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Individual growth variation and its relationship with survival in juvenile Pacific oysters, Crassostrea gigas (Thunberg)

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Abstract:

In order to study individual growth variability and its relationship with survival in juvenile Crassostrea gigas, parental oysters were sampled at four sites located along the French Atlantic coast and bred under controlled hatchery conditions. Four groups of larvae were obtained by crossing five males and five females from each of the four sites, and a fifth group by crossing these 20 males and 20 females together in a pool. Larvae were reared under conditions allowing the maintenance of a maximum variability of size and gave five experimental groups. Oysters were individually monitored for growth and survival from 3 to 10 months after fertilization. The individual growth performances were relatively stable over time and no noticeable compensation for growth occurred. Early growth rate was a very good predictor of size later in life: 66% of variation in the live weight at 10 months could be explained by variation in the initial growth rate calculated between 3 and 4 months. A significant group effect was observed on survival and on growth rate. Mortality mostly occurred between 3 and 5 months and appeared to affect the slow-growing animals more. However, two groups can be distinguished among those which died during the experimental period, one which showed a decrease in weight and the other whose growth was similar to surviving oysters. These results are discussed in the light of usual oyster farming practices and selective breeding.

Keywords: Aquaculture - *Crassostrea gigas* - Growth - Oysters - Survival

Introduction

Growth potential and survival aptitude are key traits for most species of aquacultural interest (Mahon, 1983). Growth and survival are the result of numerous physiological and environmental parameters. Growth has often been reported to be highly variable in oysters, even between individuals of the same age reared under common conditions (e.g. Medcof, 1961; Galtsoff, 1964; Haley and Newkirk, 1977; Singh and Zouros, 1978; Fujio *et al.*, 1979; Zouros *et al.*, 1980). Survival rates are also highly variable and dependent on many internal and external factors.

Oysters usually reach a marketable size between 12 and 48 months. This depends on the temperature and the quantity of food available in the rearing environment (Héral and Deslous-Paoli, 1991) and has also a genetic component (Langdon *et al.* in press). The market demands uniformity and therefore the reduction of variability in size in oyster batches. This requires intensive labour for shellfish farmers and increases the handling and stress on the oysters. Askew (1978) assessed that it is economically more useful to cull slow growing individuals rather than to keep all the size classes, but the genetic consequences of such practices are poorly known. However, selective breeding for improved growth has been shown to be feasible (see for review Sheridan, 1997; Nell *et al.*, 2000; Ward *et al*., 2000, Langdon *et al*., in press). One possibility for improvement of oyster production would be to identify the best growing individuals as early as possible. Selection could be applied at an early stage because the high fecundity permits a high selective pressure for size to be applied simply by sieving the young oysters (Collet *et al*., 1999). However, the validity of early size or early growth rate as criteria for selection for growth remained to be investigated.

Survival can be very low at early stages in oysters, both in the wild and in hatcheries (Robert and Gérard, 1999). The relationship between mortality and growth has not been precisely examined despite its potential economic importance. The correlation between growth and survival also requires further investigation, in order to know whether selection made on growth at an early stage

would lead to batches with higher survival rates. This is likely to depend on the etiology of the mortalities (Beattie *et al*., 1980; Hersherber *et al*., 1984). It has often been observed that mortality does not affect the individuals uniformly (Cheung, 1993; Silina, 1994; Nie *et al*., 1996). According to observations made on shellfish farms, resistance to diseases or stress is believed to be positively related to size and therefore links between mortality and growth are to be expected. To our knowledge however, there has been no direct investigation of the relationship between growth rate and mortality based on individual monitoring of oysters reared under controlled conditions.

In the present paper, growth was individually monitored from 3 to 10 month-old in several groups of oysters, produced in order to exhibit maximal variability in size and maintained under controlled hatchery conditions. The objectives were to assess whether growth was sufficiently stable over time and if early growth rate could be used as a predictor of size later in life. Interaction between growth and survival was also studied.

Materials and methods

Biological material

In order to generate groups with a large genetic base, 40 parental oysters (20 males, 20 females) originating from 4 sites located along the French Atlantic coast (Arcachon ARC, Seudre SEU, Port des Barques PBD and Bonne-Anse BA) were crossed together to produce firstly 'within population' groups where all the parents came form the same origin and secondly a pool where all the oysters were crossed with each other. The parental stocks were first conditioned to the hatchery environment *i.e.* filtered seawater was pumped from the Seudre estuary and supplemented with S*keletonema costatum*, *Isochrysis galbana*, *Pavlova lutheri* and *Tetraselmis suecica* algae. Sexual maturation was obtained by artificial increase of the seawater temperature at a rate of 1 °C.day^{-1} up to 20 °C. Gametes were collected by striping of the gonads and *in vitro* fertilizations were

performed in beakers. Crosses were made individually between pairs of oysters within site groups, so 5 males were crossed with 5 females. Within each of these 4 groups, the 25 crosses were then pooled together 3 hours after fertilization, leading to 4 groups named ARC, SEU, PDB and BA, according to the origin of the parents. The detailed procedure of the crosses is described in Collet *et al*., (1999). These 4 intra-site crosses (4 times 5 males x 5 females) were performed in such a way as to ensure an equal gametic contribution from each parent. In the pooled cross (20 males by 20 females), 400 factorial crosses were performed separately with the same parents and then mixed to produce a single pool.

The age of the individuals in this paper refers to the date of fertilization and is given in Days Post Fertilization (DPF). Larvae from the pool were successively sieved as detailed in Collet *et al.* (1999). The sieving made at 26 DPF was chosen for the present study because its settlement occurred on the same day as that of the other 4 groups: ARC, SEU, PDB and BA. This means that all the groups could be compared without bias potentially caused by differences in the timing of settlement. Larvae and spat were cultured according to Walne (1974). The individuals were settled on cultch, at low density to avoid competition for food and space.

Individual growth and survival monitoring

At DPF 91, a total of 1,009 individuals (193 individuals from ARC, 237 from BA, 238 from PDB, 133 from SEU and 208 from the pool), still too small to be individually tagged, were separated out in trays, each with 72 individual rearing cells. These rearing structures enabled individual monitoring of growth and mortality at early stages and prevented trophic and spatial competition. The animals were identified by the numbers of the cells in the tray structure. These trays were placed in raceways and moved daily in order to avoid position effects. At 175 DPF, all animals were large enough to be individually tagged by sticking a computer-printed plastic label on the upper shell with non-toxic epoxy resin. They were then transferred into a 800-litre rearing tank

using baskets for around 75 individuals each. Water flow was maintained at 800 $l.h^{-1}$. The rearing density was low throughout the experiment. The position of the baskets within the tank was also changed daily in order to avoid position effects. The oysters were fed with *Skeletonema costatum* produced in underground saltwater as described by Baud and Bacher (1990).

Live weight was recorded weekly from 3 to 10 months after fertilization (DPF 91 and 307 respectively) to a precision of 0.01 g, after having wiped the water from the shell. Mortality was monitored daily and all dead animals were identified and removed immediately from the tank.

Data analyses

Initial and final growth rates (IGR and FGR respectively) were calculated individually for every animal using a linear regression of live weight against time (using the 'SLOPE' function of Microsoft Excel). Specifically, IGRs were computed between DPF 91 and 126 and FGRs between DPF 227 and 307, as these two periods corresponded to linear growth. The fit of the linear model was checked by plotting the residuals of the regression of size on time. These showed no evidence of non-linear patterns.

Growth rate differences between groups over the total trial period were analysed by ANOVA for repeated measures (SYSTAT 9.0 GLM) according to Fisher and van Belle (1993), using only the individuals that survived throughout the trial in order to avoid the potential bias due to the interaction between growth and survival.

Stability of weight ranking over the trial period was tested for by computing Kendall's correlation coefficient between weight ranks at different ages (Sokal and Rohlf, 1995) and by performing a linear regression between initial (91 DPF) and final (307 DPF) weight. To test whether growth performance was conserved over the study period, two complementary analyses were also performed. Firstly, a linear regression was computed between IGR and FGR. Secondly, the intraclass correlation coefficient ρ_{intra} was extracted from an ANOVA on IGR and FGR with

time as a fixed class effect and individuals as a random class effect (PROC MIXED, SAS Software, SAS Institute 1988). This correlation coefficient is computed as $\rho_{intra} = V_{between} / (V_{between} + V_{within})$ where $V_{between}$ is the between individual variance and $V_{within} = V_{error}$ is the within individual variance. The intraclass correlation coefficient should be close to 1 if the within individual variation is negligible, indicating that the rank of IGR is almost the same as for FGR. It should be close to 0 if the reverse holds true. In order to build a confidence interval for this statistic, we computed its bootstrap standard error σ_B by taking 1000 samples with replacement from the original data (Manly, 1997). As the bootstrap distribution of the statistic was normal and no bias was observed, the confidence interval to the risk $\alpha = 0.001$ was computed as $\rho_{intra} \pm 3.29\ \sigma_B$. Finally, the impact of initial growth performance on future weight was tested for by performing a linear regression between IGR and final weight.

Survival data were analysed with combined G and χ^2 tests (Sokal and Rohlf, 1995) to assess the significance of the differences between the groups. To study the relationship between early growth and survival at a finer scale, the survival rate (at 6 months) of the smallest 10% of individuals at DPF 91 (3 months) was compared with the remaining 90 %. This was done firstly across all groups, and then individually per group using χ^2 tests to compare number of surviving animals in each size class between the two dates (3 and 6 months). As previously proposed by Newkirk (1981), the same comparison was repeated, this time considering the smallest 25 % at DPF 91 and the remaining 75 %. To further investigate the relationship between mortality and growth, two survival groups were distinguished: group S, consisting of the oysters which survived throughout the trial and group D consisting of the oysters which died during the time-span of the trial (between the second measurement at DPF 98 and the end of the experiment). Growth rate differences between groups S and D during the first 3 measurements (*i.e.* the period from DPF 91 to DPF 105 that preceded the peak mortality) were also analysed by repeated measures ANOVA (SYSTAT 9.0 GLM).

Results

Growth

The live weight measurements of the different groups are summarised in Table 1 and the mean growth curves are presented in Figure 1a. The Initial Growth Rate (IGR) and the Final Growth Rate (FGR) were computed between DPF 91 and 126 and between DPF 227 and 307, respectively. For IGR, the linear relation was highly significant ($p < 0.0001$) for all individuals and the coefficients of determination were always higher than 0.94. For FGR, the linear relation was significant to at least $p < 0.05$ for 612 individuals out of 634 and the coefficient of determination for these individuals was always higher than 0.58. The lower rate of significance for FGR than for IGR was due to lower growth in the final period of the study (Fig. 1a.). A high variability for both IGR and FGR was observed among individuals (coefficient of variation = 48.3 % for IGR and 59.7 % for FGR). Group had a significant effect on both IGR ($p < 0.0001$) and FGR ($p < 0.0001$). This analysis identified slow-growing groups (ARC: IGR = 0.093 ± 0.060, FGR = 0.040 ± 0.023 $g.d^{-1}$) and fast-growing groups (BA and SEU: IGR = 0.167 ± 0.069, FGR = 0.061 ± 0.035, and IGR = 0.175 ± 0.062, FGR = 0.047 ± 0.028 $g.d^{-1}$, respectively) over the whole study. In contrast, the pool (IGR = 0.111 ± 0.052, FGR = 0.050 ± 0.027) changed from a slow-growing to a fast-growing group between the initial and the final period while PDB (IGR = 0.144 ± 0.061, FGR = 0.042 ± 0.024) did the opposite.

Between DPF 126 and 227, individual growth curves were clearly not linear. Figure 1a clearly illustrates that oysters had not reached an asymptotic weight by the end of the studied period. This means that the direct application of commonly used growth models, as in Baud et al., (1997), is not appropriate here. In addition, irregularities in the growth curves were observed, such as between DPF 170 and DPF 190 (see figure 1a), probably due to variation in environmental

conditions, such as temperature and/or trophic conditions. Over the whole period, the interaction of group x DPF was highly significant ($p < 0.0001$ repeated measures ANOVA, Table 2) indicating the divergence of the groups over time (Figure 1a). Furthermore, this analysis gave significant results of polynomial tests up to the seventh level, indicating the complex non-linearity of the curves.

In order to test for the stability of weight ranking over time, a rank correlation analysis between initial and final size ranking was first performed. Kendall's coefficient ($\tau = 0.46$; $t = 25.7$) was found to be highly significant ($p < 0.0001$) indicating that live weight is well conserved over time and that even in non non-competitive circumstances the smallest oysters tend to remain the smallest. Secondly, the correlation between total weight at DPF 91 and 307 was calculated. It was found to be positive ($r = 0.61$) and highly significant ($p < 0.0001$; Figure 2a) confirming the previous result, but only 37 % of variation in final total weight was explained by the initial total weight at DPF 91.

To test whether the observed conservation of weight ranking was due to conservation of individual growth performances over time, the correlation between IGR and FGR was first computed. It was found to be positive ($r = 0.40$) and highly significant ($p < 0.0001$, Figure 2b) indicating a relative conservation of growth performance, but IGR explained only 16% of variation in FGR. The intraclass correlation coefficient confirmed these results. It was found equal to 0.30 and its bootstrap confidence interval [0.23; 0.36] showed that it was significantly different from 0, suggesting some conservation in growth performance over time, but that it was also significantly different from 1, indicating that conservation was not complete.

Finally, in order to test whether the initial growth rate had a significant impact on final weight, despite the partial instability of growth rates over time, the correlation between the initial growth rate and the final weight was computed. It was found to be positive ($r = 0.81$) and highly significant ($p < 0.0001$; Figure 2c). In this case, 66 % of the variability in final weight was

explained by the initial growth rate. IGR was therefore a good predictor of live weight at 10 months.

Survival

All groups endured a sharp early mortality event, recorded between DPF 91 and 126 (Figure 1b), with a maximum mortality of 11.89 % between DPF 105 and 112. Mortality decreased after this period, for all of the 5 groups of animals. The overall survival at DPF 307 was 65.7 %. BA showed the highest survival at DPF 307 with 83.1 % followed by the pool with 75.5 %, SEU with 64.66 %, ARC with 56.0 % and PDB with 48.3 %. Survival was significantly different between groups according to the G and χ^2 test ($p < 0.0001$).

Interaction between growth and survival

Firstly, no clear inter-group relationship between growth and survival appeared in this study. The ranking of growth and survival across the groups is not the same (figures 1a and 1b).

Secondly, we compared, across all groups, and then individually per group, the survival rate (at 6 months) of the smallest 10% (or 25%) of individuals at DPF 91 (3 months) with the remaining 90 % (or 75%). The results show that in both analyses (10-90 % and 25-75 %), across most groups, the smallest individuals at DPF 91 (3 months) have a lower subsequent survival than the remaining ones (Table 3). The slower growing groups (ARC and the pool) however, did not show any significant differences in survival between the 10 % smallest and the remaining. Fast-growing groups (PDB, BA and SEU) however, showed significant differences in survival between the 10 % or 25 % smallest, which had higher mortality, and the larger animals (Table 3). Tests made on survival at ten months (data not shown) showed the same patterns in significance of the tests, probably due to the low level of mortality between 6 and 10 months.

Thirdly, the individuals of all groups were divided into groups S and D, made up of those oysters which survived and died respectively during the experimental period and growth was then compared between these groups. Oysters which died had generally shown slower growth. Significant weight differences ($p < 0.001$) between oysters the two groups appeared within the period DPF 91 to DPF 105 (*i.e.* 3 successive measurements) (Figure 3). To see if animals grew in the same manner between those that subsequently died and those that survived, the effect of group (S or D) was also tested on the weight between DPF 91 and DPF 105 using a repeated measures ANOVA model. The effect of group (S or D) was highly significant ($p < 0.001$).

The relationship between live weight at 91 DPF and 98 DPF, representing increase in growth at the beginning of the study, was compared between the survival groups S and D (Figure 4) and two subgroups were distinguished within group D. The first subgroup showed the same relationship between live weight at DPF 91 and DPF 98 as group S, with a mean increase in total weight of 50 % between these two dates. This was significantly lower for the second subgroup (covariance analysis; $p < 0.001$), with a mean decrease in total weight of - 8 %.

Discussion

The importance of individual monitoring

Most papers reporting individual monitoring of marine organisms deal with growth curve modelling (e.g. in fish: Sainsbury, 1980; Hampton, 1991) or comparison of growth kinetics (Baud *et al*., 1997). In commercially important bivalve species, most of the studies aim to identify the effects of rearing site on growth of a group (Stirling and Okumus, 1995; Puigcerver, 1996) or to compare growth performance of two species (Mallet and Carver, 1995). Individual tagging has been used to study growth, reproduction and mortality of bivalves in the context of spatial (Dolmer, 1998) or temporal variation (Bologna, 1998), or the effect of competition (Brichette *et al*., 2001). Some papers report results from genetic improvement programs with the use of individual data from

animal tagging (*Ostrea chilensis*, Toro *et al*., 1995; 1996; *Tapes decussatus*, Puigcerver, 1996). In the present study, groups of Pacific oyster *Crassostrea gigas* showed a high variability for growth. Similarly, groups of cupped oysters obtained in hatcheries, with the same age and maintained in common environmental conditions, also expressed a high variability in size (e.g. Fujio *et al*., 1979; Singh and Zouros, 1978). Because of the effect of density on growth in most bivalve species (Neudecker, 1981; Rawson and Hilbish, 1990; Fréchette and Bacher, 1998), it is very important to avoid spatial competition in experimental rearing procedures in order to minimise variation for growth (Baud *et al.* 1997). Some food variation within rearing tanks has been reported (Hadley and Manzi, 1984) and it is well known that quantity of food is one of the more important factors affecting the growth of bivalves (Laing *et al.*, 1987). In our experiment, the trays of oysters were moved daily in order to prevent any bias to the estimation of growth performance linked to food availability variation in the raceways or rearing tank.

Variability in size obtained by this rearing method and without any selective sieving is very high compared to hatcheries, where economical constraints lead farmers to reduce it by culling (Bardach and Ryther, 1972; Newkirk, 1981). Similarly, previous authors have observed similar coefficients of variation (from 40 % to 60 % in *Ostrea edulis*: Newkirk and Haley, 1983; and *Crassostrea virginica*: Singh and Zouros, 1978; 1981). In our study, large differences in the growth rate were also found between groups of oysters with a significant group effect appearing at an early stage.

Each individual group, except the pool, is the result of a cross with only 10 parents which gives a rather limited genetic base. It must be borne in mind that the differences observed between the progenies from the different populations are highly influenced by these few genitors and not necessarily representative of the wild populations from which they come. It is probable however that the observed differences in growth performance are due to genetic differences between the groups, *i.e*. heritable differences in growth performances of the parents.

Genetic differences could be the reason for changes over time between groups in the present study. The similarity in IGR between the ARC group and the pool raises the question of how much the Arcachon parents contributed to the pool. Because a large variance in reproductive success in *C. gigas* (Boudry *et al*., 2002), a higher contribution of Arcachon parental oysters, relative to the other origins, could be one reason for this similarity. However, the FGR results showed the pool to be more similar to BA and SEU. A possible explanation could be that the genetic composition of the pool changed over time due to differential survival. Following this hypothesis, at the beginning of the study, during the period when IGR was measured, the progeny of the Arcachon parents might have been the most common in the pool. However, this was followed by high mortality of slower growing animals, such as those of the ARC group, the composition of the pool would become dominated by faster growing individuals, such as those of the BA and SEU groups. This would give the pool a higher FGR at the end of the study period and would decouple IGR and FGR. However, this explanation do not hold for the PDB group, that exhibited the lowest survival, but where IGR and FGR show the reverse situation. This clearly demonstrates that the interaction between growth and survival is complex.

Early growth rate as a predictor of size later in life: stability of individual growth performance over time and compensation for growth

One of the difficulties in selective breeding for growth is the accurate identification of genetically superior oysters (Smith *et al*., 1995). As it is possible to breed with one-year-old oysters (O'Beirn *et al*., 1996; personal observations), selection before this age could greatly improve the efficiency and speed of selective breeding programs. From this perspective, it is of particular interest to know whether selection for rapid growth at an early stage of the life cycle would produce corresponding rapid growth (*i.e.* rapid attainment of marketable size) and/or bigger size at later

stages. The study of stability of individual growth performance over time and of the impact of early growth rate on size at later stages is a first step in the assessment of this question. Newkirk *et al.* (1977) reported evidence which indicates that the growth rates of larvae and spat (9 months old) of *C. virginica* were positively correlated. Losee (1979), in the same species, reported that larvae settling earliest produced faster-growing spat (7-month-old juveniles). More recently, Collet *et al.* (1999) showed a positive relationship between larval and post-larval growth rate of *C. gigas*. In older animals, Haley and Newkirk (1977) reported high correlation between live oyster weight (*C. virginica*) at different ages (2 to 5-year-old-oysters) and Toro and Newkirk (1990), working with *Ostrea edulis*, observed a high correlation of both the value of shell height and live weight between 14 and 22 months.

Our study showed that, in *C. gigas*, individual growth performances were relatively conserved over time during the first year. Indeed, the correlation coefficient between the growth rate observed between 3 and 4 months old (IGR) and the growth rate observed between 8 and 11 months old (FGR) was significantly positive and their intra-class correlation coefficient was significantly different from 0. However, individual growth rates were not completely stable over time since IGR only explained 12% of variation in FGR and their intra-class correlation coefficient was also significantly different from 1. This may be related to the observation that the pool and the PDB group changed growth rank between the 3rd and the 8th month. Nevertheless, the partial instability of growth rate over time did not result in any noticeable compensation for growth between the initial and final period. Indeed, compensation for growth, a physiological mechanism suggested by Ricker (1975) and already observed in some other species of bivalve (*Argopecten irradians* Auster and Stewart, 1984; *Mercenaria mercenaria* Eldridge and Eversole, 1982), would have led to a poor or negative correlation between early growth rate and later size. In contrast, we observed that 66 % of the total weight of an 11-month-old oyster was still explained by its growth rate calculated in the 3rd month. To conclude, early growth rate appears to be a very good predictor

for size later in life in *C. gigas* due to a relatively good conservation of growth performance over time and no significant compensation mechanism for the part of individuals that vary in growth performance with time. This corpus of results supports the use of early growth measurements, made in the juvenile stage, for the estimation of subsequent size attained later in life and therefore suggests that this form of measurement would be useful in breeding programs for *C. gigas*.

However, considering previous studies, the age at which growth is measured and manner in which measurements are taken are clearly important because contrasting results were found in the European flat oyster, where Newkirk and Haley (1982) reported that the length of larval period showed no correlation with individual size after four growing seasons (40-month-old oysters). A similar conclusion was reported by Newkirk (1981) who found that growth rate at 5 months in the same species is a poor predictor of growth at later stages (29- and 41-month-old oysters). This lack of clarity could also be generated by genotype x environment interaction, when rearing conditions change over time, favouring different genotypes successively (Scheiner, 1993).

The relationship between early growth and early mortality

The present work shows that, in *Crassostrea gigas*, the early mortality occurring under hatchery conditions is significantly greater for smaller individuals. Mortality at early stages is often observed in the wild but rarely assessed precisely in animals reared under controlled conditions. Cheung (1993) found that mortality associated with pollution tends to be higher in larger animals of green-lipped mussel *Perna viridis*. Brey and Gage (1997) examined data from different species of bivalves and found a negative correlation between growth and mortality. Nie *et al*. (1996) and Silina (1994) both observed a link between increased growth rate and reduced mortality with an estimation of a critical-limit size for survival in *Mizuhopecten yessoensis* and in *Haliotis discus hannai* respectively. However, most of these studies were done in the wild with no strict control of

density which can affect growth and mortality or interact with one to influence the other. Studies under controlled conditions can help unravel the reasons for mortality by reducing environmental variability, however results obtained under husbandry or in the laboratory are likely to be different from the wild because the stresses on the animals are different. In hatcheries, causes of mortality can be multiple: pathogens, crowding resulting in accumulation of excretion products and decrease in oxygen concentration, stresses due to rapid alterations in water quality. Whatever the cause of hatchery-based mortality, this often leads to a degradation of the physiological state of the individual before death. The first consequence of this degradation would be a reduced growth rate, which could explain the negative relationship between growth and mortality found in our own and previous studies. Bamber (1990), observed a loss of weight and a reduction in feeding activity as the consequences of a strong reduction in pH of the water and these effects were associated with mortality, in *Crassostrea gigas*, *Ostrea edulis* and *Mytilus edulis*. Consequences of infestations with parasites (Byrne *et al*., 1995) or viruses (Pipe and Coles, 1995) have been also studied and corresponded to a degradation of the physiological state followed by death.

In our study, it is difficult to know whether the observed difference in growth rate between the 2 subsets of individuals (those which survived throughout the trial and those which died during this time) is a cause or a consequence of the early peak in mortality. The causes of this mortality are undetermined, although they might be related to the change in rearing conditions on transfer between trays and baskets. Another hypothesis would be that susceptibility to mortality in early life is dependent on size. In this case, culling the smaller individuals at an early stage would select for more robust animals. However, growth and mortality are not necessarily inter-dependent and so the practice of discarding smaller individuals would not be advantageous. Further work is needed to find the causes of death in the smallest individuals. This would provide a starting point for work on prophylaxis or selection for stress or disease resistance**.**

In our results, all animals which lost weight died during the trial. However, some individuals which died during the trial showed normal increases in growth. This means that at least two different types of mortality may be detected, firstly mortality associated with weight loss or leading to slow growth, and secondly background mortality without any detectable effect on growth. Further studies are needed to investigate the causes of these two different kinds of mortality.

Conclusion

Our results support the common practice of culling the smallest oysters at early stages, as commonly performed in the oyster farming industry. They also support the hypothesis that selection on size and more particularly on growth at an early stage might be an efficient strategy in a breeding program, because the relative stability of individual growth performance over time might allow an effective selection of the best growing individuals at an early stage. Furthermore, the observed positive interaction between growth and survival means that individuals selected in this way should also have better survival. Study of further correlated traits (e.g. quality, disease resistance, time to reproduction) and estimation of genetic parameters are necessary to confirm the value of this method to breeding programs.

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References

Auster, P.J. and Stewart, L.L. (1984) Compensatory growth in the Bay Scallop *Argopecten irradians*. *Journal of the Northwest Atlantic Fishery Science* **5**, 103-104.

Askew, C.G. (1978) A generalized growth and mortality model for assessing the economics of bivalve culture. *Aquaculture* **14**, 91-104

Bamber, R.N. (1990) The effects of acidic seawater on three species of lamellibranch mollusc. *Journal of Experimental Marine Biology and Ecology* **143**, 181-191.

Bardach, J.E. and Ryther, J.H. (1972) Aquaculture - The farming and husbandry of freshwater and marine organisms. Wiley Interscience, New York.

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

Beattie, J.H., Chew, K.K. and Hershberger, W.K. (1980) Differential survival of selected strains of Pacific oysters (*Crassostrea gigas*) during summer mortality. *Procedings of the National Shellfisheries Association* **70**, 184-189.

Bologna, P.A.X. (1998) Growth, production, and reproduction in bay scallops *Argopecten irradians* concentricus (Say) from the northern Gulf of Mexico. *Journal of Shellfish Research* **17**, 911-917.

Boudry, P., Collet, B., Cornette, F., Hervouet, V. and Bonhomme, F. (2002). High variance in reproductive success of the Pacific oyster (*Crassostrea gigas*, Thunberg) revealed by microsatellite-based parentage analysis of multifactorial crosses. *Aquaculture* **204**: 283-296.

Brey, T. and Gage, J.D. (1997) Interaction of growth and mortality in benthic invertebrate populations: Empirical evidence for a mortality-growth continuum. *Archive of Fisherie and Marine Research* **45**, 45-59.s

Brichette, I., Reyero, M.A. and Garcia, C. (2001) A genetic analysis of intraspecific competition for growth in mussel cultures. *Aquaculture* **192**, 155-169.

Byrne, R.A., M.L. Burleson, N.J. Smatresk and McMahon R.F. (1995) Respiratory and acid-base consequences of zebra mussel infestation on the valves of unionids. *Canadian Journal of Zoology* **73**, 1489-1494.

Cheung, S.G. (1993) Population dynamics and energy budgets of green-lipped mussel *Perna viridis* (Linnaeus) in a polluted harbour. *Journal of Experimental Marine Biology and Ecology* **168**, 1-24.

Collet, B., P. Boudry, A. Thebault, S. Heurtebise, B. Morand and Gérard. A. (1999) Relationship between pre- and post-settlement growth in the Pacific oyster *Crassostrea gigas* (Thunberg). *Aquaculture* **175**, 215-226.

Dolmer, P. (1998). Seasonal and spatial variability in growth of *Mytilus edulis* L. in brackish sound : comparisons of individual mussel growth and growth of size classes. *Fisheries Research* **34**, 17-26.

Eldridge, P.J. and Eversole, A.G. (1982) Compensatory growth and mortality of the hard clam, *Mercenaria mercenaria*. *Veliger* **24**, 276-278.

Fisher, L.D. and van Belle, G. (1993) Biostatistics. A methodology for health sciences. Wiley and Sons, New York. 991p.

Fréchette, M. and Bacher, C. (1998) A modelling study of optimal stocking density of mussel populations kept in experimental tanks. *Journal of Experimental Marine Biology and Ecology* **219**, 241-255.

Fujio, Y., Nakamura, Y., and Sugita, M. (1979) Selective advantage of heterozygotes at catalase locus in the Pacific oyster, *Crassotrea gigas*. *Japan Journal of Genetics* **54**, 359-366.

Galtsoff, P.S. (1964) The American oyster *Crassostrea virginica* Gmelin. *Fisheries Bulletin* 64. U.S.A. Fish and Wildlife Services, Government Printing Office, Washington, D.C.

Hadley, N.H. and Manzi, J.J. (1984). Growth of seed clams, *Mercenaria mercenaria* at various densities in a commercial scale nursery system. *Aquaculture* **36**, 369-378.

Haley, L.E. and Newkirk, G.F. (1977). Selecting oysters for faster growth. *Procedings of the 8th Annual Meeting, World Mariculture Society* **8**, 557-566.

Hampton, J. (1991) Estimation of southern bluefin tuna *Thunnus maccoyii* growth parameters from tagging data, using von Bertalanffy models incorporating individual variation. *Fisheries Bulletin* **89**, 577-590.

Héral, M and Deslous-Paoli, J.M. (1991) Oyster culture in European countries. In: Estuarine and marine bivalve mollusk culture (ed. W. Menzel). CRC press, U.S.A, pp.1153-191.

Hershberger, W.K., Perdue, J.A. and Beattie J.H. (1984). Genetic selection and systematic breeding in Pacific oyster culture. *Aquaculture* **39**, 237-245.

Langdon C., Evans F., Jacobson D. and Blouin M. Yields of cultured Pacific oysters Crassostrea gigas Thunberg improved after one generation of selection. *Aquaculture* (in press).

Laing, I., Utting, S.D. and Kilada R.W.S. (1987). Interactive effect of diet and temperature on the growth of juvenile clams. *Journal of Experimental Marine Biology and Ecology* **113**, 23-38.

Losee, E. (1979) Relationship between larval and spat growth rates in the oyster *(Crassostrea virginica*). *Aquaculture* **16**, 123-126.

Mahon, G.A.T. (1983) Selection goals in oyster breeding. *Aquaculture* **33**, 141-148.

Mallet, A.L. and Carver, E. (1995) Comparative growth and survival patterns of *Mytilus trossulus* and *Mytilus edulis* in Atlantic Canada. *Canadian Journal of Fisheries and Aquatic Sciences* **52**, 1873-1880.

Manly, B.J.F. (1997). Randomization, Bootstrap and Monte Carlo Methods in Biology. Second Edition. Chapman & Hall, London, UK, 399pp.

Medcof, J.C. (1961). Oyster farming in the Maritimes. *Bulletin of the Fisheries Research Board of Canada* **131**: 1-158.

Nell, J.A., Smith, I.R. and McPhee, C.C. (2000) The Sydney rock oyster *Saccostrea glomerata* (Gould 1850) breeding programme: progress and goals. *Aquaculture Research* **31**, 45-49

Neudecker, T. (1981). Experimental offshore nursery - growing of *Crassotrea gigas* Thunberg in Germany. In: Nursery Culturing of Bivalve Molluscs (eds. C. Claus, N. Depauw, and E. Jaspers). European Mariculture Society Special Publication No. 7. EMS, Bredene, Belgium, pp. 197-210.

Newkirk, G.F. (1981) On the predictability of bivalve growth rates: is a slow growing oyster a runt for life ?. *In*: Nursery Culturing of Bivalve Molluscs (eds. C. Claus, N. Depauw, and E. Jaspers). European Mariculture Society Special Publication No. 7. EMS, Bredene, Belgium, pp.211-218.

Newkirk, G.F. and Haley L.E. (1982) Phenotypic analysis of the European *oyster Ostrea edulis* L.: Relationship between length of larval period and postsetting growth rate. *Journal of Experimental Marine Biology and Ecology* **59**, 177-184.

Newkirk, G.F. and L.E. Haley (1983) Selection for growth rate in the European oyster, *Ostrea edulis*: response of second generation groups. *Aquaculture* **33**, 149-155.

Newkirk, G.F., Haley L.E., Waugh, D.L. and Doyle, R (1977). Genetics of larvae and spat growth rate in the oyster *Crassostrea virginica. Marine Biology* **41**, 49-52.

Nie, Z.Q., M.F. Ji and Yan. J.P. (1996) Preliminary studies on increased survival and accelerated growth of overwintering juvenile abalone, *Haliotis discus hannai* Ino. *Aquaculture* **140**, 177-186.

O'Beirn, F.X., Heffernan, P.B., Walker, R.L. and Jansen, M.L. (1996) Young-of-the-year oyster, *Crassostrea virginica*, reproduction in coastal Georgia. *Estuaries* **19**, 651-658.

Pipe, R.K. and Coles J.A. (1995) Environmental contaminants influencing immune function in marine bivalve molluscs. *Fish and Shellfish Immunology* **5**, 581-595.

Puigcerver, M. (1996) Analysis on individual growth of juvenile carpet shell clams, *Tapes decussatus* (L.), in three different rearing conditions employed in the Ebro's Delta, Spain. *Aquaculture Research* **27**, 399-404.

Rawson, P.D. and Hilbish, T.J. (1990). Heritability of juvenile growth for the hard clam *Mercenaria mercenaria. Marine Biology* **105**, 429-436.

Ricker, W.E. (1975) Computation and interpretation of biological statistics of fish populations. *Bulletin of the Fisheries Research Board of Canada* **191**, 1-382.

Robert, R. and Gérard, A. (1999) Bivalve hatchery technology: The current situation for the Pacific oyster *Crassostrea gigas* and the scallop *Pecten maximus* in France. *Aquatic Living Resources* **12**, 121-130.

Sainsbury, K.J. (1980) Effect of individual variability on the von Bertalanffy growth equation. *Canadian Journal of Fisheries and Aquatic Sciences* 37, 241-247.

SAS Institute Inc. (1988) *SAS/STAT User's Guide, Release 6.03*, SAS Institute Inc, SAS Campus drive, Cary, NC 27513. 1028 pp.

Scheiner, S.M. (1993) Genetics and evolution of phenotypic plasticity. *Annual Review of Ecology and Systematics* **24**, 35-68.

Sheridan, A.K. (1997) Genetic improvement of oyster production - a critique. *Aquaculture* **153**, 165-179.

Silina, A.V. (1994) Survival of different size-groups of the scallop, *Mizuhopecten yessoensis* (Jay), after transfer from collectors to the bottom. *Aquaculture* **126**, 51-59.

Singh, S.M. and Zouros, E. (1978) Genetic variation associated with growth rate in the American oyster (*Crassostrea virginica*). *Evolution* **32**, 342-353.

Singh, S.M. and Zouros E. 1981. Genetics of growth rate in oysters and its implication for aquaculture. *Canadian Journal of Genetics and Cytology* **23**, 119-130.

Smith, I.R., Sheridan, A.K., and Nell, J.A. (1995) Evaluation of growing methods for use in a Sydney rock oyster *Saccostrea commercialis* (Iredale and Roughley) selective breeding program. *Aquaculture* **131**, 189-195.

Sokal, R.R. and Rohlf, F.J. (1995) Biometry. WH Freeman and Co., New York. 887 pp.

Stirling, H.P. and Okumus I. (1995) Growth and production of mussels (*Mytilus edulis* L.) suspended at salmon cages and shellfish farms in two scottish sea lochs. *Aquaculture* 134, 193-210.

Toro, J.E. and G.F. Newkirk. (1990) Divergent selection in the European oyster (*Ostrea edulis* L.): response to selection and estimates of genetic parameters. *Marine Ecology Progress Series* **62**, 219-227.

Toro, J.E., Aguila, P. and Vergara A.M. (1996) Spatial variation in response to selection for live weight and shell length from data on individually tagged Chilean native oysters (*Ostrea chilensis* Philippi, 1845). *Aquaculture* **146**, 27-36.

Toro, J.E., Sanhueza, M.A., Winter J.E., Aguila P. and Vergara, A.M. (1995). Selection response and heritability estimates for growth in the Chilean oyster *Ostrea chilensis* (Philippi, 1845). *Journal of Shellfish Research* 14, 87-92.

Walne, P.R. (1974) Culture of bivalve molluscs: fifty years experience at Conwy, Whitefriars Press Ltd, London. 189 pp.

Ward, R.D., English, L.J., Mcgoldrick, D.J., Maguire, G.B., Nell, J.A., and Thompson, P.A. (2000). Genetic improvement of the Pacific oyster (*Crassostrea gigas* Thunberg) in Australia. *Aquaculture Research* **31**, 35-44.

Zouros, E., Singh, S.M. and Miles, H.E. (1980). Growth rate in oysters: an overdominant phenotype and its possible explanations. *Evolution* **34**, 856-867.

Abbreviations:

ARC: Arcachon

SEU: Seudre

PDB: Port des Barques

BA: Bonne Anse

DPF: Days Post Fertilization

IGR: Initial Growth Rate

FGR: Final Growth Rate

Table 1: Initial growth characteristics of the 5 groups of *Crassostrea gigas* studied. Time is represented as calendar date, DPF and months. Data are mean ± S.E.. TOT = all groups together, SEU = Seudre, PDB = Port des Barques, BA = Bonne-Anse, ARC = Arcachon

	Date	16 July 1996	20 August 1996	22 October 1996	17 February 1997
	DPF	91	126	189	307
	Month	3	4	6	10
TOT	Number	1009	703	670	663
	Mean live weight ± SE (g)	0.73 ± 0.02	6.08 ± 0.1	13.07 ± 0.24	17.79 ± 0.31
SEU	Number	133	97	87	86
	Mean live weight ± SE (g)	0.88 ± 0.05	7.7 ± 0.25	15.56 ± 0.6	19.8 ± 0.79
PDB	Number	238	118	116	115
	Mean live weight ± SE (g)	0.94 ± 0.04	7.37 ± 0.23	15.43 ± 0.52	19.82 ± 0.68
BA	Number	237	204	198	197
	Mean live weight ± SE (g)	0.86 ± 0.04	6.85 ± 0.19	15.23 ± 0.44	20.86 ± 0.6
ARC	Number	193	114	109	108
	Mean live weight ± SE (g)	0.48 ± 0.02	4.67 ± 0.21	9.31 ± 0.47	13.2 ± 0.6
POOL	Number	208	170	159	157
	Mean live weight ± SE (g)	0.44 ± 0.02	4.34 ± 0.16	9.9 ± 0.42	14.52 ± 0.53

Table 2. Results of repeated measures ANOVA on growth, based on shell length on 24 measurement dates between DPF 91 and DPF 307 for the five experimental groups

Source	Degrees of Freedom	Mean Squares	F	*p*
DPF	23	14181.627	2136.446	<0.0001
DPF x Groups	92	166.515	25.085	<0.0001
Error	14743	6.638		

Table 3: Mean live weight and survival of the smallest 10 % (A) and 25 % (B) of the 5 groups and of all groups together as determined at 3, 6 and 10 months (respectively DPF 91, 186 and 307). The data for the remaining 90 % and 75 % are given for comparison. Survival is given as (1) numbers of individuals and (2) percentages of numbers of individuals at 3 months (in italics). $\chi 2$ tests were made on survival differences between the number surviving in the smallest 10 or 25 % and the remaining 90 or 75 % between 3 and 6 months.

A

		Mean Live Weight (g) Age (Months)			Surviving Individuals Age (Months)		
Set	% of group at 3 months	3	6	10	3	6	10
All sets	10%	0.13	5.83	8.96	110	31	31
					100	*28*	*28*
	90%	0.80	13.41	18.22	899	638	632
					100	*71*	*70*
					X^2 test between 3 and 6 months: (***)		
ARC	10%	0.12	5.48	8.78	19	6	6
					100	*32*	*32*
	90%	0.52	9.54	13.46	174	103	102
					100	*59*	*59*
					X^2 test: (N.S.)		
BA	10%	0.09	5.90	7.37	24	7	7
					100	*29*	*29*
	90%	0.95	15.53	21.36	213	191	190
					100	*90*	*89*
					X^2 test: (*)		
PDB	10%	0.20	-	-	24	0	0
					100	*0*	*0*
	90%	1.02	15.43	19.82	214	116	115
					100	*54*	*54*
					X^2 test: (**)		
SEU	10%	0.18	-	-	13	0	0
					100	*0*	*0*
	90%	0.96	15.56	19.80	120	87	86
					100	*73*	*72*
					X^2 test: (*)		
POOL	10%	0.09	6.41	9.97	21	8	8
					100	*38*	*38*
	90%	0.48	10.08	14.77	187	151	149
					100	*81*	*80*
					X^2 test: (N.S.)		

B

		Mean Live Weight (g) Age (Months)			Surviving Individuals Age (Months)		
Set	% of group at 3 months	3	6	10	3	6	10
All sets	25%	0.21	7.44	11.20	252	104	104
					100	*41*	*41*
	75%	0.90	14.09	19.02	757	565	559
					100	*75*	*74*
					X^2 test between 3 and 6 months: (***)		
ARC	25%	0.19	6.27	8.66	48	14	14
					100	*29*	*29*
	75%	0.58	9.77	13.88	145	95	94
					100	*66*	*65*
					X^2 test: (*)		
BA	25%	0.22	9.26	12.92	59	31	31
					100	*53*	*53*
	75%	1.08	16.28	22.36	178	167	166
					100	*94*	*93*
					X^2 test: (N.S.)		
PDB	25%	0.33	12.89	16.69	60	14	14
					100	*23*	*23*
	75%	1.15	15.78	20.26	178	102	101
					100	*57*	*57*
					X^2 test: (*)		
SEU	25%	0.28	13.33	16.30	33	5	5
					100	*15*	*15*
	75%	1.08	15.67	20.02	100	82	81
					100	*82*	*81*
					X^2 test: (**)		
POOL	25%	0.15	5.79	9.29	52	23	23
					100	*44*	*44*
	75%	0.54	10.59	15.42	156	136	134
					100	*87*	*86*
					X^2 test: (*)		

Figure legends

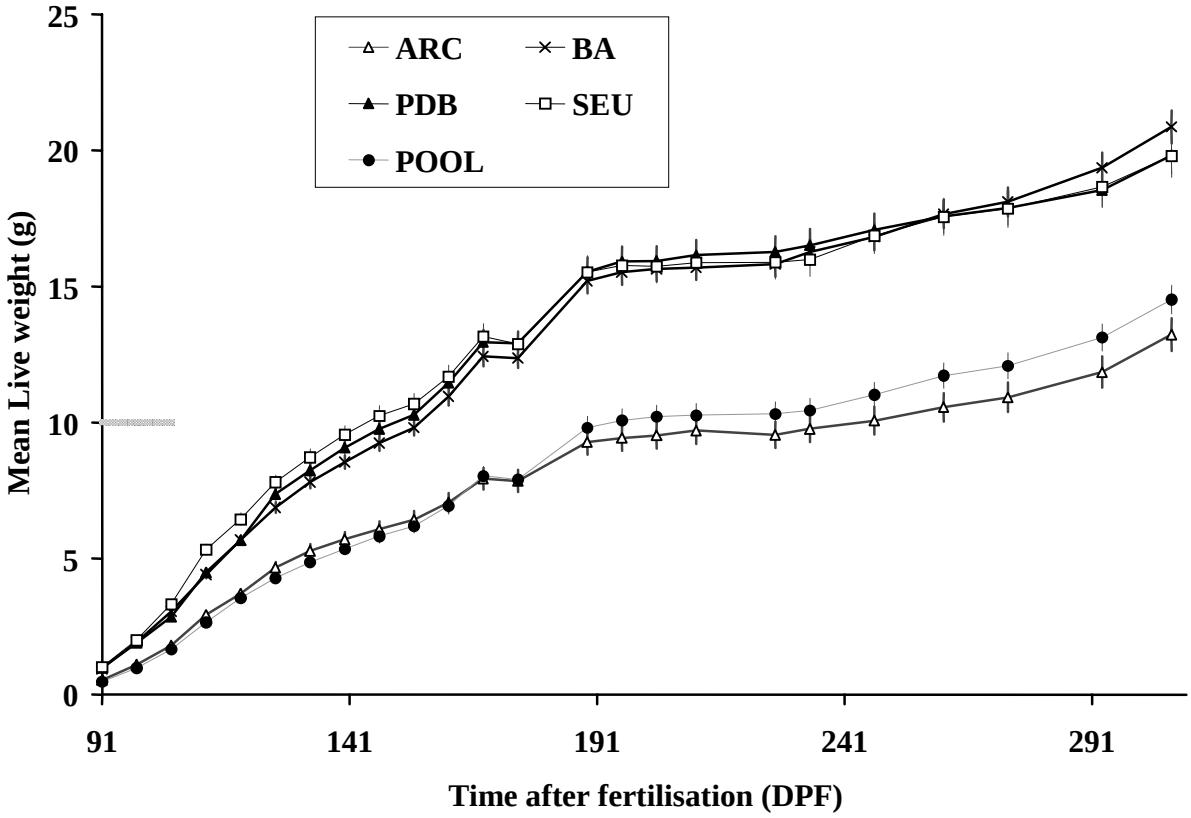
Figure 1: a) Mean growth curves per group in *Crassostrea gigas*. The error bars represent Standard Error. The horizontal bar in grey represents the period of time used for the estimation of the linear growth rate (see text). Time scale is given in number of days after fertilization (DPF). b) Evolution of survival of the 5 groups between 3 and 10 months (between DPF 91 and DPF 307) in *Crassostrea gigas* reared under controlled conditions.

Figure 2: A) Relationship of live weight between 3 and 10 months in *Crassostrea gigas* reared under controlled conditions. *n = 658*, surviving oysters at DPF 307. B) Relationship between the initial growth rate (between DPF 91 and 126) and the final growth rate (between DPF 227 and 307) between in *Crassostrea gigas* (*n = 658).* C) Relationship between the final live weight (at DPF 307) and initial growth rate (between DPF 91 and DPF 126) in *Crassostrea gigas* (*n = 658).*

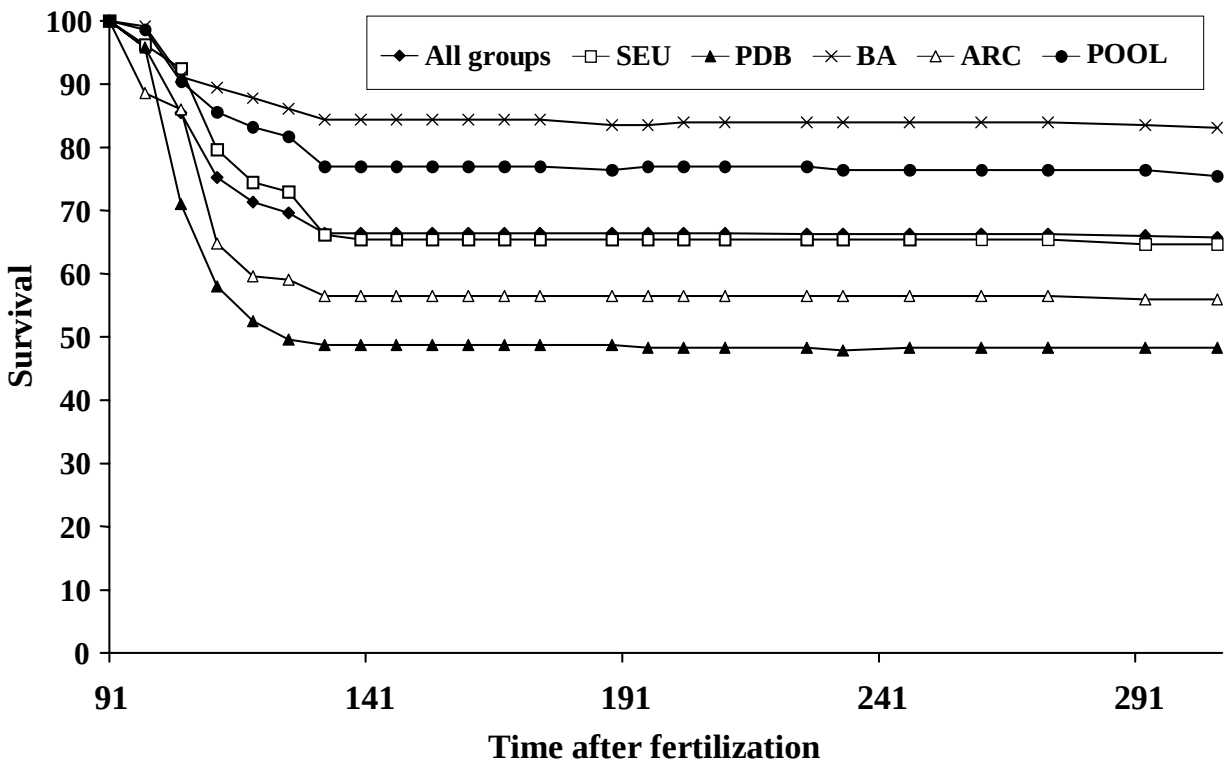
Figure 3: Interaction between growth and mortality in *Crassostrea gigas*. Comparison of the mean growth curve in the group S (oysters surviving throughout experiment) and the mean pseudo growth curve group D (oysters which died during the experiment). Only the early part of the curve is shown. Significant differences ($P < 0.0001$) between the two groups exist at the 3 first data points (between DPF 91 and DPF 126).

Figure 4: Individual growth rates of the groups S and D. Growth rate is represented as the relationship between the two first live weight records (DPF 91 and DPF 98).

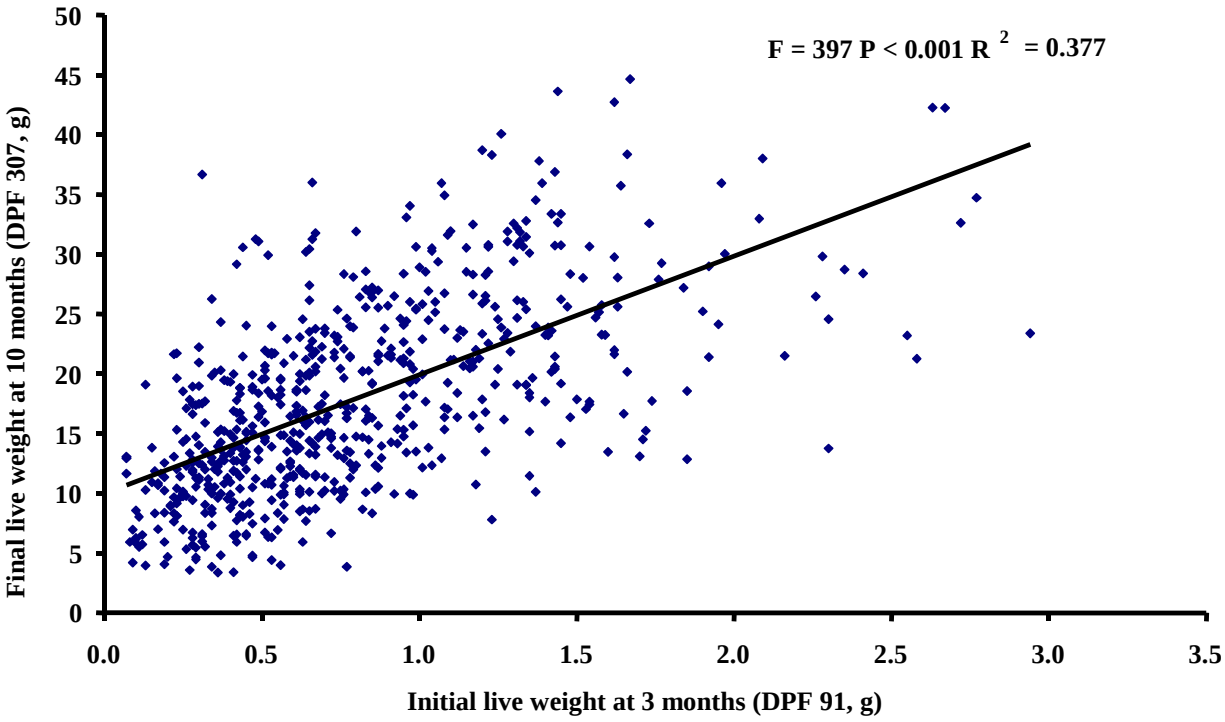
1A


ARC
BA
PDB
SEU
POOL
Mean Live weight (g)
0
5
10
15
20
25
91
141
191
241
291
Time after fertilisation (DPF)

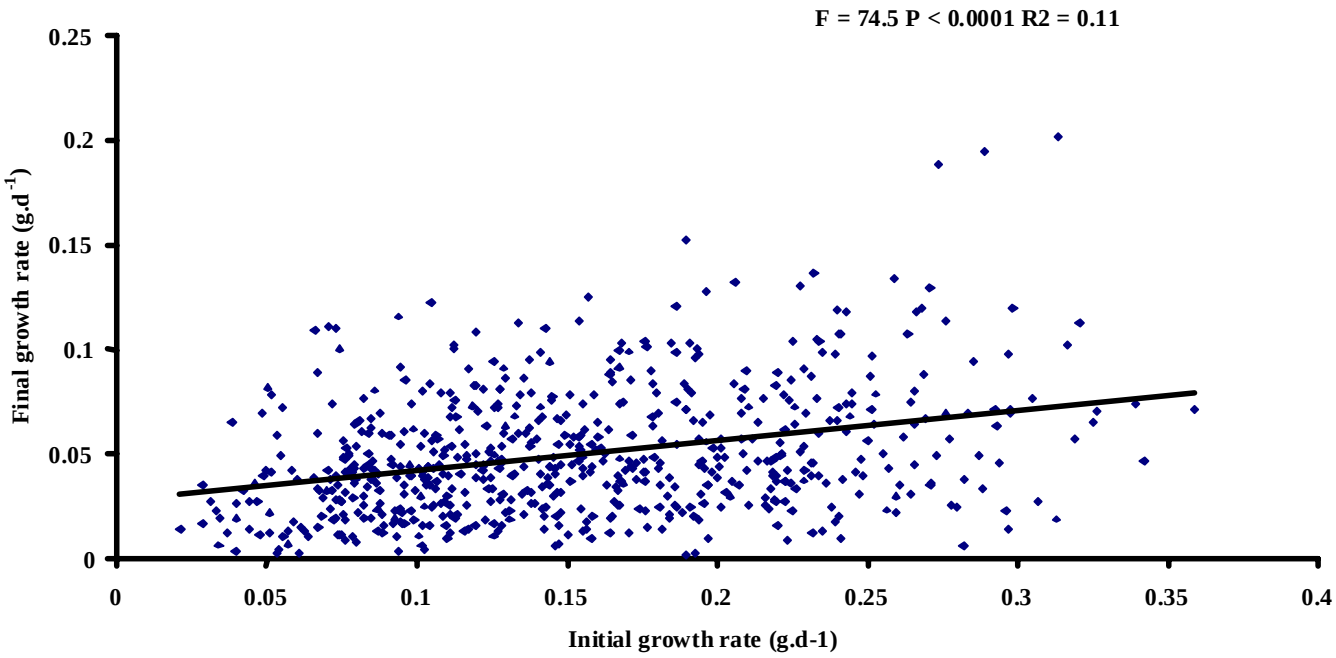
1B


All groups
SEU
PDB
BA
ARC
POOL
Survival
100
90
80
70
60
50
40
30
20
10
0
91
141
191
241
291
Time after fertilization

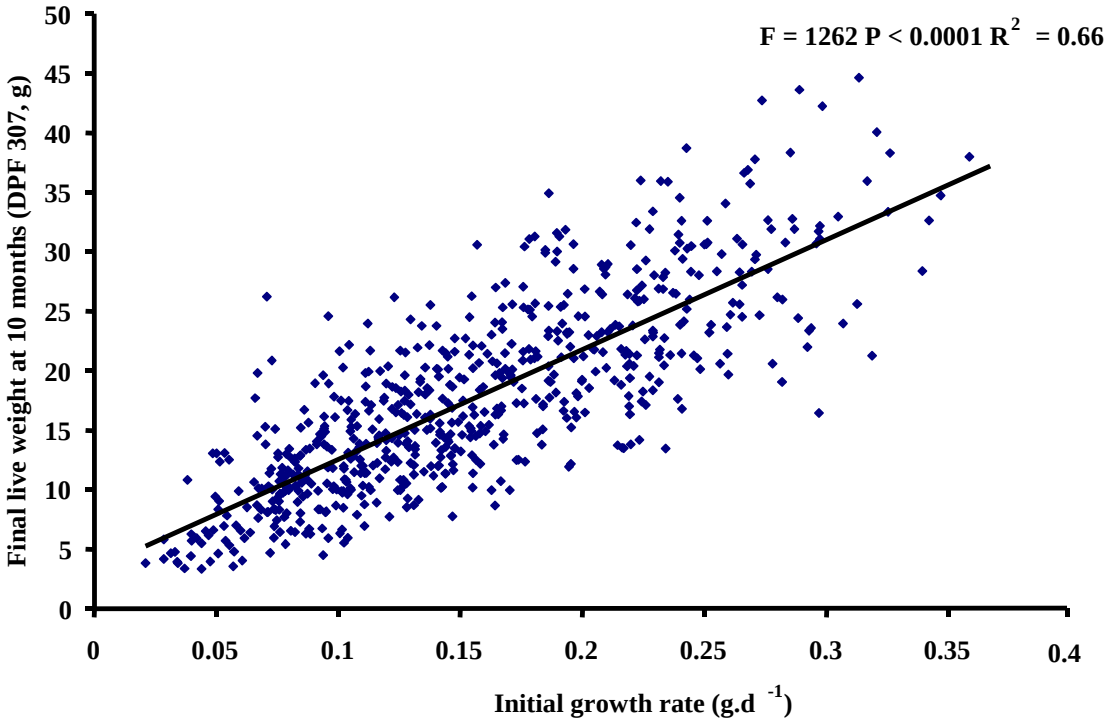
2A


F = 397 P < 0.001 R 2 = 0.377
Final live weight at 10 months (DPF 307, g)
Initial live weight at 3 months (DPF 91, g)
0
5
10
15
20
25
30
35
40
45
50
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5

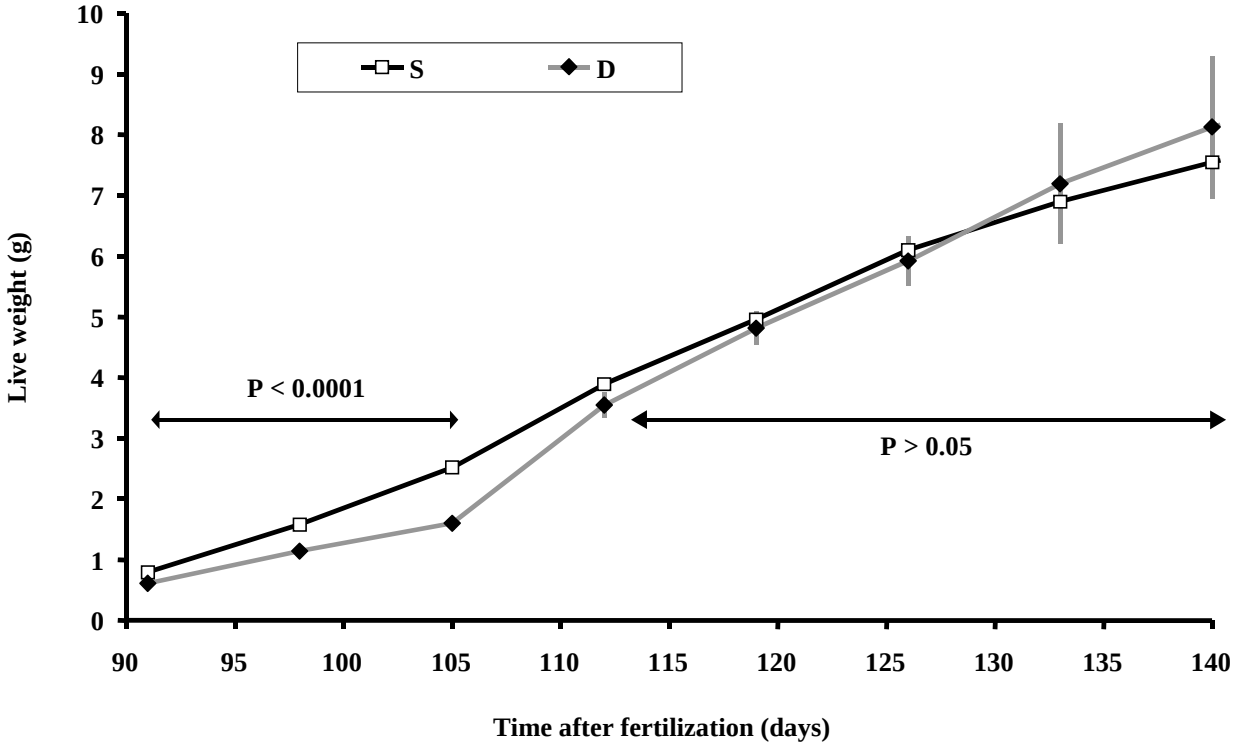
2B

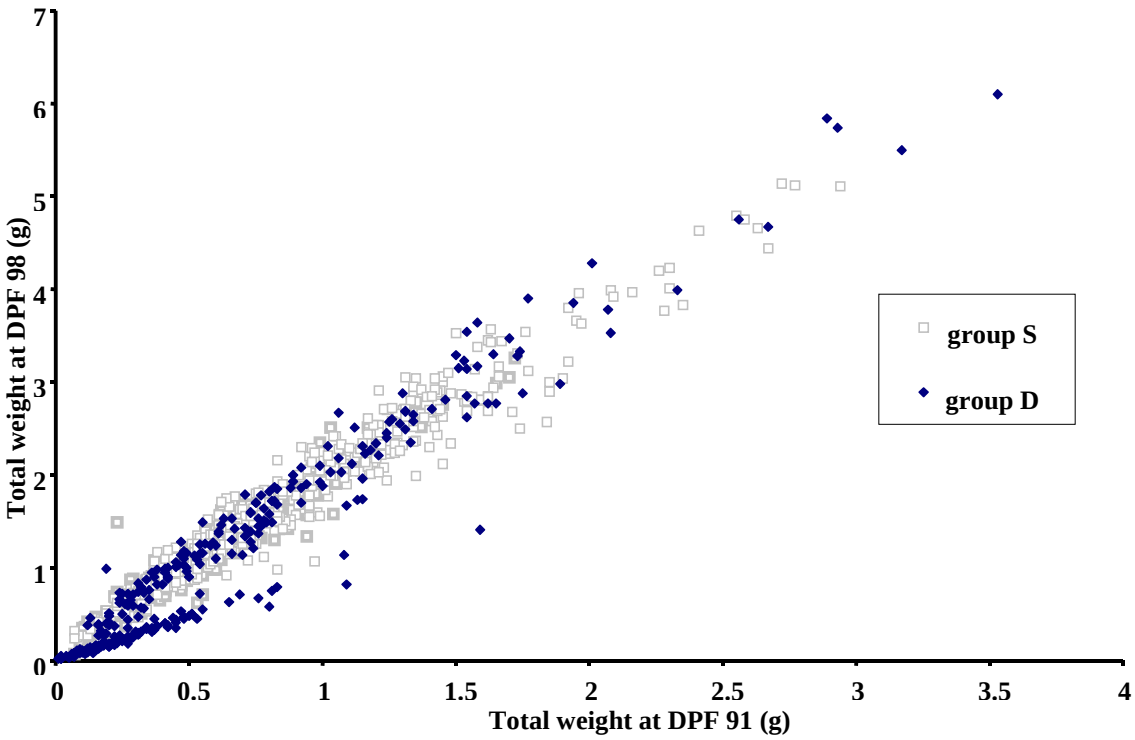

F = 74.5 P < 0.0001 R2 = 0.11
Final growth rate (g.d-1)
0.25
0.2
0.15
0.1
0.05
0
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
Initial growth rate (g.d-1)

2C


F = 1262 P < 0.0001 R2 = 0.66
Final live weight at 10 months (DPF 307, g)
50
45
40
35
30
25
20
15
10
5
0
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
Initial growth rate (g.d -1)

3


S
D
Live weight (g)
0
1
2
3
4
5
6
7
8
9
10
P < 0.0001
P > 0.05
90
95
100
105
110
115
120
125
130
135
140
Time after fertilization (days)


Total weight at DPF 98 (g)
Total weight at DPF 91 (g)
group S
group D
0
1
2
3
4
5
6
7
0
0.5
1
1.5
2
2.5
3
3.5
4

4