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Effects of inbreeding on fertilization, growth and survival in the tetraploid Pacific oyster *Crassostrea gigas*

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Abstract
Induced homologous tetraploid oysters are typically derived from a limited number of effective parents, with subsequent breeding steps likely to cause inbreeding, which may cause their own production performance to decline. To explore the performance of inbreeding, we established outbred (F0) and inbred lines (F1 and F2) in tetraploid Pacific oyster. Cleavage rate, shell height, survival rate, and total weight were compared. Inbreeding coefficients in F0, F1, and F2 were 0, 0.25, and 0.375, respectively. The results showed that there was no significant difference in cleavage rate between outbred line and inbred lines. The F1 showed distinct growth and survival depression only at larval stage, but no depression at grow-out stage. F1 growth and survival IDC values at larval stage were − 1.29% to 0.03% and − 0.31% to 0.01%, respectively. However, depressed growth and survival rate appeared in almost all F2 growth cycle; F2 growth and survival IDC values were − 1.10% to − 0.78% and − 3.14% to − 4.51% in larval stage and − 0.48% to − 0.25% and − 0.79% to − 0.64% in grow-out stage, respectively. Growth and survival rate at Longkou and Qingdao sites showed no depression in the F1 line during grow-out stage. The results indicate that inbreeding depressed growth and in particular survival in tetraploid oysters. With increased inbreeding levels, depressed growth and survival became more distinct. We provided important insights on the inbred lines construction and further breeding of tetraploid oysters.

Keywords Tetraploid *Crassostrea gigas* · Larval and grow-out stages · Growth · Survival · Inbreeding depression

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Introduction

The economic output of farming aquatic animals may be improved by artificial breeding. Selective-, cross-, and polyploid-breeding are commonly used approaches which improve animal genetics (Bartley et al. 2000; Janssen et al. 2017; Zhang et al. 2022). To genetically improve fish and shellfish, selective- and cross-breeding approaches have been predominantly used and exerted the most significant effects. In contrast, polyploid-breeding is infrequently used in the large-scale breeding of the aquatic animals (Piferrer et al. 2009), but is used for some important plants (Chen et al. 2021). In some aquatic fish, polyploid-breeding is more advanced; hybrid- and polyploid-breeding approaches have been used to generate several new and stable genetic polyploid fish lines (Liu 2010; Otterå et al. 2016). However, while polyploid breeding in most bivalve species remains at trial stages, triploid breeding in oysters has been successfully commercialized in many countries (Callam et al. 2016; Zhang et al. 2022).

The main purpose of polyploid breeding in oysters is to improve economic performance (growth, survival, and meat quality) via the triploidization of diploid oysters, and to make up for a deficiency of commercial oysters during the breeding season (Allen and Downing 1986). Triploid oysters were initially generated by diploid induction (Stanley et al. 1981), but this approach was complicated as it generated unstable triploid yields, which were inadequate for commercial production. Triploids may also be produced by crossing diploid and tetraploid oysters, and may be the best choice for large-scale production as the method is simple and provides stable triploid rates (Guo et al. 1996). Therefore, tetraploid oysters are essential prerequisites for the commercial production of triploid oysters. Tetraploid oysters can be generated by diploid (2n×2n) or triploid (3n ♀×2n♂) induction, but the approach is complicated and unstable, with high mortality rates in offspring (Benabdelmouna and Ledu 2015; Eudeline et al. 2000a). Consequently, such unstable tetraploid acquisition methods cannot ensure stable and continuous triploid production. However, previous studies have reported that induced autotetraploid oysters are fertile, produce mature gametes, and reproduce offspring, which allows for the propagation/further selection of tetraploids via self-reproduction (Guo and Allen 1997; Qin et al. 2022; Zhang et al. 2022).

Animal inbreeding occurs in both wild and cultured populations. In natural, environmental change or other factors can separate large populations into many smaller populations where inbreeding frequency increases due to a reduction in population size (Keller and Waller 2002). During artificial selection, inbreeding is more likely to occur due to the limited size of a basal population. The main method of generating tetraploid oysters is artificial induction, and to ensure high tetraploid rates, few female parents as possible should be used to improve synchronicity in egg development (Eudeline et al. 2000a, b; Gérard et al. 1999; Qin et al. 2018). Therefore, artificially induced populations are often derived from a limited number of parents, which means that inbreeding frequency may be greatly increased during continuous propagation or breeding within a population. Consequently, it is important to understand inbreeding effects during tetraploid oyster breeding.

Studies examining inbreeding in fish, crustaceans, and shellfish have reported that inbreeding mainly effects quantitative traits in populations, including growth, survival, stress resistance, and fecundity (Fang et al. 2021; Fessehaye et al. 2007; Luo et al. 2014). These quantitative trait affects are often negative and are referred to as inbreeding depression, which occurs in all kinds of organisms and is an important driving force in the evolution of mating strategies (Barrett 2003). However, in farming or breeding aquatic animals, inbreeding depression is extremely harmful. In *Crassostrea gigas* and *C. virginica*,

inbreeding depression showed obvious harm, and is manifested by slow growth, low survival rates, and weak stress resistance (Evans et al. 2004; Fang et al. 2021, 2022; Mallet and Haley 1983). Importantly, while these studies have focused on diploid oysters, inbreeding and its effects remain under-reported in triploid and tetraploid oysters. Tetraploids are the core germplasm of triploid oyster production. Exploring inbreeding effects in tetraploid oysters may facilitate targeted propagation and improved breeding strategies. In this study, important basic phenotypic traits in oysters, such as growth and survival, were used to compare inbred (F = 0.25 and 0.375) and outbred (F = 0) tetraploid *C. gigas* families.

Materials and methods

Materials

In 2019, tetraploid induced population A was established using a method described by Eudeline et al. (2000b) (male and female parents numbered > 30 and were randomly obtained from a farming population). In 2020, full-sib family B was established by randomly crossing one male and one female tetraploid from the induced population A. In 2021, inbred line F1* was established from full-sib family B through the full-sib mating, and tetraploid induced population C was established using the same induced method (Fig. 1). In 2022, 14 full-sib families (inbred line F1) were established from full-sib family B by full-sib mating. Twelve full-sib families (inbred line F2) were established from inbred line F1* by full-sib mating. Fifteen full-sib families (outbred line F0) were established from induced population C. Outbred line F0 was established using induced population C as the parent by full-sib mating. Initial numbers in full-sib F0, F1, and F2 families were 15, 14, and 12, respectively. All parents were identified as tetraploid by flow cytometry before family establishment. Inbreeding coefficient (F) values for F0, F1, and F2 lines were 0, 0.25, and 0.375, respectively.

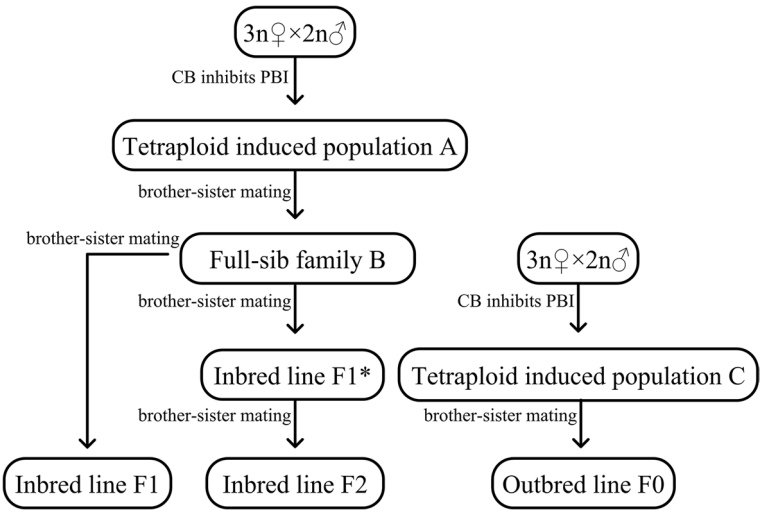

Fig. 1 Genetic background of the tetraploids used in the experiment

Family establishment

In early February 2022, oysters used to construct families were transferred from the sea to 20 m^3 tanks in hatchery for cultivation. The water temperature was maintained at 8 °C for 10 days, and then increased at 0.5 °C/day to a constant temperature of 20 °C, at 20 °C for 25 days. *Chaetoceros calcitrans* (100,000–120,000 cells/mL/day) was mainly fed to oysters four times/day (25,000–30,000 cells/mL/time). Once a day, the water (100%) was changed.

In early April 2022, parental gonads were mature and full-sib families established by artificial fertilization. Before fertilization, pure tetraploids were identified by flow cytometry. Oysters were opened, males and females identified by microscopy, and sperm and eggs from individuals collected and placed into 7 L plastic buckets. Eggs were filtered through a 48 μm nylon mesh and then washed three times through a 25 μm nylon mesh. Treated gametes were fertilized using one male to one female and we ensured that no uncontrolled spontaneous fertilization occurred before gamete mixing. Fertilized eggs were incubated in the filtered seawater at 23–24 °C and 26 ppt. D-larvae from different families were obtained after 24 h.

D-larvae were transferred to 60 L plastic buckets at a density of 4 individuals/mL, cultured in seawater (23–24 °C and 26 ppt), and fed *Isochrysis galbana* (4000–6000 cells/mL/day) three times/day before day 7. After seven days of incubation, the feeding was gradually increased to 2–4 times, and the mixture of *I. galbana* and *Chlorella vulgaris* was fed in a ratio of 1:1. Once a day, 30%–50% of the water was changed. About 18–25 days after fertilization, when 30% of larvae showed eyespots, a collector (scallop shell) was placed into buckets for 15 days. After attachment, collectors plus juveniles were transferred to an outdoor nursery, and at 90 days after gamete mixing, juveniles were transferred to mesh bags, families evenly divided into two groups, and transferred to Longkou (37.7°N, 120.3°E) and Qingdao (35.9°N, 120.3°E) sea areas, respectively (Fig. 2). On day 90 after fertilization, 300 individuals from each family were randomly selected to be transferred to the sea areas for data measurement during the grow-out stage.

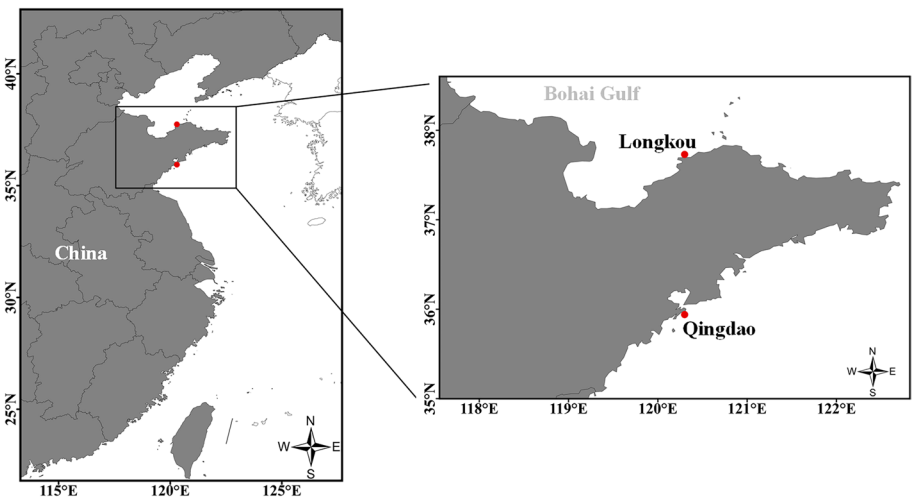

Fig. 2 The two culturing marine areas (Longkou and Qingdao) in this study

Measurements

For Cleavage rate, 50 mL of larvae were randomly collected from 60 L containers and counted under a microscope at 4 h after gamete mixing. Cleavage rate refers to the percentage of eggs that had cleaved in the total number of eggs. Shell height and survival rate were measured on days 5, 14, 22, 150, 240, and 360 after fertilization. We randomly collected 30 larvae or adult oysters from each family to measure shell height. Larval survival referred to the percentage of the number of larvae at each time point to the number of larvae at day 1, and survival rate of adult oysters was the percentage of the number of oysters at each time point to the number of juveniles at day 90. Thirty oysters from each family were randomly selected to measure total weight on day 360.

Statistical analyses

SPSS Ver. 23 was used for statistical analyses and Excel 2010 used for data calculations. Cleavage rate, shell height, survival rate, and whole weight mean values for F0, F1, and F2 lines were analyzed using one-way analysis of variance (ANOVA) and Tukey's multiple comparisons. Inbreeding generation and site influences on phenotype were analyzed using two-way ANOVA. Significance was accepted at 0.05 and extreme significance at 0.01.

Some families were not included in statistical analyses due to low larvae numbers or total larvae death. Only ten, eight, and seven families in F0, F1, and F2 lines were finally used for larval measurements. After metamorphosis, only seven, five, and five families in F0, F1, and F2 lines had > 600 spats, which were used for measurements at grow-out stage.

Inbreeding coefficients were calculated according to the following formula (Luo et al. 2014):

$$F_x = \sum \left[\left(\frac{1}{2} \right)^{n_1+n_2+1} \left(1 + F_A \right) \right]$$

n_1 represents the generation interval between the common ancestor and the male parent of the individual, n_2 represents the generation interval between the common ancestor and the female parent of the individual, and F_A represents the inbreeding coefficient of the common ancestor.

To determine the degree of inbreeding depression in different inbreeding lines, an inbreeding depression coefficient (IDC) was introduced and calculated according to the formula described by Luo et al. (2014):

$$IDC(\%) = \frac{\left(1 - \frac{\overline{W}_{inbred}}{\overline{W}} \right)}{\left(F - F_{inbred} \right)} \times 100\%$$

where $\overline{W}$ and $\overline{W}_{inbred}$ represent various traits and F and F_{inbred} represent the inbreeding coefficient for outbred (F0) and inbred (F1,F2) lines, respectively.

To determine site (Longkou and Qingdao) and generation (F0, F1, and F2) effects and their interactions on growth, survival rate, and whole weight, the following two-factor analysis model was introduced:

$$W_{SG} = M + S + G_b + \left(S_a \times G_b \right) + \delta_{ab}$$

where W_{SG} = mean of a trait for site S, generation G, M = global mean for a given trait, S_a = site effects (a = 1 or 2 for Longkou and Qingdao), G_b = generation (b = 1, 2, or 3 for F0, F1, or F2, respectively), S_a x G_b = interaction between site and generation and δ_{ab} = error.

Results

Larval stage growth and survival

Cleavage rates in fifteen F0 families ranged from 63.22%–96.46% (average = 82.77%), and it showed no significant difference when compared with F1 families (73.14%) and F2 families (75.12%) (Fig. 3A). On day 5, larval average shell heights in F0, F1, and F2 were no significant differences recorded (Fig. 3B). Survival rate in the F0 (85.89%) was not significantly different from the F1(86.52%), but was significantly higher when compared with the F2 (60.62%). Only the F2 showed survival inbreeding depression; the survival IDC value was − 0.78% (Fig. 3C).

On day 14, larval shell height in three groups showed no significant difference (Fig. 3B). When compared with day 5, mean survival rate of F1 was decreased by 30.54%, and survival rates in the seven F1 families varied from 30.97%–74.07%. On day 14, survival rate (76.37%) of F0 was significantly higher when compared with F1 (55.98%) and F2 (48.67%) (Fig. 3C). Both F1 and F2 showed distinct inbreeding depression with respect to survival rates, with corresponding survival IDC values of − 1.07% and − 0.97%, respectively (Figs. 4 and 5).

On day 22, larvae from F0 (293.46 μm) were significantly larger than F2 (269.62 μm), and corresponding IDC values were higher at − 0.23% (Figs. 3B and 5). Variation trend of larval survival rate was: The highest survival rate occurred in the F0 (67.46%), which was significantly higher when compared with F1 (45.74%) and F2(39.72%) (Fig. 3C). F1 and F2 larval survival rates also showed distinct inbreeding depression, with survival IDC values − 1.29% and − 1.10%, respectively (Fig. 5).

Growth in grow-out stage

Shell height in oysters from Qingdao was lower when compared with Longkou, but trend differences in the three lines at both sites were almost the same: F0 shell height was higher when compared withF1 and F2 at most time cut-off points (Fig. 4). This phenotypic

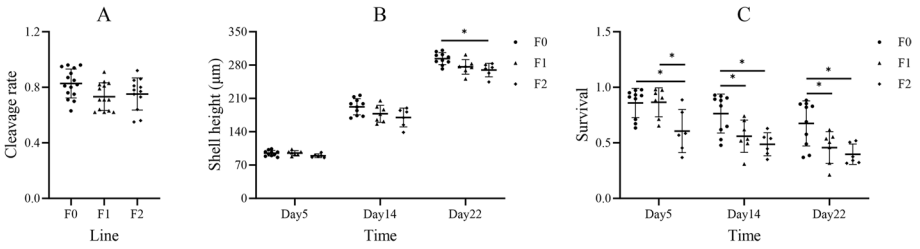

Fig. 3 Cleavage rate (**A**), shell height (**B**), and survival (**C**) values in the F0 (F = 0) outbred line and F1 (F = 0.25) and F2 (F = 0.375) inbred lines of tetraploid *C. gigas*. The asterisk and the horizontal line under it indicate a significant difference between the two groups ($P < 0.05$)

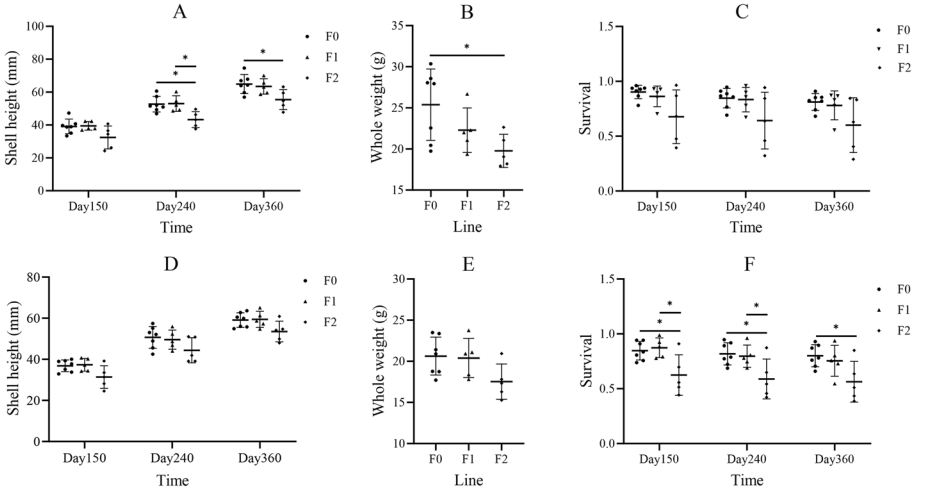

Fig. 4 Shell height, survival, and whole weight values in the F0 (F = 0) outbred line and F1 (F = 0.25) and F2 (F = 0.375) inbred lines of tetraploid *C. gigas* at grow-out stage in Longkou (**A**, **B**, and **C**) and Qingdao (**D**, **E**, and **F**). B and E refer to the whole weight of oysters at day 360. The asterisk and the horizontal line under it indicate a significant difference between the two groups ($P < 0.05$)

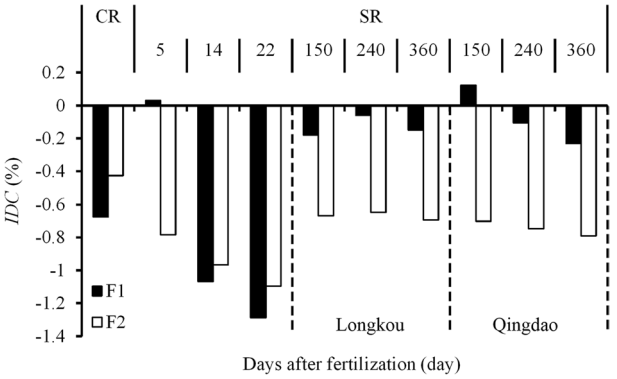

Fig. 5 Inbreeding depression coefficient (IDC) of cleavage rates (CR) and survival rates (SR) in tetraploid *C. gigas* families in F1 (F = 0.25) and F2 (F = 0.375) inbred lines at different times. Day5, day14 and day22 are the larval stages. Day 150, day 240 and day 360 are the grow-out stages

variation was mainly due to differences in inbreeding levels among generations, with significant differences, while site effects and interactions were non-significant. Although the site and different generations exerted extremely significant effects on whole weight, differences between inbreeding generations had the largest effects (Table 1).

In Longkou, no significant difference in shell height was recorded between F1 and F0 during grow-out stage, while F1 shell height was higher than F0 dimensions at days 150 and 240. Correspondingly, F1 showed almost no growth inbreeding depression. However, F2 showed slower growth, and shell height was significantly lower at days 240 and 360 when compared with F0. Shell heights on days 240 and 360 were 43.27 mm and 55.46 mm, respectively, and corresponding shell height IDC values were up to − 0.46% and − 0.42%, respectively (Figs. 4A and 6). Trend in whole weight variation was F0 > F1 > F2; F0 whole weight was significantly higher when compared with F2. Whole weight of F1 and F2 IDC values were − 0.49% and − 0.59%, respectively

Table 1 One way analysis of variance showing site (S: Longkou and Qingdao) and generation (G: F0, F1, and F2) effects on survival rate, shell height, and whole weigh values in tetraploid *C. gigas* families in the F0 (F = 0) outbred line and F1 (F = 0.25) and F2 (F = 0.375) inbred lines at grow-out stage

Character	Time	Source	*df*	*MS*	*F-value*	*P*
Survival rate	Day 150	S	1	0.009	0.489	0.490
		G	2	0.174	9.569	0.001**
		S*G	2	0.004	0.208	0.814
	Day 240	S	1	0.013	0.610	0.441
		G	2	0.157	7.331	0.003**
		S*G	2	< 0.001	0.019	0.981
	Day 360	S	1	0.005	0.228	0.636
		G	2	0.157	6.944	0.004**
		S*G	2	0.001	0.023	0.978
Shell height	Day 150	S	1	27.717	1.413	0.244
		G	2	139.527	7.115	0.003**
		S*G	2	1.213	0.062	0.940
	Day 240	S	1	17.304	0.669	0.420
		G	2	209.133	8.090	0.002**
		S*G	2	13.748	0.532	0.593
	Day 360	S	1	128.558	5.373	0.028*
		G	2	187.359	7.831	0.002**
		S*G	2	11.167	0.467	0.632
Whole weight	Day 360	S	1	72.870	8.804	0.006**
		G	2	55.394	6.692	0.004**
		S*G	2	7.508	0.907	0.415

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

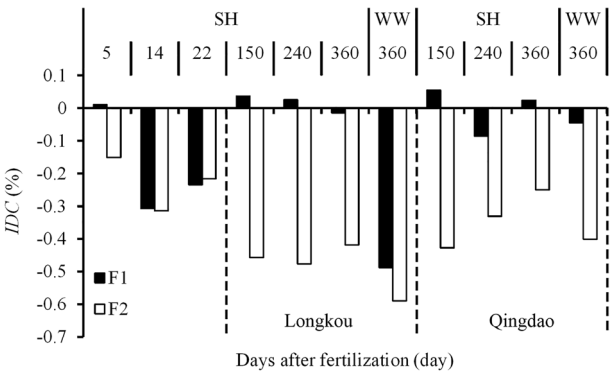

Fig. 6 Inbreeding depression coefficient (IDC) of shell height (SH) and whole weight (WW) in tetraploid *C. gigas* families in F1 (F = 0.25) and F2 (F = 0.375) inbred lines at different times

(Figs. 4B and 6). In Qingdao, oyster growth was relatively slow, F1 and F2 showed no significant growth differences when compared with F0 (Fig. 4D). Whole weight variation trend was consistent with Longkou data, but showed no significant difference at three generations (Fig. 4E).

Survival in grow-out stage

Survival rates in adult oysters from different inbreeding generations were highly significant; however, site effects and interactions with genotype had no no significant effects on survival rates (Table 1). In Longkou, survival rates showed the same variation trend during grow-out stage: F0 > F1 > F2, but with no significant differences. F2 survival rates were the lowest, with significant decreases observed from day 90 to 150, with some stability thereafter. F2 survival rates on days 150, 240, and 360 were 67.69%, 64.14%, and 60.03%, respectively, and corresponding survival IDC values were − 0.67%, − 0.65%, and − 0.69%, respectively (Figs. 4C and 5). In Qingdao, F1 survival rates also showed no distinct inbreeding depression, with no significant differences between F1 and F0. In contrast, F2 showed distinct mortality, with survival rates dropping sharply from day 90 to 150. F2 survival rates on days 150, 240, and 360 were 62.43%, 58.87%, and 56.34%, respectively, and survival IDC values were − 0.70%, − 0.75%, and − 0.79%, respectively (Figs. 4F and 5).

Discussion

Inbreeding often causes phenotypic depression in farming aquatic animals, with depression performance related to species, generation, the environment, and developmental stage. In previous studies, inbreeding depression often showed distinct interaction effects with the environment: in different environments, phenotypic inbreeding depression was completely different (Pray et al. 1994; Wang et al. 2002). In our study, the site had little influence on oyster growth and survival, but different levels of inbreeding among generations were the main sources of variation in growth and survival. This was possibly related to the fact that both sites were suitable for oyster survival and did not result in any distinct environmental stress. Additionally, due to the particularity of tetraploid oysters, ploidy reversion occurs in progeny and generates a certain proportion of diploids and triploids (de Sousa et al. 2017; Li and Li 2022; Qin et al. 2022; Zhang et al. 2022, 2014). If diploidization or triploidization did occur among these oysters, it is likely to have contributed to phenotypic differences (growth, survival, and gonadal development) (Li and Li 2022; Li et al. 2022; Qin et al. 2022; Wu et al. 2019; Zhang et al. 2022). The tetraploids of different inbred generations used in our experiment also showed varying degrees of ploidy reversion. In F2, more individuals reverse to triploids, the growth and survival of the entire family or generation will be enhanced (Li et al. 2023). Therefore, the detected inbreeding depression may be less than what actually exists.

When inbreeding occurs in an farming aquatic animal population, inbreeding depression will become more obvious with development of the organism (Gjerde et al. 1983; Kincaid 1983). In rainbow trout, inbred lines showed no significant inbreeding depression at day 160, but highly significant inbreeding depression was found at all inbreeding levels (F = 0.25, 0.375 and 0.50) when grown to 18 months (Gjerde et al. 1983). However, in our study inbreeding depression of larval survival rate was more distinct, similar to that observed with the *Argopecten circularis* (Ibarra et al. 1995). According to Fischer et al. (2003), early development is controlled by fewer loci, with dominant negative deleterious homozygous genes more pronounced at this stage, leading to more pronounced inbreeding depression. Additionally, the more severe inbreeding depression in the larval stage is also related to the greater sensitivity of this stage to environmental factors. The degree of

sensitivity to the environment is different at different developmental stages, and induces different manifestations of inbreeding depression (Armbruster and Reed 2005). In oyster larval development, umbo formation (day 5- day 10) was a dangerous period, when slow growth and a sharp decline in survival tends to occur. According to our previous farming experience, during the period, oysters are particularly sensitive to external environmental changes, with internal stress resistance levels key in determining survival. Similarly, inbred lines F1 and F2 larvae showed more distinct survival depression than other times during umbo formation, which indicated that inbreeding weakened stress resistance in tetraploid *C. gigas*.

Significant depression in F2 survival rates also occurred from day 90 to day 150, i.e., a period of dramatic environmental change when oysters are moved from nursery conditions to the natural conditions of oyster farms. During this period, F2 survival rates in Qingdao and Longkou decreased by 37.57% and 32.31%, respectively, while F1 and F0 survival rates decreased by less than 20%. This indicated that oysters had become more sensitive to the environment with increased inbreeding generation. Additionally, many inbred families were not cultivated to larvae end stage, and survival or growth rates were not quantified. These families tended to show more pronounced inbreeding depression. Therefore, our theoretical inbreeding depression estimates may be lower than actual values, which means real inbreeding depression is more severe than first estimated. Inbreeding must be avoided during tetraploid oyster artificial breeding as it will cause phenotypic depression in the population. The survival rates of the F2 families had dramatic variation, and the standard deviation of survival rate of the last 5 families in Longkou and Qingdao was more than 20% and 15%, respectively, which was higher than that of F0 and F1 (Fig. 4). It has reported that the genetic diversity of inbred population is lower than that of normal population, and the heterozygosity of gene loci is also reduced (Han et al. 2019). In our study, the heterozygosity of lethal gene loci decreased, and the homozygosity of the lethal gene loci in some F2 families was too high, resulting in decreased survival.

Although inbreeding had negative effects on tetraploid oyster progeny survival, they were small and not sustained in terms of growth. Also, no significant difference in larval shell height was observed between inbred and outbred families at early larval stage, with differences becoming significant after day 5, and F1 and F2 lines showing distinct growth depression. In bivalves, nutrient accumulation (glycogen, lipids, and proteins) in eggs primarily determines growth rates in early larvae (Cruz and Ibarra 1997; Liu et al. 2008). Oyster gonads become fully developed and matured at sufficient temperatures and nutrition levels under artificial conditions (Millican and Helm 1994). In our study, stock cultures were maintained at adequate temperatures with appropriate dietary requirements, so nutrient accumulation in eggs was practically the same. Nutrient accumulation in eggs determine growth more than inbreeding effects at early larval stage, so inbreeding effects may not be strongly evident. But with the growth, inbreeding effect gradually occupied the position, and the growth depression will gradually appear.

F1 only showed distinct growth depression at late larval stage, but no significant difference in shell height was observed between F1 and F0 lines at grow-out stage at both sites. In contrast, F2 showed distinct growth depression at some times in the grow-out stage and provided evidence that inbreeding effects on growth were related to the inbreeding generation with greater inbreeding generating more pronounced inbreeding depression. In previous studies, *Oncorhynchus kisutch* and *O. mykiss* showed significant growth depression during high inbreeding when compared with low inbreeding (Gallardo et al. 2004; Gjerde et al. 1983; Myers et al. 2001; Su et al. 1996), consistent with our conclusions. However, F1 showed no distinct growth depression under

our experimental conditions, which did not mean that low inbreeding had no effects on growth, but that these effects may only show under environmental stress (Armbruster and Reed 2005). Compared with diploid oysters, tetraploid oysters seem to show more obvious inbreeding depression. Evans et al. (2004) reported that the growth and survival IDC values of diploid *C. gigas* with inbreeding coefficient of 0.203 could reach -0.283% and -0.523% at day 157. For our data, the maximum growth and survival IDC values of tetraploid *C. gigas* reached -0.457% and -0.702% at day 150, indicating a more pronounced inbreeding depression. According to Mallet and Haley (1983), diploid *C. virginica* with inbred coefficient of 0.25 showed a maximum weight IDC value of -0.279% after one year of farming. However, the maximum weight IDC value of tetraploid *C. gigas* at day 360 in our experiment was -0.589%, which also showed more obvious growth inbreeding depression. Therefore, inbreeding in tetraploid oysters usually produce greater growth and survival depression, which is likely to affect its further selective breeding.

Conclusion

Inbred oyster growth and survival (inbreed coefficients of 0.25 and 0.375) were compared with an outbred F0 line (control) in tetraploid *C. gigas*. Inbred lines with different inbreeding coefficients showed different degrees of growth and survival depression, while inbreeding depression at different growth stage was variable. The highly inbred line (F2, F = 0.375) showed more pronounced inbreeding depression in terms of growth and survival when compared with a less inbred line (F1, F = 0.25). Survival inbreeding depression was more distinct than growth, and occurred across the whole growth cycle. Inbreeding depression in terms of survival was more pronounced at larval stage when compared with grow-out stage. Overall, inbreeding in tetraploid *C. gigas* depressed growth and survival. Manifested inbreeding depression was mainly related to inbreeding generation and developmental stages, and was less affected by the different sites. Therefore, inbreeding was highly unfavorable when establishing a stable tetraploid oyster germplasm bank. Importantly, some measures could be adopted to avoid inbreeding or reduce its degree during *C. gigas* tetraploid breeding, including expansion of the basic population, introducing allopolyploid from different species, and proceeding hybridization among strains.

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Declarations

Competing interests The authors declare no competing interests.

References

Allen SK, Downing SL (1986) Performance of triploid Pacific oysters, *Crassostrea gigas* (Thunberg). I. Survival, growth, glycogen content, and sexual maturation in yearlings. J Exp Mar Biol Ecol 102:197–208. https://doi.org/10.1016/0022-0981(86)90176-0

Armbruster P, Reed DH (2005) Inbreeding depression in benign and stressful environments. Heredity 95:235–242. https://doi.org/10.1038/sj.hdy.6800721

Barrett SCH (2003) Mating strategies in flowering plants: the outcrossing–selfing paradigm and beyond. Philos Phil Trans R Soc Lond B 358(1434):991–1004. https://doi.org/10.1098/rstb.2003.1301

Bartley DM, Rana K, Immink AJ (2000) The use of inter-specific hybrids in aquaculture and fisheries. Rev Fish Biol Fish 10:325–337. https://doi.org/10.1098/rstb.2003.1301

Benabdelmouna A, Ledu C (2015) Autotetraploid Pacific oysters (*Crassostrea gigas*) obtained using normal diploid eggs: induction and impact on cytogenetic stability. Genome 58:333–348. https://doi.org/10.1139/gen-2015-0014

Callam BR, Allen SK, Frank-Lawale A (2016) Genetic and environmental influence on triploid *Crassostrea virginica* grown in Chesapeake Bay: Growth. Aquaculture 452:97–106. https://doi.org/10.1016/j.aquaculture.2015.10.027

Chen RR, Feng ZY, Zhang XH, Song ZJ, Cai DT (2021) A new way of rice breeding: Polyploid rice breeding. Plants 10:422. https://doi.org/10.3390/plants10030422

Cruz P, Ibarra AM (1997) Larval growth and survival of two catarina scallop (*Argopecten circularis*, Sowerby, 1835) populations and their reciprocal crosses. J Exp Mar Biol Ecol 212:95–110. https://doi.org/10.1016/S0022-0981(96)02742-6

de Sousa JT, Allen SK, Wolfe BM, Small JM (2017) Mitotic instability in triploid and tetraploid one-year-old eastern oyster, *Crassostrea virginica*, assessed by cytogenetic and flow cytometry techniques. Genome 61:79–89. https://doi.org/10.1139/gen-2017-0173

Eudeline B, Allen SK, Guo XM (2000a) Delayed meiosis and polar body release in eggs of triploid Pacific oysters, *Crassostrea gigas*, in relation to tetraploid production. J Exp Mar Biol Ecol 248:151–161. https://doi.org/10.1016/S0022-0981(00)00158-1

Eudeline B, Allen SK, Guo XM (2000b) Optimization of tetraploid induction in Pacific oysters, *Crassostrea gigas*, using first polar body as a natural indicator. Aquaculture 187:73–84. https://doi.org/10.1016/S0044-8486(00)00302-1

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. https://doi.org/10.1016/j.aquaculture.2003.09.023

Fang JF, Han ZQ, 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. Aquac Rep 19:100590. https://doi.org/10.1016/j.aqrep.2021.100590

Fang JF, Xu CX, Li Q (2022) Transcriptome analysis of inbreeding depression in the Pacific oyster *Crassostrea gigas*. Aquaculture 557:738314. https://doi.org/10.1016/j.aquaculture.2022.738314

Fessehaye Y, Komen H, Rezk MA, van Arendonk JAM, Bovenhuis H (2007) Effects of inbreeding on survival, body weight and fluctuating asymmetry (FA) in Nile tilapia, *Oreochromis niloticus*. Aquaculture 264: 27–35. Aquaculture 264:27–35. https://doi.org/10.1016/j.aquaculture.2006.12.038

Fischer M, Hock M, Paschke M (2003) Low genetic variation reduces cross-compatibility and offspring fitness in populations of a narrow endemic plant with a self-incompatibility system. Conserv Genet 4:325–336. https://doi.org/10.1023/A:1024051129024

Gallardo JA, Garcia X, Lhorente JP, Neira R (2004) Inbreeding and inbreeding depression of female reproductive traits in two populations of *Coho salmon* selected using BLUP predictors of breeding values. Aquaculture 234:111–122. https://doi.org/10.1016/j.aquaculture.2004.01.009

Gérard A, Ledu C, Phélipot P, Naciri-Graven Y (1999) The induction of MI and MII triploids in the Pacific oyster *Crassostrea gigas* with 6-DMAP or CB. Aquaculture 174:229–242. https://doi.org/10.1016/S0044-8486(99)00032-0

Gjerde B, Gunnes K, Gjedrem T (1983) Effect of inbreeding on survival and growth in rainbow trout. Aquaculture 34:327–332. https://doi.org/10.1016/0044-8486(83)90212-0

Guo XM, Allen SK (1997) Sex and meiosis in autotetraploid Pacific oyster, *Crassostrea gigas* (Thunberg). Genome 40:397–405. https://doi.org/10.1139/g97-053

Guo XM, DeBrosse GA, Allen SK (1996) All-triploid Pacific oysters (*Crassostrea gigas* Thunberg) produced by mating tetraploids and diploids. Aquaculture 142:149–161. https://doi.org/10.1016/0044-8486(95)01243-5

Han ZQ, Li Q, Liu SK, Yu H, Kong LF (2019) Genetic variability of an orange-shell line of the Pacific oyster *Crassostrea gigas* during artificial selection inferred from microsatellites and mitochondrial COI sequences. Aquaculture 508:159–166. https://doi.org/10.1016/j.aquaculture.2019.04.074

Ibarra AM, Cruz P, Romero BA (1995) Effects of inbreeding on growth and survival of self-fertilized catarina scallop larvae, *Argopecten circularis*. Aquaculture 134:37–47. https://doi.org/10.1016/0044-8486(95)00022-T

Janssen K, Chavanne H, Berentsen P, Komen H (2017) Impact of selective breeding on European aquaculture. Aquaculture 472:8–16. https://doi.org/10.1016/j.aquaculture.2016.03.012

Keller LF, Waller DM (2002) Inbreeding effects in wild populations. Trends Ecol Evol 17:230–241. https://doi.org/10.1016/S0169-5347(02)02489-8

Kincaid HL (1983) Inbreeding in fish populations used for aquaculture. Aquaculture 33:215–227. https://doi.org/10.1016/0044-8486(83)90402-7

Li YG, Li Q (2022) The growth, survival and ploidy of diploid, triploid and tetraploid of the Pacific oyster (*Crassostrea gigas*) in larval and juvenile stages. Aquaculture 553:738083. https://doi.org/10.1016/j.aquaculture.2022.738083

Li YG, Xu CX, Li Q (2022) Effects of salinity and temperature on growth and survival of diploid and tetraploid larvae of the Pacific oyster. Crassostrea Gigas Aquaculture 550:737809. https://doi.org/10.1016/j.aquaculture.2021.737809

Li H, Yu R, Li Q (2023) Comparison on chromosome stability between inbred and outbred full-sib families of tetraploid *Crassostrea gigas* by cytogenetic technique. Aquaculture 569:739348. https://doi.org/10.1016/j.aquaculture.2023.739348

Liu SJ (2010) Distant hybridization leads to different ploidy fishes. Sci China Life Sci 53:416–425. https://doi.org/10.1007/s11427-010-0057-9

Liu WG, Li Q, Yuan YD, Zhang SH (2008) Seasonal variations in reproductive activity and biochemical composition of the cockle *Fulvia mutica* (Reeve) from the eastern coast of China. https://doi.org/10.2983/0730-8000(2008)27[405:SVIRAA]2.0.CO;2

Luo K, Kong J, Luan S, Meng XH, Zhang TS, Wang QY (2014) Effect of inbreeding on survival, WSSV tolerance and growth at the post larval stage of experimental full-sibling inbred populations of the Chinese shrimp *Fenneropenaeus chinensis*. Aquaculture 420–421:32–37. https://doi.org/10.1016/j.aquaculture.2013.10.030

Mallet AL, Haley LE (1983) Effects of inbreeding on larval and spat performance in the American oyster. Aquaculture 33:229–235. https://doi.org/10.1016/0044-8486(83)90403-9

Millican PF, Helm MM (1994) Effects of nutrition on larvae production in the European flat oyster, *Ostrea edulis*. Aquaculture 123:83–94. https://doi.org/10.1016/0044-8486(94)90121-X

Myers JM, Heggelund PO, Hudson G, Iwamoto RN (2001) Genetics and broodstock management of coho salmon. In: Lee CS, Donaldson EM (eds) Reproductive biotechnology in finfish aquaculture. Elsevier, Amsterdam, pp 43–62

Otterå H, Thorsen A, Karlsen Ø, Fjelldal PG, Morton HC, Taranger GL (2016) Performance of triploid Atlantic cod (*Gadus morhua* L.) in commercial aquaculture. Aquaculture 464:699–709. https://doi.org/10.1016/j.aquaculture.2016.08.018

Piferrer F, Beaumont A, Falguière J-C, Flajšhans M, Haffray P, Colombo L (2009) Polyploid fish and shellfish: Production, biology and applications to aquaculture for performance improvement and genetic containment. Aquaculture 293:125–156. https://doi.org/10.1016/j.aquaculture.2009.04.036

Pray LA, Schwartz JM, Goodnight CJ, Stevens L (1994) Environmental dependency of inbreeding depression: Implications for conservation biology. Conserv Biol 8:562–568. https://doi.org/10.1046/j.1523-1739.1994.08020562.x

Qin YP, Xiao S, Ma HT, Mo RG, Zhou ZH, Wu XW, Zhang YH, Yu ZN (2018) Effects of salinity and temperature on the timing of germinal vesicle breakdown and polar body release in diploid and triploid Hong Kong oysters, *Crassostrea hongkongensis*, in relation to tetraploid induction. Aquac Res 49:3647–3657. https://doi.org/10.1111/are.13833

Qin YP, Zhang YH, Yu ZN (2022) Aquaculture performance comparison of reciprocal triploid *C. gigas* produced by mating tetraploids and diploids in China. Aquaculture 552:738044. https://doi.org/10.1016/j.aquaculture.2022.738044

Stanley JG, Allen SK, Hidu H (1981) Polyploidy induced in the American oyster, *Crassostrea virginica*, with cytochalasin B. Aquaculture 23:1–10. https://doi.org/10.1016/0044-8486(81)90002-8

Su GS, Liljedahl L-E, Gall GAE (1996) Effects of inbreeding on growth and reproductive traits in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 142:139–148. https://doi.org/10.1016/0044-8486(96)01255-0

Wang SZ, Hard JJ, Utter F (2002) Salmonid inbreeding: a review. Rev Fish Biol Fish 11:301–319. https://doi.org/10.1023/A:1021330500365

Wu XW, Zhang YH, Xiao S, Qin YP, Ma HT, Yu ZN (2019) Comparative studies of the growth, survival, and reproduction of diploid and triploid Kumamoto oyster, *Crassostrea Sikamea*. J World Aquac Soc 50:866–877. https://doi.org/10.1111/jwas.12596

Zhang YH, Qin YP, Yu ZN (2022) Comparative study of tetraploid-based reciprocal triploid Portuguese oysters, *Crassostrea angulata*, from seed to marketsize. Aquaculture 547:737523. https://doi.org/10.1016/j.aquaculture.2021.737523

Zhang ZR, Wang XL, Zhang QQ, Allen SK (2014) Cytogenetic mechanism for the aneuploidy and mosaicism found in tetraploid Pacific oyster *Crassostrea gigas* (Thunberg). J Ocean Univ China 13:125–131. https://doi.org/10.1007/s11802-014-2318-x