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RESEARCH

Effects of temperature on broodstock conditions, gonadal development and survival of tetraploid and diploid oysters of 'Haida No. 3' line (*Crassostrea gigas*)

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Abstract
Tetraploid oysters are valuable germplasm resources for the production of triploid oysters. However, the broodstock condition and gonadal development of tetraploid oysters are poorly understood. To assess the effects of temperature on gamete quantity and quality, tetraploid and diploid oysters were held at controlled temperatures of 18, 20 and 22 °C for 120 days. The gametogenesis process in tetraploid and diploid oysters showed an accelerated trend in response to increased conditioning temperatures. Nonetheless, the gonadal development of tetraploid oysters was slower than that of diploid oysters. Histomorphometric analysis of gonadal tissues showed variations in observed sex ratio under different conditioning temperatures. The proportions of tetraploid and diploid males increased with elevated temperature. However, survival rates of tetraploid and diploid oysters at 22 °C were significantly lower than those at 18 and 20 °C ($P < 0.05$). In terms of the number of oocytes and spermatozoa, tetraploid oysters were fewer than diploid oysters. Overall, these results demonstrated the differences in broodstock conditioning and gonadal development between tetraploid and diploid oysters, suggesting the need for specialized broodstock cultivation strategies for tetraploid oysters.

Keywords Conditioning temperature · Tetraploid · *Crassostrea gigas* · Gonadal development · Broodstock

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Introduction

The Pacific oyster (*Crassostrea gigas*) is native to Northern Asia and has been introduced to many countries due to its rapid growth and environment adaptability. Currently, *C. gigas* has become the most significant commercial oyster species with high economic value (FAO 2023). However, diploid *C. gigas* generally exhibits thin bodies and low marketability after spawning during the breeding season. Due to the reduction of gametogenesis, triploid oysters exhibit increased marketability and improved meat quality (Nell 2002). Nowadays, triploid *C. gigas* is particularly popular with farmers, accounting for an estimated 90% of oyster spat production in France (Dégremont et al. 2010), and one third of aquaculture production on the west coast of North America (Nell 2002). The mating of tetraploid and diploid oysters can produce 100% triploid oysters, therefore tetraploid oysters hold significant importance within the global oyster industry.

In the oyster industry, seed production of triploid *C. gigas* originates from hatcheries. Broodstock conditioning of tetraploid oysters is regarded as a pivotal step in artificial triploid seed production. The manipulation of environmental factors has been demonstrated to have a clear impact on oyster fecundity (Gonzalez Araya et al. 2012). In recent decades, optimal parameters of temperature, photoperiod, supplemental feeding, and salinity have been established for diploid *C. gigas* broodstock conditioning (Muranaka and Lannan 1984; Fabioux et al. 2005). Understanding the impact of temperature on gametogenesis in tetraploid oysters is essential for the improvement of conditioning protocols in hatcheries (Chávez-Villalba et al. 2002). However, while triploid *C. gigas* is widely cultivated globally, little is known about the physiological and regulatory processes involved in the reproduction of tetraploid *C. gigas*.

Temperature is a main factor that affects metabolism, accelerates gametogenesis and gonadal development (Fabioux et al. 2005). Manipulation of temperature under controlled conditions can be a useful tool to improve the rate of gametogenesis and influence sex ratio in oysters (Alyssa et al. 2013). The male-to-female ratio tended to increase due to the inhibitory effect of elevated temperatures on ovarian differentiation (Baroiller and D'Cotta 2001; Hayashi et al. 2010). Consequently, understanding the impact of temperature on gametogenesis in tetraploid *C. gigas* is crucial for enhancing conditioning protocols in hatcheries.

In order to assess the effects of temperature on broodstock conditions, gonadal development and survival of tetraploid and diploid oysters, this study determined the gonadal development stage, sex ratio, survival rate, diameter of mature oocytes, and number of oocytes and spermatozoa in tetraploid and diploid *C. gigas* conditioned at 18, 20 and 22 °C for 120 days. The ultimate objective of the present study was to better understand the broodstock conditions and gonadal development of tetraploid *C. gigas*.

Materials and methods

Experimental animals

In January 2023, one-year-old diploid and tetraploid oysters of 'Haida No. 3' line were taken from Jiaonan (Shandong Province, China) and subsequently transferred to an oyster hatchery located in Laizhou (Shandong Province, China). In Jiaonan, the farming area

has an annual water temperature range of 3.7–26.9 °C and an annual water salinity range of 28.8–31.8 psu. The seawater temperature and salinity were downloaded from National Marine Data Center (http://mds.nmdis.org.cn/). Diploid oysters were derived from 'Haida No. 3' line of *C. gigas*, with breeding targets of black shell color and rapid growth (Xu et al. 2019). Triploid oysters of 'Haida No. 3' line were induced by chemical treatments that suppressed the extrusion of the second polar body (PB2) from fertilized eggs of the same 'Haida No. 3' line. Tetraploid oysters were obtained by inhibiting the release of polar body 1 (PB1) in eggs from triploids of 'Haida No. 3' line fertilized with the haploid sperm of 'Haida No. 3' line. After three generations of mass selection of tetraploids from 'Haida No. 3' line, tetraploid oysters used in the experiment were obtained. Individuals of similar size were selected, cleaned to remove attached organisms, and placed in 24 m^3 concrete ponds with continuous aeration at constant temperature (7 ± 1 ℃) and salinity (30 ± 1) for one week of conditioning.

To determine the ploidy of individuals sampled from 3000 tetraploid oysters and to exclude other ploidy individuals from further histological analyses, a flow cytometer was used to detect the DNA content of all tetraploids. All samples were processed as described fully in a previous study (Zhou et al. 2023). The DNA content of gill cells of diploid oysters was determined as the control.

Temperature treatments

The broodstock conditioning was evaluated as previously described with a minor modification (Chávez-Villalba et al. 2002). Three temperatures (18, 20 and 22 °C) were used in the experiment from January 2023 to April 2023. Nine polyethylene tanks (300 L) were used for each temperature. Six tanks were used to observe gonadal development and three tanks were used to measure survival of tetraploids and diploids. Each tank was divided into two equal experimental blocks by polyethylene mesh. One hundred oysters of each ploidy were placed in two trial blocks of a tank. The temperature was raised at a rate of 0.5 °C d^{-1} from the initial temperature (7 °C), paused for one week when the temperature reached 10 °C, and then raised at a rate of 0.5 °C d^{-1} until the target temperatures (18, 20, 22 °C) were reached using immersion heaters. As a result, 27 trial blocks (3 temperatures × 9 tanks) were used in this experiment. The oysters were kept aerated with air stones and received daily water changes. All experimental groups were fed fresh *Phaeodactylum tricornutum* Bohlin and *Chlorella vulgaris* six times a day, with the amount of microalgae gradually increasing with increasing temperature until satiation. Tanks were drained daily and cleaned with a 0.5 g/L *potassium permanganate* ($KMnO4, \geq 99.0\%$) solution once a week. Dead oysters were counted and removed daily, and then the cumulative survival rates of tetraploids and diploids were calculated.

Sampling and histological analysis

Thirty oysters from each group were randomly sampled twice a month during broodstock conditioning. The oocyte and sperm development status of tetraploid and diploid oysters at different temperatures was determined by sampling gonadal tissues. For histological examination, 3-mm cross section of the visceral mass was excised anterior to the pericardial region. The gonad tissue from each sample was fixed in Bouin's solution for 24 h and then stored in 70% ethanol. The samples were dehydrated using a series of ethanol solutions (70%, 80%, 90%, and 100%), were embedded in paraffin, sectioned to a thickness of

5 µm, mounted on glass slides, and stained with hematoxylin and eosin. The gonad sections were examined using a light microscope (Olympus BX53, Japan) to determine the stages of gonadal development. The recorded images were processed by means of digital image analysis.

C. gigas is typically a protandrous alternate hermaphrodite. Oysters were classified as female, male, hermaphrodite and undetermined based on the types of gametes present. Based on the qualitative classification (5 stages: 0 to 4) of Lango-Reynoso et al. (2000) and Normand et al. (2008), the reproductive stages were identified as follows:

Resting stage (S0): The follicles are absent or elongated and consist of undifferentiated germ cells; no trace of sexuality.
Early growth stage (S1): The follicles are small and isolated with numerous spermatogonia or oogonia along the follicle wall. In the male reproductive system, spermatocytes and few spermatid balls were present, while in the female reproductive system, developing and attached oocytes were predominance connected to developing follicles.
Late growth stage (S2): The follicles are actively developing with primary gametocytes and some secondary spermatozoa and oocytes. In the male reproductive system, there was a prevalence of spermatocytes and spermatid clusters, with a limited presence of spermatozoa clusters within the follicle lumen. In the female reproductive system, medium-sized oocytes undergoing vitellogenesis were predominantly observed, while post-vitellogenesis oocytes were less numerous and found freely within the follicle lumen.
Mature stage (S3): Near-ripe or ripe follicles are densely covered with maturing gametes; presence of mature gametes. In the male and female lines, numerous spermatozoa balls and mature oocytes filled the follicular lumen.
Spawning and reabsorption stages (S4): The follicles are distended and some are ruptured, but many gametes may still remain. In some cases, re-development occurs with increased numbers of primary oocytes and spermatocytes.

Measurements

To assess the effect of temperature on oocyte diameter, 100 oocytes from 10 randomly selected females in each group were measured for diameter range. Histological sections were observed under a 100× microscope and photographed with a digital camera (Olympus BX53, Japan). To maximize field coverage, cross-sections of gonadal specimens should always be traced along the largest axis containing the oocyte. The pictures were processed using Cellsens Standard software.

To measure the number of oocytes and spermatozoa, 30 females and 30 males were randomly chosen from each group. The oocytes were counted using the method described (Li et al. 2022). The fully matured female gonad was boiled in 1 L filtered seawater by stirring evenly, and then aliquots of 10 µL were counted under the microscope. The number of spermatozoa was determined using the hemocytometer method (Momin and Memis 2023). The gonad was completely crushed using a 38-µm nylon screen. The sperm was diluted in a beaker with 1 L of filtered seawater. The diluted sperm samples were examined under a light microscope using a haemocytometer (depth = 0.1 mm; volume = 0.00025 mm^3). The cell counts were determined by counting the number of individual spermatozoa in 5 small squares (0.2 mm × 0.2 mm; each with 16 squares) and calculating the total average cell

Table 1 The measurable traits and relative DNA content and coefficient of variation (CV) for DNA content of 60 tetraploid and diploid *C. gigas*

Oyster type	Shell height (mm)	Shell length (mm)	Shell width (mm)	Wet weight (g)	Relative DNA content	CV (%) for DNA content
Tetraploids	68.00 ± 9.30^{a}	48.31 ± 5.04^{a}	26.38 ± 4.24^{a}	50.88 ± 13.99^{a}	400	15.17
Diploids	71.20 ± 8.09^{a}	50.92 ± 6.23^{a}	24.79 ± 5.08^{a}	50.52 ± 14.29^{a}	200	10.60

Different superscripts indicate significant differences ($P < 0.05$) between tetraploids and diploids

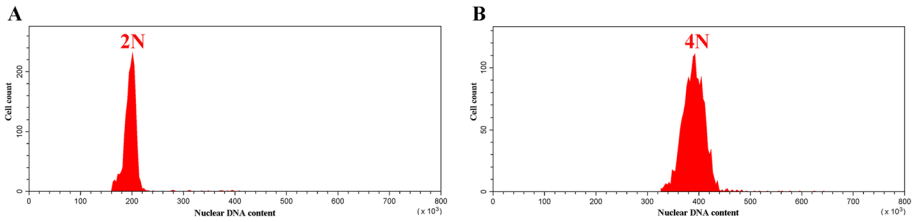

Fig. 1 The relative DNA content and ploidy level of tetraploid and diploid *C. gigas* determined by flow cytometry. **A** Tetraploid oysters; **B** Diploid oysters

count using the formula below: Total spermatozoa = (Number of spermatozoa counted/0.02 $\mu L \times 10^{6}$ μL).

Data analysis

Reproductive stages were calculated based on the corresponding oocyte proportion in 30 oysters (Lango-Reynoso et al. 2000). The data on phenotypic traits (shell height, shell length, shell width, wet weight, oocyte diameter, and the number of oocytes and spermatozoa) were presented as mean ± standard deviation. Homogeneity of variances was assessed using Levene's tests. Shell height, shell length, shell width and wet weight of tetraploid and diploid oysters were compared using Student's t-test. Comparison of survival rate, oocyte diameter, and the number of oocytes and spermatozoa of tetraploids and diploids was analysed using one-way analysis of variance (ANOVA), and Tukey's multiple comparison test. All statistical tests were performed using IBM SPSS Statistics for Windows (version 25.0). Differences with a significance level of $P < 0.05$ were considered statistically significant.

Results

Measurement of appearance and DNA content

Prior to the experiment, the shell lengths of tetraploid and diploid oysters were 68.00 ± 9.30 mm and 71.20 ± 8.09 mm, respectively, and the differences were not significant ($P > 0.05$) (Table 1). The average wet weight of tetraploids was 50.88 ± 13.99 g, which was similar to that of diploids (50.52 ± 14.29 g). The DNA content of the tetraploid oysters

was twice that of the diploid oysters, suggesting that the tetraploids used in this experiment were tetraploid oysters (Fig. 1 and Table 1).

Effect of different temperatures on gonadal development

Changes in gametes indicate temperature effects on gonadal development in *C. gigas* (Fig. 2). At the beginning of experiment, histological sectioning showed that tetraploid and diploid oysters were classified as resting stage (S0, Fig. 3). At 18 °C, gonadal maturation progressed slowly in tetraploid oysters during the first 45 days, mainly at S0 and S1, with about 16.7% at S2. The gonadal development process was rapid in diploid oysters, and most diploids reached maturity within 60–75 days. At the end of treatment, the proportion of tetraploids at S3 was 53.3%, and 40% of tetraploids were classified as S4.

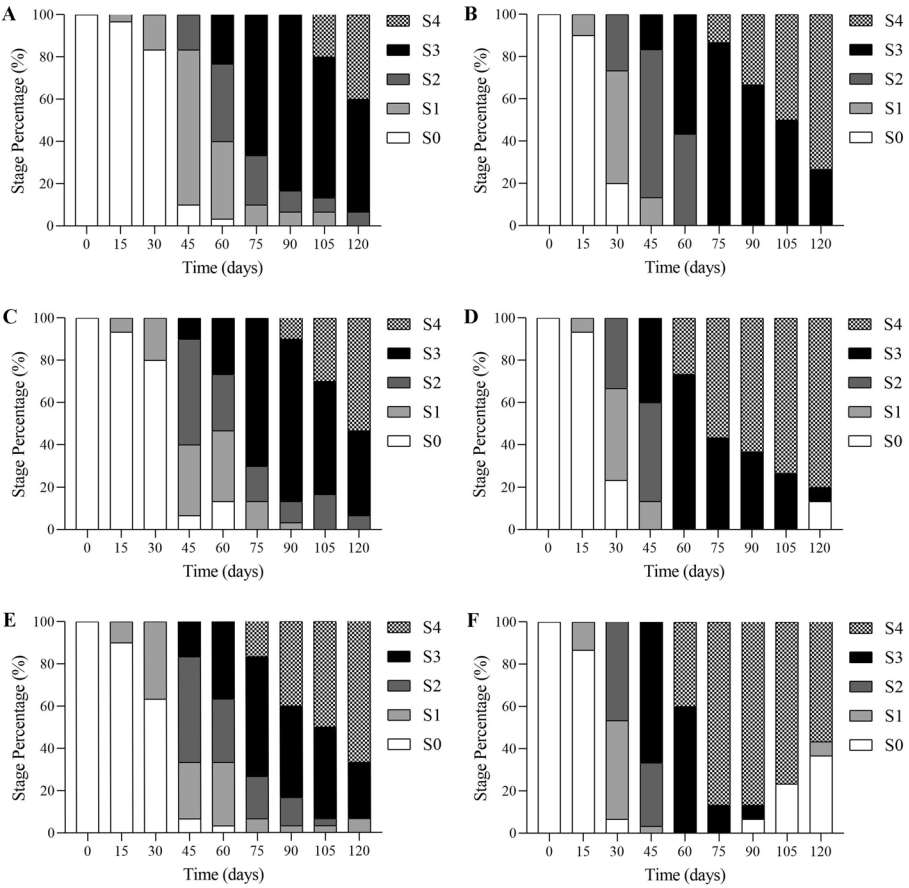

Fig. 2 Proportion of 60 tetraploid and diploid oysters at different stages of gonad development under different temperature regimes (18, 20 and 22 °C) during 120 days. **A** Tetraploid oysters at 18 °C; **B** Diploid oysters at 18 °C; **C** Tetraploid oysters at 20 °C; **D** Diploid oysters at 20 °C; **E** Tetraploid oysters at 22 °C; **F** Diploid oysters at 22 °C

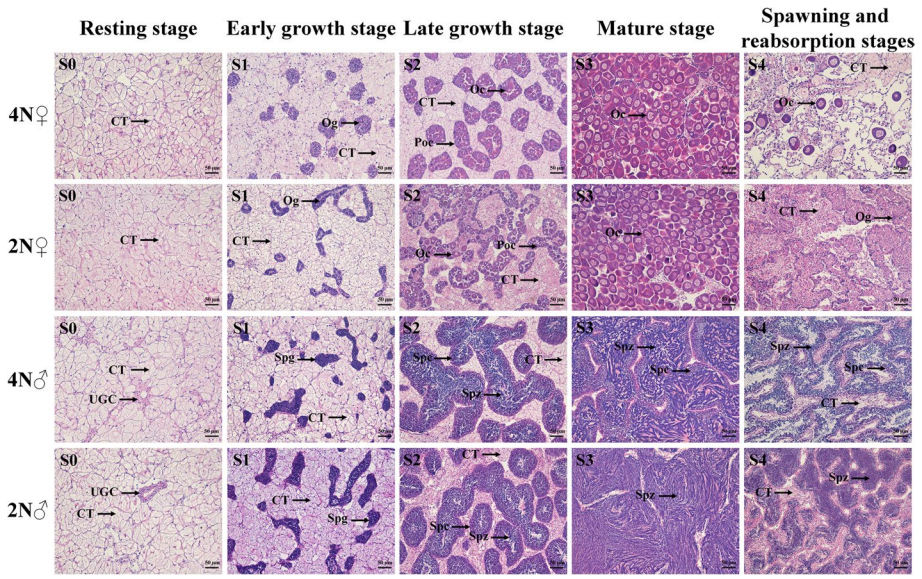

Fig. 3 Histological identification of the five stages of gonadal development in tetraploid and diploid oysters (*C. gigas*) under 20 × objective lens. Scale bar = 50 μm. CT: conjunctive tissues; UGC: undifferentiated germ cells; Og: oogonia; Poc: previtollogenic oocyte; Oc: oocyte; Spg: spermatogonia; Spc: spermatocyte; Spz: spermatozoa

At 20 °C, the acceleration of gametogenesis was more apparent. The proportions of S0, S1 and S2 in the first month were similar in all groups compared to 18 °C (Fig. 2). Subsequently, the tetraploid and diploid follicles were predominantly filled with oogonia and spermatogonia, and the follicles became progressively larger with an increase in germ cells (Fig. 3). In the second month, the percentage of S3 increased to 73.3%, and the spawning and reabsorption stages (S4) were observed for the first time in diploid oysters. At 75–90 days, a high proportion of ripe stage was observed in tetraploid oysters (Fig. 2). Diploids with gonads in the resting stage (S0) were found at 120 days, indicating that some diploids were ready to start a new gametogenic cycle.

At 22 °C, the development of gametogenesis was faster than at 18 and 20 °C. At this temperature, tetraploid oysters reached the mature phase in 75 days and diploid oysters in 45 days. Tetraploid and diploid gonads were filled with mature oocytes and spermatozoa without connective tissue (Fig. 3). The proportion of S0 in diploids increased from 90 to 120 days, with a small proportion of S1 appearing at the end of the experiment.

Effect of different temperatures on sex ratio

Based on histological examination of gonadal tissues, sex ratio varied throughout experiment, suggesting that temperature affected sex ratio. At 18 °C, the sex ratio of tetraploid oysters was predominantly undetermined during the first month. The proportion of females and males in tetraploid oysters was not different from approximately 1:1 at 60–90 days, but it was different with sex ratio of approximately 3:2 at 105–120 days (Fig. 4). The proportion of diploid females exceeded 50% at 45–120 days, suggesting that the sex ratio was stable at 18 °C. At 20 °C, the proportion of tetraploid males increased during the first

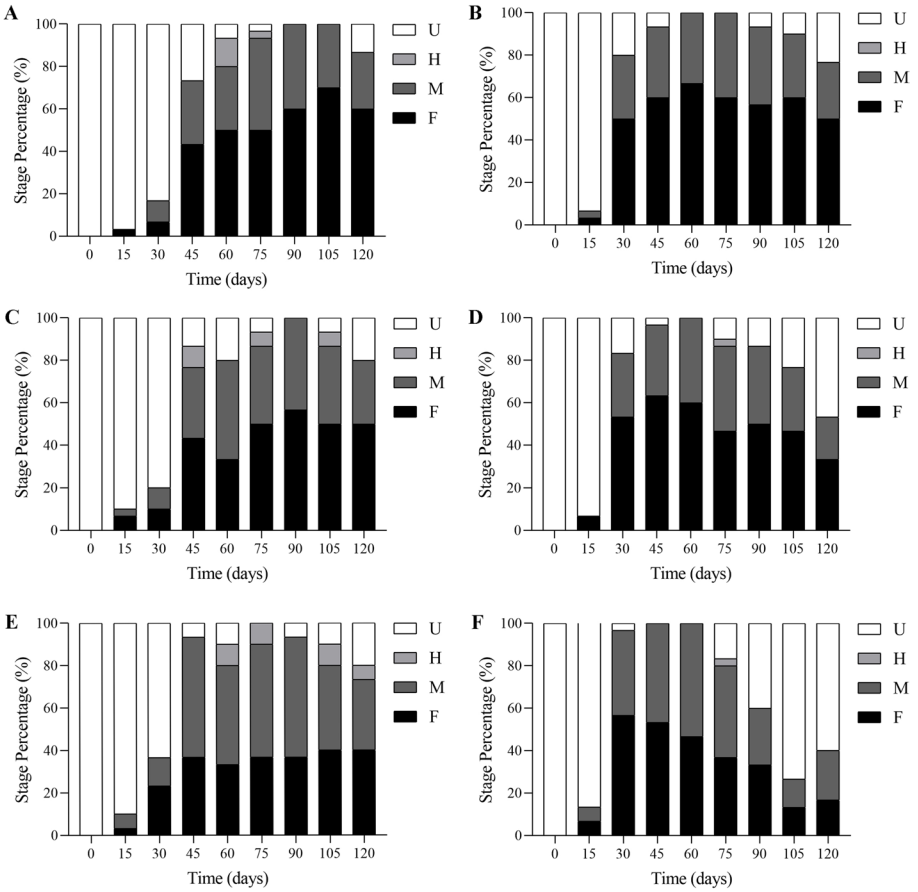

Fig. 4 Proportion of tetraploid and diploid oysters at different stages of sex ratio under different temperature regimes (18, 20 and 22 °C) during 120 days. Oysters were classified according to the gametes present as females (F), males (M), hermaphrodite (H) and undetermined (U). **A** Tetraploid oysters at 18 °C; **B** Diploid oysters at 18 °C; **C** Tetraploid oysters at 20 °C; **D** Diploid oysters at 20 °C; **E** Tetraploid oysters at 22 °C; **F** Diploid oysters at 22 °C

60 days. The ratio of females to males in the tetraploid oysters was approximately 1:1 at 75–120 days. The percentage of diploid females decreased between 45 and 120 days, with 46.7% of undetermined diploids at 120 days. At 22 °C, the proportions of males were higher in most groups compared to 20 °C. At 45–120 days, the proportion of tetraploid females was approximately 40% and the percentage of tetraploid hermaphrodites was about 10%. The proportion of diploid females decreased at 30–105 days, while the percentage of undetermined diploids increased at 75–105 days.

Effect of different temperatures on survival rate

Survival rates of tetraploids and diploids decreased with increasing temperature, with significant differences between 22 °C and the other two temperature treatments ($P < 0.05$).

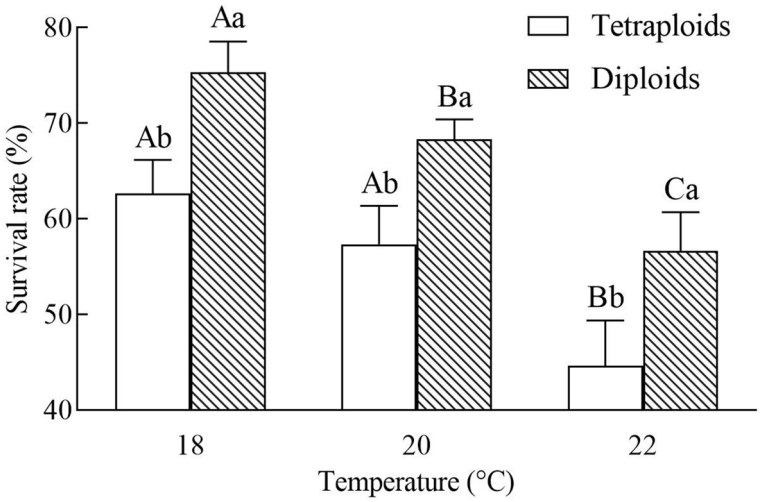

Fig. 5 Survival rate of 200 tetraploid and diploid *C. gigas* at 18, 20 and 22 °C. Different uppercase letters indicate significant differences ($P < 0.05$) at the same ploidy, while different lowercase letters indicate significant differences ($P < 0.05$) at the same temperature

The survival rate of diploids was significantly higher than that of tetraploids in three temperatures ($P < 0.05$). At 22 °C, survival rates of tetraploids and diploids were $44.67 \pm 4.73\%$ and $56.67 \pm 4.04\%$, respectively (Fig. 5).

Oocyte diameter and number of oocytes and spermatozoa in tetraploid and diploid oysters

The diameter of oocytes at 18 °C was larger than those at 20 and 22 °C, but not significant ($P > 0.05$) (Table 2). In the three temperature regimes, the diameters of oocytes in tetraploids ranged from 58.63 ± 3.83 to 59.30 ± 4.51 μm. However, the oocyte diameters of diploid oysters ranged from 47.16 ± 2.80 to 48.14 ± 2.29 μm. In terms of the number of oocytes, tetraploid oysters were significantly fewer than diploid oysters. The number of oocytes in tetraploids was from 2.77 ± 0.41million to 3.41 ± 0.30 million. The number of oocytes in diploids ranged from 6.94 ± 0.37 million to 7.37 ± 0.21 million (Table 2). Meanwhile, tetraploid oysters had significantly lower sperm counts than diploid oysters ($P < 0.05$). The number of sperm in tetraploids was from $0.623 \pm 0.$ 07 billion to 0.745 ± 0.08 billion. The number of sperm in diploids ranged from 1.685 ± 0.09 billion to 1.758 ± 0.12 billion (Table 2).

Discussion

Effect of different temperatures on gonadal development

Temperature plays a pivotal role for many species in regulating the initiation and duration of gametogenesis, the pace of gamete maturation, and the induction of spawning. In

Table 2 Oocyte diameter and number of tetraploids and diploids oocytes and sperm in 60 *C. gigas* from different temperature regimes

Oyster type	Oocyte diameter 18 ℃ (μm)	Oocyte diameter 20 ℃ (μm)	Oocyte diameter 22 ℃ (μm)	Total oocytes 18 ℃ ($\times 10^6$)	Total oocytes 20℃ ($\times 10^6$)	Total oocytes 22 ℃($\times 10^6$)	Total sperms 18 ℃ ($\times 10^8$)	Total sperms 20℃ ($\times 10^8$)	Total sperms 22 ℃($\times 10^8$)
Tetraploids	59.30 ± 4.51^{Aa}	58.80 ± 3.34^{Aa}	58.63 ± 3.83^{Aa}	2.77 ± 0.41^{Cb}	3.13 ± 0.34^{Bb}	3.41 ± 0.30^{Ab}	6.23 ± 0.70^{Bb}	6.98 ± 0.89^{Ab}	7.45 ± 0.76^{Ab}
Diploids	48.14 ± 2.29^{Ab}	47.16 ± 2.80^{Ab}	47.46 ± 2.90^{Ab}	6.94 ± 0.37^{Ba}	7.37 ± 0.2^{Aa}	7.34 ± 0.34^{Aa}	16.85 ± 0.86^{Aa}	17.58 ± 1.16^{Aa}	17.08 ± 0.77^{Aa}

Different uppercase letters indicate significant differences ($P < 0.05$) at the same ploidy and different temperatures, while different lowercase letters indicate significant differences ($P < 0.05$) between tetraploids and diploids

this study, the effect of temperature on gametogenesis was evident in tetraploid and diploid oysters maintained between 18 and 22 °C, as oocyte growth was observed to accelerate with increasing temperature. When oysters were reared at 20 and 22 °C, oocytes continued to develop until vitellogenesis but without the formation of new oocytes (Chávez-Villalba et al. 2007). In previous studies gonadal development in bivalves was also found to be accelerated at higher temperatures when both temperature and ingestion rate were increased (Zapata-Restrepo et al. 2019).

Tetraploid oysters reached the mature phase more slowly than diploid oysters at the same temperature. In cyprinid loach *Misgurnus anguillicaudatus*, tetraploids also showed delayed gonadal development compared to diploids (Zhou et al. 2022). Experiments conducted in controlled environments on the reproductive conditioning of different species at different ploidy levels supported the hypothesis that the time required for gametogenesis to be satisfactorily completed was different at the same temperature (Heasman et al. 1996; Martínez and Pérez 2003). These results indicated that tetraploid oysters could require more energy to reach sexual maturity (Qin et al. 2022; Francesc et al. 2009). These results provided a foundation for studying gonadal development in tetraploid oysters and enhancing our knowledge of this process in polyploid oysters.

Effect of different temperatures on sex ratio

C. gigas was a species that exhibited protandric hermaphroditism, with sex determination controlled by genetic factors and environmental conditions (Guo et al. 1998). In this study, a higher proportion of males was found in most groups of tetraploid and diploid oysters kept at 22 °C compared to 20 °C and 18 °C. Sex determination in *C. gigas* was strongly influenced by temperature, resulting in a high proportion of males at high temperatures (Santerre et al. 2013). Prior studies had also demonstrated that temperature affected the sex ratio of both *Ostrea edulis* and *C. gigas*, with more females observed at lower temperatures (Alyssa et al. 2013; Santerre et al. 2013).

The current study showed that the hermaphrodite rate of tetraploids was higher than that of diploids. Researchers have proposed that polyploidy disrupted sex determination in animals, which was supported by previous studies on triploid oysters (Muller 1925; Yang et al. 2022). However, tetraploid *C. gigas* exhibited sexual characteristics similar to those of diploid oysters, with an even distribution of males and females and a low percentage of hermaphroditism (Guo and Allen 1997). Hermaphroditism may be caused by genetic abnormalities occurring in ploidy change, including triploidy, aneuploidy, unequal crossover, or heterologous translocation affecting major sex-determining genes (Guo et al. 1998). However, some studies argued that polyploidy was not a problem for sex under conditions of strong (or dominant) genetic determinism (White 1978). Despite the limited understanding of the mechanisms of genetic determination, it was widely believed that the protandric dioecy observed in *C. gigas* was genetically controlled, probably by dominant genes (Guo and Allen 1997).

Effect of different temperatures on survival rate

In the study, the survival of tetraploid and diploid oysters decreased with increasing temperature, with a significant difference between oysters exposed to 22 °C and the other two temperature treatments. Several studies indicated that prolonged exposure of bivalves to high temperatures leads to increased stress levels in adult organisms, with

stress intensity positively correlating with temperature magnitude (Robinson 1992). In fact, the oyster's metabolic demands for reproduction, growth and maintenance were maximized as temperatures increased. As a result, many oysters may not have enough energy reserves to defend against pathogens or environmental disruptions (Berthelin et al. 2000).

In the present study, the survival rate of tetraploids was significantly lower than that of diploids in three temperatures ($P < 0.05$). In our previous research, tetraploid oysters had lower cumulative survival than diploid oysters in all periods of 480 days (Zhou et al. 2023). The first two generations of tetraploid oysters experienced severe mortalities associated with early gametogenesis (Guo 2012). The low survival rate of tetraploid oysters may be attributed to the increased energy requirements for giant cell division, inappropriate broodstock conditions, and different culture techniques (Qin et al. 2022). In general, neo-polyploid organisms exhibited reduced survival or viability as a result of rapid adaptation to novel environments, whereas tetraploid organisms had advantages in terms of heterosis, genetic redundancy, and asexual reproduction (Comai 2005).

Oocyte diameter and number of oocytes and spermatozoa in tetraploid and diploid oysters

Female reproductive traits played a key role in ensuring reproductive efficiency in aquaculture species (Ren et al. 2020). In oysters, the relative size of oocytes is generally considered to be a valid indicator of gametes quality (Lango-Reynoso et al. 2000). In this study, the diameter of oocytes in tetraploid oysters was larger than that in diploid oysters. The results were similar to the previous study, where the diameter of tetraploid oocytes (62.5 μm) was significantly larger than that of normal diploid oocytes (53.4 μm) (Guo et al. 1996). In loach *Misgurnus anguillicaudatus* and Catarina scallop *Argopecten ventricosus*, tetraploid oocytes were significantly larger compared to diploids for all cells measured (Li et al. 2012; Ibarra et al. 2017). A larger oocyte diameter could provide more nutrients, resulting in reduced mortality during the processes of fertilization, hatching and initial feeding (Bromage et al. 1992; Kian et al. 2004). Commercial production with high reproductive capacity could reduce the cost of breeding programs (Caballero-Zamora et al. 2015).

This study found that tetraploid oysters had significantly fewer oocytes than diploid oysters ($P < 0.05$). The phenomenon of reduced fecundity or sterility in neo-polyploids has been documented in plants and some animals. In higher vertebrates such as fish, the increased cellular volume in polyploid organisms was frequently compensated for by a reduction in cell number, so that the overall size of the organs remained constant (Benfey and Sutterlin 1984). Considering the increased oocyte volume of tetraploids, the gonadal output of tetraploid females was approximately the same as normal diploids (Guo and Allen 1997). Tetraploid oysters were developed for efficient production of triploid oysters through the process of diploid sperm crossing with regular diploid oysters (Guo et al. 1996). In this study, the number of spermatozoa in tetraploids was lower than that in diploids. Similarly, previous research indicated that tetraploid males in *C. gigas* had a 50% reduction in the number of spermatozoa per unit of wet gonad weight compared to diploid males (Dong et al. 2005a). The linear dimension of tetraploid sperm was about 1.25 times larger than that of diploid sperm, resulting in a doubled volume of tetraploid sperm compared to diploids (Dong et al. 2005b).

Conclusion

In the present study, the effects of three rearing temperatures on broodstock conditioning, gonadal development and survival of both tetraploid and diploid *C. gigas* was assessed. The gametogenesis process in tetraploid and diploid oysters exhibited an acceleration trend in response to elevated conditioning temperatures, but tetraploids showed a comparatively delayed onset of maturation compared to diploids. The sex ratio (male: female) exhibited fluctuations throughout the experiment, indicating that the ratio was influenced by temperature treatments. At 22 °C, the proportions of males were usually higher compared to the other two temperature treatments. However, tetraploid and diploid oysters had the lowest survival rates at 22 °C. In terms of the number of oocytes and spermatozoa, tetraploid oysters were fewer than diploid oysters. Base on the above results, the present study has established the appropriate temperature (20 °C) for broodstock conditioning of tetraploid oysters of 'Haida No. 3' line. Overall, this study provides a better understanding of broodstock conditions and gonadal development of tetraploid *C. gigas*.

Author contributions All authors contributed to the study conception and design. JZ completed methodology, investigation, data curation, and writing—original draft. GJ designed methodology. CX provided supervision and resources. GC performed data curation. Supervision, conceptualization, resources, writing—review and editing, and funding acquisition were performed by QL.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate Not applicable.

Human and animal ethics The present study was performed according to the standard operation procedures (SOPs) of the Guide for the Use of Experimental Animals of the Ocean University of China. All animal care and use procedures were approved by the Institutional Animal Care and Use Committee of Ocean University of China.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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