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Short communication

# Growth, survival and reproductive traits of two genetically improved allotriploid oysters derived from *Crassostrea gigas* and *C. angulata*

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ABSTRACT

Allotriploid oysters between *Crassostrea gigas* and *C. angulata* have been utilized in commercial culture in China. The prevalence of this allotriploids is attributed to fast growth and extensive temperature adaptability, however its drawbacks are low meat ratio and slightly restored fecundity. To tackle these problems, two types of allotetraploids were produced in a previous study. Here, a comparative assessment of the growth, survival and reproductive traits was made for genetically improved allotriploids (based on allotetraploids), traditional allotriploids, and autotriploids (*C. gigas* and *C. angulata*). The results showed that the survival rates of larvae for all crosses were >80% on day 15. Genetically improved allotriploids survived better than autotriploids during juvenile and adult stages. The whole weights, wet meat weights, and meat ratios of genetically improved allotriploids were larger than traditional allotriploids on day 390 and day 510. The condition indexes, infertile percentages and fecundity of genetically improved allotriploids were lower than *C. gigas* autotriploids but higher than that of traditional allotriploids. At the end of the experiment, genetically improved allotriploids (GIT1) had the largest yield (56.79 kg) among all the six crosses. Overall, this study demonstrated that GIT1 exhibited superior growth and survival characteristics, as well as higher sterility compared with traditional allotriploids, which could be used as a potential variety in northern China.

## 1. Introduction

Oysters dominate over all other shellfish species in terms of global distribution and aquaculture output (FAO, 2023). The rapid increase in global oyster production for nearly three decades can be attributed to technological innovations in the aquaculture industry (Botta et al., 2020). Especially important here are the development of triploid breeding technology (Stanley et al., 1984; Guo and Allen, 1994a). Triploid oysters have often offered many benefits to the oyster industry, such as faster growth, improved meat quality and nutritional value, and high sterility permitting year-round marketability (Wadsworth et al., 2019; Brianik and Allam, 2023; Jiang et al., 2023). Nowadays, triploids have been widely adopted by some major oyster farming areas worldwide, such as North America, Europe, Australia, and China (Wadsworth et al., 2019; Botta et al., 2020).

The combination of ploidy manipulations and other breeding technologies has become a popular approach in genetic improvement of oyster species. Since triploids are usually sterile, they cannot be directly improved by successive selective breeding (Piferrer et al., 2009). One potential approach of genetic improvement for triploid production traits is selective breeding within the parent population, including diploid and tetraploid parental lines. Guo (2012) conducted a 6-year successive breeding on tetraploid *Crassostrea virginica*, which resulted in a 109% increase in the yield of triploid progeny. Mass selection has also been reported in tetraploid *C. gigas* (Miller et al., 2014; Wan et al., 2023). Moreover, favorable performance in growth and shell color was found in triploids obtained from genetically selected diploid and tetraploid strains (Zhou et al., 2023). The integration of ploidy breeding with crossbreeding is another indirect way to improve the traits of triploids. The resulting triploid hybrids (allotriploids) are expected to show improved heterosis in growth, survival, yield and disease resistance relative to autotriploids (Yao et al., 2013; Jiang et al., 2022a). In fish, triploid hybrids derived from *Salmo labrax* × *Oncorhynchus mykiss* (Akhan et al., 2011), *Megalobrama amblycephala* × *Xenocypris davidi* (Hu et al., 2012), *Ctenopharyngodon idellus* × *M. amblycephala* (He et al., 2013) exhibited significantly higher growth rates compared to their parents. Allotriploids between *C. idellus* and *Erythroculter ilishaeformis* exhibited superiorities in terms of wet weight and total amino acid

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content in muscle compared to the parents (Wu et al., 2019). In oyster, allotriploids obtained from *C. hongkongensis* and *C. gigas* (Zhang et al., 2014), as well as *C. ariakensis* and *C. gigas* (Li et al., 2022), exhibited positive advantages in survival and growth in the spat and adult stages. Allotriploids between *C. hongkongensis* and *C. ariakensis* exhibited positive growth advantages at different sites (Qin et al., 2020). However, very few of the allotriploid oysters has been commercially successful due to the low viability of embryos and larvae with respect to the parental species.

Pacific oysters *C. gigas* and Fujian oysters *C. angulata* are two closely related species adapted to relatively cold and warm habitats in northern and southern China, respectively (Wang et al., 2010; Ren et al., 2010). It has been reported that *C. gigas* and *C. angulata* can be crossed to produce viable diploid and triploid hybrids with heterosis in growth, survival, thermal resistance and lipid nutritional quality (Jiang et al., 2021, 2022a; Tan et al., 2019, 2020). Recently, this allotriploids has attracted interest for aquaculture because of its rapid growth and extensive temperature adaptability, and has been commercially applied in China (Qin et al., 2023). Nonetheless, a disconcerting point to consider is the lower meat ratio and slightly restored fecundity in this allotriploids grown in northern China. Thus, further genetic improvements are required for this allotriploids in aquaculture.

Two genetically improved allotetraploids derived from *C. gigas* and *C. angulata* were obtained using chemical method in a previous study (Jiang et al., 2024). Laboratory-based testing revealed phenotypic advantages of allotetraploids relative to autotetraploid *C. gigas* and *C. angulata* at the larval stage (Jiang et al., 2023b). In this study, the growth, survival and reproductive traits of triploid oysters obtained from improved allotetraploids and traditional autotetraploids were compared in northern China. The objective was to assess the breeding potentialities of the genetically improved allotetraploids.

## 2. Materials and methods

### 2.1. Broodstock collection

In April 2021, allotetraploid oysters (GGAG–allotriploid GGA ♀ × diploid *C. gigas* ♂ and AAGG–allotriploid AAG ♀ × diploid *C. gigas* ♂), autotetraploid oysters (GGGG–triploid *C. gigas* ♀ × diploid *C. gigas* ♂ and AAAA–triploid *C. angulata* ♀ × diploid *C. angulata* ♂) and diploid oysters (GG–diploid *C. gigas* ♀ × diploid *C. gigas* ♂ and AA–diploid *C. angulata* ♀ × diploid *C. angulata* ♂) were produced (Jiang et al., 2023b; Jiang et al., 2024). G and A represent a set of chromosomes for *C. gigas* and *C. angulata*, respectively. In May 2022, sexually mature one-year-old diploid GG (shell heights: 87.41 ± 9.16 mm, total weight 44.47 ± 8.50 g) and AA (shell heights: 74.99 ± 7.06 mm, total weight 28.20 ± 3.26 g), and tetraploid GGAG (shell heights: 69.23 ± 6.26 mm, total weight 27.83 ± 3.26 g), AAGG (shell heights: 66.37 ± 6.58 mm, total weight 26.04 ± 3.96 g), GGGG (shell heights: 58.64 ± 6.61 mm, total weight 22.51 ± 4.61 g) and AAAA (shell heights: 53.92 ± 6.78 mm, total weight 20.71 ± 3.06 g) broodstocks were collected from populations cultured in Rongcheng, Shandong province (37°11′N, 122°35′E) and reared at a hatchery in Laizhou, Shandong Province (37°31′N, 119°90′E). Broodstocks were acclimated in a 24-m3 concrete pond filled with aerated seawater (salinity 30, temperature 21 °C) for two weeks, with a stocking density of 50 individuals per basket. Thirty percent of the seawater was replaced daily, and the oysters were fed with *Phaeodactylum tricornutum* (~ 150,000 cells·ml$^{-1}$) three times a day. Ploidy of tetraploids (gill tissue) was confirmed using flow cytometer (Beckman Coulter) prior to spawning.

### 2.2. Production of auto- and allotriploids

Eggs extracted from five female diploid *C. gigas* were divided into four sublots and crossed with the pooled sperm from five male tetraploid GGAG, tetraploid AAGG, tetraploid *C. angulata*, and tetraploid *C. gigas*, respectively; eggs extracted from five female diploid *C. angulata* were divided into two sublots and crossed with the pooled sperm from five male tetraploid *C. gigas* and tetraploid *C. angulata*, respectively. Fertilization was conducted in 5-l polyethylene plastic barrels, with a sperm-to-egg ratio of approximately 15:1. Six different crosses were thereby obtained, including two genetically improved allotriploid crosses (abbreviated as GIT1–diploid *C. gigas* ♀ × tetraploid GGAG ♂ and GIT2–diploid *C. gigas* ♀ × tetraploid AAGG ♂), two traditional allotriploid crosses (GAA–diploid *C. gigas* ♀ × tetraploid *C. angulata* ♂; AGG–diploid *C. angulata* ♀ × tetraploid *C. gigas* ♂), and two autotriploid crosses (GGG–diploid *C. gigas* ♀ × tetraploid *C. gigas* ♂; AAA–diploid *C. angulata* ♀ × tetraploid *C. angulata* ♂) (Table 1). G and A represent a set of chromosomes for *C. gigas* and *C. angulata*, respectively for traditional allotriploid and autotriploid crosses. Three replicate groups were set using different parental oysters.

### 2.3. Larval rearing and spat grow-out

Prior to larval rearing, ploidy of D-larvae was confirmed using flow cytometer (Jiang et al., 2024). For each cross, ~3000 D-larvae were gathered into a 1.8-ml centrifuge tube containing 1 ml 1 × phosphate buffer solution. The suspensions were disaggregated by repeated aspiration using a 1-ml syringe fitted with a 26-G needle. Afterwards, 200 μL DAPI solution (Sigma) was added into the centrifuge tube for 2 min of staining. Samples were filtered through a 48-μm nylon sieve, transferred to a 96-well plate, and analyzed using flow cytometry. Details of larval and spat rearing are described fully in a previous study (Jiang et al., 2021). Hatched larvae from three replicates within each cross were pooled and stocked into three identical 100-l buckets. Seawater temperature and salinity were maintained at 21–23 °C and 29–31, respectively, during the nursery phase. During the first 7 days, larvae were fed *Isochrysis galbana* three times a day, followed by a 1:1 mixture of *Isochrysis galbana* and *Platymonas helgolandica*. The daily feeding amount increased from ~20,000 to ~80,000 cells·ml$^{-1}$ as the larvae grew. Thirty percent of the seawater was changed once a day. On day 25 post-fertilization, the attachment scallop shells were suspended in the buckets for larval settlement.

Spat were initially deployed at a commercial oyster farming area in Sanggou Bay, Rongcheng, Shandong Province (37°11′N, 122°35′E) were cultivated using the long-line method. When the shell height of spat reached 10 mm, three replicate 8-layer lantern nets were set for each cross and ten spat were placed in each layer to avoid the potential effects of overcrowding.

### 2.4. Hatching index, growth and survival measurement

The hatching index includes both fertilization rate and hatching rate. At 2 h post-fertilization, three samples of eggs were taken to assess the fertilization rate. Fertilization rate was calculated as the proportion of fertilized eggs to the total number of eggs. On day 1 post-fertilization, three samples of larvae were collected to evaluate the hatching rate.

**Table 1**
Experimental design for the crosses of autotriploids (GGG and AAA), allotriploids (GAA and AGG) and improved allotriploids (GIT1 and GIT2).

| | Parents | GGGG♂ | AAAA♂ | GGAG♂ | AAGG♂ |
|---|---|---|---|---|---|
| Rep1 | GG♀ | GGG | GAA | GIT1 | GIT2 |
| | AA♀ | AGG | AAA | – | – |
| Rep 2 | GG♀ | GGG | GAA | GIT1 | GIT2 |
| | AA♀ | AGG | AAA | – | – |
| Rep 3 | GG♀ | GGG | GAA | GIT1 | GIT2 |
| | AA♀ | AGG | AAA | – | – |

GG♀ and AA♀ indicates the female diploid *C. gigas* and female diploid *C. angulata*, respectively; GGGG♂ and AAAA♂ indicates the male tetraploid *C. gigas* and male tetraploid *C. angulata*, respectively; GGAG♂ and AAGG♂ indicates the two types of male allotetraploids.

Hatching rate was calculated as the proportion of D-larvae among fertilized eggs. On day 15 post-fertilization, three 50-ml samples were taken to calculate shell height and survival rate of larvae.

Oysters were sampled from 5 to 17 months of age to encompass a full reproductive season. Live and dead oysters were recorded in each lantern net in October 2022 (day 150), February (day 270), June (day 390) and October 2023 (day 510) and incremental survival and cumulative survival were calculated. The incremental survival was the ratio of live spat at the current sampling point to those at the previous sampling point. The cumulative survival rate was the ratio of the number of live spat at the current sampling point to the total number of spat initially deployed. Meanwhile, shell heights of 30 individuals were randomly measured to the nearest 0.01 mm using a vernier caliper. Whole weights, shell weights and wet meat weights of the same individuals used for measuring shell height were recorded using an electronic scale (0.01 g) in June and October 2023. Meat ratio was calculated as the percentage of wet meat weight to total weight. Yield (kg) at the last sampling was expressed as survival rate × total weight × total number of oysters initially deployed for each replicate lantern net (Rawson and Feindel, 2012). Subsequently, whole flesh was taken, dried at 80 °C for 48 h to measure dry meat weight. The condition index ($CI$) was determined as the followings (Abbe and Albright, 2003):

$$CI = \frac{\text{dry meat weight} \times 100}{\text{total weight–shell weight}}$$

### 2.5. *Reproductive trait*

The ploidy of individuals was identified by flow cytometry and only triploid oysters were used for fecundity determination. Ninety oysters from each cross were randomly selected to evaluate the sex ratio using an optical microscope. The types of sex were classified as female, male, hermaphrodite and asexual (Jiang et al., 2022a). Fifteen female and 15 male individuals were randomly chosen for fecundity determination. The egg numbers were detected as previously described (Cox and Mann, 1992). The whole tissue was completely crushed using a 57-μm mesh sieves and the eggs were rinsed into a 300-ml beaker with filtered seawater; the liquid containing the eggs was filtered through a 15-μm mesh sieves to removed impurities; additional filtered seawater was added to bring the volume of liquid up to 100 ml. Three replicate 100-μl aliquots were taken to count the number of eggs under the microscope (100× magnification). The number of sperm was determined using the hemocytometer method (Dascanio, 2021). The filtered liquid containing sperm was brought to a volume of 1 L. Three replicate 10-μl aliquots were taken and loaded the hemocytometer to count the number of sperms under the microscope (400× magnification).

### 2.6. *Statistical analyses*

Analyses were carried out using SPSS 26.0 software, with $P < 0.05$ considered significant. An arcsine transformation for hatching index, survival rate and meat ratio, and a logarithmic transformation for shell height, weight, yield and condition index were performed prior to further analysis. Differences in all growth-related metrics among six crosses were assessed with one-factor ANOVA followed by multiple comparison Tukey test.

## 3. Results

### 3.1. *Hatching index and larval development*

No significant differences were found in the fertilization and hatching rates among the genetically improved allotriploid, allotriploid and autotriploid crosses (Table 2). The triploid rates of the six crosses were 100% on day 1 post-fertilization. Average survival rates of larvae for all crosses were equal or >81.09% ± 5.52% on day 15, with no significant difference among them. Shell height of the allotriploid GAA was significantly ($P < 0.05$) higher than the two autotriploids on day 15 (Table 3).

### 3.2. *Growth and survival*

There were no significant differences in cumulative survival and incremental survival between genetically improved allotriploid crosses and allotriploid crosses (Table 2). The allotriploid GIT1 and GIT2 survived better than autotriploids during the whole experiment.

The allotriploid GAA had a significantly larger shell height ($P < 0.05$) than autotriploid AAA and GGG (Table 3) and even showed better shell height than GIT1 and GIT2. However, the whole weights, wet meat weights, and meat ratios of GIT1 and GIT2 were larger than GAA and AGG on day 390 and day 510 (Table 4). Especially, the wet meat weights and meat ratios of GIT1 and GIT2 were significantly higher than those of GAA and AGG after the spawning season (day 510). On day 390, the

**Table 3**
Shell height and yield of genetically improved allotriploids (GIT1 and GIT2), allotriploids (GAA and AGG) and autotriploids (AAA and GGG).

| Crosses | Shell height | | | | | Yield |
|---|---|---|---|---|---|---|
| | Day 15 (μm) | Day 150 (mm) | Day 270 (mm) | Day 390 (mm) | Day 510 (mm) | Day 510 (kg) |
| GIT1 | $286.65 \pm 13.43^{ab}$ | $60.01 \pm 5.90^{ab}$ | $75.36 \pm 7.01^{ab}$ | $104.70 \pm 11.82^{ab}$ | $113.41 \pm 11.60^{ab}$ | $56.79 \pm 3.64^{a}$ |
| GIT2 | $281.15 \pm 14.31^{ab}$ | $59.29 \pm 5.39^{ab}$ | $73.53 \pm 6.34^{ab}$ | $98.77 \pm 8.87^{bc}$ | $112.00 \pm 9.19^{ab}$ | $53.79 \pm 3.06^{a}$ |
| GAA | $290.57 \pm 11.00^{a}$ | $61.63 \pm 4.76^{a}$ | $77.80 \pm 5.90^{a}$ | $109.81 \pm 9.37^{a}$ | $117.95 \pm 9.06^{a}$ | $53.94 \pm 8.64^{a}$ |
| AGG | $283.82 \pm 12.61^{ab}$ | $59.80 \pm 5.56^{ab}$ | $74.40 \pm 5.83^{ab}$ | $96.15 \pm 7.80^{c}$ | $110.05 \pm 7.15^{b}$ | $47.50 \pm 1.98^{ab}$ |
| AAA | $258.53 \pm 13.50^{c}$ | $45.58 \pm 6.32^{c}$ | $56.46 \pm 7.40^{c}$ | $69.37 \pm 6.77^{d}$ | $71.38 \pm 6.90^{c}$ | $31.86 \pm 1.68^{c}$ |
| GGG | $277.90 \pm 11.18^{b}$ | $55.65 \pm 7.56^{b}$ | $70.26 \pm 6.45^{b}$ | $94.55 \pm 7.92^{c}$ | $108.46 \pm 10.09^{b}$ | $42.03 \pm 3.25^{b}$ |

**Table 2**
Hatching index (fertilization rate and hatching rate), incremental survival, and cumulative survival of genetically improved allotriploids (GIT1 and GIT2), allotriploids (GAA and AGG) and autotriploids (AAA and GGG).

| Crosses | Hatching index (%) | | Cumulative survival (%) | | Incremental survival (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | Fertilization rate | Hatching rate | Day 15 | Day 510 | Day 150 | Day 270 | Day 390 | Day 510 |
| GIT1 | $91.11 \pm 3.27^{a}$ | $94.17 \pm 3.12^{a}$ | $85.20 \pm 0.27^{a}$ | $73.33 \pm 3.28^{a}$ | $92.92 \pm 2.12^{a}$ | $96.86 \pm 0.61^{a}$ | $97.21 \pm 1.19^{a}$ | $83.78 \pm 1.60^{a}$ |
| GIT2 | $91.85 \pm 4.57^{a}$ | $95.00 \pm 2.04^{a}$ | $82.93 \pm 4.52^{a}$ | $70.42 \pm 1.56^{ab}$ | $90.42 \pm 2.36^{a}$ | $94.47 \pm 2.30^{ab}$ | $97.10 \pm 1.15^{a}$ | $84.95 \pm 0.77^{a}$ |
| GAA | $92.22 \pm 5.05^{a}$ | $92.92 \pm 2.36^{a}$ | $84.60 \pm 2.10^{a}$ | $73.75 \pm 3.06^{a}$ | $93.33 \pm 1.56^{a}$ | $95.58 \pm 3.12^{a}$ | $96.74 \pm 0.60^{a}$ | $85.50 \pm 3.15^{a}$ |
| AGG | $92.22 \pm 6.54^{a}$ | $94.58 \pm 5.03^{a}$ | $84.78 \pm 3.45^{a}$ | $67.92 \pm 2.57^{abc}$ | $89.58 \pm 3.12^{a}$ | $93.51 \pm 1.68^{ab}$ | $96.98 \pm 1.31^{a}$ | $83.62 \pm 1.48^{a}$ |
| AAA | $91.48 \pm 2.92^{a}$ | $92.92 \pm 3.28^{a}$ | $81.09 \pm 5.52^{a}$ | $61.25 \pm 2.70^{bc}$ | $90.83 \pm 2.95^{a}$ | $82.06 \pm 3.25^{b}$ | $96.69 \pm 1.31^{a}$ | $85.26 \pm 6.49^{a}$ |
| GGG | $91.11 \pm 1.81^{a}$ | $94.17 \pm 2.12^{a}$ | $83.56 \pm 2.30^{a}$ | $59.17 \pm 4.25^{c}$ | $85.00 \pm 2.70^{a}$ | $97.02 \pm 1.30^{a}$ | $97.45 \pm 0.77^{a}$ | $74.01 \pm 9.10^{a}$ |

Different superscripted letters in each column indicate significant differences ($P < 0.05$).

**Table 4**
Whole weight, wet meat weight, meat ratio, and condition index of genetically improved allotriploids (GIT1 and GIT2), allotriploids (GAA and AGG) and autotriploids (AAA and GGG).

| Crosses | Whole weight (g) | | Wet meat weight (g) | | Meat ratio (%) | | Condition index | |
|---|---|---|---|---|---|---|---|---|
| | Day 390 | Day 510 | Day 390 | Day 510 | Day 390 | Day 510 | Day 390 | Day 510 |
| GIT1 | $89.49 \pm 10.17^{a}$ | $96.72 \pm 11.79^{a}$ | $24.29 \pm 6.43^{a}$ | $29.73 \pm 5.47^{a}$ | $27.23 \pm 6.83^{ab}$ | $31.10 \pm 6.06^{a}$ | $6.04 \pm 0.83^{b}$ | $6.60 \pm 1.14^{ab}$ |
| GIT2 | $87.00 \pm 13.49^{ab}$ | $95.44 \pm 11.45^{ab}$ | $22.76 \pm 6.10^{a}$ | $29.06 \pm 5.96^{a}$ | $26.25 \pm 6.06^{abc}$ | $30.41 \pm 4.90^{a}$ | $5.84 \pm 1.38^{b}$ | $6.18 \pm 1.08^{b}$ |
| GAA | $86.52 \pm 8.51^{ab}$ | $91.54 \pm 7.15^{ab}$ | $20.04 \pm 4.17^{ab}$ | $22.35 \pm 4.95^{b}$ | $23.07 \pm 3.58^{cd}$ | $24.49 \pm 5.29^{b}$ | $4.81 \pm 1.06^{c}$ | $3.46 \pm 0.63^{d}$ |
| AGG | $83.91 \pm 12.07^{ab}$ | $87.42 \pm 8.36^{b}$ | $17.83 \pm 3.82^{bc}$ | $19.16 \pm 3.02^{bc}$ | $21.20 \pm 3.23^{d}$ | $21.90 \pm 2.60^{b}$ | $4.62 \pm 0.82^{c}$ | $3.36 \pm 0.53^{d}$ |
| AAA | $64.16 \pm 7.45^{c}$ | $65.01 \pm 5.11^{c}$ | $15.80 \pm 3.96^{a}$ | $16.60 \pm 2.41^{c}$ | $24.57 \pm 5.23^{bcd}$ | $25.57 \pm 3.49^{b}$ | $6.41 \pm 1.17^{ab}$ | $4.84 \pm 0.86^{c}$ |
| GGG | $80.87 \pm 7.38^{b}$ | $88.75 \pm 10.78^{ab}$ | $23.79 \pm 6.13^{a}$ | $29.41 \pm 6.15^{a}$ | $29.30 \pm 6.69^{a}$ | $33.07 \pm 5.19^{a}$ | $7.09 \pm 1.09^{a}$ | $7.18 \pm 0.90^{a}$ |

Different superscripted letters in each column indicate significant differences ($P < 0.05$).

condition index was ranked in the following order: GGG > AAA > GIT1 > GIT2 > GAA > AGG. From June (day 390) to October (day 510) in 2023, the condition index decreased from 4.81 ± 1.06 to 3.46 ± 0.63 for GAA, from 4.62 ± 0.82 to 3.36 ± 0.53 for AGG, and from 6.41 ± 1.17 to 4.84 ± 0.86 for AAA, concomitant with oyster spawning in the field. However, the condition indexes of GIT1 (6.60 ± 1.14), GIT2 (6.18 ± 1.08) and GGG (7.18 ± 0.90) obviously increased, to the highest levels during the entire experiment.

At the end of the experiment, the yield of GIT1 was approximately 2.85 kg (~5.3%) higher than GAA, 9.29 kg (~19.6%) higher than AGG, 14.76 kg (~35.1%) higher than GGG ($P < 0.05$), and 24.93 kg (~78.2%) higher than AAA ($P < 0.05$) (Table 3).

### 3.3. Reproductive trait

The percentages of infertile individuals of the six crosses ranked in the following order: GGG (81.1%) > GIT1 (72.2%) > GIT2 (68.9%) > AGG (62.2%) > GAA (56.7%) > AAA (47.8%) (Table 5). In four allotriploid crosses, male individuals were more abundant than females, with a ratio of female to male ranging from 1: 2.7 to 1: 4.5. In contrast, the female and male ratios were 6.2: 1 and 3.7: 1 for AAA and GGG, respectively.

Differences of fecundity in females and males were significant among the crosses (Table 5). Progenies from AAA were the most fecund ((25.56 ± 3.18) × $10^4$ eggs), followed by AGG ((13.20 ± 1.43) × $10^4$ eggs) and GAA ((10.19 ± 1.22) × $10^4$ eggs); GIT2 ((7.66 ± 0.80) × $10^4$ eggs) and GIT1 ((6.08 ± 0.68) × $10^4$ eggs) were inferior, with the worst fertility found in GGG ((2.54 ± 0.26) × $10^4$ eggs). The fecundity of males characterized by sperm number was ranked in approximately the same order.

**Table 5**
Sex ratio and fecundity of genetically improved allotriploids (GIT1 and GIT2), allotriploids (GAA and AGG) and autotriploids (AAA and GGG).

| Crosses | Sex ratio | | | | Fecundity | |
|---|---|---|---|---|---|---|
| | Female No. (%) | Male No. (%) | Hermaphrodite No. (%) | Asexual No. (%) | Egg No. ($\times 10^4$) | Sperm No. ($\times 10^7$) |
| GIT1 | 4(4.4) | 18 (20.0) | 3(3.3) | 65 (72.2) | $6.08 \pm 0.68^{e}$ | $2.85 \pm 0.27^{b}$ |
| GIT2 | 6(6.7) | 16 (17.8) | 6(6.7) | 62 (68.9) | $7.66 \pm 0.80^{d}$ | $2.87 \pm 0.36^{b}$ |
| GAA | 7(7.8) | 25 (27.8) | 7(7.8) | 51 (56.7) | $10.19 \pm 1.22^{c}$ | $3.14 \pm 0.29^{a}$ |
| AGG | 7(7.8) | 22 (24.4) | 5(5.6) | 56 (62.2) | $13.20 \pm 1.43^{b}$ | $3.19 \pm 0.24^{a}$ |
| AAA | 37 (41.1) | 6(6.7) | 4(4.4) | 43 (47.8) | $25.56 \pm 3.18^{a}$ | $3.19 \pm 0.29^{a}$ |
| GGG | 11 (12.2) | 3(3.3) | 3(3.3) | 73 (81.1) | $2.54 \pm 0.26^{f}$ | $2.52 \pm 0.18^{c}$ |

No. (%): number of individuals of certain sex type (percentage of certain sex type); No. (×104): number of eggs was value × 104; No. (×107): number of sperms was value × 107.

## 4. Discussion

Allotriploids are expected to combine triploid advantage and heterosis, thus obtaining further improvements in aspects such as growth, survival and disease resistance (Bingham et al., 1994; Yao et al., 2013; Jiang et al., 2022a). The first experiment of allotriploid oysters was reported by Allen et al. (1993), who obtained a few abnormal allotriploid larvae. In this study, alloploid GAA and AGG grew better than autotriploid GGG and AAA in regard to shell height. However, it was observed that the faster growing allotriploids showed lower wet meat weight and meat ratio compared with autotriploids, which paralleled the phenomenon observed in northern China. The situations were even worse for condition index. Significantly, the genetically improved allotriploids significantly outperformed traditional allotriploids on day 390 to day 510 in all meat parameters. In addition, the higher yield supported the assumption that improved allotriploids can have a larger biomass and commercial value than autotriploids.

Triploids with three alleles at a single locus (triploid triple heterozygotes) may show a fitness superiority over diploids with two alleles (diploid heterozygotes) (Piferrer et al., 2009). Therefore, it is not surprising that the overall survival rates among the six types of triploids were relatively high and not significantly different in the field trial. Nonetheless, the summer survival (day 150 and 510) at Rongcheng was lowest for GGG, while the lowest winter survival (day 270) was found in AAA. A possible explanation is that *C. angulata* is mainly cultivated in southern China and has good adaptability to subtropical warm water compared to *C. gigas*, indicating the importance of local adaptation of species. In contrast, four allotriploids showed stable survival advantages throughout the growing seasons. Studies noted that alloploypoids can have combinations of desirable traits of two parental species, thus showing an improved environmental adaptation (Comai, 2005; Bodt et al., 2005). The survival advantage has also been reported in several allotriploid fishes (Wang et al., 2020). One of the keys for successful production of genetically improved strains is the viability from embryos to adults. In this sense, GIT1 and GIT2 can considered to be good candidates for oyster aquaculture, as their survival performance was comparable to that of traditional allotriploids.

Functional gametes have been confirmed in several triploid mollusks, including triploid *Mulinia lateralis* (Guo and Allen, 1994b), triploid *C. ariakensis* (Erskine and Allen, 2003), triploid *Haliotis discus hannai* (Li et al., 2004), and triploid *Pinctada fucata martensii* (Komaru and Wada, 1990). Gonadal development also noted in all types of triploids in this study, corresponding to observations for auto- and allotriploid oysters in previous studies (Allen and Downing, 1986; Degremont et al., 2012; Jiang et al., 2022a; Li et al., 2022; Zhang et al., 2022. Qin et al., 2022, 2023). Among them, the highest and lowest fertility ratio and fecundity (number of sperm and eggs) were observed in AAA and GGG, respectively. This may be attributed to the fact that diploid *C. angulata* usually invests more energy into reproduction during growing process compared to diploid *C. gigas* (Jiang et al., 2022b). It is worth noting that the reproductive capacity of GIT1 and GIT2 was inferior to that of AGG and GAA, albeit it was larger than GGG. Given the superior reproductive characteristic of *C. angulata*, the decreased fecundity in genetically improved allotriploids may be related to the reduced genome proportion of *C. angulata*, which lowers the expression of genes related to gonadal development. Differences in the origin of an additional set of chromosomes is important because it may affect differences in triploid performance (Callam et al., 2016; Wang et al., 2002). However, differences in fecundity might also be due to asynchrony in gonadal development and spawning events (Cox and Mann, 1992). Therefore, estimates of fecundity for the different types of triploids in this study may be affected by the time of sampling relative to spawning activity.

Gonadal development impacts morphological characteristics, yield and meat quality (glycogen, flavor, texture) by transferring energy to reproduction in shellfish (Piferrer et al., 2009). For oysters, gamete generation relies on nutritional health, as gamete accumulation occurs at the expense of depleting stored glycogen (Mann, 1979; Cox and Mann, 1992). Here, reduced condition indexes for GAA, AGG and AAA were recorded from day 390 to day 510, indicating deterioration in meat quality after the spawning season. This perhaps can be attributable to gonadal development or spawning by a proportion of triploids in late summer and early autumn. Importantly, however, the meat parameter and condition index of allotriploids were altered by using genetically improved tetraploid parents. Within all but the GGG, GIT1 had a significantly higher meat ratio and condition index than the other triploids (with a similar pattern within GIT2).

Interestingly, opposite female-male ratios were observed between autotriploids and allotriploids. As revealed by previous reports (Qin et al., 2022; Zhang et al., 2022), fertile individuals in autotriploid AAA and GGG showed a high proportion of feminization in the present study. Triploidization may change sex ratio owing to imbalances in epistatic and autosomal sex determinants (Devlin and Nagahama, 2002). However, the four types of alloploids had sex ratios skewed in favor of males, which differs from what was found by other studies about allotriploid oysters (Zhang et al., 2014; Qin et al., 2020, 2023; Li et al., 2022). This seems to suggest a different strategy in allotriploids, where fertile individuals develop first as males. The high male proportion was also reported in allotriploid *Megalobrama amblycephala* × *Xenocypris davidi*, which is thought to be possibly linked to genomic incompatibility or abnormal expression and regulation of genes related to gonad development (Hu et al., 2012). In addition to genetic effects, the environment can also change the sex of oysters. Females are favored when warm and well-nourished, and vice versa toward males (Quayle, 1988). It has been reported that females of mollusks expend 1.5-fold more energy to generate eggs than males to generate sperms (Russell-Hunter, 1979). Therefore, it is reasonable to speculate that allotriploids allocate more energy to growth rather than reproduction compared to autotriploids, resulting in sex ratios biased toward males. The lower food abundance (Gao et al., 2006) and temperature (Jiang et al., 2022a) at Rongcheng may also explain the larger male proportion in allotriploids compared to other studies.

In summary, this study suggested that all six triploids exhibited favorable performance in terms of hatching index as well as larval growth and survival. The wet meat weights and meat ratios of GIT1 and GIT2 were larger than GAA and AGG on day 510. The condition indexes and infertile percentages of GIT1 and GIT2 were lower than GGG but higher than that of GAA and AGG. The fecundity of GIT1 and GIT2 were higher than GGG but lower than that of GAA and AGG. At harvest, GIT1 had the largest yield among all the six crosses. Overall, the present study indicated that the genetically improved allotriploids GIT1 can be used as a new potential variety for oyster industry.

## CRediT authorship contribution statement

**Gaowei Jiang:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft. **Chengxun Xu:** Resources, Supervision. **Qi Li:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing.

## Declaration of competing interest

The authors declare no conflict of interest.

## Data availability

Data will be made available on request.

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