



# Comparative study of growth, survival and yield of selected, inbreeding and wild populations in Pacific oysters

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## ARTICLE INFO

### Keywords:

Selective breeding  
Inbreeding depression  
Growth  
Survival  
Yield

## ABSTRACT

In order to assess the overall progress of our long-term selective breeding program and quantify the effects of inbreeding on production traits in selected line of Pacific oyster *Crassostrea gigas*, we systematic analyzed the growth, survival and yield of selected, inbreeding and wild populations for 440 days in northern China. During the larval stage, selected line exhibited high superiority in both survival rate and shell height. Compared with wild population, the shell height and survival rate of selected line increased by 19.10% and 57.15% at day 20, respectively. Meanwhile, the shell height and individual weight of the selected line were significantly higher than wild population at whole grow-out stage. Notably, the cumulative survival rate of selected line increased by 71.35% compared with wild population at harvest. Additionally, the final yield of selected line increased by 157.98% compared with wild population and it would be of great benefits to oyster industry. In this study, the effects of inbreeding on shell height were not significant ( $P > 0.05$ ) at whole larvae stage, ranged from  $-0.68\%$  to  $-5.01\%$ . On the contrary, there were significant inbreeding depression ( $-5.14\%$  to  $-22.46\%$ ) on larvae survival and the estimated coefficient were  $-2.06\%$  to  $-8.98\%$  per 10% increase of inbreeding coefficient of F. During grow-out stage, inbreeding depression on survival were not significant ( $P > 0.05$ ), ranged from  $-0.83\%$  to  $-5.04\%$ . On the contrary, there were significant ( $P < 0.05$ ) inbreeding depression on growth traits and tend to increase with age. The estimated inbreeding depression coefficient of individual weight ranged from  $-1.54\%$  to  $-5.91\%$  per 10% increase of inbreeding coefficient of F, which was higher than the estimate obtained for shell height ( $-0.64\%$  to  $-5.52\%$ ). For yield, the estimated inbreeding depression coefficient was  $-8.37\%$  per 10% increase of inbreeding coefficient of F ( $P < 0.05$ ). The above results indicate that substantial genetic progress was achieved in our selective breeding program, and it is necessary to maintain pedigree records to avoid the harmful effects of inbreeding depression.

## 1. Introduction

The Pacific oyster, *Crassostrea gigas*, is considered as one of the most important commercial oyster species all over the world, supporting the development of worldwide shellfish industry (Ruesink et al., 2005). Since the goal of oyster farming industry is to meet growing world demand, it is necessary to significantly increase production. Worldwide oyster production is largely based on un-improved populations, which may limit profitability and productivity due to the unrealized potential for genetic improvement (De Melo et al., 2016). In order to develop the oyster industry, it is necessary to establish a selective breeding program to improve seed quality and meet the growing spat demand from farmers and commercial enterprises.

Selective breeding has proven to be a simple but effective method to increase the important economic traits of oysters, even after only a few generations of selection (Gjedrem and Rye, 2018). The hatchery-based seed production to support oyster culture has promoted breeding programs seeking to develop genetically improved lines with superior production performance (Rawson and Feindel, 2012). In fact, some of selective breeding programs focused on the improvement of economically important traits of oysters have been initiated in different parts of world, and have obtained encouraging results (Kube et al., 2018; Dégremont et al., 2010a; Dégremont et al., 2015; Li et al., 2011; Langdon et al., 2003; De Melo et al., 2016). Undoubtedly, genetic improvement in these production traits would be beneficial for oyster culture and farmers.

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<https://doi.org/10.1016/j.aquaculture.2023.739232>

Received 8 October 2022; Received in revised form 29 December 2022; Accepted 1 January 2023

Available online 3 January 2023

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In northern China, the production of *C. gigas* has often been limited by summer massive mortalities and a short growing season. To improve the increasing valuable Pacific oyster industry in China, a selective breeding of *C. gigas* was initiated in 2007 (Li et al., 2011). After nine generations of mass selection, remarkable genetic gains for growth rate were achieved in the selected line (Zhang et al., 2019). Recently, summer mortality events were commonly observed in oyster farms and often result in high mortality. Therefore, selection for summer survival was incorporated into our selective breeding program in 2019, and good genetic progress for summer survival has been achieved through two generations of family selection (Chi et al., 2022). Our goal is to breed a new variety with good growth performance and high survival rate in northern China. Understanding the overall progress is critical for long-term selective breeding program. Wild individuals are supposed to be unaffected by artificial selection, which makes wild population an ideal control for assessing the genetic progress in selective breeding programs (Li et al., 2018).

The goal of selective breeding programs is to maximize superior performances, which may lead to inbreeding. In fact, a small degree of inbreeding is often inevitable in order to balance the need for maximum selection intensity with the need to retain a sufficient potential parent (Falconer and Mackay, 1996). Inbreeding tends to affect traits related to reproductive fitness and environment adaptability, resulting in a reduction in the average phenotypic value of these traits, a phenomenon known as inbreeding depression (Lynch and Walsh, 1998). Inbreeding depression of oyster has been well documented in the progeny of self-fertilization and full-sib matings (Beattie et al., 1987; Bierne et al., 1998; Mallet and Haley, 1983; Evans et al., 2004; Fang et al., 2021; Launey and Hedgecock, 2001). However, the effects of inbreeding on production traits in selected *C. gigas* population at different grow-out stages remain uncertain. Acceptable inbreeding rate may vary from different selective breeding programs (Pante et al., 2001). For selective breeding programs of oysters to be successful and economically viable, it is necessary to quantify the impact of inbreeding and incorporate this information into the design of the breeding programs.

In the present study we report the comparative growth, survival and yield performance of selected, inbreeding and wild populations for 440

days in northern China. The purposes of this study were: 1) to evaluate the gross progress of our selective breeding program; 2) to quantify the effects of inbreeding on production traits in selective *C. gigas* line.

## 2. Material and method

### 2.1. Experiments oysters

We have developed two fast-growing strains of *C. gigas* through over eight generations of mass selection. Fig. 1 shows the pedigrees of the families used. In 2019, a base population (G1) was established using the two fast-growing strains, according to Chi et al. (2021) to produce a total of 49 families. Subsequently, families with high summer survival were pair-spawned to create the G2 families. In this study, a selected line (SL) was established by crossing of unrelated individuals of the G2 family ( $F = 0$ ). Full-sibling (brother-sister) crosses were used to generate inbreeding line (IL) using the 12 G2 families ( $F = 0.25$ ). The selected line and inbreeding line consisted of 12 full-sib families, respectively. In addition, 12 families of wild control population (WP) were established by crossing one female and one male using wild individuals collected from Rongcheng as parents (Fig. 2).

### 2.2. Family construction and offspring culture

Larval rearing was conducted according to the standard operating procedure described by Chi et al. (2022). The hatched larvae of each family were reared individually in a 100-L bucket using filtered seawater. D-larvae were fed enough *Isochrysis galbana* before the umbo stage, after which the larvae were fed enough algae diet of *I. galbana* and *Platymonas* sp. according to the larvae density. The seawater temperature of each family is 23–25 °C and the salinity is 30–32 psu. When 30% or more of the pediveliger larvae appears eyespots, the scallop shells were placed in bucket to provide a substrate for eyed larvae to set. When all attachments were complete, each family was tagged and transferred to an outdoor nursery pond for temporary rearing.

The fertilization rate was defined as the ratio of fertilized eggs to total eggs, hatching rate was measured as the proportion of D-shaped

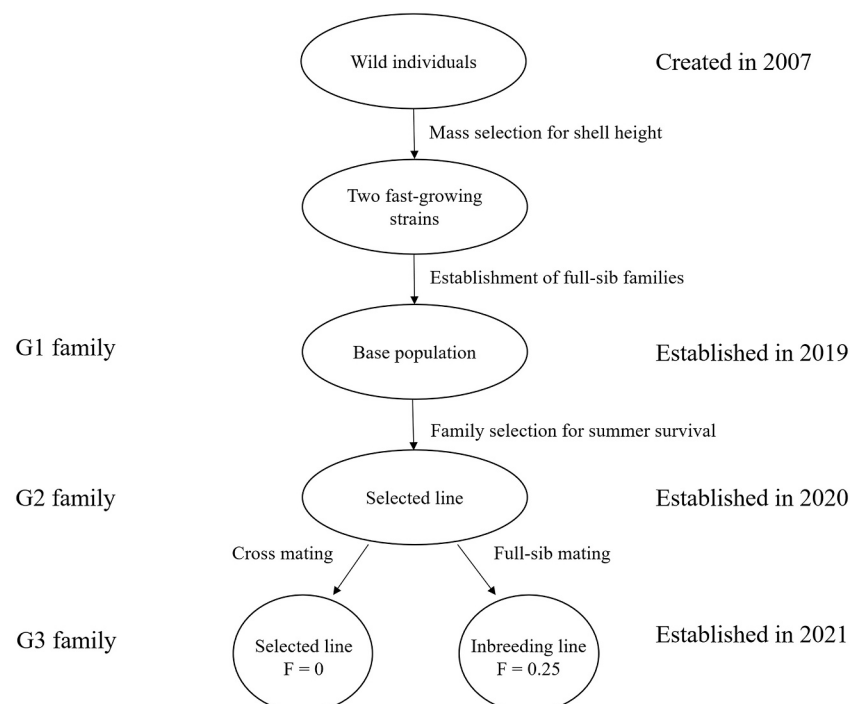


Fig. 1. Pedigrees of families used in this study.

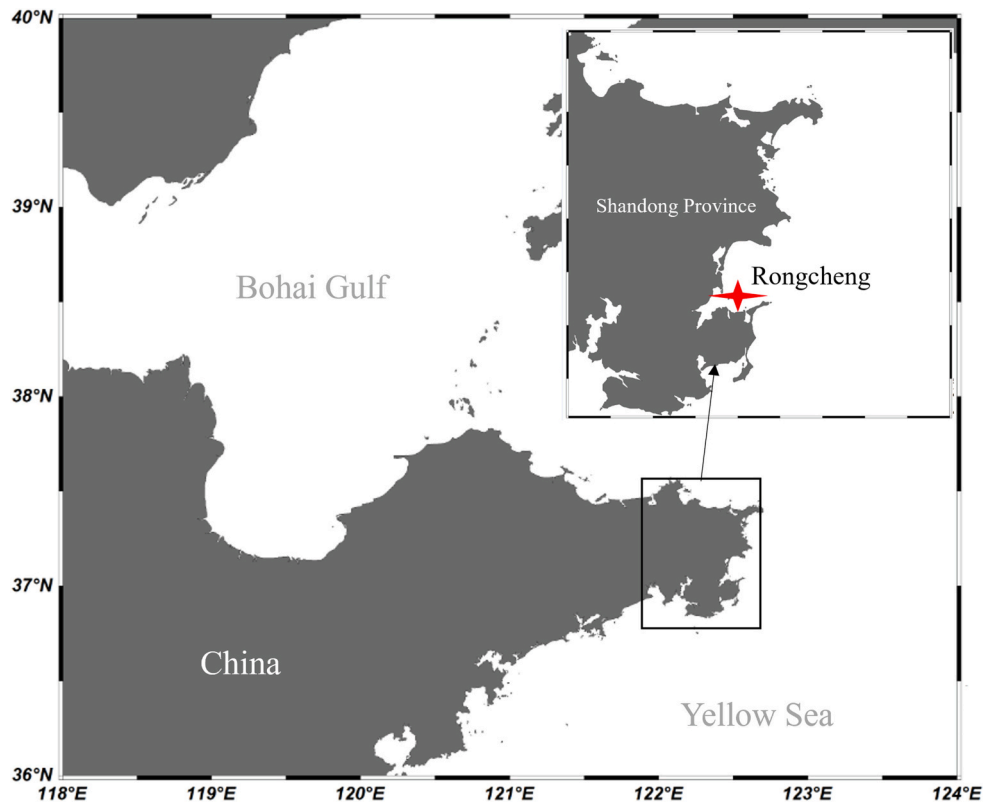


Fig. 2. Map of the planting site in Rongcheng, Shandong province of China.

larvae to total zygotes (24 h of insemination). The larval shell height (30 individuals) was measured by Image-ProPlus image analysis software 6.0 on day 1, 5, 10, 15 and 20 according to Xu et al. (2019). Larvae survival was defined as the ratio of live larvae at the sampling day to that of D-larvae stage.

### 2.3. Field deployment and monitoring

In May 2021, families from each population were transported and cultivated in Rongcheng, Shandong province of China. 120 oysters from each family were placed into three replicate lantern nets. During grow-out stage, 30 oysters were randomly selected from each family on day 110, 220, 330 and 440 to record growth-related parameters. Cumulative survival rate was calculated based on the number of surviving oysters from one lantern net at each sampling date. At day 440, all live oysters were weighed to determine yield. The shell height and individual weight were measured with a vernier calipers (accurate to 0.01 mm) and electronic scales (accurate to 0.01 g), respectively.

### 2.4. Data analysis

All data were expressed as mean  $\pm$  standard deviation and were analyzed with one-way analysis of variance followed by multiple comparison Tukey test using SPSS 22.0 software. Differences were considered statistically significant if  $P < 0.05$ .

The incremental survival rate was evaluated as follows (Qin et al., 2020):

$$I_{t+1}(\%) = (N_{t+1}/N_t) \times 100$$

where  $I_{t+1}$  is the incremental survival rate at time  $t + 1$ ;  $N_t$  is the number of surviving oysters at time  $t$ ;  $N_{t+1}$  is the number of surviving oysters at time  $t + 1$ ;  $t + 1$  was the next measurement time point of  $t$ .

The inbreeding depression coefficient was evaluated as follows (Keys et al., 2004):

$$IDC(\%) = \frac{1 - (W_{inbred}/W)}{(F - F_{inbred})} \times 100$$

where  $W_{inbred}$  and  $W$  represent the mean value of inbreeding line and the selected line, respectively.  $F_{inbred}$  and  $F$  represent the inbreeding coefficients of inbreeding line and the selected line.

The superiority of selected line was calculated as follows:

$$S(\%) = \frac{X_s - X_w}{X_w} \times 100$$

where  $X_s$  is the mean value of the selected line,  $X_w$  is the mean value of the wild population.

## 3. Results

### 3.1. Growth and survival at larvae stage

The difference of initial shell height of the three populations was not significant ( $P > 0.05$ ) (Fig. 3A). With the extension of culture period, the shell height of the three populations increased gradually. The shell height of wild population was lower than those of the other two populations at larval stage, with the order of WP < IL < SL. From day 5, the shell height of the selected line was significantly higher than wild populations ( $P < 0.05$ ). However, no significant difference was observed in shell height between selected line and inbreeding line throughout the larvae stage ( $P > 0.05$ ). Compared to the wild population, the shell height of the selected line was increased by 0.28%–19.10% at larvae stage (Table 1).

The survival traits of three populations are shown in Fig. 3B. The fertilization and hatching rates of the selected line were slightly higher than those of the two other populations, but no significant difference was observed among three populations ( $P > 0.05$ ). From day 10, the selected line was significantly higher than two other populations in

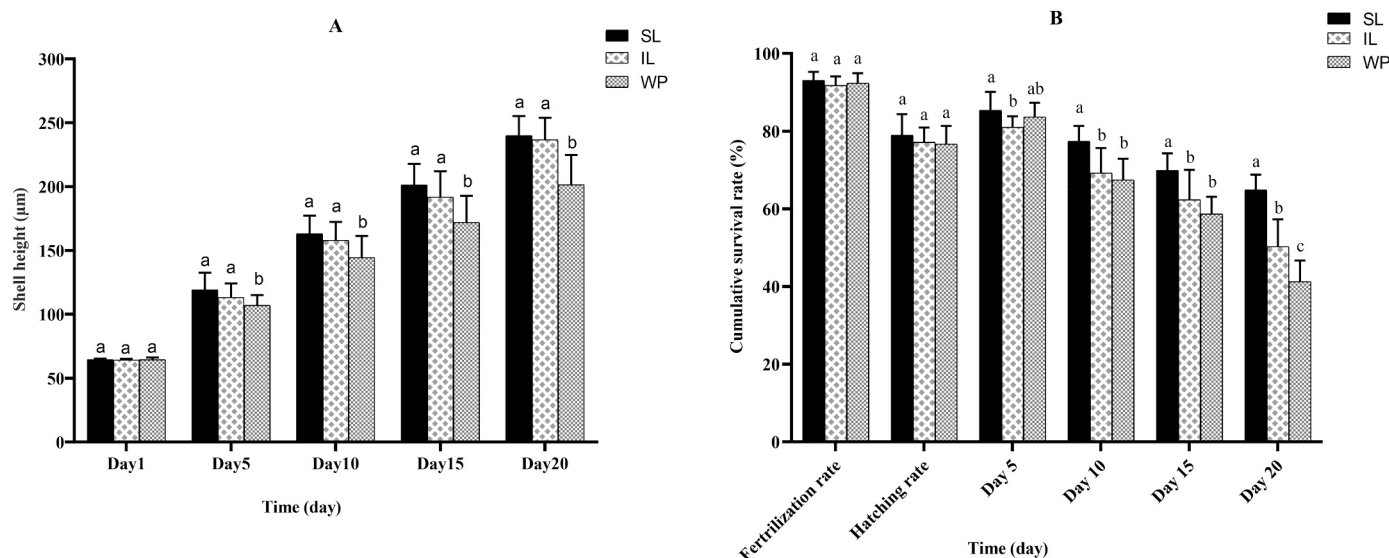


Fig. 3. Shell height (mm) (Fig. A) and survival traits (%) (Fig. B) for selected line, inbreeding line and wild population at larvae stage. Different superscript letters at the same time indicate significant difference ( $P < 0.05$ ).

survival ( $P < 0.05$ ), with the order of  $SL > IL > WP$ . From day 5 to 15, there was no significant difference between that of inbreeding line and wild population ( $P > 0.05$ ). On day 20, the inbreeding line was significantly higher than wild population ( $P < 0.05$ ). Notably, the survival rate of selected line was significantly higher than wild population throughout the larvae stage ( $P < 0.05$ ). Compared to the wild population, the survival of the selected line was increased by 2.04%–57.15% at larvae stage (Table 1).

### 3.2. Growth, survival and yield at grow-out stage

The selected and inbreeding lines exhibited excellent performance than wild population during whole grow-out stage ( $P < 0.05$ ) (Fig. 4). High mortality of *C. gigas* was observed during the summer months (day 110 and day 440), with few deaths in other months. Interestingly, the incremental survival rate of the three populations at day 110 (first summer) was higher than these populations at day 440 (second summer) (Fig. 4A). At harvest, the cumulative survival rate of wild population was only 32.99%, while the other two populations was higher than 50% (Fig. 4B). It is noteworthy that the cumulative survival rate of the selected line increased by 71.35% at harvest (440 days in the field) compared to the wild population (Table 2).

At all periods, the selected line grew faster than the other two populations followed the order of  $SL > IL > WP$  (Fig. 5). From day 110 to day 440, the shell height and individual weight of selected line were significantly higher than wild population ( $P < 0.05$ ), though not always significantly higher than inbreeding line. From day 220 to 440, shell height and individual weight were significantly higher ( $P < 0.05$ ) in the selected line than in the inbreeding line. The yield of selected line were significantly higher than that of inbreeding line and wild population at harvest ( $P < 0.05$ ) (Fig. 6). Notably, the yield of inbreeding line varied considerably between families, with yields ranging from 1788 g to 6773 g.

Table 1

The observed performance superiority of the selected line over the wild population of the *C. gigas* at larvae stage.

Traits	Day 1	Day 5	Day 10	Day 15	Day 20
Shell height (mm)	0.28	11.48	13.06	17.05	19.10
Survival rate (%)	–	2.04	14.78	19.11	57.15

### 3.3. Inbreeding depression

In this study, there were no significant inbreeding depression of fertilization and hatching rate ( $P > 0.05$ ). Notably, significant inbreeding depression for larvae survival was found from day 10 to day 20 ( $P < 0.05$ ), with the inbreeding depression ranged from –10.61% to –22.46%. And the estimated inbreeding depression coefficient per 10% increase of inbreeding coefficient of F ranged from –2.06% to –8.98% (Table 3). Additionally, inbreeding depression of survival tended to increase with age during the larval stage. Interestingly, no significant inbreeding depression of shell height was found throughout the larvae stage (–0.68% to –5.01%) ( $P > 0.05$ ).

The grow-out survival of the selected line was not significantly higher than that of the inbreeding line ( $P > 0.05$ ). The inbreeding depression for survival ranged from –0.83% to –5.04% at different grow-out stages, and the estimated inbreeding depression coefficient for survival ranged from –0.33% to –2.02% per 10% increase of inbreeding coefficient of F (Table 4). The estimated inbreeding depression coefficient of shell height and individual weight was from –0.64% to –5.52% and –1.54% to –5.91% per 10% increase of inbreeding coefficient of F, respectively. At day 110 and day 220, shell height and individual weight of the selected line was not significantly different from inbreeding line ( $P > 0.05$ ). However, at days 330 and 440, there were significant inbreeding depression ( $P < 0.05$ ) of shell height and individual weight in inbreeding line. Overall, inbreeding depression for growth traits tended to increase with age. Notably, the estimated inbreeding depression coefficient for yield at harvest was –8.37% per 10% increase of inbreeding coefficient of F.

## 4. Discussion

### 4.1. Effect of selective breeding on production traits

Like in most aquaculture genetic improvement programs, growth performance is the important trait in *C. gigas* selective breeding programs. In this study, regardless of larvae or grow-out stage, the selected line significantly grew faster than the wild population. Compared with wild population, the shell height of the selected line increased by 46.69% at harvest, showing that the effect of selective breeding of *C. gigas* was obvious. Moreover, the individual weight of the selected line was also higher than that of wild population at harvest, suggesting that individual weight got a positive correlation response when selection for

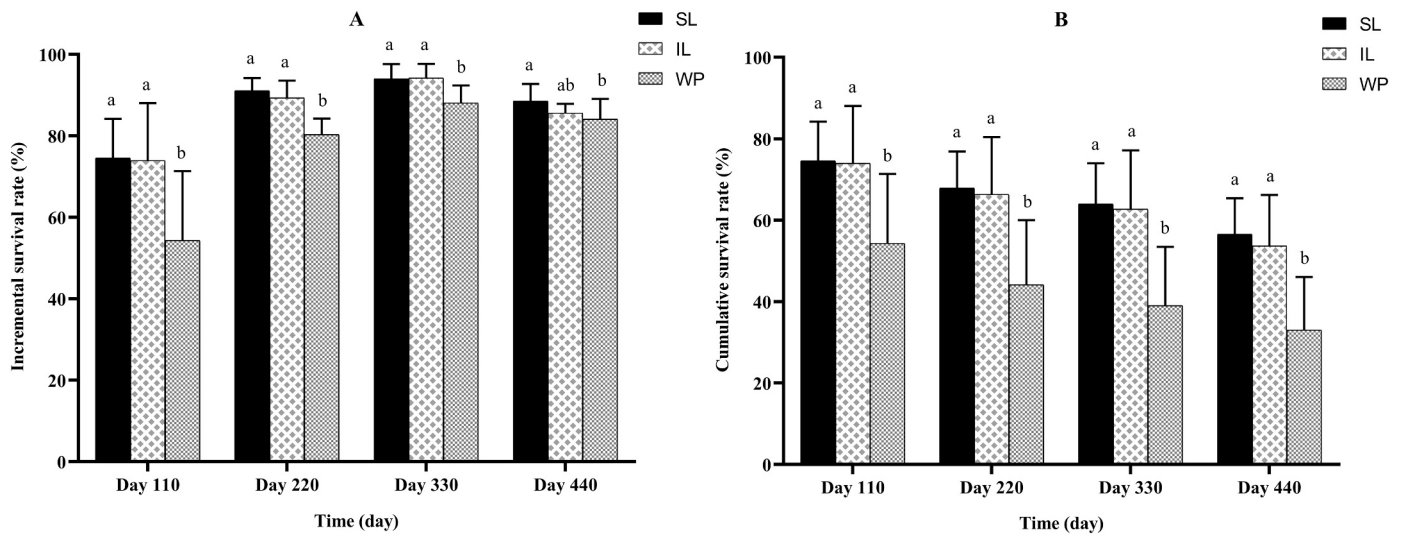


Fig. 4. Incremental survival rate (%) (A) and cumulative survival rate (%) (B) for selected line, inbreeding line and wild population at different grow-out stages. Different superscript letters at the same time indicate significant difference ( $P < 0.05$ ).

Table 2

The observed performance superiority of the selected line over the wild population of the *C. gigas* at different grow-out stages.

Traits	Day 110	Day 220	Day 330	Day 440
Shell height (mm)	31.24	39.55	36.29	46.69
Individual weight (g)	56.60	41.21	48.66	46.43
Survival rate (%)	37.32	53.85	63.87	71.35
Yield (g)	–	–	–	157.98

shell height was performed. Similarly, in European oyster *Ostrea edulis*, Toro and Newkirk (1990) found a high genetic correlation ( $> 0.90$ ) between body weight and shell height in a strain selected for body weight. Individual weight is a commercially important characteristic because oysters are graded by weight and those that weigh more achieve the best prices (O'Connor et al., 2019). The above results clearly showed that our selective breeding program has greatly improved the growth traits of oysters. Other researchers have also obtained promising results of improved growth traits in different oyster species. For instance, in Sydney rock oysters (*Saccostrea commercialis*), Nell et al. (1996, 1999) found that live weight increased by 4% and 18% in mass-selected oyster

after one and two generations of selection, respectively, compared to wild controls. Another study that used family selection to improve harvest whole weight in Portuguese oyster (*Crassostrea angulata*), achieved a significant direct response by 17.4% after three generations (Vu et al., 2020). In the mass selected Iwagaki oyster (*Crassostrea nippona*), 11.07%–12.46% genetic gain for body weight were observed after three successive generations of selection (Hu et al., 2022).

Previous studies have showed that it is possible to improve the survival rate in oyster through selective breeding (Lipovsky and Chew, 1972; Hershberger et al., 1984; Dégremont et al., 2010a; Dove et al., 2013). In our study, the survival rate of the selected line was significantly higher than wild population in both larvae and growth-out stage. The cumulative survival rate of the selected line increased by 71.35% at harvest compared to the wild population, showing that the genetic improvement of survival was successful. In this study, high oyster mortality occurred primarily in summer, suggesting that mass mortality in summer remains a major challenge for *C. gigas* in China. Therefore, farmers could use selected oysters that resistance to the summer mortality to increase survival rate and thus increase the output of oysters. In addition, the selected line had high incremental survival rate in the first and second summers, which suggested that the superior genetic-based

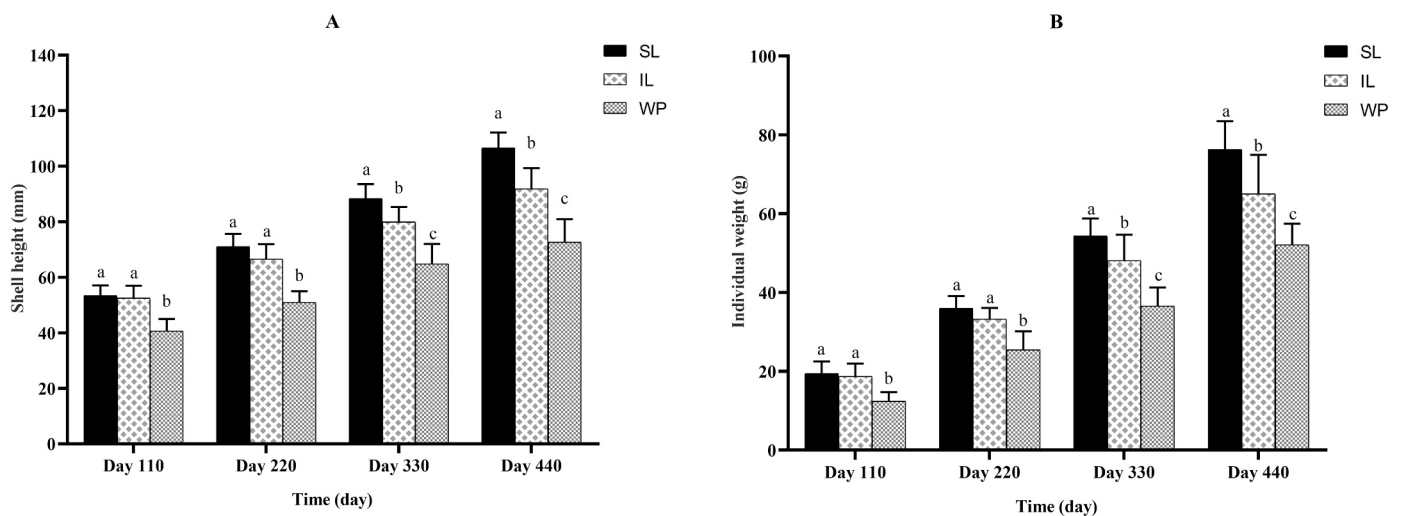


Fig. 5. Shell height (mm) (A) and individual weight (g) (B) for selected line, inbreeding line and wild population at different grow-out stages. Different superscript letters at the same time indicate significant difference ( $P < 0.05$ ).

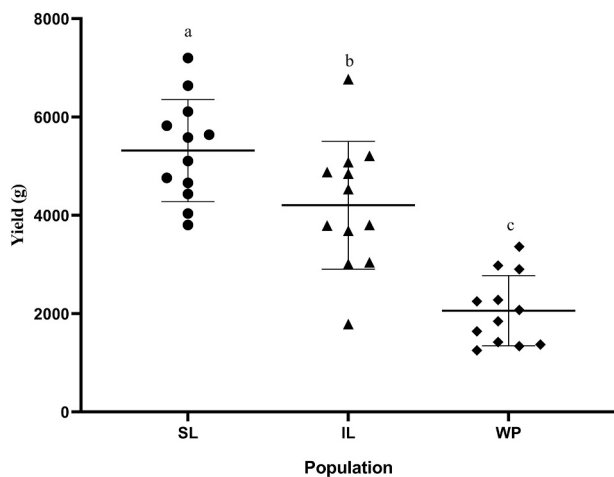


Fig. 6. Harvest yield (g) for selected line, inbreeding line and wild population. Different superscript letters indicate significant difference ( $P < 0.05$ ).

traits can survive into the second summer. It should be noted that the oyster mortality was higher in the first summer than in the second summer. For wild population, the low survival rate of oysters recorded in the first summer may lead to the elimination of sensitive oysters from this population. This result is in agreement with a study conducted in 2001–2003 in France, in which the surviving susceptible oysters of summer massive mortality outbreak had higher survival rate and performed as well as resistant oysters in the following summer (Dégremont et al., 2010b). Some studies have also reported that younger oysters are more susceptible to summer mortality than older oysters because of their higher metabolic demand (Maurer et al., 1986; Brown and Hartwick, 1988; Azéma et al., 2017; Hick et al., 2018).

Oyster farmers are most interested in yield, which is a composite characteristic determined by survival and growth (Blum, 1988). On the west coast of the USA, MBP using family selection to improve individual weight, which has obtained 10–20% gains per generation for yield in

different environments (Langdon et al., 2003). In France, Dégremont et al. (2010a) found that selection to improve summer survival would automatically increase yield of juvenile *C. gigas*. Our selective breeding program initially focused on shell height before incorporating selection for summer survival. Encouragingly, simultaneous improvements in both survival rate and growth traits has achieved in our selective breeding program. The cumulative survival rate of selected line (56.53%) were approximately two times of that of wild population (32.99%) at harvest. In addition, faster growth of the selected line throughout the whole grow-out stage was an important cause of yield differences. As expected, yield of the selected line increased by about 160% compared to wild population, which will be of great benefit to farmers. The great yield advantage of the selected line indicate great potential to oyster farming industry.

#### 4.2. Inbreeding depression for production traits

In this study, all populations exhibited high fertilization rates (> 91%), indicating that there was no sperm-egg recognition barrier for inbreeding line. Survival is considered a fitness trait and inbreeding depression typically affects these types of traits more than growth traits (Lynch and Walsh, 1998; Falconer and Mackay, 1996). Inbreeding may reduce population fitness by expressing recessive and deleterious alleles in homozygous form (Wang et al., 2002; Nakadate et al., 2003). In this study, inbreeding had a significant depression (−5.14% to −22.46%) on survival rate at larvae stage. The possible reason is that oyster carry a very high genetic load and mortality due to inbreeding depression lethal recessive genes mainly occurs in the early stage (Evans et al., 2004; Bierre et al., 1998). Similarly, Longwell and Stiles (1973) reported that inbred line ( $F = 0.25$ ) had significantly inbreeding depression on larval survival in *Crassostrea virginica*. Interestingly, inbreeding had significant effects on survival at larvae stag, but had no significant effects on grow-out survival. It is consistent with those observed by Evans et al. (2004) who found the low depression in survival of inbred families during two growing seasons. A possible explanation for low inbreeding depression in grow-out survival in this study was that selection for summer survival masking the true magnitude of inbreeding depression. If most

Table 3  
Inbreeding depression on growth and survival of selected *C. gigas* at larvae stage.

Traits	Day 1		Day 5		Day 10		Day 15		Day 20	
	Inbreeding depression	Inbreeding depression (per 10% increase in F)	Inbreeding depression	Inbreeding depression (per 10% increase in F)	Inbreeding depression	Inbreeding depression (per 10% increase in F)	Inbreeding depression	Inbreeding depression (per 10% increase in F)	Inbreeding depression	Inbreeding depression (per 10% increase in F)
Shell height (μm)	−0.68	−0.27	−5.01	−2.01	−3.21	−1.28	−4.64	−1.86	−1.36	−0.54
Survival (%)	–	–	−5.14	−2.06	−10.61	−4.24	−10.79	−4.32	−22.46	−8.98

Table 4  
Inbreeding depression on growth, survival and yield of selected *C. gigas* at different grow-out stages.

Traits	Day 110		Day 220		Day 330		Day 440	
	Inbreeding depression	Inbreeding depression (per 10% increase in F)	Inbreeding depression	Inbreeding depression (per 10% increase in F)	Inbreeding depression	Inbreeding depression (per 10% increase in F)	Inbreeding depression	Inbreeding depression (per 10% increase in F)
Shell height (mm)	−1.61	−0.64	−6.36	−2.54	−9.55	−3.82	−13.82	−5.52
Individual weight (g)	−3.86	−1.54	−7.50	−3.00	−11.48	−4.59	−14.79	−5.91
Survival (%)	−0.83	−0.33	−2.25	−0.90	−1.95	−0.78	−5.04	−2.02
Yield (g)	–	–	–	–	–	–	−20.91	−8.37

inbreeding depression is due to deleterious recessive alleles, it is possible that direct selection can counteract inbreeding depression by purging deleterious alleles from inbreeding populations (Su et al., 1996; Swindell and Bouzat, 2006). In a similar situation, Hörstgen-Schwark (1990) found that no different growth performance between inbred line and selected line when selected for individual weight of rainbow trout. Furthermore, one hypothesis to explain this phenomenon is that when oysters from inbreeding line cultured in the field, oysters with inbreeding depression lethal recessive alleles may have already died at the larvae stage. Therefore, further studies are needed to confirm whether purging reduces or eliminates these effects of survival in *C. gigas*.

Many studies have found that inbreeding had significant effects in growth traits of *C. gigas* (Beattie et al., 1987; Hedgecock et al., 1995; Langdon et al., 2003; Evans et al., 2004), which is consistent with significant nonadditive gene action determining performance characters in *C. gigas*. In this study, the sensitivity of individual weight in *C. gigas* to inbreeding at harvest (5.91% depression for a 10% increase in F) was similar to those observed by Evans et al. (2004) who found an average phenotypic decrease of 8.8% per 10% increase in F. It is well documented that the effect of inbreeding on growth traits increases with age (Gjerde et al., 1983; Kincaid, 1983; Su et al., 1996). Similarly, inbreeding depression coefficient (per 10% increase in F) in growth traits showed a clear trend of increasing with age between different grow-out stages in this study. This can be attributed to the fact that cubic nature of the growth curve amplifying differences in growth rate over time (Kincaid, 1983). This is the first report to quantify statistically inbreeding depression of different grow stages in *C. gigas*, which is important for selecting breeding strategies to implement effective genetic improvement programs.

The genetic complexity of yield has been widely reported (Baker, 1987; Ceccarelli et al., 1991). In this study, a 10% increase in F will result in an 8.37% reduction in yield. Our results were slightly lower than those observed by Evans et al. (2004) who found that a 12.2% depression in harvest yield (570 days in field) for a 10% increase in F in adult *C. gigas* families. Since the inbreeding depression coefficients for growth traits showed a clear tendency to increase with age at different growth-out stages, the relatively short time (440 days) that the oysters spent in the field in this study may have resulted in slightly lower inbreeding depression. The effects of inbreeding depression on growth and survival rate had a cumulative effect on depression of yield (Evans et al., 2004). In the present study, there were significant difference ( $P < 0.05$ ) in individual weight between the selected and inbreeding lines of *C. gigas* during growth-out stage, which contributed significantly inbreeding depression in yield. Therefore, selective breeding programs should carefully manage inbreeding regardless of the trait selected. Our results highlight the need to manage inbreeding in conjunction with genetic improvement, as inbreeding in oyster can lead to yield reduction through inbreeding depression.

#### 4.3. Application prospect of inbreeding populations for further breeding

Inbreeding depression and heterosis have been studied in detail for dozens of decades in plants and mammals. However, such studies have not been conducted enough for aquaculture species (Nakadate et al., 2003). Heterosis is complementary to the phenomenon of inbreeding depression and is thought to be an effect of it (Falconer and Mackay, 1996). Strong non-additive (heterosis) for growth and survival have been reported for crosses among inbred lines of *C. gigas*, and the heterosis for yield is a common phenomenon (Beattie et al., 1987; Hedgecock et al., 1995; Bayne et al., 1999; Hedgecock and Davis, 2007). In this study, high phenotypic variation for yield was observed among inbreeding families, suggesting that there may be potential for exploiting inbreeding and heterosis for line development in the future breeding program. Improvement of commercial oyster seed can be achieved through a combination of selection among inbred families and

hybridization of excellent inbred families (Hedgecock and Davis, 2007).

## 5. Conclusion

In summary, the growth, survival and yield of selected, inbreeding and wild populations were systematically assessed. The selected line exhibited huge superiority of performance traits in both larval and grow-out stages. Notably, the yield of selected line increased by 157.98% compared with wild population at harvest, which has important application value for commercial oyster cultivation. In this study, inbreeding significant depressed growth traits and yield in selected line of *C. gigas* during different grow-out stages. Interestingly, grow-out survival was not affected by inbreeding, but inbreeding depression had occurred before the metamorphosis stage. Therefore, the effects of inbreeding must be considered in the design of *C. gigas* breeding programs to limit inbreeding depression of important production traits.

## Author statement

**Yong Chi:** Completion of the experiment, data analysis, and manuscript drafting. **Qi Li:** Experimental design and coordination and manuscript revision. **Chengxun Xu:** Data analysis.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

The authors do not have permission to share data.

## Acknowledgments

This work was supported by the grants from the Agriculture Research System of China Project (CARS-49), and the Earmarked Fund for Agriculture Seed Improvement Project of Shandong Province (2021LZGC027, 2020LZGC016).

## References

- Azéma, P., Maurouard, E., Lamy, J.B., Dégremont, L., 2017. The use of size and growing height to improve *Crassostrea gigas* farming and breeding techniques against OsHV-1. *Aquaculture* 471, 121–129.
- Baker, R.J., 1987. Differential response to environmental stress. In: Weir, B.S., Goodman, M.M., Eisen, E.J., Namkoong, G. (Eds.), *Proceedings of the 2nd International Conference on Quantitative Genetics*. Sinauer Associates, Sunderland, MA, USA, pp. 492–504.
- Bayne, B.L., Hedgecock, D., McGoldrick, D., Rees, R., 1999. Feeding behavior and metabolic efficiency contribute to growth heterosis in Pacific oysters [*Crassostrea gigas* (Thunberg)]. *J. Exp. Mar. Biol. Ecol.* 233, 115–130.
- Beattie, J.H., Perdue, J., Hershberger, W., Chew, K., 1987. Effects of inbreeding on growth in the Pacific oyster (*Crassostrea gigas*). *J. Shellfish Res.* 6, 25–28.
- Bierne, N., Launey, S., Naciri-Graven, Y., Bonhomme, F., 1998. Early effect of inbreeding as revealed by microsatellite analysis on *Ostrea edulis* larvae. *Genetics* 148, 1893–1906.
- Blum, A., 1988. *Plant Breeding for Stress Environments*. CRC Press, Boca Raton, FL, USA, p. 223.
- Brown, J.R., Hartwick, E.B., 1988. Influences of temperature, salinity and available food upon suspended culture of the Pacific oyster, *Crassostrea gigas*. 2. Condition index and survival. *Aquaculture* 70, 253–267.
- Ceccarelli, S., Acevedo, E., Grando, S., 1991. Breeding for yield stability in unpredictable environments: single trait, interaction between traits, and architecture of genotypes. *Euphytica* 56, 169–185.
- Chi, Y., Li, Q., Liu, S.K., Kong, L.F., 2021. Genetic parameters of growth and survival in the Pacific oyster *Crassostrea gigas*. *Aquac. Res.* 52, 282–290.
- Chi, Y., Jiang, G.W., Liang, Y.X., Xu, C.X., Li, Q., 2022. Selective breeding for summer survival in Pacific oyster (*Crassostrea gigas*): genetic parameters and response to selection. *Aquaculture* 556, 738271.
- De Melo, C.M.R., Durland, E., Langdon, C., 2016. Improvements in desirable traits of the Pacific oyster, *Crassostrea gigas*, as a result of five generations of selection on the west coast, USA. *Aquaculture* 460, 105–115.

- Dégremont, L., Bédier, E., Boudry, P., 2010a. Summer mortality of hatchery-produced Pacific oyster spat (*Crassostrea gigas*). II. Response to selection for survival and its influence on growth and yield. *Aquaculture* 299, 21–29.
- Dégremont, L., Boudry, P., Ropert, M., Samain, J.-F., Bédier, E., Soletchnik, P., 2010b. Effects of age and environment on survival of summer mortality by two selected groups of the Pacific oyster *Crassostrea gigas*. *Aquaculture* 299, 44–50.
- Dégremont, L., Nourry, M., Maurouard, E., 2015. Mass selection for survival and resistance to OsHV-1 infection in *Crassostrea gigas* spat in field conditions: response to selection after four generations. *Aquaculture* 446, 111–121.
- Dove, M.C., Nell, J.A., O'Connor, W.A., 2013. Evaluation of the progeny of the fourth generation Sydney rock oyster *Saccostrea glomerata* (Gould, 1850) breeding lines for resistance to QX disease (*Marteilia sydneyi*) and winter mortality (*Bonamia roughleyi*). *Aquac. Res.* 44, 1791–1800.
- Evans, F., Matson, S., Brake, J., Langdon, C., 2004. The effects of inbreeding on performance traits of adult Pacific oysters (*Crassostrea gigas*). *Aquaculture* 230, 89–98.
- Falconer, D.S., Mackay, T.F., 1996. *Introduction to Quantitative Genetics*, 4th edn. Pearson Education Limited, Essex, UK.
- Fang, J.F., Han, Z.Q., Li, Q., 2021. Effect of inbreeding on performance and genetic parameters of growth and survival traits in the Pacific oyster *Crassostrea gigas* at larval stage. *Aquaculture Reports* 19, 100590.
- Gjedrem, T., Rye, M., 2018. Selection response in fish and shellfish: a review. *Rev. Aquac.* 10, 168–179.
- Gjerde, B., Gunnes, K., Gjedrem, T., 1983. Effect of inbreeding on survival and growth in rainbow trout. *Aquaculture* 34, 327–332.
- Hedgecock, D., Davis, J.P., 2007. Heterosis for yield and crossbreeding of the Pacific oyster *Crassostrea gigas*. *Aquaculture* 272, S17–S29.
- Hedgecock, D., McGoldrick, D.J., Bayne, B.L., 1995. Hybrid vigor in Pacific oysters: an experimental approach using crosses among inbred lines. *Aquaculture* 137, 285–298.
- Hershberger, W.K., Perdue, J.A., Beattie, J.H., 1984. Genetic selection and systematic breeding in Pacific oyster culture. *Aquaculture* 39, 237–245.
- Hick, P.M., Evans, O., Rubio, A., Dhand, N.K., Whittington, R.J., 2018. Both age and size influence susceptibility of Pacific oysters (*Crassostrea gigas*) to disease caused by Ostreid herpesvirus-1 (OsHV-1) in replicated field and laboratory experiments. *Aquaculture* 489, 110–120.
- Hörstgen-Schwark, G., 1990. Prospect of producing inbred lines for consolidation of growth performance. In: *Proceedings of the 4th World Congress on Genetics Applied to Livestock Production*, XVI, pp. 163–166.
- Hu, Y., Li, Q., Xu, C., Liu, S., Kong, L., Yu, H., 2022. Response to selection for growth in successive mass selected generations of Iwagaki oyster *Crassostrea nippona*. *Aquaculture* 560, 738575.
- Keys, S.J., Crocos, P.J., Burrige, C.Y., Coman, G.J., Gerard, D.P., Preston, P.P., 2004. Comparative growth and survival of inbred and outbred *Penaeus (marsupenaeus) japonicus*, reared under controlled environment conditions: indications of inbreeding depression. *Aquaculture* 241, 151–168.
- Kincaid, H., 1983. Inbreeding in fish populations used for aquaculture. *Aquaculture* 33, 215–227.
- Kube, P., Dove, M., Cunningham, M., Kirkland, P., Gu, X., Hick, P., O'Connor, W., Elliott, N., 2018. Genetic selection for resistance to Pacific oyster mortality syndrome. In: *CSIRO Marine and Atmospheric Research, FRDC and Seafood CRC Project 2012/760*, p. 55. Hobart, Australia.
- Langdon, C., Evans, F., Jacobson, D., Blouin, M., 2003. Yields of cultured Pacific oysters *Crassostrea gigas* Thunberg improved after one generation of selection. *Aquaculture* 220, 227–244.
- Launey, S., Hedgecock, D., 2001. High genetic load in the Pacific oyster *Crassostrea gigas*. *Genetics* 159, 255–265.
- Li, J., Wang, M., Fang, J., Liu, X., Xue, S., Mao, Y., Liu, G., 2018. A comparison of offspring growth and survival among a wild and a selected strain of the Pacific abalone (*Haliotis discus hannai*) and their hybrids. *Aquaculture* 495, 721–725.
- Li, Q., Wang, Q., Liu, S., Kong, L., 2011. Selection response and realized heritability for growth in three stocks of the Pacific oyster *Crassostrea gigas*. *Fish. Sci.* 77, 643–648.
- Lipovsky, V.P., Chew, K., 1972. Mortality of Pacific oysters (*Crassostrea gigas*): the influence of temperature and enriched seawater on survival. *Proc. Natl. Shellfish. Assoc.* 62, 72–82.
- Longwell, A.C., Stiles, S., 1973. Gamete cross incompatibility and inbreeding in the commercial American oyster, *Crassostrea virginica* Gmelin. *Cytologia* 38, 521–533.
- Lynch, M., Walsh, B., 1998. *Genetics and Analysis of Quantitative Traits* Sinauer Associates, Massachusetts, USA, p. 980.
- Mallet, A.L., Haley, L.E., 1983. Effects on inbreeding on larval and spat performance in the American oyster. *Aquaculture* 33, 229–235.
- Maurer, D., Comps, M., His, E., 1986. Caractéristiques des mortalités estivales de l'huître *Crassostrea gigas* dans le bassin d'Arcachon. *Haliotis* 15, 309–317.
- Nakadate, M., Shikano, T., Taniguchi, N., 2003. Inbreeding depression and heterosis in various quantitative traits of the guppy, *Poecilia reticulata*. *Aquaculture* 220, 219–226.
- Nell, J.A., Sheridan, A.K., Smith, I.R., 1996. Progress in a Sydney rock oyster, *Saccostrea commercialis* (Iredale and Roughley), breeding program. *Aquaculture* 144, 295–302.
- Nell, J.A., Smith, I.R., Sheridan, A.K., 1999. Third generation evaluation of Sydney rock oyster *Saccostrea commercialis* (Iredale and Roughley) breeding lines. *Aquaculture* 170, 195–203.
- O'Connor, W., Dove, M., O'Connor, S., In, V.V., Lien, V.T.N., Van, P.T., 2019. *Enhancing Bivalve Production in Northern Vietnam and Australia. Final Report. Australian Centre for International Agricultural Research FIS/2010/100.*
- Pante, M.J.R., Gjerde, B., McMillan, I., 2001. Inbreeding levels in selected populations of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 192, 213–224.
- Qin, Y.P., Li, X.Y., Noor, Z., Li, J., Zhou, Z.H., Ma, H.T., Yu, Z.N., 2020. A comparative analysis of the growth, survival and reproduction of *Crassostrea hongkongensis*, *Crassostrea ariakensis*, and their diploid and triploid hybrids. *Aquaculture* 520, 734946.
- Rawson, P., Feindel, S., 2012. Growth and survival for genetically improved lines of eastern oysters (*Crassostrea virginica*) and interline hybrids in Maine, USA. *Aquaculture* 25, 61–67.
- Ruesink, J.L., Lenihan, H.S., Trimble, A.C., Heiman, K.W., Micheli, F., Byers, J.E., Kay, M.C., 2005. Introduction of non-native oysters: ecosystem effects and restoration implications. *Annu. Rev. Ecol. Syst.* 36, 643–689.
- Su, G.S., Liljedahl, L.E., Gall, G., 1996. Effects of inbreeding on growth and reproductive traits in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 142, 139–148.
- Swindell, W.R., Bouzat, J.L., 2006. Selection and inbreeding depression: effects of inbreeding rate and inbreeding environment. *Evolution* 60, 1014–1022.
- Toro, J.E., Newkirk, G.F., 1990. Divergent selection for growth rate in the European oyster *Ostrea edulis*: response to selection and estimation of genetic parameters. *Mar. Ecol. Prog. Ser.* 62, 219–227.
- Vu, S.V., Knibb, W., Nguyen, N.T., Vu, I.V., O'Connor, W., Dove, M., Nguyen, N.H., 2020. First breeding program of the Portuguese oyster *Crassostrea angulata* demonstrated significant selection response in traits of economic importance. *Aquaculture* 518, 734664.
- Wang, S.Z., Hard, J.J., Utter, F., 2002. Salmonid inbreeding: a review. *Rev. Fish Biol.* 11, 301–319.
- Xu, H.Q., Li, Q., Han, Z.Q., Liu, S.K., Yu, H., Kong, L.F., 2019. Fertilization, survival and growth of reciprocal crosses between two oysters, *Crassostrea gigas* and *Crassostrea nippona*. *Aquaculture* 507, 91–96.
- Zhang, J.X., Li, Q., Xu, C.X., Han, Z.Q., 2019. Response to selection for growth in three selected strains of the Pacific oyster *Crassostrea gigas*. *Aquaculture* 503, 34–39.