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Growth, survival and thermotolerance of diploids, triploids and tetraploids of the Fujian oyster *Crassostrea angulata* with normal, golden and black shell colors

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ABSTRACT

Triploids have received increasing attention in the industrialization of oysters due to their fast growth rate, tolerance, and their sterility to be marketed year-round. Cultivating varieties or lines with special colors could be crucial for germplasm enrichment as well as traceability and intellectual property protection during the commercialization process. However, triploids and tetraploids with special colors have not been studied in the Fujian oyster *Crassostrea angulata*, which has seriously limited their commercialization and scalability. In this study, diploid and tetraploid *C. angulata* with normal, golden and black shell colors were used as parents and their corresponding triploids were constructed. The growth and survival of the nine groups were comprehensively evaluated during the larval and grow-out stages. Sex ratio, ploidy composition and thermotolerance were also statistically compared. All three triploid groups (NDT, GDT and BDT) exhibited significant advantages in growth and survival, compared to their diploid (NDD, GDD and BDD) and tetraploid (NTT, GTT and BTT) counterparts in all three sites. The triploid advantages in survival rate among NDT, GDT and BDT were 38.36%, 40.38% and 36.00%, respectively, at Jiaonan at day 480. Moreover, higher thermotolerances were exhibited in NDT, BDT and GDT, with the values of LT_{50} at 41.77 °C, 42.28 °C and 42.13 °C, respectively. On the contrary, the three tetraploid *C. angulata* lines (NTT, GTT and BTT) exhibited poor growth, survival and thermotolerances. The values of LT_{50} for GTT and NTT were comparable and lower than this of BTT (GTT: 39.85 °C; BTT: 41.05 °C; NTT: 39.57 °C). It is encouraging to note that the tetraploid rates also remained above 80% at day 480 in all three tetraploid lines. Based on our study, the three triploid *C. angulata* lines with normal, black, and golden shell colors showed great potential and can be industrialized as new varieties to improve the oyster industry. However, the inferior performance of tetraploids should not be overlooked and demands extra concern in the future breeding process.

1. Introduction

In recent years, polyploid aquatic animals have developed rapidly, making tremendous impacts on the aquaculture industry and generating huge profits. For the oyster, triploidization was successfully achieved for the first time in American oyster *Crassostrea virginica* through the suppression of the emission of polar bodies from diploid fertilized eggs by cytochalasin B (Stanley et al., 1981). Subsequently, the successful breeding of viable tetraploids made the production of 100% mated triploids (obtained by crossing between female diploids and male tetraploids) turn from an idea into a reality (Guo and Allen, 1994b; Guo, 1991). Compared to diploids, triploids showed faster growth rates and improved stress resistance (Nell, 2002; Guo et al., 1996; Piferrer et al., 2009; Wadsworth et al., 2019b). In addition, the sterility of triploids allows them to be marketed year-round and maintain favorable taste during the summer (Allen and Downing, 1986; Liang et al., 2023). Furthermore, it has been reported that the summer mass mortality of diploid oysters can be linked to their higher reproductive effort (Cowan et al., 2023; Cotter et al., 2010; Samain et al., 2007; Callam et al., 2016; Piferrer et al., 2009; Guo and Allen, 1994a). Triploids do not suffer from these defects due to a decrease of gonadal development (Piferrer et al., 2009). Therefore, breeding triploid oysters might offer the possibility to solve the mass mortality of oysters in summer. To date, the industrialized triploid oysters include *C. gigas* (Zhou et al., 2023), *C. virginica*

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(Walton et al., 2013; Callam et al., 2016; Bodenstein et al., 2023), and *C. angulata* (Zhang et al., 2022; Liang et al., 2023).

Environmental factors have significant impacts on the growth, survival, resistance and other aquaculture characteristics of mollusks (Swan et al., 2007). *C. gigas* has significant variability in growth or survival rate among different farming sites (Swan et al., 2007; Dégremont et al., 2005; Liang et al., 2022). Significant genotype by environment (G × E) interactions have also been reported in the genetic improvement project of *C. gigas* in the USA (Langdon et al., 2003). Although subsequent studies have revealed that G × E interactions for body weight, survival and yield were all minor, they were statistically significant in the total variance (Evans and Langdon, 2006). Significant effects of G × E effects on growth and/or survival were also observed in the studies of the American oyster *C. virginica* and the Hong Kong oyster *C. hongkongensis* (Mallet and Haley, 1983; Zhang et al., 2017a). Thus, to fully utilize the production potential of the oysters, it is necessary to find the optimal sites for their cultivation.

Oysters are economically valuable marine species in China. Four species of oysters in the genus *Crassostrea*, namely *C. angulata*, *C. hongkongensis*, *C. gigas*, *C. ariakensis*, were naturally distributed along the long coastline of China (Li et al., 2022). Of these, the Fujian oyster *C. angulata* has been cultivated mainly in southern China (Fujian, Guangdong and Guangxi provinces). Its annual production is higher than that of other farmed oyster species, with 2.13 million tonnes of oysters produced annually in 2022, accounting for 34% of China's total oyster production (BOF (Bureau of Fisheries), 2023). Although the yield of this oyster is higher than that of other species, its disadvantages such as small size and bland flavor seriously affect its market value. In addition, the diploid *C. angulata* can be fertile all year round, which severely compromises its growth as well as its market availability. Therefore, development of triploid *C. angulata* may be an effective way to improve its quality. In our previous work, we have successfully bred tetraploid *C. angulata* (Liang et al., 2023) and the genetic improvement program for the tetraploid and diploid lines of *C. angulata* with normal, golden and black shell colors was initiated in 2022. However, the aquaculture traits (growth, survival, sex composition, etc.) of diploids, tetraploids and their mated triploids with normal, golden and black shell colors of *C. angulata* have not been systematically studied, which severely limits their industrialization. Moreover, cultivating strains with special colors helps to increase species diversity, helps to protect intellectual property rights, and helps to distinguish the strain from other strains to avoid mixing.

In this study, the growth, survival, ploidy stability and sex composition of diploid, triploid and tetraploid *C. angulata* with normal, golden and black shell colors were systematically compared; and the effects of environments, ploidies, populations and their interactions on the performance of *C. angulata* were comprehensively analyzed. In addition, the thermotolerance of *C. angulata* and *C. gigas* was thoroughly compared. This study was expected to provide supporting fundamental data for investigating the breeding potential of different ploidy of *C. angulata* with different shell colors.

2. Materials and methods

2.1. Parental oyster collecting and rearing

In March 2022, the one-year-old diploids and tetraploids *C. angulata* with normal, golden, and black shell colors were collected from Jiaonan, Shandong Province, China (335.88°N, 119.97°E). In this study, strains that did not undergo directional selection for shell color, with or without heterochromatic stripes, were defined as oysters with normal shell color. Oysters with solid golden or black colors on both the right and left shells were defined as the golden or black shell lines (Fig. 1). Tetraploids were produced by inhibiting the release of the first polar body (PB1) in fertilization eggs from female triploids and male diploids in 2021 (Liang et al., 2023). Mass selection for all diploids and tetraploids lines with normal, golden, and black shell colors started from 2022 (Fig. 2). These parental oysters were then transported to an oyster hatchery in Yantai, Shandong Province (37.3°N, 119.9°E). All groups were temporarily maintained in conditioned water (temperature: 24.0 ± 2.0 °C; salinity: 28.0 ± 2.0 psu) until gonadal maturation.

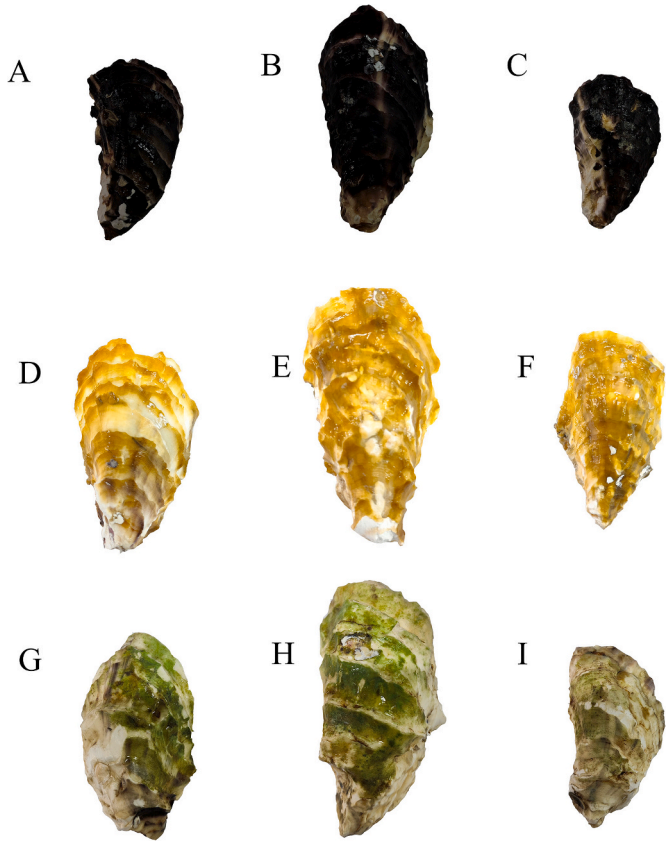

Fig. 1. Phenotypes of each group of *C. angulata* at day 480. Note: A, B and C indicate BDD, BDT and BTT, respectively. D, E, and F indicate GDD, GDT and GTT, respectively. G, H and I indicate NDD, NDT and NTT, respectively.

2.2. Mating strategies and artificial insemination

Experimental parents with well-developed gonads were selected based on shell height and shell color. Diploids and tetraploids *C. angulata* with normal shell color were opened and the sexes were identified under a light microscope. Subsequently, the ploidy of gills and hemolymph from each tetraploid were verified by flow cytometry (FCM) to identify the true tetraploids (Allen, 1983; Li and Li, 2022). Only individuals with tetraploid ploidy for gills and hemolymph were retained. Eggs from each female diploid or tetraploid were collected separately and matured in 5-L beakers for approximately 40 min. Once maturation was complete, the eggs were divided into two portions evenly. Artificial insemination was accomplished by the following methods: (1) Eggs from diploids were fertilized with sperm from diploids and tetraploids to produce the NDD (normal diploid ♀ × normal diploid ♂) and NDT (normal diploid ♀ × normal tetraploid ♂) groups, respectively; (2) Eggs from tetraploids were fertilized with sperm from tetraploids to generate the NTT (normal tetraploid ♀ × normal tetraploid ♂) group. The diploid, tetraploid and triploid *C. angulata* with the golden and black shell colors was generated in the same manner. For oysters with the golden shell color, GDD (golden diploid ♀ × golden diploid ♂), GDT (golden diploid ♀ × golden tetraploid ♂) and GTT (golden tetraploid ♀ × golden tetraploid ♂) were generated. For oysters with the black shell color, BDD (black diploid ♀ × black diploid ♂), BDT (black diploid ♀ × black tetraploid ♂) and BTT (black tetraploid ♀ × black tetraploid ♂) were generated. During

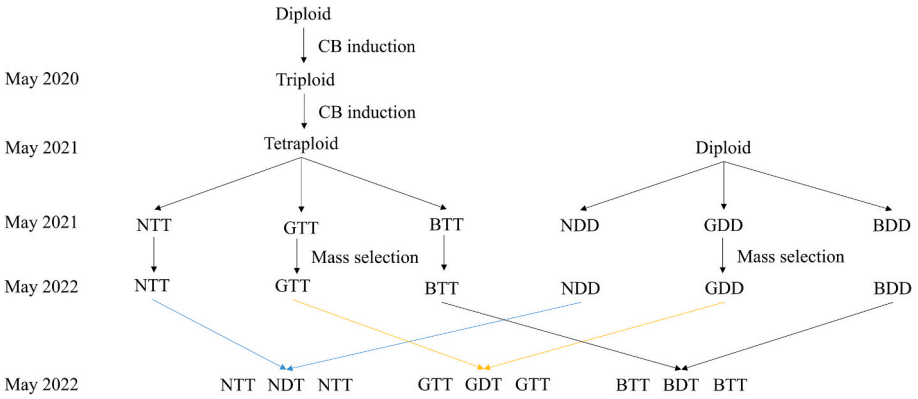

Fig. 2. The graphic represents the parental origin and mating strategy in this study.

fertilization, the appropriate number of sperm was added to the eggs, with 8–10 sperm retained around each egg. Each group was set up with three biological replicates, each replicate was completed using three males and three females for a single pair of fertilization.

2.3. Larvae and juvenile rearing

Larvae and juveniles were reared by referring to the method described by Li et al. (2011). Briefly, when fertilization was completed, each group of fertilized eggs was transferred separately to 100 L polyethylene plastic buckets for incubation. After a 24-h incubation, the larvae were selected with 300 mesh sieves and reared in conditioned water set at a temperature of 25.0 ± 0.5 °C and salinity of 30.0 ± 0.5 psu. The initial density of larvae was regulated to 2 larvae per ml and gradually reduced to 0.5 larvae per ml with larval growth. The larvae were fed with *Isochrysis galbana* and proportionate *Platymonas* sp. was progressively increased at later stage. When 30% of the larvae developed eye spots, clean oyster shells were threaded and hung in buckets for larvae to attach and metamorphose. Once the attachment was completed, the spats were transferred to an outdoor pond for 2–3 weeks to prevent wild oyster contamination. All groups were transferred to three farming areas in Shandong Province in July 2022: Rongcheng (37.11°N, 122.35°E), Rushan (36.45°N, 121.42°E) and Jiaonan (35.35°N, 119.30°E). In October 2022, each group was then placed into 10 layers of lantern nets with 30 oysters in each layer.

2.4. Measurement of phenotype-related parameters

During the larval stage, the shell height of 30 individuals per group was photographed with a microscope (Olympus BX53) and measured with image analysis software (image - pro plus 6.0) at days 1, 5, 10, 15, 20 and 25 after fertilization. The larval survival rate was computed as the proportion of surviving larvae on the sampling day to the total number of larvae in the D-larval stage (Liang et al., 2022).

A random sample of 30 oysters from each replicate was selected to assess growth parameters (shell height and wet weight) on days 120, 240, 360 and 480 during the grow-out stage. The shell height was measured with vernier calipers (accuracy of 0.01 mm) and the wet weight was monitored with an electronic scale (accuracy of 0.01 g).

The survival rate of juveniles was calculated as the proportional difference between the living oysters at time T compared to the living oysters at time 0.

$SR_T\ (\%) = N_{(T)} \times 100 / N_0$.

Here, SR_T denotes the survival rate of each group at time T; $N_{(T)}$ represents the total number of living oysters in each lantern net at time T; and N_0 is the total number of oysters in each lantern net for each group in October 2022.

2.5. Comparative analysis of gonadal development and sex composition

One hundred oysters were randomly sampled from each group and ploidy was determined on days 120, 240, 360 and 480. To ensure the reliability of the determinations of ploidy, repeat tests were carried out on tetraploid gills and hemolymph (Li and Li, 2022).

Two hundred individuals in each group were sampled for comparative analysis of gonadal development and sex composition in July 2023. The gonadal tissue of each oyster was fixed in Bouin's solution for 24 h and then preserved in 70% ethanol. Afterward, the sections were dehydrated, paraffin-embedded, 5-μm sectioned and stained with hematoxylin. The sections were observed and photographed using an Olympus BX53 microscope (Olympus, Japan). On the basis of the type of germ cells, oysters are classified as male, female, hermaphrodite and undeveloped asexual.

2.6. Thermotolerance

Diploid, triploid and tetraploid *C. angulata* with normal, black and golden shell colors and *C. gigas* with normal shell colors were gathered for temperature tolerance experiments at day 420. Acute heat treatment was used to determine the thermotolerance range and LT_{50} among different groups (Ghaffari et al., 2019). The experiments were carried out in 100 L polyethylene plastic buckets. The temperature ranges (35–45 °C) were determined based on previous experiments and seawater temperature fluctuations in Fujian and Shandong provinces (Ghaffari et al., 2019; Jiang et al., 2021). Each group was gradually raised to the target temperature (from 35 °C to 45 °C with a gradient of 0.5 °C) using electric heaters (accuracy of 0.1 °C) at a rate of 0.5 °C/h with different initiation times. Therefore, the groups simultaneously reached the preset temperature. The oysters in each group were treated at the target temperature for 1 h and then were transferred to seawater at 25 °C for 10 days. During this period, their mortality was recorded. Oysters were considered dead if they did not close after being exposed to external stimuli. The water temperature of the control group was set at 25 °C and no oysters died during the experiment. One hundred oysters were included in each group. In this study, the temperature at which all experimental animals survived (100% survived) was defined as the sublethal temperature. The temperature at which all experimental animals died (100% died) was the lethal temperature. And LT_{50} was calculated by probit regression prediction (Ghaffari et al., 2019; Jiang et al., 2021). The LT_{50} of each line was compared to judge the temperature tolerance of each line, with higher LT_{50} values representing higher temperature tolerance.

2.7. Statistical analyses

Data are presented as mean ± standard deviation (SD). Growth parameters (shell height and wet weight) were log-transformed on a base

of 10 and the survival rate for each group was arcsine transformed to improve normality and homoscedasticity. Differences in growth or survival between groups at the same site were compared by one-way ANOVA and Tukey's test for multiple comparisons in SPSS 26.0. Significance was set at $P < 0.05$.

To determine the effects of the ploidies (diploids vs. triploids vs. tetraploids), environmental factors (Rongcheng vs. Rushan vs. Jiaonan) and populations (normal shell line vs. black shell line vs. golden shell line) on the characteristics of the *C. angulata* during the grow-out stage, a three-factor ANOVA was carried out.

To assess the phenotypic traits of triploids, the mid-parental heterosis was calculated through the following equation (Hallauer et al., 2010).

M (%) = [(TR - MP) × 100] / MP.

Where TR and MP represent the mean values of aquatic traits (shell height, wet weight or survival) for the triploids (NDT, GDT or BDT) and purebred groups (diploids: NDD, GDD or BDD; tetraploids: NTT, GTT or BTT) respectively. M indicates the mid-parent heterosis of triploids.

To further investigate the profits in phenotype of the triploids compared with diploids, the triploid advantage rate (T) was calculated using the following model adapted from Wang et al. (2011):

T (%) = (M_{DT} - M_{DD}) × 100 / M_{DD}.

Here, M_{DT} is the mean phenotypic value (shell height, survival rate or wet weight) of the triploids (NDT, GDT or BDT); M_{DD} indicates the mean phenotypic value of the diploid counterparts (NDD, GDD or BDD). T is the triploid advantage rate of the triploids (NDT, GDT or BDT).

3. Results

3.1. Growth, survival and ploidy variations during the larval stage

After a 24-h hatching duration, the shell heights of tetraploid and triploid D-larvae were significantly greater than those of diploids for *C. angulata* with normal, black and golden shell colors Fig. 3). The shell height of GTT were significantly larger than those of GDD and GDT from day 1 to day 15. GDT exhibited triploid advantages from day 15, with values of triploid advantage T_{GDT} from 8.59% to 22.11% (Table 1). Moreover, NDT and BDT larvae were significantly larger than their diploid and tetraploid counterparts, with middle-parent heterosis for shell height at 26.34% and 17.47%, respectively.

All three triploid groups exhibited higher survival rate throughout the whole larval stage except for GDT at day 5 (Fig. 3). The triploid advantages for NDT, BDT and GDT were 75.72%, 77.78% and 70.73%, respectively. In addition, the survival rate of three tetraploid groups decreased dramatically with larval growth, with values at 34.46%, 33.00% and 35.00% for NTT, BTT and GTT, at day 25.

No significant variations existed among the ploidy composition of these groups during the larval stage (Table 2). The DNA content examination showed NDD, BDD and GDD were 100% diploids, and NDT, BDT and GDT showed 100% triploids in the whole larval stage. The ploidy composition of NTT, BTT and GTT were changed in the eyed-stage, with tetraploid rates at 99%, 95% and 98%, respectively.

3.2. Growth, survival and ploidy variations at three sites

Shell heights and wet weights of diploids, triploids and tetraploids of *C. angulata* were significantly different at three sites regardless of shell color (Table 3). The growth tendencies of all groups at the three sites were: Jiaonan > Rushan > Rongcheng (Figs. 4–6). Triploid groups were significantly larger than their diploid and tetraploid parents ($P < 0.05$). The middle-parent heterosis of shell height and wet weight for BDT were 27.83% and 63.22%, respectively, at Rushan (Fig. 5; Table 4).

Significant triploid advantages were detected in the survival rate for NDD, BDD and GDD (Table 4). The survival rate for NDD, BDD and GDD at day 480 were 60.62%, 73% and 68%, respectively, at Jiaonan. And the triploid advantages for these three groups were 38.36%, 40.38% and 36%, respectively. However, the survival rate of the tetraploid group (NTT, BTT and GTT) was not so favorable. The survival rate of NTT was only 33.89% in Rongcheng at day 480.

The DNA content of diploids and triploids did not undergo any changes from the larval stage to the grow-out stage according to flow cytometry (Table 2). NDD, BDD and GDD were all 100% diploids, and NDT, BDT, and GDT were all 100% triploids during the whole life stage. The ploidy composition of NTT was 83% for tetraploids, 7% for diploids, 7% for triploids and 3% for aneuploids at day 480. As for BTT, the ploidy was composed by 82% tetraploids, 3% diploids, 10% triploids and 5%

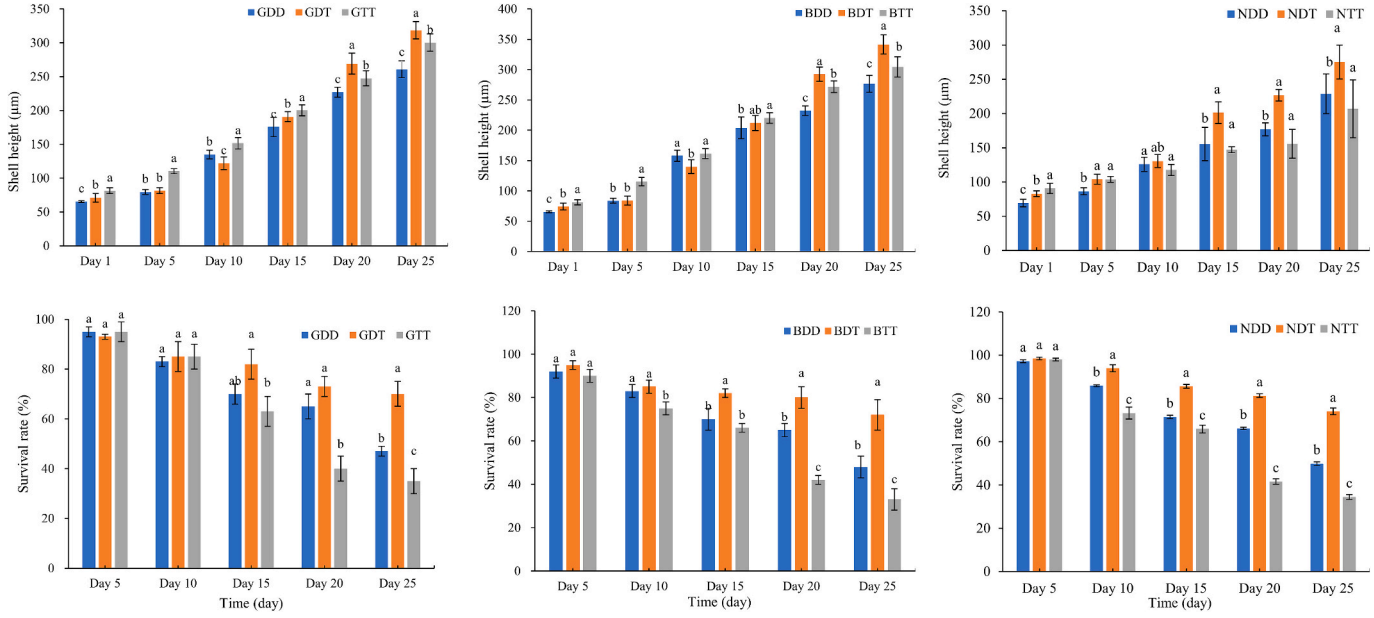

Fig. 3. The shell height and survival rate for the nine experimental groups of *C. angulata* at the larval stage. GDD, GDT and GTT indicate diploid, triploid and tetraploid *C. angulata* with golden shell color, respectively. BDD, BDT and BTT indicate diploid, triploid and tetraploid *C. angulata* with black shell color, respectively. NDD, NDT and NTT indicate diploid, triploid and tetraploid *C. angulata* with normal shell color, respectively. Different superscript letters at the same time indicate significant difference ($P < 0.05$).

Table 1
The middle-parent heterosis (M) and triploid advantage (T) for shell height, wet weight and survival rate for the three triploid lines (NDT, BDT and GDT) during the larval stage.

Item	Shell height						Survival rate				
	Day 1	Day 5	Day 10	Day 15	Day 20	Day 25	Day 5	Day 10	Day 15	Day 20	Day 25
M_{NDT}	19.18	9.35	7.35	32.97	36.3	26.34	0.91	18.05	24.62	50.86	75.72
T_{NDT}	30.75	20.42	3.85	29.39	28.08	20.25	1.27	9.36	19.81	22.86	48.57
M_{BDT}	1.24	−15.44	−12.35	−0.01	16.01	17.47	4.40	7.59	20.59	49.53	77.78
T_{BDT}	13.50	0.52	−11.40	3.96	25.89	23.39	3.26	2.41	17.14	23.08	50.00
M_{GDT}	−3.26	−14.04	−14.84	1.51	13.45	13.55	−2.11	1.19	23.31	39.05	70.73
T_{GDT}	8.59	2.59	−9.55	8.59	18.57	22.11	−2.11	2.41	17.14	12.31	48.94

Table 2
The ploidy variations for the diploid, triploid and tetraploid *C. angulata* with normal, black, and golden shell colors at different phases.

		Day 1	Day 25	Day 120	Day 240	Day 360	Day 480
NDD	2 N	100%	100%	100%	100%	100%	100%
	3 N	/	/	/	/	/	/
	4 N	/	/	/	/	/	/
	AN	/	/	/	/	/	/
NDT	2 N	/	/	/	/	/	/
	3 N	100%	100%	100%	100%	100%	100%
	4 N	/	/	/	/	/	/
	AN	/	/	/	/	/	/
NTT	2 N	/	/	2%	3%	3%	7%
	3 N	/	1%	2%	3%	7%	7%
	4 N	100%	99%	96%	92%	90%	83%
	AN	/	/	/	2%	/	3%
BDD	2 N	100%	100%	100%	100%	100%	100%
	3 N	/	/	/	/	/	/
	4 N	/	/	/	/	/	/
	AN	/	/	/	/	/	/
BDT	2 N	/	/	/	/	/	/
	3 N	100%	100%	100%	100%	100%	100%
	4 N	/	/	/	/	/	/
	AN	/	/	/	/	/	/
BTT	2 N	/	2%	1%	3%	2%	3%
	3 N	/	3%	1%	5%	3%	10%
	4 N	100%	95%	95%	91%	87%	82%
	AN	/	/	3%	1%	8%	5%
GDD	2 N	100%	100%	100%	100%	100%	100%
	3 N	/	/	/	/	/	/
	4 N	/	/	/	/	/	/
	AN	/	/	/	/	/	/
GDT	2 N	/	/	/	/	/	/
	3 N	100%	100%	100%	100%	100%	100%
	4 N	/	/	/	/	/	/
	AN	/	/	/	/	/	/
GTT	2 N	/	1%	/	/	2%	9%
	3 N	/	/	3%	10%	11%	6%
	4 N	100%	98%	95%	90%	86%	84%
	AN	/	1%	2%	/	1%	1%

2 N, 3 N, 4 N and AN indicate diploids, triploids, tetraploids and aneuploids, respectively.

aneuploids, respectively. Moreover, the tetraploid rate of 84% was achieved for GTT at day 480.

3.3. Sex composition

Gonadal identification demonstrated that the female-to-male ratio in NDD was close to 1:1 (Table 5). However, the sex ratio was not well equalized in the other groups. For tetraploids, the proportions of males were 75%, 72% and 81% for NTT, BTT and GTT, respectively. The NDT sex consisted of 35% females, 5% males, 5% hermaphrodites and 55% asexual individuals, while BDT sex consisted of 38% females, 8% males, 1% hermaphrodites and 53% asexual individuals. As for GDT, the ratio among females: males: hermaphrodites: asexual individuals were 48%: 2%: 2%: 48%. Moreover, of a total of 600 triploids of *C. angulata*, only 90 (15%) were identified as being in gametogenesis (with a large number of observable gametes). Although some triploid oysters were able to generate a few gametes, with the exception of tetraploids produced by crossing triploid and diploid, most of these gametes were useless spermatozoa.

3.4. Comparison of temperature tolerance

No oysters were dead at 35 °C in all groups. When exceeding 45 °C, oysters in all groups had a mortality rate of 100%. Therefore, the LT_{50} was determined for all groups in this experiment within the temperature ranges of between 35 °C and 45 °C. No oyster mortality was observed when exposed to temperatures below 37.5 °C, in the DD, DT, and NDD (Table 6). The lethal temperatures for GDT, BDT and NDT groups were higher than this in DT, with values at 41.50 °C, 44.00 °C, 44.00 °C and 43.5 °C for DT, GDT, BDT and NDT, respectively. Moreover, the lethal temperature for BTT was highest among the four tetraploid groups (TT: 41.00 °C; GTT: 43.00 °C; BTT: 44.00 °C; NTT: 43.00 °C). The probit regression predictions showed that the LT_{50} of the triploid groups were all higher than their corresponding diploid and tetraploid parents with values of LT_{50} at 42.13 °C, 42.28 °C and 41.77 °C for GDT, BDT and NDT, respectively. Compared to diploids and triploids, tetraploids showed inferior temperature tolerance. The values of LT_{50} for GTT and NTT were comparable and lower than this of BTT (GTT: 39.85 °C; BTT: 41.05 °C; NTT: 39.57 °C).

4. Discussion

4.1. Effect of ploidy on characteristics of C. angulata

Polyploids of fish and shellfish have received increasing attention from farmers and scholars in recent years, and some triploid shellfish have been successfully used in production. In this study, the triploid *C. angulata* significantly outperformed their diploid and tetraploid counterparts in traits such as shell height, living weight and/or survival. The superior performance of triploids over diploids and tetraploids was prevalent in shellfish. Compared to the diploid *C. virginica*, triploids exhibited higher shell height and dry tissue weights, and oyster farmers were expected to benefit from rearing triploid oysters in the USA (Walton et al., 2013; Callam et al., 2016). The triploids also exhibited higher growth rates compared to the diploids in a Mexico study of *C. virginica* (Wadsworth et al., 2019a). In addition, recent work on other oyster species also shown that triploids exhibited superior aquatic characteristics than their diploid and/or tetraploid parents, such as *C. gigas* (Allen and Downing, 1986; Guo et al., 1996; Nell, 2002; Zhou et al., 2023), *C. sikamea* (Wu et al., 2019) and *C. hongkongensis* (Zhang et al., 2017b; Qin et al., 2019). On average, the mated triploids grew 20% faster than diploids in terms of shell height and 49% faster in terms of whole wet weight (Wadsworth et al., 2019b). The outstanding performance of triploids in this study may be due to three factors: firstly, due to the partial sterility of triploids, which allows their energy to be reallocated from gonadal development to somatic cell growth; secondly, the gigantism of triploid cells; and the hybrid vigor caused by the overall

Table 3
The effects of the ploidies (diploids vs. triploids vs. tetraploids), environmental factors (Rongcheng vs. Rushan vs. Jiaonan) and populations (normal shell line vs. black shell line vs. golden shell line) on the characteristics of the *C. angulata* during the grow-out stage.

Day	Items	d.f.	Shell height			Wet weight			Survival rate		
			MS	F-value	*P*-value	MS	F-value	P-value	MS	F-value	P-value
Day 120	E	2	17,961.448	499.021	<0.001	2507.446	130.447	<0.001	/	/	/
Day 240		2	19,018.509	248.960	<0.001	12,689.691	200.982	<0.001	65.812	9.006	<0.001
Day 360		2	27,315.133	261.755	<0.001	20,484.056	118.046	<0.001	136.821	17.923	<0.001
Day 480		2	47,532.941	459.677	<0.001	30,427.643	143.873	<0.001	240.315	30.342	<0.001
Day 120	P	2	10,074.583	279.901	<0.001	1431.374	74.466	<0.001	/	/	/
Day 240		2	47,296.606	619.131	<0.001	20,249.801	320.720	<0.001	1795.631	245.724	<0.001
Day 360		2	66,038.862	632.835	<0.001	52,359.603	301.738	<0.001	3227.446	422.789	<0.001
Day 480		2	53,677.455	519.099	<0.001	59,694.156	282.256	<0.001	6570.905	829.647	<0.001
Day 120	Po	2	4799.895	133.355	<0.001	1529.677	79.580	<0.001	/	/	/
Day 240		2	8215.156	107.540	<0.001	7081.233	112.154	<0.001	520.753	71.263	<0.001
Day 360		2	49,250.757	471.959	<0.001	49,366.433	284.489	<0.001	178.068	23.326	<0.001
Day 480		2	28,941.714	279.887	<0.001	47,220.047	223.274	<0.001	244.820	30.911	<0.001
Day 120	E * P	4	566.711	15.745	<0.001	144.093	7.496	<0.001	/	/	/
Day 240		4	1367.758	17.904	<0.001	822.257	13.023	<0.001	27.717	3.793	0.009
Day 360		4	533.584	5.113	<0.001	1110.152	6.398	<0.001	98.628	12.920	<0.001
Day 480		4	244.785	2.367	**0.051**	2684.165	12.692	<0.001	30.924	3.904	<0.001
Day 120	E * Po	4	8564.288	237.941	<0.001	1391.632	72.398	<0.001	/	/	/
Day 240		4	24,377.816	319.115	<0.001	8949.928	141.751	<0.001	84.521	11.566	<0.001
Day 360		4	11,832.827	113.391	<0.001	11,503.548	66.293	<0.001	122.984	16.111	<0.001
Day 480		4	7612.559	73.619	<0.001	4971.113	23.505	<0.001	102.755	12.974	<0.001
Day 120	P * Po	4	215.721	5.993	<0.001	480.412	24.993	<0.001	/	/	/
Day 240		4	1411.411	18.476	<0.001	825.784	13.079	<0.001	99.416	13.605	<0.001
Day 360		4	2230.117	21.371	<0.001	2791.055	16.084	<0.001	123.502	16.178	<0.001
Day 480		4	404.721	3.914	0.004	1131.149	5.348	<0.001	11.417	1.441	**0.233**
Day 120	E * P * Po	8	447.686	12.438	<0.001	844.125	43.915	<0.001	/	/	/
Day 240		8	2942.597	38.520	<0.001	1075.438	17.033	<0.001	44.604	6.104	<0.001
Day 360		8	798.584	7.653	<0.001	629.671	3.629	<0.001	100.103	13.113	<0.001
Day 480		8	134.208	1.298	**0.241**	1903.101	8.999	<0.001	8.672	1.095	**0.381**

E, P and Po indicate environments, ploidies and populations, respectively. Bolded figures are stated as having no significant effect ($P > 0.05$).

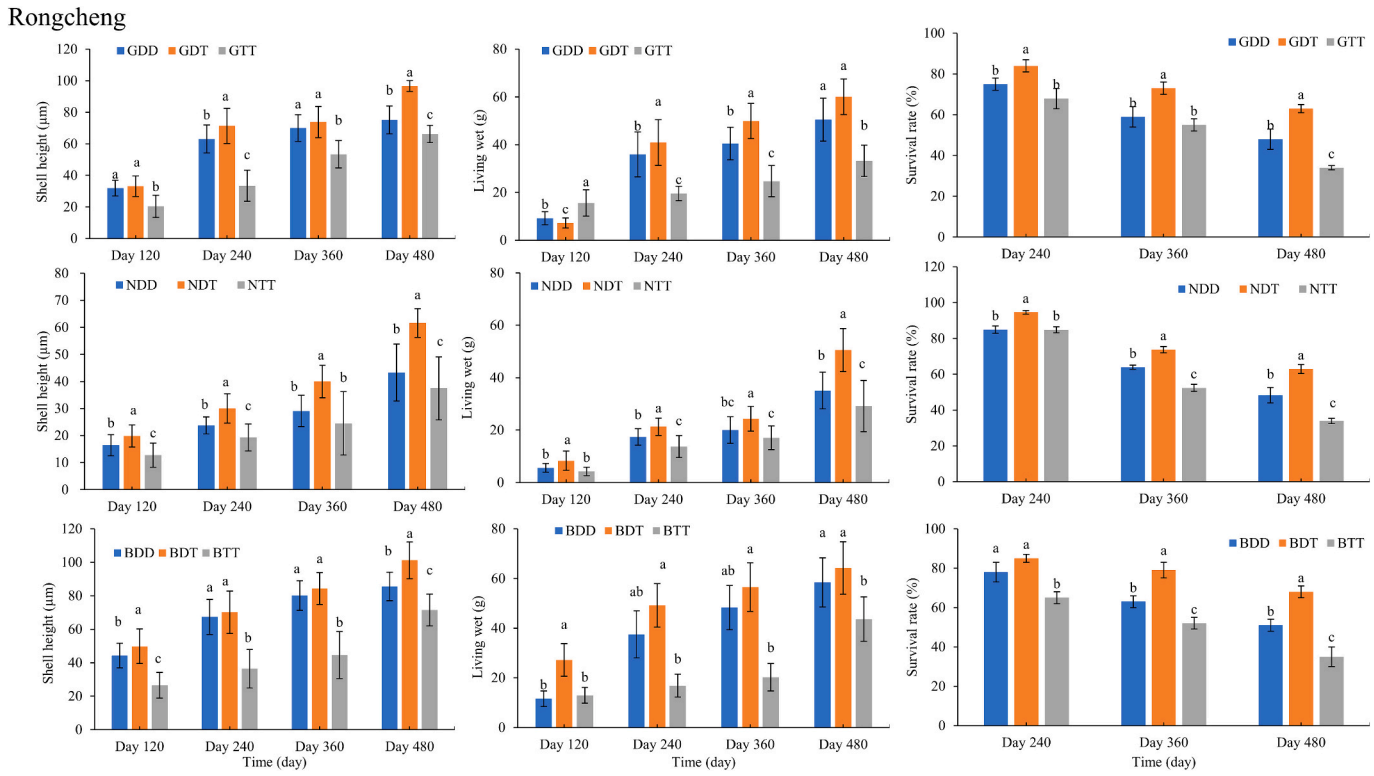

Fig. 4. The shell height, wet weight and survival rate for the nine groups of *C. angulata* in Rongcheng during the grow-out stage. Different superscript letters at the same time indicate significant difference ($P < 0.05$).

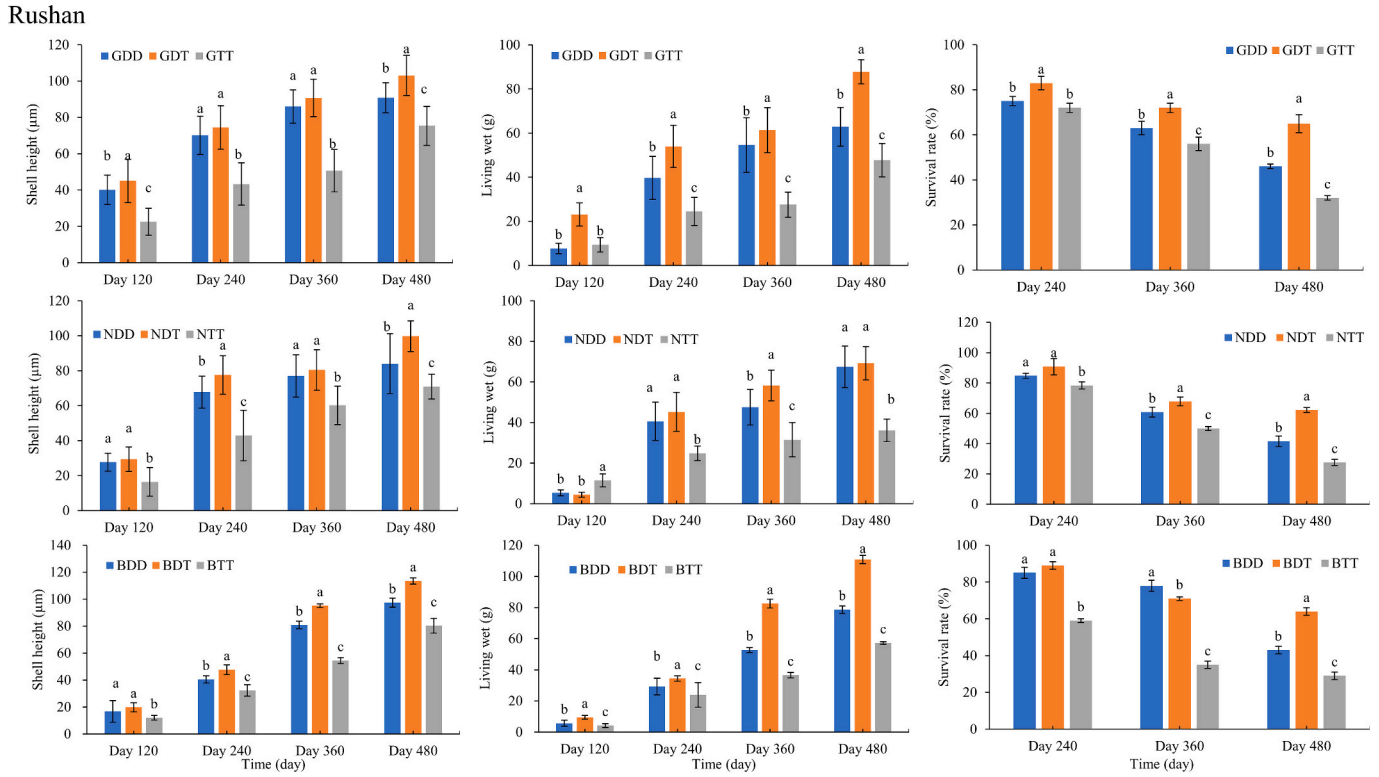

Fig. 5. The shell height, wet weight and survival rate for the nine groups of *C. angulata* in Rushan during the grow-out stage. Different superscript letters at the same time indicate significant difference ($P < 0.05$).

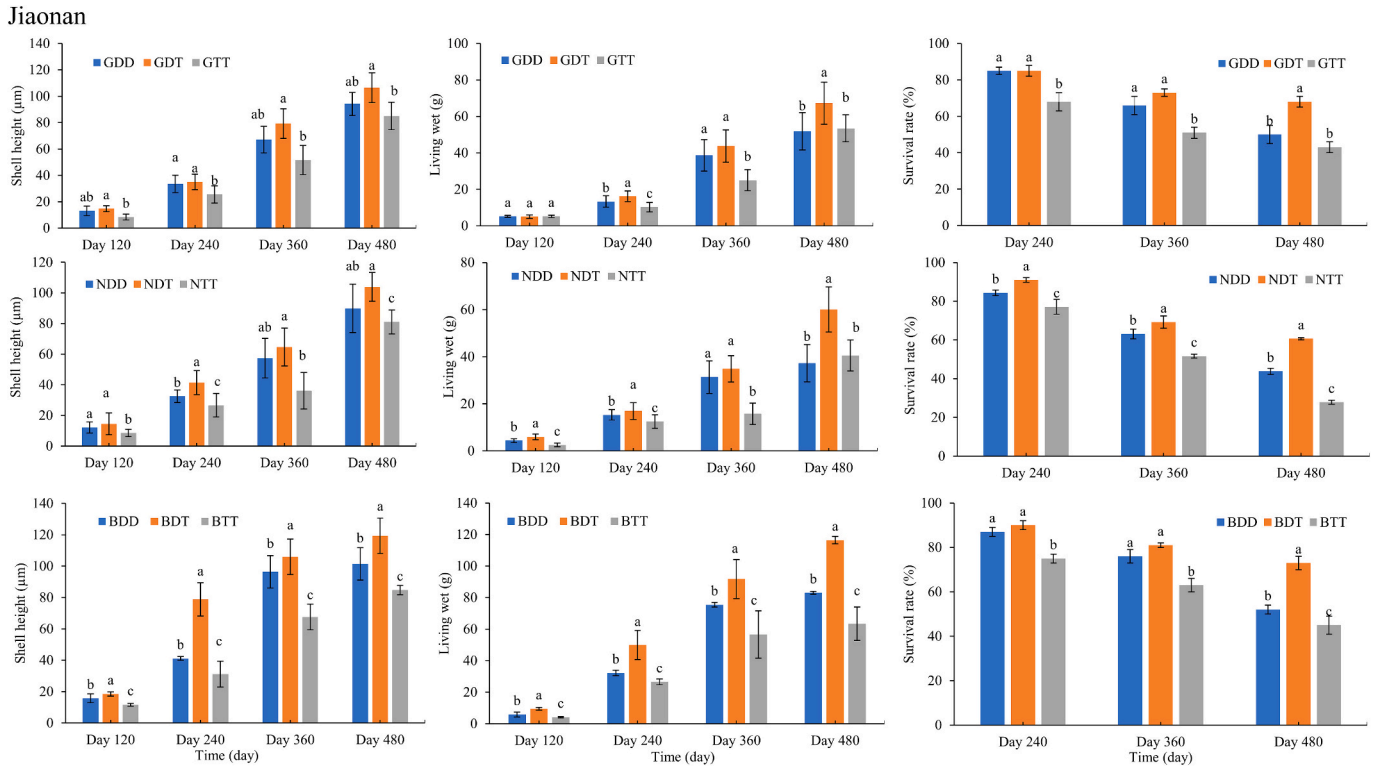

Fig. 6. The shell height, wet weight and survival rate for the nine groups of *C. angulata* in Jiaonan during the grow-out stage. Different superscript letters at the same time indicate significant difference ($P < 0.05$).

Table 4
The middle-parent heterosis (M) and triploid advantage (T) for shell height, wet weight and survival rate for the three triploid lines (NDT, BDT and GDT) during the grow-out stage.

Sites	Items	Shell height				Wet weight				Survival rate		
		Day 120	Day 240	Day 360	Day 480	Day 120	Day 240	Day 360	Day 480	Day 240	Day 360	Day 480
Rushan	M_{NDT}	33.35	40.22	17.16	28.73	−47.69	38.36	47.21	33.62	11.31	22.44	80.36
	T_{NDT}	6.18	14.54	4.34	18.66	−17.72	11.47	22.29	2.56	7.07	11.54	50.02
	M_{BDT}	37.62	31.05	40.71	27.83	91.5	29.6	84.82	63.22	23.61	25.66	77.78
	T_{BDT}	18.01	17.78	17.74	16.65	66.03	17.72	56.47	40.99	4.71	−8.97	48.84
	M_{GDT}	43.69	31.29	32.73	24.00	169.68	67.92	49.42	58.72	12.93	21.01	66.67
	T_{GDT}	12.2	6.16	5.46	13.39	198.2	35.76	12.43	39.63	10.67	14.29	41.3
Jiaonan	M_{NDT}	40.76	39.92	38.23	21.57	73.27	21.83	47.93	54.54	12.72	20.66	69.24
	T_{NDT}	19.71	27.33	12.53	15.63	35.64	10.56	11.16	61.2	7.88	9.7	38.36
	M_{BDT}	35.41	117.91	29.22	28.15	89.48	70.01	38.91	59.06	11.11	16.55	50.52
	T_{BDT}	17.92	91.43	9.92	17.58	62.41	55.03	21.56	40.23	3.45	6.58	40.38
	M_{GDT}	36.78	18.73	33.55	18.85	−3.71	37.68	37.52	27.6	11.11	24.79	46.24
	T_{GDT}	11.83	4.64	18.11	13.04	−3.35	21.85	13.18	29.47	0	10.61	36
Rongcheng	M_{NDT}	36.04	39.48	49.21	52.69	69.32	36.69	31.17	57.27	11.44	26.82	53.11
	T_{NDT}	20.88	26.43	37.43	42.35	49.61	22.24	21.62	44.11	11.37	15.43	30.32
	M_{BDT}	40.76	35.25	35.22	28.85	121.53	80.88	65.02	25.85	18.88	37.39	58.14
	T_{BDT}	12.52	4.14	5.26	18.28	134.24	31.07	17.02	9.94	8.97	25.4	33.33
	M_{GDT}	26.69	47.77	19.63	36.78	−41.78	47.28	53.01	43.5	17.48	28.07	53.66
	T_{GDT}	3.81	12.96	5.45	28.68	−21.55	13.72	23.23	18.97	12	23.73	31.25

Table 5
The sex composition of the nine groups at gamete maturity phase.

Group	Female	Male	Hermaphrodite	Asexual	Total
NDD	116 (58%)	84 (42%)	/	/	200 (100%)
NDT	70 (35%)	10 (5%)	10 (5%)	110 (55%)	200 ((100%)
NTT	46 (23%)	150 (75%)	4 (2%)	/	200 (100%)
BDD	50 (25%)	150 (75%)	/	/	200 (100%)
BDT	76 (38%)	16 (8%)	2 (1%)	106 (53%)	200 (100%)
BTT	54 (27%)	144 (72%)	2 (1%)	/	200 (100%)
GDD	146 (73%)	54 (27%)	/	/	200 (100%)
GDT	96 (48%)	4 (2%)	4 (2%)	96 (48%)	200 (100%)
GTT	38 (19%)	162 (81%)	/	/	200 (100%)

Table 6
The sublethal temperature, lethal temperature and LT_{50} for the twelve groups.

Group	Sublethal temperature (°C)	Lethal temperature (°C)	LT_{50} (°C)
DD	37.50	42.50	39.77
DT	37.50	41.50	38.99
TT	37.00	41.00	38.72
GDD	37.00	43.00	41.28
GDT	37.00	44.00	42.13
GTT	37.00	43.00	39.85
BDD	37.00	43.50	41.91
BDT	37.00	44.00	42.28
BTT	37.00	44.00	41.05
NDD	37.50	43.50	41.58
NDT	37.00	43.50	41.77
NTT	37.00	43.00	39.57

DD, DT and TT indicate diploid, triploid and tetraploid *C. gigas* with normal shell color, respectively. GDD, GDT and GTT indicate diploid, triploid and tetraploid *C. angulata* with golden shell color, respectively. BDD, BDT and BTT indicate diploid, triploid and tetraploid *C. angulata* with black shell color, respectively. NDD, NDT and NTT indicate diploid, triploid and tetraploid *C. angulata* with normal shell color, respectively.

increased genomic heterozygosity of triploids (Allen and Downing, 1986; Guo and Allen, 1994a; Wang et al., 2002; Piferrer et al., 2009; Leary et al., 1985).

Apart from the fact that triploids showed faster growth and higher tolerance, high infertility was an essential factor in enabling triploids to be used in industrial breeding. Because a lower reproductive effort of triploids takes place in summer, more energy can be used to maintain growth and/or survival, and they remain palatable in summer (Allen and Downing, 1986; Piferrer et al., 2009; Normand et al., 2008). Suppressed gonadal development in triploids was also reported in *C. gigas* (Guo and Allen, 1994a; Jouaux et al., 2010; Hermabessiere et al., 2016) and *C. virginica* (Peachey and Allen, 2016; Matt and Allen, 2021). The high proportion of sterile triploids may correspond to its high survival characteristics. This may be due to the fact that energy in diploid oysters is mainly used for gamete formation leading to slow growth and increased mortality (Callam et al., 2016; Guo and Allen, 1994a). Triploid oysters suffer from disrupted spermatogenesis and interruptions of mitosis and meiosis, occurring early and at all stages of the gametogenic cycle (Maillard et al., 2022). In addition, even though triploids produce motile spermatozoa and fertilize them, the resulting larvae failed to survive (Guo and Allen, 1994a; Komaru et al., 1994).

Traits such as growth and survival in the tetraploids were significantly inferior to those of the diploids and triploids in this study. The tetraploids performed poorly than the diploids and triploids also reported in *C. gigas* (Li et al., 2022; Qin et al., 2022; Li and Li, 2022; Zhou et al., 2023) and *C. virginica* (Guo, 2012). In contrast, Guo and Allen (1994b) confirmed that tetraploids grow faster than diploids and triploids and attributed the opposite result to a genetic defect in tetraploids, whereby the tetraploid parents were derived from the same family and were sibling crosses. As the number of tetraploid generations increased, the tetraploid population will become less genetically diverse and show an inbreeding depression. Such an explanation is partial, however, as tetraploid growth has also been attributed directly to chromosome stability and environmental conditions such as food abundance and/or pollution level (Piferrer et al., 2009; de Sousa et al., 2016). In addition, the slow growth and survival rate of tetraploids were also associated with the increased energy required to separate the four sets of chromosomes and develop giant cells during meiosis and mitosis (McCombie et al., 2005). Despite the poor performance of tetraploids, it is possible to improve their traits through selective breeding (Wan et al., 2023; Li and Li, 2022; Zhou et al., 2023).

The unbalanced sex ratio is prevalent in triploids and tetraploids in this study, which could be caused by incompatible and aberrant genetic

control of sex differentiation or conflicting expression and regulation of genes concerned with gonadal development (Dheilly et al., 2014). Moreover, the tetraploid rate in yearling stage of *C. angulata* with normal, black and golden shell colors in this study were higher than other tetraploid oyster species, such as *C. gigas* (Qin et al., 2022). In addition, a study on *C. angulata* conducted by Zhang et al. (2022) reveled the tetraploid rate decreased from 100% in the D-larval stage to 33.6% in the yearling stage. This did not occur in the present study, probably due to the fact that we have greatly minimized the factors that may affect ploidy stability in the breeding of tetraploids (Liang et al., 2023).

4.2. Ploidy stability

Some tetraploids were converted to aneuploids in this study. The formation of these aneuploids may involve an underlying genetic background (Zhang et al., 2014; de Sousa et al., 2017). McCombie et al. (2005) have reported significant differences in the ploidy compositions of the offspring of six tetraploid families with different somatic ploidy qualities, in which the aneuploid tetraploid parents were more likely to produce offspring that lost chromosomes and reverted to lower ploidy levels. Li et al. (2023) also found that higher proportions of aneuploidy occurred in F1 and F2 of tetraploid *C. gigas* than in F0 (aneuploidy rates in somatic cells: 14.2% in F0; 25.5% in F1; 29.7% in F2). Although the tetraploids used in this study were double-checked for gills and hemolymph, aneuploid cells are very small in number in most individuals, undetectable by flow cytometry, and are therefore often overlooked. The formation of aneuploids may also be linked to their physiological state, such as growth rate. Rapidly growing tissues, such as gills or membranes, with active cell division, could rapidly develop large chromosome clumps in mitosis, resulting in chromosome loss and aneuploid cell formation (Zhang et al., 2010). Similarly, Zhou et al. (2023) have reported higher aneuploidy rates in the embryonic stage of tetraploids *C. gigas* than in the adult stage. Embryonic cells division very speedily and were more likely to form aneuploids after the loss of chromosomes. Although mosaic tetraploids appeared to have little influence on the producing of triploids, it is obviously detrimental to the succeeding of tetraploids (Matt and Allen, 2014; de Sousa et al., 2017).

Chromosome loss was observed in tetraploid *C. angulata* in all three sites, reversing into triploids, mosaics and aneuploids in this study. Chromosome loss in polyploids leading to their conversion to triploids, mosaics or aneuploids have also been recorded in tetraploid *C. gigas* (Zhang et al., 2010; McCombie et al., 2005; Li et al., 2023), tetraploid *C. virginica* (de Sousa et al., 2017), and triploid *C. gigas* and *C. ariakensis* (Zhang et al., 2010). Low cell volume to nucleus ratio has been reported to be the cause of mitotic developmental failure in hyperploid structures, thus resulting in alterations in their ploidy levels (Guo and Allen, 1994a). In addition, the mitotic observations of triploids and tetraploids indicated that unnatural clumping of chromosomes caused mitosis to fail, probably resulting in the elimination of some chromosomes during division (Zhang et al., 2010; Zhang et al., 2002). Furthermore, inbreeding depression has been more popularly regarded as the main contributor to tetraploid chromosome instability (Li et al., 2023). The ancestral parents for tetraploids were very limited in number, and a limited number of parents significantly increased the probability of inbreeding during subsequent breeding. Although chromosome loss occurred prevalently in tetraploids, it was not irreversible. The genetic improvement project for tetraploid *C. gigas* indicated that its performance can be significantly improved through selective breeding (Wan et al., 2023; Zhou et al., 2023; Li and Li, 2022).

4.3. Effect of environmental factors

Environmental factors, such as temperature, food abundance and pollution levels, significantly influence the traits of aquatic animals. In this study, the same-ploidy Fujian oysters exhibited extensive variations in growth, survival and other traits in different sites. Such high genotype and environmental (G × E) interactions have also been reported in *C. gigas* (Swan et al., 2007; Dégremont et al., 2005; Han et al., 2020; Liang et al., 2022; Langdon et al., 2003; Evans and Langdon, 2006), *C. virginica* (Rawson and Feindel, 2012; Mallet and Haley, 1983) and *C. hongkongensis* (Zhang et al., 2017a). Temperature and salinity were comparable at all three culture sites in this study. Food diversity and abundance may be the main contributors to the differential performance of oyster traits at different sites (Cruz et al., 1998). Although G × E significantly affected the aquatic traits in oysters, the studies showed that G × E variance accounted for only a minor proportion of the total phenotypic variance (Swan et al., 2007). In other words, lines or strains of oysters that performed productively in one location also tend to perform productively in another location (Kvingedal et al., 2010). This was also supported in this study.

4.4. Different temperature tolerances in C. angulata and C. gigas

Compared to the diploids and tetraploids *C. gigas*, their *C. angulata* counterparts exhibited excellent temperature tolerance with a higher survival rate when exposed to thermal conditions in this study. The favorable characteristics of the parents could be inherited by the offspring (Leeds and Weber, 2019; Sacobie et al., 2012; Hand et al., 2004; Callam et al., 2016). The ancestral tetraploids were obtained by inhibiting the emission of the polar bodies of the fertilized eggs from female triploids and male diploids, and the triploids used to produce tetraploids were bred by suppressing the polar bodies of the diploid fertilized eggs in this study (Liang et al., 2023). Thus, the high temperature tolerance capacity of diploids can be inherited to tetraploids and transmitted to triploids by hybridization with tetraploids. In addition, tetraploids exhibited weakened temperature tolerance compared to diploids and triploids. This coincides with the low survival rate of tetraploids, indicting strict temperature control should be practiced when culturing tetraploids to avoid exceeding the tolerance range, either in the nursery or at sea.

4.5. Application prospects

The diploid oysters became less palatable due to their reproductive activity in summer. Additionally, gonadal development may also be responsible for the massive summer mortality of oysters (Cowan et al., 2023; Cotter et al., 2010; Samain et al., 2007). However, sterile triploids enable them to be available to the market even in summer (Normand et al., 2009), and the growth rate and tolerance of triploids were better than diploids (Nell, 2002; Wadsworth et al., 2019b). Therefore, triploid oysters have received increasing attention from farmers and scientists. The triploid *C. angulata* with normal, black, and golden shell colors breed in this study demonstrated significantly growth and survival advantages compared to the diploids and tetraploids. Moreover, compared to the triploid *C. gigas*, three triploid *C. angulata* lines in this study exhibited higher temperature tolerance. Therefore, the triploid *C. angulata* with normal, black, and golden shell colors in this study showed great aquaculture potential and could be used as new high-quality oyster varieties. Moreover, the inferior performance of the three tetraploid lines of *C. angulata* also alerts us to consider extra efforts in selecting and breeding those fragile but precious populations.

5. Conclusions

Three triploid *C. angulata* lines with normal, black, and golden shell colors were developed through crossing the corresponding diploids and tetraploids. The three triploid groups exhibited significant advantages in terms of growth and survival rates in all three sites. BDT were larger than GDT and NDT, which exhibited greater breeding potential. Moreover, compared to their diploid and tetraploid parents and *C. gigas*, triploid *C. angulata* exhibited higher thermal tolerance. Although the

ploidy composition of the three tetraploid lines varied during the grow-out stage, the tetraploid rate remained above 80% at day 480. This fact is certainly encouraging. However, the poor growth, survival and temperature tolerance of the three tetraploid *C. angulata* lines with normal, black and golden shell colors implies that more work should be devoted to breed those three populations. By comparing the aquaculture performance among the three lines of *C. angulata* with the normal, black and golden shell colors in this paper, we suggested that the black shell line of *C. angulata* has a higher potential to be used in commercial production. Furthermore, independent production investigations have shown that oysters with black shell color are also more popular in both farmers and the market, and have a broader market potential.

CRediT authorship contribution statement

Yuanxin Liang: Writing – original draft, Investigation, Conceptualization. **Hong Hu:** Data curation. **Geng Cheng:** Data curation. **Chengxun Xu:** Resources. **Qi Li:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

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.
lyx raw data 20240504 (Original data) (Mendeley Data)

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