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Influence of ploidy, genetic and environment on production traits of the Pacific oyster *Crassostrea gigas*

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ABSTRACT

Pacific oysters, *Crassostrea gigas*, are one of the most widely farmed shellfish. To meet demand, the advantages of growth and increased sterility are currently being exploited in the commercial culture of triploid *C. gigas*. However, the extent of the triploid advantage can vary, environmental conditions and rearing methods. In the present study, the performance of diploid and triploid oysters was compared at two sites (Rongcheng and Rushan) to determine whether the triploidization would result in any advantage on production traits that could be utilized for aquaculture of this species. Triploid oysters were also produced using the wild broodstock and selected line to verify whether selectively bred oysters maintain their improved traits after triploidization. In this study, we found that the survival advantage of triploid is "site-dependent" and that Rongcheng's triploids did not exhibit significantly higher cumulative survival than diploids. Notably, incremental survival of triploid oysters was consistently higher than that of diploids during the second summer, which may be related to gonad development and spawning in diploids. At both sites, growth traits (shell height and individual weight) were always higher for triploids than in diploids in both the selected and control groups, which is strong evidence of a superior growth advantage for triploids. In addition, we found that the production performance (growth, survival and yield) of the selected group was consistently better than that of the control group (either diploid or triploid), suggesting that the superior traits of diploids can be converted to triploids through ploidy manipulation. Our results support the notion that yield can be further optimized by reducing mortality through a selective breeding program coupled with triploid production to increase growth rates. This study provides meaningful information on the potential of triploid *C. gigas* for oyster culture in northern China.

1. Introduction

The Pacific oyster (*Crassostrea gigas*) is native to Northeast Asia including China, Korean peninsula, and Japan, and has been introduced to different countries since the 1920s (Mann, 1979). About 40 years ago, triploid oysters were developed to increase oyster production (Stanley et al., 1981), and they are now widely cultured in France (Dégremont et al., 2015), China (Qin et al., 2023), Australia (Nell and Perkins, 2005), and North America (Guo, 2009). For the last 20 years, oyster production in most countries is dominated by the use of triploids (Nell, 2002; FAO, 2018; Wadsworth et al., 2019). The demand for triploid oysters is mainly due to the generally increased growth rate of triploid oysters, which is approximately 30% faster than diploid oysters, and the consistently good meat quality due to the sterility of triploid oysters (Matthiessen and Davis, 1992; Nell, 2002; Wadsworth et al., 2019). Various explanations have been proposed for the triploid effect in oysters: differences in energy allocation due to increased genetic heterozygosity, additive genetic effects of an additional set of chromosomes, suppression of reproduction, and triploid cell gigantism (Allen and Downing, 1986; Hawkins et al., 1994; Guo and Allen, 1994; Hand et al., 1998; Mallia et al., 2006).

The production of hatchery-bred triploid oysters imposes additional costs on farmers, which must be justified by improved product value or increased yields. Selective breeding and polyploid breeding are two important avenues for genetic improvement of oysters, and their cross-application promises new benefits for oyster breeding. Several studies have shown that the performance of triploid oysters can be improved using selected diploids (Dégremont et al., 2014; Hand et al., 2004; Callam et al., 2016), This suggests that improved traits (growth or survival) can be transferred from desirable diploids to triploids. Hand et al.

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(2004) suggested that combining triploids with selection may result in "positive feedback or synergistic effects" leading to additional phenotypic gains. Therefore, the use of selective breeding and polyploidy techniques is expected to improve production performance in oyster culture.

China is the largest oyster culture country in the world, accounting for 86% of the global production (Botta et al., 2020). In 2020, China's *C. gigas* aquaculture production was approximately 1.57 million tons, accounting for 28.90% of China's total oyster aquaculture production (BOF, 2021). Driven by the remarkable culture benefits, triploid oyster cultivation developed rapidly in China in recent years, presenting a promising development prospect (Yu and Li, 2021). However, in recent years, some oyster farmers have reported large numbers of oyster deaths, and many have associated the deaths with triploidy. These concerns triggered this quantitative study to compare the performance of diploids and triploids at two commercial oyster sites in northern China. In previous work, we included selection for summer survival in a selective breeding program, and genetic progress for summer survival has been good through two generations of family selection (Chi et al., 2022). Although selective breeding for disease resistance traits is effective, it is unclear whether selectively-bred *C. gigas* maintain their improved resistance after triploidization.

In the scientific literature, the advantages and disadvantages of triploidization are inconsistent, regardless of which triploid production method is used (Wadsworth et al., 2019; Brianik and Allam, 2023). Consistency in triploid oyster performance is considered an important goal since the use of triploid oyster seed increases the cost of culture for growers (Mizuta et al., 2021). Notably, most studies on ploidy in *C. gigas* have been conducted using crossbred triploid oysters, a method that only produces half-siblings of different parentage, thereby increasing genetic variability between diploids and triploids (Gagnaire et al., 2006; Walton et al., 2013; Zhou et al., 2023; Bodenstein et al., 2023). The best way to compare diploids and triploids is to use combinations with the same genetic background, which can be achieved by chemical induction.

Triploid and diploid oysters from the same genetic background, as well as unselected or selected oysters, were used in this study. We systematically compared the growth, survival and yield of sibling triploid and diploid *C. gigas* grown at two different sites in northern China. The objectives of this study were: 1) to compare the production performance of diploid and triploid *C. gigas*; 2) to assess whether selective breeding could be transferred to polyploids.

2. Materials and method

2.1. Parent oyster collection

Two broodstocks were used in this study. The first broodstock was the third generation of full-sib families bred in our selective breeding program in 2019, which aimed to improve the summer survival of *C. gigas* in northern China (Chi et al., 2021). The selection criterion was for a higher summer survival rate for juvenile oysters (Chi et al., 2022). The second broodstock was a wild population sampled from a non-farming area in Rongcheng, Shandong Province (Fig. 1) in 2021, which was used to produce the controls. In December 2021, the diploid broodstocks of selected families and wild population were transported to Laizhou breeding base, Shandong Province (Fig. 1).

2.2. Production of families and larval rearing

Oysters were stripped for spawning in March 2022 after maturation promotion at the Laizhou breeding base. For the selected families, eggs from one female oyster were obtained and divided equally into two beakers. The first beaker was fertilized with sperm from one male to produce the batch named 2NS (S indicates selection). The second beaker was used to produce triploids, which were chemically induced with cytochalasin B (CB) according to a protocol modified from Yang et al.

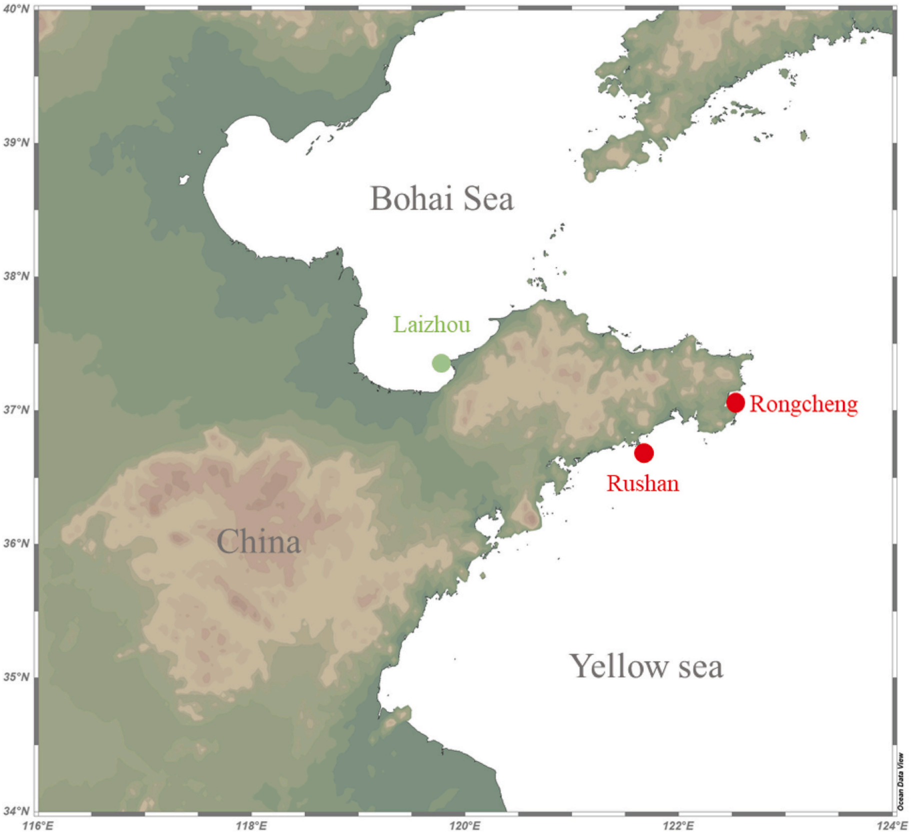

Fig. 1. Location of the broodstocks origin and oyster farming environments in China.

(2018). Briefly, 0.5 mg/L CB was used to inhibit the second polar body of fertilized eggs when the first polar body was present in about 30% of the embryos. After CB treatment for 15 min, the embryos were collected by filtration through a sieve and immersed in 0.005% DMSO solution for 40 min. Finally, the residual solution is rinsed off and the fertilized eggs were placed in a bucket for incubation. The offspring obtained were named 3NS. Similarly, 2NC and 3NC were bred from wild populations using one male and one female (C indicates control) (Fig. 2). Triploid rates for each induction group were analyzed using flow cytometry, and combinations with triploid rates >95% were selected for the experiment. Finally, three different replicates (6 sires and 6 dams) were successfully conduced, each consisting of four groups (2NS, 3NS, 2NC and 3NC) (Fig. 2). Each group has three full-sib families, for a total of twelve full-sib families.

Larvae were cultured as described by Chi et al. (2022). After fertilization, each group of larvae was individually transferred to a 300-L plastic bucket containing filtered seawater at 22–24 °C. The seawater in the bucket was changed daily. Beginning at 24 h after fertilization, larvae were fed three times a day with *Isochrysis galbana* during the first week, followed by a mixture of *Platymonas* sp. and *I. galbana*. When 50% or more of the pediveliger larvae develop eyespots, a string of scallop shells is placed in a bucket to serve as a substrate for their settling.

2.3. Ploidy assessment

The DNA ploidy level of larvae at 7 days after fertilization were verified using a flow cytometer. Ploidy was determined according to the protocols developed by Jiang et al. (2022) and Allen (1983). 5000–6000 larvae per family ($n = 12$) were collected to determine their composite ploidy. A small number of free-swimming larvae from each triploid group were taken into centrifuge tubes containing 1 × phosphate buffer solution. The larvae were first disintegrated by repeated aspiration using a 1 mL syringe. The samples were subsequently filtered through a 48 μm nylon sieve and then stained by adding 0.80 mL DAPI (4′,6-diamidino-2-phenylindole) staining buffer for 10 min. Then, the ploidy of each triploid group was examined by flow cytometry.

2.4. Field test and measurements

In May 2022, spat were transferred to two major oyster commercial culture sites (Rongcheng and Rushan) in Shandong Province (Fig. 1). At both sites, spat were first secured with nylon ropes and then cultured for 2 months using the long line method. In July 2022, each group was placed in three cages at each site as replicates, with 100 oysters per cage placed for field grow-out. Cages were cleaned monthly and dead oysters in the cages were discarded. Surface seawater temperature and salinity were measured in situ during each observation using a mercury thermometer and a portable refractometer.

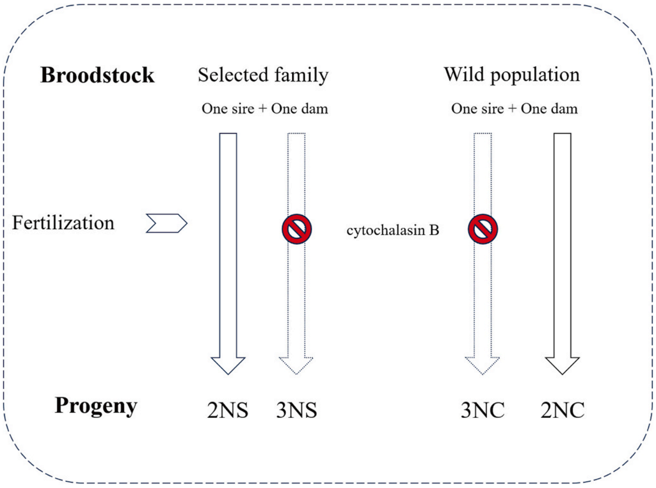

Fig. 2. Schematic overview of the family mating design.

Individual oyster size (shell height and individual weight) and survival were monitored periodically in October 2022, January, May and August 2023 for each group. Survived oysters of each cage were recorded and cumulative survival rate was calculated according to the following formula:

$$C_t\% = \frac{N_t}{N_0} \times 100$$

where C_t represents the cumulative survival rate at sampling time t; N_t is the number of survived oysters at sampling point t; N_0 represents the total number of oysters per cage in October 2022.

The incremental survival rate was calculated according to the following formula:

$$I_{t+1}\% = \frac{N_{t+1}}{N_t} \times 100$$

where I_t represents the incremental survival rate of oyster at time t + 1; N_{t+1} is the number of survived oysters at time t + 1; t + 1 was the next time after time t.

The yield was calculated according to the following formula (Rawson and Feindel, 2012):

$$Y = C \times IW \times N_0$$

where C and IW is the cumulative survival rate and the individual weight of oyster at the last time (August 2023).

Thirty oysters were randomly selected from each group and shell height was measured using vernier calipers (0.01 mm) and individual weight was measured using electronic scales (0.01 g).

2.5. Data analysis

To improve the normality and homogeneity of the raw data, survival rates were arcsine transformed, and shell heights and individual weights were logarithmic transformed. One-way analysis of variance (ANOVA) was performed using SPSS 25.0, followed by multiple comparisons tests for different groups based on growth traits and survival traits. When the homogeneity of variance was still not satisfied after data transformation, the Kruskal-Wallis nonparametric test was used for pair-wise comparison between groups. $P < 0.05$ was considered as significant difference. A generalized linear model was used to determine the effects of the genotype (selection and control), ploidy (diploid and triploidy) and the environment (Rongcheng and Rushan) and their interactions on growth traits, survival rates and yield with the following statistical mode:

$$Y_{ijk} = \mu + G_i + P_j + E_k + \left(G_i \times P_j\right) + \left(G_i \times E_k\right) + \left(P_j \times E_k\right) + \left(G_i \times P_j \times E_k\right) + \delta_{ijk}$$

where Y_{ijk} = dependent variable (shell height, individual weight, incremental survival rate, cumulative survival rate or yield); μ = common mean; G_i = genotype effect (selection or control); P_j = ploidy effect (diploid or triploid);E_k = environment effect (Rongcheng or Rushan);$G_i \times P_j$ = interaction effect between genotype and ploidy; $G_i \times E_k$ = interaction effect between genotype and environment;$P_j \times E_k$ = interaction effect between ploidy and environment;$G_i \times P_j \times E_k$ = interaction effect between genotype, ploidy and environment; δ_{ijk} = residual error.

Triploid advantage (*TA*) was defined as the percentage difference in growth traits or survival between diploids (2 N) and the triploids (3 N) and was calculated as follows (Qin et al., 2019):

$$TA\% = \left(\frac{3N - 2N}{2N}\right) \times 100$$

where positive *TA* indicates that the triploid oysters performed better than diploid oysters and negative *TA* indicates that triploids oysters performed worse.

Selected advantage (SA) is defined as the percent difference in growth traits or survival rate between the selected group (SG) and the control group (CG) and is calculated as follows (Zhou et al., 2023):

$$SA\% = \left(\frac{SG - CG}{CG}\right) \times 100$$

where positive *SA* indicates that the selected oysters performed better than the control oysters, and negative TA indicates that the selected oysters performed worse.

3. Results

3.1. Temperature and salinity in two sites

Seawater temperature and salinity were similar at both sites (Fig. 3). The temperature ranged from 1.9 °C to 25.6 °C in Rongcheng and from 2.3 °C to 27.1 °C in Rushan. The salinity ranged from 29.5 psu to 31.5 psu in Rongcheng and from 29.9 psu to 32.2 psu in Rushan.

3.2. Growth traits at two sites

Growth traits, including shell height and individual weight, were measured for four groups at both sites and are shown in Fig. 4. During the grow-out stages, all groups in Rushan grew faster than those in Rongcheng. Overall, the triploid oysters grew faster than the diploid oysters in both environments, and the growth of the selected groups was also faster than that of the wild groups. Thus, growth performance was ranked in the following order: 3NS > 2NS > 3NC > 2NC. The triploid advantage for growth traits was consistently positive, ranging from 7.13% to 33.85% and 10.97% to 29.36% for Rushan and Rongcheng, respectively (Table 3). Three-way ANOVA showed that the genotype × ploidy interaction effects and genotype × environment × ploidy interaction effects had significant ($P < 0.05$) effects on shell height in August 2023 ($P < 0.05$) (Table 1). The selected advantage of growth traits in Rongcheng and Rushan ranged from 0.67% to 37.90% and 9.21% to 42.68%, respectively (Table 5).

3.3. Survival traits at the two sites

In Rongcheng site, the incremental survival rate of diploids was significantly higher than that of triploids (either selection or wild) in the summer of 2022 ($P < 0.05$) (Fig. 5A). In the summer of 2023, incremental survival rate was significantly higher for 3NC than for 2NC ($P < 0.05$), but no significant differences were found between 2NS and 3NS ($P > 0.05$). At the remaining two time points, no significant differences were found between the different groups. The triploid advantage and selective advantage of incremental survival rate ranged from −20.81% to 34.62% and − 2.92% to 26.10%, respectively (Table 4 and Table 6). In August 2023, the cumulative survival rates for 2NS, 3NS, 2NC and 3NC were 64.00%, 60.22%, 44.00% and 43.78%, respectively (Fig. 5B). Meanwhile, the triploid advantage and selective advantage of cumulative survival rate ranged from −5.90% to −0.51% and 37.56% to 45.45%, respectively.

In Rushan site, there were no significant differences in the incremental survival of 2NC and 3NC over the four monitoring periods (Fig. 5C). In May and August 2023, the incremental survival rate of 3NS was significantly higher than that of 2NS ($P < 0.05$), but no significant difference was found between 2NS and 3NS ($P > 0.05$). The triploid advantage and selective advantage of incremental survival rate ranged from −1.65% to 14.01% and − 0.10% to 23.24%, respectively (Table 4 and Table 6). During the final monitoring period, cumulative survival was significantly higher in the triploid group than in the diploid group, and significantly higher in the selection group than in the control group, in the order 3NS > 2NS > 3NC > 2NC (Fig. 5D). The triploid advantage and selective advantage of cumulative survival rate ranged from 14.41% to 19.73% and 43.34% to 50.00%, respectively.

Three-way ANOVA results showed significant effects of environment × ploidy interaction on incremental survival in October 2022 ($P < 0.001$), January 2023 ($P < 0.01$) and May 2023 ($P < 0.01$) (Table 2). In August 2023, significant genotype × environmental interaction effects ($P < 0.01$) and genotype × environment × ploidy interaction effects ($P < 0.05$) were observed for incremental survival rate. For cumulative survival rate, a significant environment × ploidy interaction effect was observed at all four time points ($P < 0.001$).

3.4. Yield at two sites

At both sites, the yield of 3NS was higher than the other three groups in the order of 3NS > 2NS > 3NC > 2NC (Fig. 6). In Rushan, the yield of triploids was significantly higher than that of diploids ($P < 0.05$), in contrast, the difference was not significant in Rongcheng ($P > 0.05$). Significant environment × ploidy interaction effects and genotype × environment interaction effects were found in the three-way ANOVA for yield ($P < 0.001$). The triploid advantage and selective advantage of

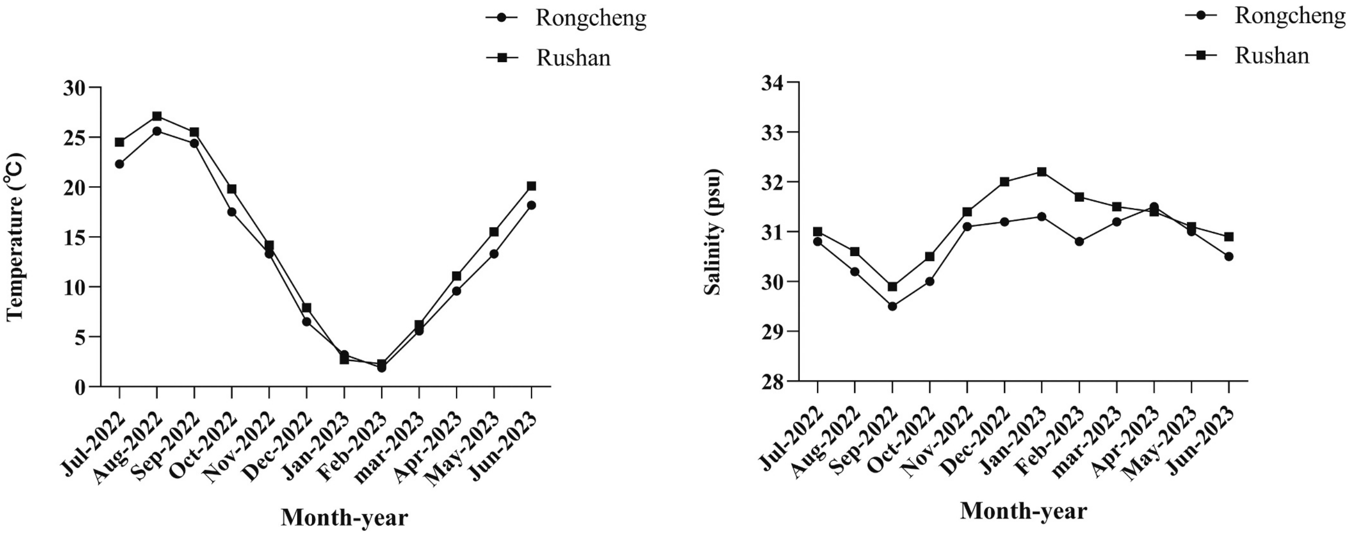

Fig. 3. Seasonal variation in surface seawater temperature and salinity in Rongcheng and Rushan from July 2022 to June 2023.

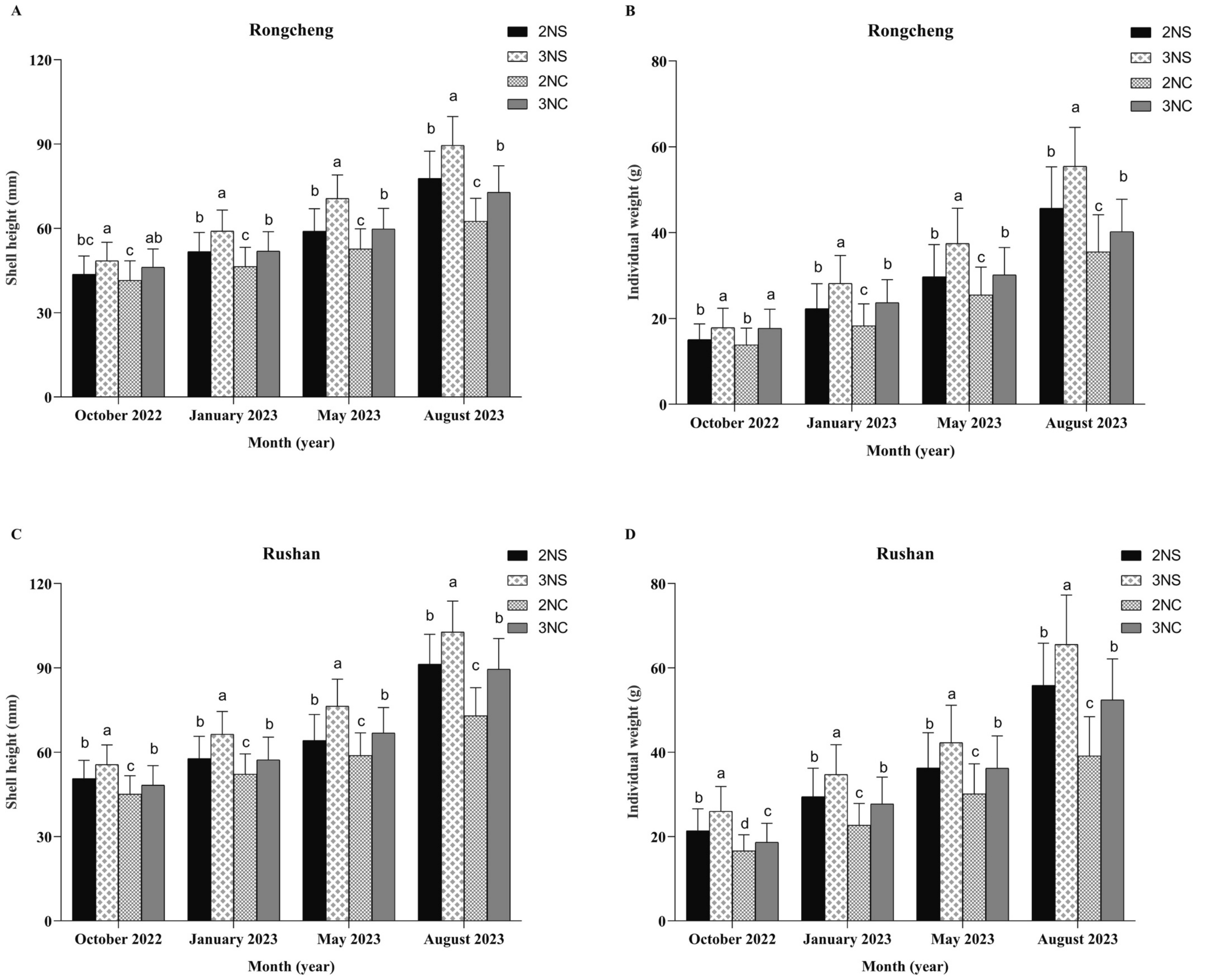

Fig. 4. Shell height and individual weight for four groups (2NS, 3NS, 2NC, 3NC) *C. gigas* at Rongcheng and Rushan from October 2022 to August 2023. Different superscript letters at the same time indicate significant difference ($P < 0.05$).

yield ranged from 12.69% to 59.18% and 80.00% to 112.57%, respectively (Table 2).

4. Discussion

Over the past few decades, genetic improvement technologies have developed and expanded the aquaculture industry (Nascimento-Schulze et al., 2021; Regan et al., 2021). Selective breeding and polyploid breeding are two major technological tools that have been used to improve performance and sensory quality in aquaculture (Guo, 2009; Gjedrem and Rye, 2018). In this study, we systematically evaluated the effects of genotype, environment, ploidy and their interactions on production traits (growth, survival and yield) of the *C. gigas*. Our results can provide valuable guidance information for the oyster industry, especially in light of the current scarce and conflicting knowledge.

4.1. Triploid advantage on production traits

Aquaculture is a highly volatile industry, often subject to unpredictable abiotic and biotic factors. For oysters, survival is an important characteristic that affects profitability. One of the biggest constraints to oyster aquaculture is disease-related losses, which amount to millions of dollars globally due to disease (e.g., summer mortality of *C. gigas*) (Lacoste et al., 2001; Cotter et al., 2010; Alfaro et al., 2019; Brianik and Allam, 2023). When comparing the survival rate of diploids and triploids at the culture sites, our results varied with culture time.

Several researchers have demonstrated that survival rates of diploid and triploid bivalves vary greatly depending on the culture site and stage of gonad development (Nell, 2002; Francesc et al., 2009). Incremental survival rates provide a more intuitive and accurate reflection of the effects of genotype, ploidy and environment than cumulative survival rates. In the present study, temporal differences were evident, with triploids experiencing lower mortality in the second summer and higher mortality in the first summer. Different developmental stages of triploids are sensitive to different stresses, which may lead to different periods of mortality. In addition, environmental factors may also be different between years, which may also contribute to the differences between the two summers. Some studies have reported that the most mortality-prone stages of bivalves and many fish species are the larval and juvenile stages (Brianik and Allam, 2023). Therefore, oyster farmers need to be aware that the summer to fall maybe a particularly vulnerable period for juvenile triploids.

Rongcheng triploids had higher mortality in the first summer, whereas Rushan diploids and triploids did not differ in mortality. The

Table 1
Three-way analyses of variance testing for the interaction effects of genotype, environment and ploidy on shell height and individual weight. The *P*-value associated with each F-value are indicated by asterisks (* – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$; ns – not significant).

Day	Effect	Shell height		Individual weight	
		d.f.	F-value	d.f.	F-value
October 2022	Genotype	1	71.685***	1	86.130***
	Environment	1	94.965***	1	168.748***
	Ploidy	1	77.283***	1	97.798***
	Genotype × Environment	1	14.040***	1	43.444***
	Genotype × Ploidy	1	0.240^{ns}	1	0.003^{ns}
	Environment ×Ploidy	1	1.451^{ns}	1	1.943^{ns}
	Genotype × Environment × Ploidy	1	0.445^{ns}	1	4.676*
January 2023	Genotype	1	141.753***	1	138.170***
	Environment	1	117.493***	1	147.155***
	Ploidy	1	137.067***	1	147.450***
	Genotype × Environment	1	0.112^{ns}	1	2.636^{ns}
	Genotype × Ploidy	1	2.721^{ns}	1	0.656^{ns}
	Environment ×Ploidy	1	0.112^{ns}	1	3.552^{ns}
	Genotype × Environment × Ploidy	1	0.354^{ns}	1	0.006^{ns}
May 2023	Genotype	1	159.128***	1	106.035**
	Environment	1	91.334***	1	97.289***
	Ploidy	1	238.061***	1	120.746***
	Genotype × Environment	1	2.053^{ns}	1	0.098^{ns}
	Genotype × Ploidy	1	6.482*	1	0.138^{ns}
	Environment ×Ploidy	1	0.024^{ns}	1	1.067^{ns}
	Genotype × Environment × Ploidy	1	0.059^{ns}	1	2.350^{ns}
August 2023	Genotype	1	467.679***	1	309.141***
	Environment	1	324.435***	1	128.122***
	Ploidy	1	279.993***	1	171.214***
	Genotype × Environment	1	2.834^{ns}	1	0.000^{ns}
	Genotype × Ploidy	1	6.376*	1	1.780^{ns}
	Environment ×Ploidy	1	0.698^{ns}	1	4.255*
	Genotype × Environment × Ploidy	1	4.668*	1	10.158**

reason for the higher triploid mortality is unknown but is likely related to differences in environmental conditions. Seawater temperature and salinity were similar at both sites, but phytoplankton abundance and primary productivity were reported to be higher in Rushan than in Rongcheng (Gao et al., 2006). Several studies have shown that in unfavorable environments (low dissolved oxygen, low salinity, poor food quality and availability, high disease pressure), triploids survive less than diploids (Stanley et al., 1984; Goulletquer et al., 1996; Cheney et al., 2000). In addition, Brianik and Allam (2023) reported that triploid oysters had a higher risk of mortality than diploid oysters under multiple stressors, especially when they occurred together. In future studies, experimental tests should be conducted at all sites and important potential stressors should be monitored.

Several studies have reported that the survival of triploid oysters is usually similar to that of diploid oysters, because of the absence of reproductive effort, as observed in this study during the first summer in Rushan (Dégremont et al., 2016; Matt et al., 2020; Matt and Allen Jr., 2021). Indeed, summer mortality in diploid oysters is often attributed to stress caused by intense physiological changes during gonad development and spawning (Samain et al., 2007; Huvet et al., 2010; Wendling and Wegner, 2013). Most triploid oysters show gonadal atrophy during the breeding season, suggesting that triploid induction significantly reduces diploid fecundity. Notably, a positive correlation between reproductive effort and summer mortality in the *C. gigas* has been reported (Cotter et al., 2010; Huvet et al., 2010). Thus, triploids may provide a more stable energy reserve because of the absence of reproductive effort, thereby increasing tolerance to other stresses. However, the advantage of triploidy in survival may be lost when sterility is incomplete (Duchemin et al., 2007; Houssin et al., 2019; Melo et al., 2020). For instance, abnormally pronounced gonad development has been suggested as a factor contributing to the significantly higher summer mortality of triploids *C. gigas* in France (Houssin et al., 2019).

In this study, we found that the survival advantage of triploid was "site-dependent", and in Rongcheng, there was no significant difference in cumulative survival between triploids and diploids. Genotype × environment interactions are known to influence the success of bivalve aquaculture, so it is not surprising that triploid dominance is "site-dependent" and is frequently observed in many studies (Brake et al., 2004; Guévélou et al., 2019; Melo et al., 2020). Notably, further differences may arise from different genetic backgrounds (selected or control), even within a breeding program as these oysters have not been selected for triploid performance. Therefore, when conducting experiments to assess the effect of ploidy on production traits, it should be ensured that the genetic backgrounds of diploid and triploid oysters used for comparisons are as close as possible, as genotype × environment interactions may blur signals generated by ploidy differences (Dégremont et al., 2016; Brianik and Allam, 2023). In our study, the main advantage of chemically induced triploids is that they do not confound ploidy and germplasm factors.

This study also tested whether triploids have a growth advantage in commercial culture environments. Our results showed that the growth traits of triploid were significantly higher than those of diploid *C. gigas*, which is in agreement with the majority of studies on triploid oysters (Matthiessen and Davis, 1992; Nell, 2002; Zhang et al., 2017; Zhou et al., 2023). Although several explanations for the growth advantage of the triploid oyster have been offered, the mechanisms and reasons for the rapid growth of triploids remain unclear (Allen and Downing, 1986; Hawkins et al., 1994; Guo and Allen, 1994; Guo et al., 1996; Mallia et al., 2006). It is worth noting that a significant growth advantage for triploids was not observed in all studies.

The triploid advantage was not evident at sites where environmental conditions were unfavorable for oyster growth or where feeding practices increased (Barber and Mann, 1991; Stone et al., 2013). Our findings support current oyster culture practices and a number of hypotheses regarding the growth of triploid and diploid oysters. Not surprisingly, increased growth rates of triploid oysters are highly favorable as they result in higher meat yields and allow populations to reach market size months earlier (Dégremont et al., 2012; Wadsworth et al., 2019).

Yield was the result of survival and growth. Previous studies have shown that survival is the most important parameter in explaining yield variation (Dégremont et al., 2005; Freeman and Dickie, 1979). The growth advantage of triploids was observed at both sites, but there was no significant survival advantage at Rongcheng. Thus, the survival and growth patterns of the above groups resulted in significant differences in yield. Low survival may reduce yields as the number of surviving individuals becomes the limiting factor.

4.2. *Selected advantage on production traits*

Favorable selection responses for diploid survival performance have been well documented in *C. gigas* populations, typically exceeding 10% per generation (Dégremont et al., 2010; Dégremont et al., 2015; Divilov et al., 2021; Chi et al., 2022). However, the extent to which these genetic gains are maintained in triploid oysters remains largely unresolved. In this study, cumulative survival was much higher in both diploids and triploids when parents were selected for higher summer survival, suggesting that selective breeding advances for summer mortality resistance in diploid oysters could be transferred to improve summer survival rate in triploids. In another study, triploid oysters produced by crossing diploid females selected for resistance to summer mortality with tetraploid males had higher survival rates than that produced by unselected diploid females (Dégremont et al., 2012). In addition, similar results

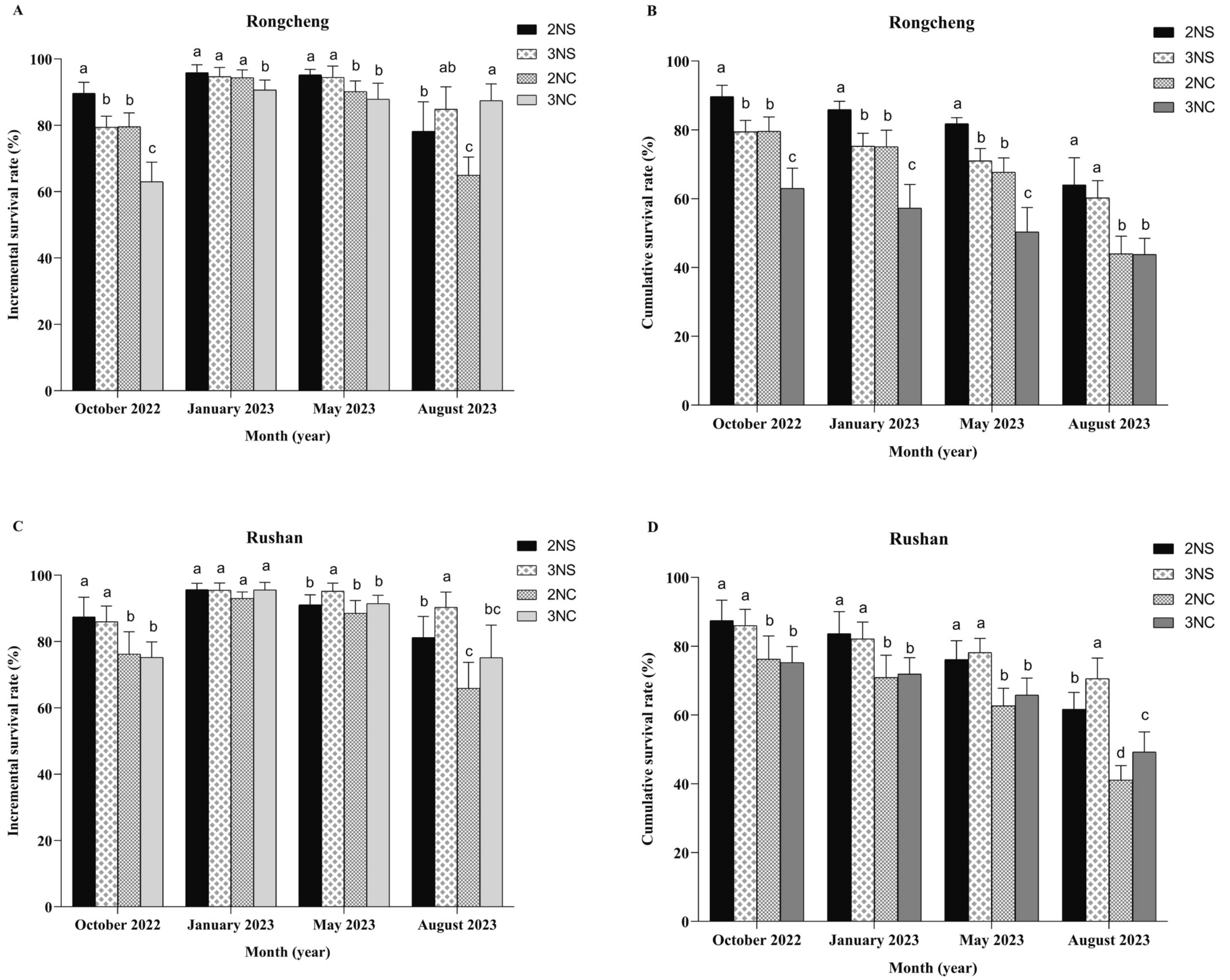

Fig. 5. Incremental survival rate and cumulative survival rate for four groups (2NS, 3NS, 2NC, 3NC) *C. gigas* at Rongcheng and Rushan from October 2022 to August 2023. Different superscript letters at the same time indicate significant difference ($P < 0.05$).

have been reported for resistance to pathogens in fish species (Dorson et al., 1991; Weber et al., 2013). Given that triploids cannot be used as broodstock, this is encouraging from a breeding perspective.

As with most aquaculture genetic improvement programs, growth performance is an important metric in oyster selective breeding programs. The selected families used in this study were derived from two fast-growing lines that were mass-selected for shell height for ten and eight generations, respectively, prior to being selected for summer survival (Chi et al., 2021). In this study, the growth rate of the selected line was significantly faster than that of the wild population regardless of ploidy. A study by Hand et al. (2004) showed that chemically induced triploids *Saccostrea glomerata* using an improved diploid line can substantially improve triploid growth compared to unselected line. Callam et al. (2016) recently provided more support for the selective advantage of triploid oysters as they found that diploid parents contributed significantly to the performance of triploid progeny. All of these studies suggest that selection of diploid parents is applicable to improving production traits associated with triploid progeny.

5. Conclusion

This study confirmed the significant growth advantage of triploid oysters over diploid oysters in terms of both shell height and individual weight, and provides support for the commercial production of triploid oysters in northern China. Notably, we found that the triploid survival advantage is "site-dependent" and that triploids have a significant survival advantage only in Rushan. In the long term, it is important to identify the root causes of triploid mortality and find solutions to limit triploid mortality at all sites. We also found that during the second summer, the incremental survival of triploids was consistently higher than that of diploid *C. gigas*, which may be related to physiological disorders caused by diploid gonadal development and spawning. Encouragingly, we found that the production performance of the selected group was consistently better than that of the control group, suggesting that improved traits can be transferred from desirable diploids to triploids. In conclusion, triploidization based on improved *C. giga* strains could further improve economically important traits and offer great potential for commercial oyster farming.

CRediT authorship contribution statement

Yong Chi: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing – original draft. **Chengxun Xu:** Conceptualization, Methodology, Resources, Supervision. **Qi**

Table 2

Three-way analyses of variance testing for the interaction effects of genotype, environment and ploidy on incremental survival, cumulative survival and yield. The P-value associated with each F-value are indicated by asterisks (* – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$; ns – not significant).

Day	Effect	Incremental survival		Cumulative survival		Yield	
		d.f.	F-value	d.f.	F-value	d.f.	F-value
October 2022	Genotype	1	98.259***	1	98.259***	–	–
	Environment	1	5.944**	1	5.944*	–	–
	Ploidy	1	34.949***	1	34.949***	–	–
	Genotype × Environment	1	0.202^{ns}	1	0.202^{ns}	–	–
	Genotype × Ploidy	1	0.092^{ns}	1	0.092^{ns}	–	–
	Environment ×Ploidy	1	21.262***	1	21.162***	–	–
	Genotype × Environment × Ploidy	1	0.489^{ns}	1	0.489^{ns}	–	–
January 2023	Genotype	1	11.245**	1	107.324***	–	–
	Environment	1	2.496^{ns}	1	7.675**	–	–
	Ploidy	1	0.766^{ns}	1	33.000***	–	–
	Genotype × Environment	1	1.240^{ns}	1	0.439^{ns}	–	–
	Genotype × Ploidy	1	0.092^{ns}	1	0.038^{ns}	–	–
	Environment ×Ploidy	1	9.324**	1	27.472***	–	–
	Genotype × Environment × Ploidy	1	4.579*	1	1.822^{ns}	–	–
May 2023	Genotype	1	35.080***	1	186.727***	–	–
	Environment	1	0.431^{ns}	1	5.417*	–	–
	Ploidy	1	2.441^{ns}	1	25.398***	–	–
	Genotype × Environment	1	2.770^{ns}	1	2.915^{ns}	–	–
	Genotype × Ploidy	1	1.108^{ns}	1	0.370^{ns}	–	–
	Environment ×Ploidy	1	9.873**	1	53.909***	–	–
	Genotype × Environment× Ploidy	1	0.102^{ns}	1	0.884^{ns}	–	–
August 2023	Genotype	1	33.666***	1	215.396***	1	579.327***
	Environment	1	0.098^{ns}	1	4.418*	1	87.342***
	Ploidy	1	49.349***	1	6.020*	1	94.156***
	Genotype × Environment	1	9.726**	1	1.388^{ns}	1	10.581**
	Genotype × Ploidy	1	3.031^{ns}	1	0.092^{ns}	1	2.454^{ns}
	Environment ×Ploidy	1	1.949^{ns}	1	16.874***	1	29.776***
	Genotype × Environment × Ploidy	1	6.632*	1	1.417^{ns}	1	0.013^{ns}

Table 3

Triploid advantage (TA, %) for shell height and individual weight in selection and wild populations during grow-out stages.

Population	Site	Shell height				Individual weight			
		Oct 2022	Jan 2023	May 2023	Aug 2023	Oct 2022	Jan 2023	May 2023	Aug 2023
Selection	Rongcheng	10.97	14.18	19.73	15.18	18.31	26.56	26.07	21.43
	Rushan	9.81	14.99	18.99	12.49	21.70	17.93	16.60	17.43
Wild	Rongcheng	11.18	11.91	13.50	16.44	28.07	29.36	18.18	13.20
	Rushan	7.13	9.80	13.66	22.62	12.40	22.34	20.24	33.85

Table 4

Triploid advantage (TA, %) for increment survival, cumulative survival and yield in selection and wild populations during grow-out stages.

Population	Site	Incremental survival rate				Cumulative survival	Yield
		Oct 2022	Jan 2023	May 2023	Aug 2023	Aug 2023	Aug 2023
Selection	Rongcheng	−11.40	−1.20	−0.84	8.54	−5.90	13.77
	Rushan	−1.65	−0.17	4.55	11.20	14.41	34.78
Wild	Rongcheng	−20.81	−3.96	−2.55	34.62	−0.51	12.69
	Rushan	−1.31	2.77	3.31	14.01	19.73	59.18

Table 5

Selected advantage (SA, %) for shell height and individual weight in diploid and triploid populations during grow-out stages.

Population	Site	Shell height				Individual weight			
		Oct 2022	Jan 2023	May 2023	Aug 2023	Oct 2022	Jan 2023	May 2023	Aug 2023
Diploid	Rongcheng	5.14	11.36	12.02	24.34	8.98	21.71	16.57	28.54
	Rushan	12.31	10.70	9.21	25.13	28.78	29.83	20.42	42.68
Triploid	Rongcheng	4.94	13.62	18.17	23.00	0.67	19.07	24.34	37.90
	Rushan	15.12	15.94	14.33	14.79	39.45	25.14	16.77	25.06

Table 6

Selected advantage (SA, %) for increment survival, cumulative survival and yield in diploid and triploid populations during grow-out stages.

Population	Site	Increment survival				Cumulative survival	Yield
		Oct 2022	Jan 2023	May 2023	Aug 2023	Aug 2023	Aug 2023
Diploid	Rongcheng	12.71	1.54	5.65	20.41	45.45	87.92
	Rushan	14.72	2.85	2.82	23.24	50.00	112.57
Triploid	Rongcheng	26.10	4.46	7.51	−2.92	37.56	89.72
	Rushan	14.33	−0.10	4.05	20.21	43.34	80.00

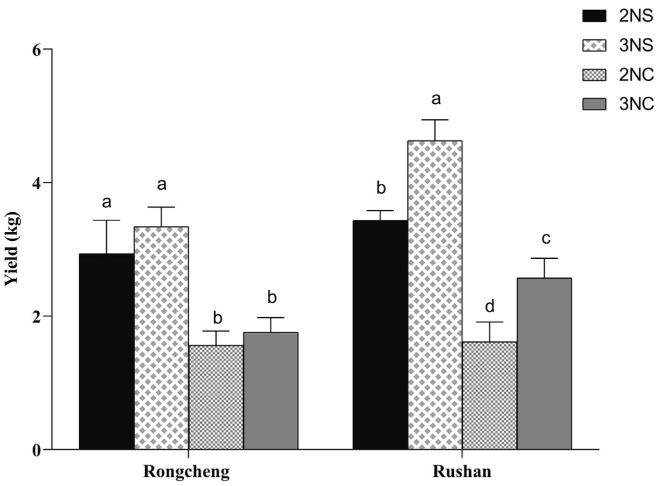

Fig. 6. Yield of four groups (2NS, 3NS, 2NC, 3NC) *C. gigas* at Rongcheng and Rushan in August 2023. Different superscript letters at the same time indicate significant difference ($P < 0.05$).

Li: Funding acquisition, Project administration, Supervision, Writing – review & editing.

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

Data will be made available on request.

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