean phenotypes as function of inbreeding in S89I and S89 populations. Each point represents a family. Lines are linear regressions.

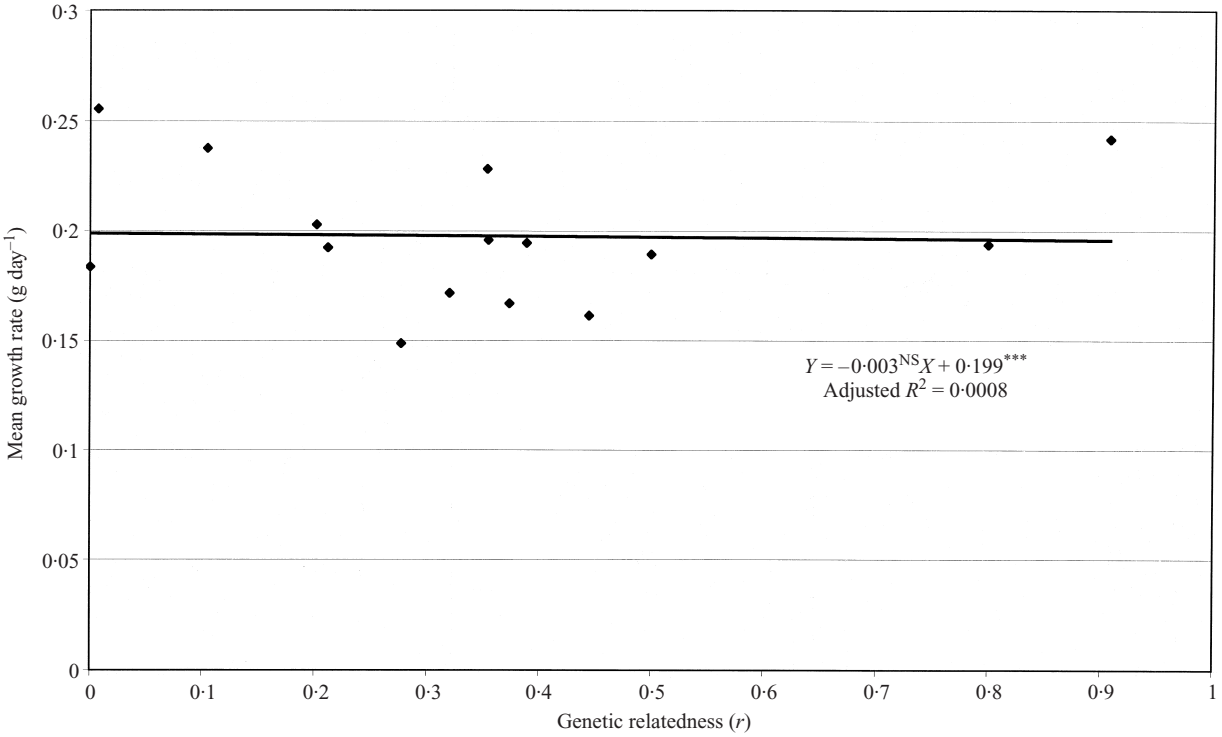

Fig. 3. Changes in mean phenotypes as function of genetic relatedness in S85 population. Each point represents a family.

(iv) *Influence of relatedness in S85 families*

Fig. 3 shows the relationship between relatedness and family means for growth rate within the S85 population. Only 15 of 17 families were used in the analysis because two genitors died before genotyping, which means that the genetic relatedness (r) could not be calculated for two of the families. The regression line appears to be slightly negative but the slope was not significantly different from 0. The adjusted R^2 was

https://doi.org/10.1017/S0016672300004663 Published online by Cambridge University Press

null, indicating a large dispersion of the data, mainly due to two families that had high growth rates (0·194 and 0·242) coupled with high relatedness (0·80 and 0·91, respectively).

(v) *Estimating inbreeding depression*

For the S89 populations, the value of inbreeding depression (ID) was estimated using the regression analyses between *f* and family growth rates: ID was 1 for S89I, 0·44 for S89W and 0·55 when the populations were pooled. For the S85 populations, the value of ID was estimated using the regression analysis between the relatedness *r* and family growth rates: in this case, ID was very small (0·02).

4. Discussion

Bonamia ostreae is unlikely to have affected the results. The Baie de Bourngneuf, where the experiment was conducted, is known to be weakly contaminated by *B. ostreae*. Baud *et al.* (1997) and Naciri-Graven *et al.* (1999) have shown that no parasite infection was recorded in 1-year-old oysters and that 13% of the selected oysters were infected by the end of the second year of the experiment versus 20% for the Quiberon controls. In this study, the monitored oysters were too young to experience significant parasite pressure. This is in agreement with the results of Culloty & Mulcahy (1996) who showed that *B. ostreae* is typically observed in oysters that are at least 2 years old.

(i) *A better growth for the selected populations?*

In our study, the selected populations grew better than the control population. The mean growth rates of S89I and S89W populations were about 18% and 20% higher than that of the Quiberon control. S85 performed 7% better than the control but the difference was not significant. Naciri-Graven *et al.* (1999) suggested that the better growth of the resistant populations in experimental conditions might be the consequence of hatchery and nursery breeding. The environmental conditions imposed by cultivation might have indirectly selected those animals more able to grow in a confined and/or a warm environment, while the Quiberon control is better adapted to cold waters and an open environment. Baud *et al.* (1997) and Naciri-Graven *et al.* (1998) have indeed shown that the difference between a crossbred G1G2 population (involving animals from S85, S89I and S89W populations) and a control was present in a confined environment but not in the open sea. Alternatively, selection for resistance to *B. ostreae* could have resulted in a selection, through hatchery practice, for oysters which escape infection by faster growing. We cannot exclude the third hypothesis that the selection for improved resistance to *B. ostreae* indirectly enhanced the growth rate in the case where the two traits were positively correlated. Whatever the explanation, it is interesting to notice that, at least under experimental conditions, the third generation of S85 performed as well as the control and that the second generations of S89I and S89W did even better. The results indeed demonstrate that there is a potential for growth improvement in *Ostrea edulis*, even though inbreeding depression might impair the effect of a further selection.

(ii) *Heterosis versus inbreeding depression*

The crossbred population performed much better than the control population (+28%), confirming the results obtained on similar populations by Baud *et al.* (1997) and Naciri-Graven *et al.* (1999) and on other bivalves (Hedgecock *et al.*, 1998). Moreover, the crossbred population grew better than the selected populations from which the parents were derived (+13%). This is not a direct estimation of heterosis for growth since the cross mean is compared with the progeny means of the parents and not with the means of the parents themselves; 13% is therefore an overestimate of the actual heterosis. Heterosis must, however, exist since inbreeding depression was found, at least in the S89 population. In the S85 population indeed, the low estimated inbreeding depression is due to the very good performance of two families, derived from parents with high relatedness, and that might have already purged their genetic load. A new analysis excluding these two families gave an ID value of 0·43 which is quite similar to the S89 ID value (0·55).

The high ID values estimated across the S89 populations (55%) and potentially in the S85 population (43%) are in agreement with the finding of Bierne *et al.* (1998), who showed that the genetic load is high in *Ostrea edulis*. Similar results have also been reported by Beattie *et al.* (1987), Ibarra *et al.* (1995) and McGoldrick & Hedgecock (1997) on other bivalves. Finding a significant inbreeding depression for growth, a character which has usually been considered as a component of fitness in bivalves, is in agreement with DeRose & Roff's (1999) results. Following earlier observations reviewed in Falconer & Mackay (1996), these authors have indeed proved that life-history traits usually experience significant inbreeding depression, in contrast with purely morphological traits which experience much lower depression.

(iii) *What about a further round of selection?*

The high heritability value within each selected population indicates that selection for higher growth rates at the family level would be effective, and this is

https://doi.org/10.1017/S0016672300004663 Published online by Cambridge University Press

all the more true with the crossbred population (S89-G1 × S85-G2). Accordingly, Newkirk & Haley (1982) had an encouraging response for weight at 2 years after one generation of selection in the European flat oyster, with the mass-spawned selected groups being on average 23% heavier than the control line. The response in growth rate after a second round of selection was, however, lower than after the first one, and Newkirk & Haley (1983) suggested that inbreeding may have contributed to these lower performances. Our results are in agreement with the former ones, showing that there is still a large variation for growth within populations, despite the high selection pressures that were used for selecting the resistance to *Bonamia ostreae* (Naciri-Graven *et al*., 1998), and the occurrence of inbreeding.

(iv) *Significance of heritability values*.

The heritability values at the family level (h^2) and at the family mean level (h^2_{FM}) show that consistent variability still exists within each selected population. h^2_{FM} corresponds to the ratio of $(\sigma^2_A + \sigma^2_D)$ to mean phenotypic variance (σ^2_{mP}) and it estimates the likelihood that a mean phenotype will reflect the genotype (Gallais, 1990). In our study, this likelihood is very high, indicating that the environmental conditions were well controlled and that a selection on a mean family basis would be very efficient. h^2 corresponds to the ratio of twice the covariance between full-sibs $(\sigma^2_A + \frac{1}{2}\sigma^2_D)$ to phenotypic variance (σ^2_P) and therefore lies between the strict-sense heritability ($h^2_{ss} = \sigma^2_A/\sigma^2_P$) and the broad-sense heritability ($h^2_{bs} = (\sigma^2_A + \sigma^2_D)/\sigma^2_P$). It is tendentious to compare heritabilities from different experiments since this parameter depends on the environment, the population under study and the experimental conditions. However, it is interesting to notice that the significant heritabilities we obtained for the selected populations ($0{\cdot}27 < h^2 < 0{\cdot}84$) are in the same range as heritabilities for growth performance found in natural populations of other bivalves ($h^2_{ss} \pm SD = 0{\cdot}5 + 0{\cdot}2$ and $h^2_{bs} \pm SD = 0{\cdot}8 \pm 0{\cdot}3$ in *Mytilus edulis* (Strömgren & Nielsen, 1989), $h^2_{ss} \pm SE = 0{\cdot}72 \pm 0{\cdot}32$ to $0{\cdot}91 \pm 0{\cdot}17$ in *Mercenaria mercenaria* (Rawson & Hilbish, 1990) and $h^2_{ss} = 0{\cdot}43 \pm 0{\cdot}18$ to $0{\cdot}69 \pm 0{\cdot}11$ in *Ostrea chilensis* (Toro, 1995)).

Heritability estimates differ consistently between the populations studied here. The relatedness coefficient, together with the number of studied families, explained 46% (51%) of the variation in h^2 (h^2_{FM}), and were positively correlated with heritability values. This might explain why the lowest heritability was found for the Quiberon control, which was represented by the smallest number of families (5) and which showed the lowest genetic relatedness ($r \pm SE = 0{\cdot}048 \pm 0{\cdot}002$; Launey *et al*., submitted). On the contrary, S85, S89I and S89W have significantly higher relatedness coefficients and a higher number of families (from 7 to 17). The significant and relatively high heritabilities for growth in the selected populations might be viewed as the consequence of inbreeding, which is expected to increase the genetic variance between individuals or families while decreasing the mean of quantitative characters (Gallais, 1990; see also Fowler & Whitlock, 1999).

(v) *Further insights*

Finding a high genetic load for growth has implication in population genetics of bivalves. A correlation between multilocus heterozygosity (MLH) and fitness traits as measured by growth has indeed often been observed in natural populations of marine bivalves (Zouros *et al*., 1980; Koehn & Gaffney, 1984; Gaffney *et al*., 1990; Zouros & Pogson, 1994). David *et al*. (1995) suggested that a high genetic load was a necessary condition to explain MLH–fitness correlation by the general effect hypothesis. This hypothesis also required a small level of inbreeding (or at least linkage disequilibrium between markers and selected genes) that has not yet been clearly assessed in natural populations. Several factors, however, such as large variation in reproductive success (Hedgecock, 1994), non-random mating or restricted gamete and larvae dispersal (David *et al*., 1997*a*), could easily result in such level of inbreeding. Our results, following those of Bierne *et al*. (1998) and David *et al*. (1997*b*) on *Spisula ovalis*, therefore suggest that the general effect hypothesis (David *et al*., 1995) might be relevant in explaining the MLH–fitness correlation in natural populations of marine bivalves.

We wish to thank all the staff members of the various laboratories for their help with maintenance, sampling and measurements, especially C. Ledu, P. Phélipot, S. Heurtebise and M. Nourry, for hatchery and nursery production. This work was supported by grants from the French State, the Brittany Region, the Pays de la Loire and the Poitou-Charentes Regions. Y.N.G. was supported by the Swiss Science Foundation while writing this article (grant no. 32-55036-98).

References

Bacher, C. & Baud, J. P. (1992). Intensive rearing of juvenile oysters *Crassostrea gigas* in an upwelling system: optimization of biological production. *Aquatic Living Resources* **5**, 89–98.

Baud, J.-P. & Bacher, C. (1990). Use of ground saline water for intensive rearing of *Ruditapes philippinarum* juveniles in a nursery system. *Aquaculture* **88**, 157–178.

Baud, J. P., Gérard, A. & Naciri-Graven, Y. (1997). Comparative growth of *Bonamia ostreae* resistant and wild flat oysters, *Ostrea edulis*, in an intensive upwelling system. I. First year of experiment. *Marine Biology* **130**, 71–79.

Beattie, J. H., Purdue, J., Hershberger, W. & Chew, K. (1987). Effect of inbreeding on growth in the Pacific

oyster *Crassostrea gigas*. *Journal of Shellfish Research* **6**, 25–28.
Becker, W. A. (1992). *Manual of Quantitative Genetics*, 5th edn. Pullman, WA: Academic Enterprise.
Bierne, N., Launey, S., Naciri-Graven, Y. & Bonhomme, F. (1998). Early effect of inbreeding as revealed by microsatellite analyses on *Ostrea edulis* larvae. *Genetics* **148**, 1893–1906.
Charlesworth, D. & Charlesworth, B. (1987). Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* **18**, 237–268.
Comps, M., Tigé, G. & Grizel, H. (1980). Etude ultrastructurale d'un protiste parasite de l'huître plate *Ostrea edulis* L. *Comptes Rendus de l'Académie des Sciences, Paris* **290**, 383–384.
Culloty, S. & Mulcahy, M. F. (1996). Season-, age- and sex-related variations in the prevalence of Bonamiasis in flat oysters (*Ostrea edulis* L.) on the south coast of Ireland. *Aquaculture* **144**, 53–63.
David, P., Delay, B., Berthou, P. & Jarne, P. (1995). Alternative models for allozyme-associated heterosis in the marine bivalve *Spisula ovalis*. *Genetics* **139**, 1719–1726.
David, P., Perdieu, M. A., Pernot, A. F. & Jarne, P. (1997*a*). Fine-grained spatial and temporal population genetic structure in the marine bivalve *Spisula ovalis*. *Evolution* **51**, 1318–1322.
David, P., Delay, B. & Jarne, P. (1997*b*). Heterozygosity and growth in the marine bivalve *Spisula ovalis*: testing alternative hypotheses. *Genetical Research* **70**, 215–223.
DeRose, M. A. & Roff, D. A. (1999). A comparison of inbreeding depression in life-history and morphological traits in animals. *Evolution* **53**, 1288–1292.
Elston, R. A., Farley, C. A. & Kent, M. L. (1986). Occurrence and significance of Bonamiasis in European flat oysters *Ostrea edulis* in North America. *Disease of Aquatic Organisms* **2**, 49–54.
Falconer, D. S. & Mackay, T. F. C. (1996). *Introduction to Quantitative Genetics*, 4th edn. Harlow, UK: Longman.
Figueras, A. J. (1991). *Bonamia* status and its effects in cultured flat oysters in the Ria de Vigo Galicia (NW Spain). *Aquaculture* **93**, 225–233.
Fowler, K. & Whitlock, M. C. (1999). The distribution of phenotypic variance with inbreeding. *Evolution* **53**, 1143–1156.
Friedman, C. S. & Perkins, S. O. (1994). Range extension of *Bonamia ostreae* to Maine, USA. *Journal of Invertebrate Pathology* **64**, 179–181.
Friedman, C. S., McDowell, T., Groff, J. M., Hollibaugh, J. T., Manzer, D. & Hedrick, R. P. (1989). Presence of *Bonamia ostreae* among populations of the European flat oyster, *Ostrea edulis* L., in California, USA. *Journal of Shellfish Research* **8**, 133–137.
Gaffney, P. M., Scott, T. M., Koehn, R. K. & Diehl, W. J. (1990). Inter-relationships of heterozygosity, growth rate and heterozygote deficiencies in the coot clam *Mulinia lateralis*. *Genetics* **124**, 687–699.
Gallais, A. (1990). *Théorie de la Sélection en Amélioration des Plantes*. Paris: Masson.
Grizel, H., Mialhe, E., Chagot, D., Boulo, V. & Bachère, E. (1988). Bonamiasis: a model study of diseases in marine molluscs. In *Disease Processes in Marine Bivalve Molluscs* (ed. W. S. Fisher), pp. 1–4. AFS Special Publication. Bethesda, MD: American Fisheries Society.
Haure, J. & Baud, J. P. (1993). Intensive rearing of flat oyster *Ostrea edulis* in raceways using underground saltwater: an approach to validate a methodology. In *From Discovery to Commercialization*. Torremolinos World Aquaculture 93 (ed. M. Carillo, L. Dahle, J. Morales, P. Soorgeloos, N. Svennevig & J. Wyban, pp. 60–61. Special publication no. 19. Ostend, Belgium: European Aquaculture Society.
Hedgecock, D. (1994). Does variance in reproductive success limit effective population sizes of marine organisms? In *Genetics and Evolution of Aquatic Organisms* (ed. A. R. Beaumont), pp. 122–134. London: Chapman & Hall.
Hedgecock, D., McGoldrick, D. J. & Bayne, B. L. (1998). Hybrid vigor in the Pacific oysters: an experiment approach using crosses among inbred lines. *Aquaculture* **137**, 285–298.
Ibarra, A. M., Cruz, P. & Romero, B. A. (1995). Effect of inbreeding on growth and survival of self-fertilized catarina scallop larvae, *Argopecten circularis*. *Aquaculture* **134**, 37–47.
Knapp, S. J., Stroup, W. W. & Ross, W. M. (1985). Exact confidence intervals for heritability on a progeny mean basis. *Crop Science* **25**, 192–194.
Koehn, R. K. & Gaffney, P. M. (1984). Genetic heterozygosity and growth rate in *Mytilus edulis*. *Marine Biology* **82**, 1–7.
Launey, S. (1998). Marqueurs microsatellites chez l'huitre plate *Ostrea edulis* (L.): caractérisation et application à un programme de sélection pour une résistance au parasite *Bonamia ostreae* et à l'étude de populations naturelles. Doctoral thesis, l'INA-PG, Paris.
Launey, S., Barré, M., Gérard, A. & Naciri-Graven, Y. (submitted). Population bottleneck and effective size in *Bonamia ostreae*-resistant populations of *Ostrea edulis* as inferred by microsatellite markers. *Genetical Research*.
Lynch, M. (1988). Design and analysis of experiments on random drift and inbreeding depression. *Genetics* **120**, 791–807.
Lynch, M. & Walsh, B. (1998). *Genetics and Analysis of Quantitative traits*. Sunderland, MA: Sinauer Associates.
Manly, B. J. F. (1997). *Randomization, Bootstrapping and Monte-Carlo Methods in Biology*, 2nd edn. London: Chapman & Hall.
Martin, A. G., Gérard, A., Cochennec, N. & Langlade, A. (1993). Selecting flat oysters, *Ostrea edulis*, for survival against the parasite *Bonamia ostreae*: assessment of the resistance of a first selected generation. In *Production, Environment and Quality*. Bordeaux Aquaculture 92 (ed. G. Barnaby & P. Kestemont), pp. 547–554. Special publication no. 18. Ghent, Belgium: The European Aquaculture Society.
McGoldrick, D. J. & Hedgecock, D. (1997). Fixation, segregation and linkage of allozyme loci in inbred families of the Pacific oyster *Crassostrea gigas* (Thunberg): implication for the cause of inbreeding depression. *Genetics* **146**, 321–334.
Naciri, Y., Vigouroux, Y., Dallas, J., Desmarais, E., Delsert, C. & Bonhomme, F. (1995). Identification and inheritance of (Ga/TC)*n* and (AC/GT)*n* repeats in the European flat oyster *Ostrea edulis* (L.). *Molecular Marine Biology and Biotechnology* **4**, 83–87.
Naciri-Graven, Y., Martin, A. G., Baud, J. P., Renault, T. & Gérard, A. (1998). Selecting flat oyster *Ostrea edulis* for survival when infected with the parasite *Bonamia ostreae*. *Journal of Experimental Marine Biology and Ecology* **224**, 91–107.
Naciri-Graven, Y., Haure, J., Gérard, A. & Baud, J. P. (1999). Comparative growth of *Bonamia ostreae* resistant and wild flat oyster *Ostrea edulis* in an intensive system. II. Second year of experiment. *Aquaculture* **171**, 195–208.

Newkirk, G. F. (1986). Controlled mating of the European oyster *Ostrea edulis*. *Aquaculture* **57**, 111–116.
Newkirk, G. F. & Haley, L. E. (1982). Progress in selection for growth rate in the European oyster, *Ostrea edulis*. *Marine Biology Progress Series* **10**, 77–79.
Newkirk, G. F. & Haley, L. E. (1983). Selection for growth rate in the European oyster, *Ostrea edulis*: response of second generation groups. *Aquaculture* **33**, 149–155.
Polanco, E., Montes, J., Outon, M. J. & Melendez, M. I. (1984). Situation pathologique du stock d'huitres plates en Galice (Espagne) en relation avec *Bonamia ostreae*. *Haliotis* **14**, 91–95.
Queller, D. C. & Goodnight, K. F. (1989). Estimating relatedness using genetic markers. *Evolution* **3**, 258–275.
Rawson, P. D. & Hilbish, T. J. (1990). Heritability of juvenile growth for the hard clam *Mercenaria mercenaria*. *Marine Biology* **105**, 429–436.
Roff, D. A. (1997). *Evolutionary Quantitative Genetics*. New York: Chapman & Hall.
SAS Institute (1988). *SAS/STAT User's Guide*, release 6.03. Cary, NC: SAS Institute.
Sokal, R. R. & Rohlf, F. J. (1995). *Biometry: The Principles and Practice of Statistics in Biological Research*, 3rd edn. New York: Freeman.
Statistical Sciences (1995). S-Plus guide to statistical and mathematical analysis, version 3.3. Seattle: Statsci, a division of MathSoft.
Strömgren, T. & Nielsen, M. V. (1989). Heritability of growth in larvae and juveniles of *Mytilus edulis*. *Aquaculture* **80**, 1–6.
Toro, J. E. (1995). Response to artificial selection and realized heritability estimates for live weight and shell length for the Chilean native oyster (*O. chilensis* Philippi, 1845). *Aquaculture* **137**, 272.
Van Banning, P. (1985). Control of *Bonamia* in Dutch oyster culture. In *Fish and Shellfish Pathology* (ed. A. E. Ellis), pp. 393–396. London: Academic Press.
Wada, K. T. & Komaru, A. (1994). Effect of selection for shell coloration on growth rate and mortality in the Japanese pearl oyster, *Pinctada fucata martensii*. *Aquaculture* **125**, 59–65.
Walne, P. R. (1974). *Culture of Bivalve Molluscs: Fifty Years Experiment at Conwy*. London: Whitefriars.
Zouros, E. & Pogson, G. H. (1994). Heterozygosity, heterosis and adaptation. In *Genetics and Evolution of Aquatic Organisms* (ed. A. R. Beaumont), pp. 135–146. London: Chapman & Hall.
Zouros, E., Singh, S. M. & Miles, H. E. (1980). Growth rate in oysters: an overdominant phenotype and its possible explanations. *Evolution* **42**, 1332–1334.