Aquaculture International (2024) 32:6097–6113
https://doi.org/10.1007/s10499-024-01457-9

RESEARCH

Effects of temperature and salinity on the larval early development, growth, and settlement of the diploid, triploid, and tetraploid Pacific oyster "Haida No. 2" strain

Geng Cheng[1] · Yuanxin Liang[1] · Haining Zhang[1] · Chengxun Xu[1] · Qi Li[1,2]

Received: 26 January 2024 / Accepted: 29 February 2024 / Published online: 6 March 2024

Abstract
Tetraploids play a crucial role in the large-scale production of all-triploid Pacific oyster, *Crassostrea gigas* by mating with diploids. For nursery farms, research related to larval tolerance to the environment appears to be extremely important. For this goal, fertilization, embryonic development, growth, and settlement from fertilization stage to metamorphosis stage of the diploid, triploid, and tetraploid larvae of *C. gigas* "Haida No. 2" strain were studied under controlled conditions of temperature (20, 24, 28, and 32 ℃) and salinity (18, 21, 24, 27, and 30 psu). The results showed that increasing temperature and lowering salinity reduced survival time of sperm while increasing temperature and raising salinity accelerated the germinal vesicle breakdown (GVBD) ratio of stripped eggs in diploids and tetraploids for the first time. The appropriate condition for hatching of four crosses (DD, DT, TD, and TT, males were listed first, D for diploid, T for tetraploid) were listed as follows: 20–28 ℃/27–30 psu for DD, 24 ℃/27–30 psu for DT, 24–28 ℃/27–30 psu for TD, and 24 ℃/27–30 psu for TT. Diploids and triploids had similar performance but were all better than tetraploids under different temperature and salinity. The settlement rate increased with rising temperature while salinity and ploidy had little influence on settlement. The information obtained in this study can contribute to increasing the yield of triploid and tetraploid "Haida No. 2" of *C. gigas*.

Keywords *Crassostrea gigas* · Ploidy · Temperature-salinity condition · Embryonic development · Growth trait

Handling Editor: Gavin Burnell

✉ Qi Li
qili66@ouc.edu.cn

[1] Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao 266003, China

[2] Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, China

Introduction

Polyploid breeding, with its great advantages such as fast growth and triploid gonad sterility, has become one of the important breeding tools and has been successfully practiced in several species (Fraser et al. 2021; Pongtippatee et al. 2012; Qin et al. 1998; Xie et al. 2023). Oyster, as one of the economically important aquatic animals, its polyploidy was first reported in 1981 and has developed rapidly over the last three decades (Stanley et al. 1981). At present, several species of oysters, including *Crassostrea gigas* (Zhou et al. 2023), *C. angulata* (Zhang et al. 2022), and *C. virginica* (Bodenstein et al. 2021), have been successfully polyploidized and industrialized, achieving great production benefits.

As a kind of wide-temperature and wide-salt species, the Pacific oyster *C. gigas* is distributed in the coastal waters in northern China, and deeply favored by farmers because of its fast growth and strong environmental adaptability (Wang et al. 2012; Martínez-García et al. 2022). However, the Pacific oyster suffered from summer mass mortality, which can cause substantial economic losses and severely hamper the development of the oyster aquaculture industry. Studies have shown that summer mass mortality in the Pacific oyster can be attributed in part to gonadal development and substantial spawning activity (Cotter et al. 2010; Li et al. 2007; Samain 2011). Triploidization of oysters, on the other hand, could be a good solution to these problems because it exhibits poor fertility and gamete emission difficulties and shows advantage in survival ability due to sterility (Yang et al. 2022; Jouaux et al. 2013). In order to obtain triploid oysters, the access to tetraploids is particularly important, and 100% triploids can be obtained by using the sperm of tetraploids crossed with the eggs of diploids (Guo et al. 1996). Since tetraploid Pacific oyster have relatively high fecundity (Guo and Allen 1997), after the first generation of tetraploids has been induced, a large number of offspring can be obtained by self-breeding without repeating the laborious and complicated inducing process (Eudeline et al. 2000). At present, the acquisition of tetraploid *C. gigas* is already a common technique, and many researchers have conducted experiments on tetraploid *C. gigas* to understand their biological characteristics and production performance. (McCombie et al. 2005; Miller et al. 2014; Zhang et al. 2014; Qin et al. 2022; Zhou et al. 2023).

Although a great deal of research has been conducted on growth characteristics (Qin et al. 2022; Zhou et al. 2023), gonadal development (Yang et al. 2022), and the immune system (Zhang et al. 2023) in juvenile and adult triploid and tetraploid *C. gigas*, fewer study has been performed on their larval stage, which is part of great interest to breeders in the nursery. Li et al. (2022) studied the effects of temperature and salinity on the growth and survival of tetraploid *C. gigas*, which are two very important factors affecting the growth and survival of shellfish larvae (Li et al. 2021; Rico-Villa et al. 2009; Matsubara et al. 2023). However, the information was rather limited in that it only dealt with growth and survival of tetraploid *C. gigas* larvae from hatching out of D-stage larvae to 16th day after fertilization. Therefore, in order to understand the growth characteristics of polyploid larvae of the Pacific oyster in detail, we investigated the effects of temperature and salinity on triploid and tetraploid larvae of "Haida No. 2" strain of *C. gigas* from fertilization to settlement.

Materials and methods

Experimental materials

Diploid oysters were derived from the selected strain "Haida No. 2" of *C. gigas* (13th generation), with golden color and rapid growth (Ge et al. 2016). Tetraploid oysters were obtained by inhibiting the release of polar body of the fertilization eggs from female triploid and male diploid "Haida No. 2" of *C. gigas*.

In April 2023, 1-year-old oysters of diploid and tetraploid were collected from Rushan and transported to Laizhou, Shandong Province, China. Both broodstocks were maintained with conditioning water (temperature 23 ± 1 ℃; salinity 29 ± 1 psu) and fed with *Nitzschia closterium* until gonadal maturity. The polidy of tetraploids was identified by flow cytometry (Beckman CytoFLEX) as described in Yang et al. (2022) before the experiment.

Design of experiment and measurement

In this study, five levels of salinities (18, 21, 24, 27, and 30 psu) and four levels of temperatures (20, 24, 28, and 32 ℃) were set according to the range of salinity and temperature changes in the coastal aquaculture areas in China (Chu et al. 2005; Liu et al. 2008). The temperature was at 24 ℃ in the salinity treatments, and the salinity was at 27 psu in the temperature treatments. Each experiment was repeated three times.

To explore the effects of salinity and temperature on sperm motility of diploid and tetraploid *C. gigas*, the sperm adaptation time (AT), fast movement time (FT), total movement time (TT), and sperm viability (V) were recorded by the light microscope (Li et al. 2021). Sperm motility was assessed by the number of dead spermatozoa to total spermatozoa within the field of view using a blood cell counting plate. To facilitate counting of the total number of spermatozoa, spermatozoa could be mixed with an equal volume of fresh water resulting in sperm death and recounted. Specially, adaption time was defined as the time when about 50% of the sperms changed from essentially immobile to fast-moving after mixing with seawater. Fast movement time was defined as the time from the end of the adaptation time to the time when about 70% of the fast-moving sperm stopped moving or moved slowly. Total movement time was defined as the time from the mixing of sperm with gradient seawater to the cessation of movement of more than 95% of the sperm. Sperm viability was defined as the ratio of FT to TT. Sperm of diploids and tetraploids was filtered with a 48-μm nylon screen and then immersed in the seawater of the under the temperature of 20, 24, 28, or 32℃ and the salinity of 27 psu, or under the temperature of 24 ℃ and the salinity of 18, 21, 24, 27, or 30 psu respectively. Three males of diploids and tetraploids were used at each replication.

To explore the effects of salinity and temperature on the development of stripped eggs of diploid and tetraploid *C. gigas*, germinal vesicle breakdown (GVBD) ratio of stripped eggs was observed. GVBD is an important sign of stripped eggs maturation since spawned oocytes of many marine mollusks need a period to start breakdown of the germinal vesicle (Osanai 1985; Qin et al. 2018; Li et al. 2021). Eggs of diploids were collected by stripping the gonads of diploids and then sifting with a 53-μm nylon screen, washed on a 25-μm screen and finally immersed in the seawater under the temperature of 20, 24, 28, or 32℃ and the salinity of 27 psu, or under the temperature of 24℃ and the salinity of 18, 21, 24, 27, or 30 psu respectively, while eggs of tetraploids were sifted with a 96-μm nylon screen

and then resuspended in the seawater of the corresponding temperature and salinity. GVBD ratio was defined as the percentage of the number of oocytes without visible germinal vesicles to the total of all normal oocytes. Three female diploids and tetraploids were used at each replication.

To explore the effects of salinity and temperature on fertilization and incubation ability of diploid, triploid, and tetraploid *C. gigas*, fertilization rate, embryonic development, and hatching rate were observed. Ten males and ten females from diploid and tetraploid broodstocks were prepared for the experiment, consisting of four different crosses, diploid ♀ × diploid ♂ (DD), diploid ♀ × tetraploid ♂ (DT), tetraploid ♀ × diploid ♂ (TD), and tetraploid ♀ × tetraploid ♂ (TT). Eggs of ten females of diploids were pooled together and then divided equally into eighteen 5-L plastic buckets (temperature 24 ℃; salinity 27 psu). Then, the eggs were added into seawater under different salinities (18, 21, 24, 27, and 30 psu) or different temperatures (20, 24, 28, and 32 ℃), respectively. Also, sperm of ten males of diploids and tetraploids were striped and pooled together, respectively, and then mixed with the nine types of seawater containing eggs of diploids as described above to obtain DD and DT crosses. Similarly, egg pools of tetraploids mixed with sperm pools of diploids and tetraploids to obtain TD and TT crosses. At the same time, the different developmental stage of embryos was recorded by fixing in 2% formaldehyde at 0.5 h, 1.5 h, 12 h, and 20 h respectively. The embryos of the four crosses under different salinity and temperature levels were sampled at 1 h and 24 h after fertilization, respectively, to calculate the fertilization rate and hatching rate. The fertilization rate was defined as the percentage of the number of fertilized eggs to the total number of eggs, and the hatching rate was defined as the percentage of the number of D-larvae to the number of fertilized eggs at 24 h.

To explore the effects of salinity and temperature on larval rearing, the shell height and survival rate of larvae of the three crosses (DD, DT, and TT) under different salinity and temperature were quantified. The three crosses were incubated firstly in 24-m^3 tanks with a temperature of 24 ℃ and a salinity of 27 psu, and the D-larvae were then collected after 24 h. Eggs of ten females and sperm of ten males were used in each cross. The D-larvae of the three crosses were transferred into a volume of 150-L tanks with an initial larval density of 2 ind/mL. Specifically, the ploidy of three crosses was identified by flow cytometry (Beckman CytoFLEX) to ensure the correctness of the ploidy of the D-larvae. The salinity and temperature of each tank were adjusted gently to the corresponding level over the next 12 h. Larvae were fed with *Isochrysis galbana* three times and 1/3 of the water was replaced every day. The shell height was measured on day 2, 6, 10, 14, and 18 after fertilization. The relative growth rate (RGR) and accumulative survival rate (ASR) were calculated according to equations:

$$\mathrm{RGR}(\%) = (H_t - H_0)/H_0 \times 100\%$$

$$\mathrm{ASR}(\%) = N_t/N_0 \times 100\%$$

where H_0 and H_t are the average shell height of 30 larvae randomly sampled from each tank on day 2 and time t, respectively; N_0 and N_t are the number of larvae on day 2 and time t, respectively.

To explore the effects of salinity and temperature on larval metamorphosis of the three crosses (DD, DT, and TT), the settlement rate (SR) of larvae under different salinity and temperature levels were measured. When more than 50% larvae in each 160-L tank were eyed, they were transferred to a 20-L tank to the corresponding salinity and temperature with eyed larval density of 0.2 ind/mL. The salinity was at 27 psu in the temperature

treatments; the temperature was at 24 ℃ in the salinity treatments. One day after stabilization, strings of scallop shells were put in the tanks as substrates. Three days later, the settlement rate (SR) was calculated according to equations:

$$\mathrm{SR}(\%) = (1 - N_r/N_e) \times 100\%$$

where N_r is the number of larvae remaining in the seawater and N_e is the number of initially eyed larvae in the tank, and the number of spat attached to scallop shells and tank walls helped confirm the results (Rico-Villa et al. 2009).

Statistical analyses

The results of the experiment were presented as the means ± standard deviation (SD) and the data were analyzed using SPSS 25.0. In order to improve normality and homoscedasticity, the sperm motility data (the sperm adaptation time, fast movement time, and total movement time) and shell height were log-transformed on a base of 10 and the GVBD ratio, the fertilization rate, hatching rate, survival rate, and settlement rate were arcsine transformed. Differences of the data among different temperatures or salinities within a certain cross were compared by one-way ANOVA followed by least significant difference (LSD) analysis. Significance was set as $P < 0.05$.

Results

Effects of salinity and temperature on sperm movement and GVBD ratio

The sperm adaptation time of tetraploids was significantly higher than that of diploids under 18 psu or 20 ℃ ($P < 0.05$) (Table 1). The fast movement time and total movement time of diploid and tetraploid sperm significantly decreased with elevated temperature whereas these the two indexes increased with rising salinity. Furthermore, the sperm vitality of diploids was higher than that of tetraploids under every controlled condition.

Elevated salinity and temperature both accelerated GVBD ratio for both diploids and tetraploid (Fig. 1). Low salinity is inimical to GVBD regardless of ploidy. The value of GVBD ratio, however, was higher in the seawater under higher salinity, which was 94.00% and 95.33% at the salinity of 27 and 30 psu, respectively, for diploids, and was 84.67% and 85.00% at the salinity of 27 and 30 psu, respectively, for tetraploids. GVBD ratio of diploids was significantly higher than that of tetraploids at each temperature at 60 min ($P < 0.05$).

Effects of salinity and temperature on incubation

The fertilization rate was significantly lower for all four crosses under low salinity condition (18 and 21 psu, $P < 0.05$) (Fig. 2). The fertilization rate was the lowest under extreme temperature condition (32 ℃), with the value of 70.00%, 60.33%, 67.33%, and 67.33% in DD, DT, TD, and TT group, respectively. The hatching rate basically increased with rising salinity in the four crosses, while high temperature did not favor the hatching rate, as the value was the lowest in 32 ℃. However, the TD group had the worst performance in hatching rate compared to the other groups under all the controlled conditions though the

Table 1 The comparison of sperm movement between diploids and tetraploids (mean ± SD, $n = 3$ replicates)

Item	Temperature (℃)	Diploid	Tetraploid	Salinity (psu)	Diploid	Tetraploid
AT (min)	20	8.66 ± 3.05^{a}	11.67 ± 2.52^{b}	18	8.67 ± 2.08^{b}	10.67 ± 1.53^{c}
	24	7.00 ± 2.00^{a}	8.67 ± 2.52^{ab}	21	8.33 ± 1.53^{b}	8.67 ± 2.52^{bc}
	28	5.33 ± 1.15^{a}	5.67 ± 0.58^{a}	24	5.67 ± 0.58^{a}	7.67 ± 1.53^{abc}
	32	4.66 ± 1.52^{a}	5.33 ± 1.53^{a}	27	4.67 ± 1.53^{a}	5.67 ± 1.15^{ab}
				30	4.67 ± 0.58^{a}	5.33 ± 0.58^{a}
FT (min)	20	710.00 ± 36.06^{d}	523.33 ± 25.17^{d}	18	183.33 ± 15.28^{a}	96.67 ± 5.77^{a}
	24	510.00 ± 40.00^{c}	330.00 ± 30.00^{c}	21	436.67 ± 25.17^{b}	246.67 ± 20.82^{b}
	28	366.67 ± 25.17^{b}	193.33 ± 40.41^{b}	24	546.67 ± 25.17^{c}	380.00 ± 36.06^{c}
	32	18.33 ± 3.51^{a}	12.33 ± 2.52^{a}	27	666.67 ± 35.12^{d}	386.67 ± 15.28^{c}
				30	680.00 ± 26.46^{d}	393.33 ± 11.55^{c}
TT (min)	20	1376.67 ± 75.05^{d}	1223.33 ± 55.08^{d}	18	600.00 ± 30.00^{a}	416.67 ± 15.28^{a}
	24	960.00 ± 30.00^{c}	723.33 ± 40.41^{c}	21	813.33 ± 47.26^{b}	546.67 ± 25.17^{b}
	28	546.67 ± 30.55^{b}	450.00 ± 30.00^{b}	24	920.00 ± 40.00^{c}	683.33 ± 30.55^{c}
	32	33.33 ± 10.41^{a}	25.00 ± 5.00^{a}	27	956.67 ± 35.12^{c}	696.67 ± 25.17^{c}
				30	973.33 ± 30.55^{c}	696.67 ± 11.55^{c}
V (%)	20	51.57	42.78	18	30.56	23.20
	24	53.13	45.62	21	53.69	45.12
	28	67.07	42.96	24	59.42	55.61
	32	55.00	49.33	27	69.69	55.50
				30	69.86	56.46

Different letters in each column indicate significant differences ($P < 0.05$). AT, FT, TT, and V, respectively, represent the sperm adaptation time, sperm fast movement time, sperm total movement time, and sperm vitality. The temperature was at 24 ℃ in the salinity treatments, and the salinity was at 27 psu in the temperature treatments

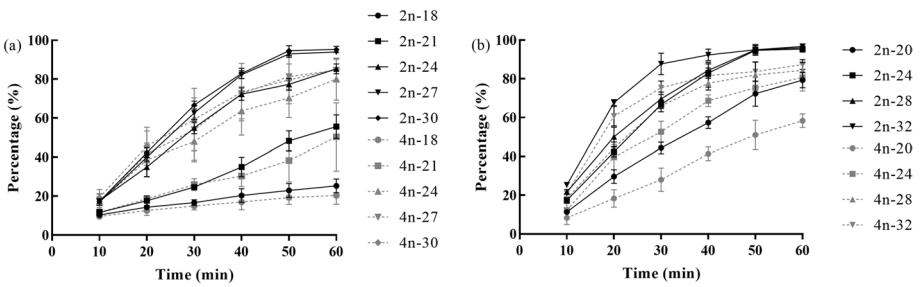

Fig. 1 GVBD ratio over time of stripped oocytes from diploid and tetraploid Pacific oyster, *Crassostrea gigas* "Haida No. 2" strain under different salinities (**a**) and temperatures (**b**)

fertilization rate had no significant difference with the other three crosses under the same condition.

The embryonic development was affected by ploidy (DD, DT, TD, and TT) and environmental factors (salinity and temperature) (Fig. 3, Supplement Fig. S1). The embryonic development rate increased with rising salinity and the highest values were observed under the salinity of 27 and 30 psu for the four crosses. The same tendencies were found in DD and DT groups under different temperature levels. But too high

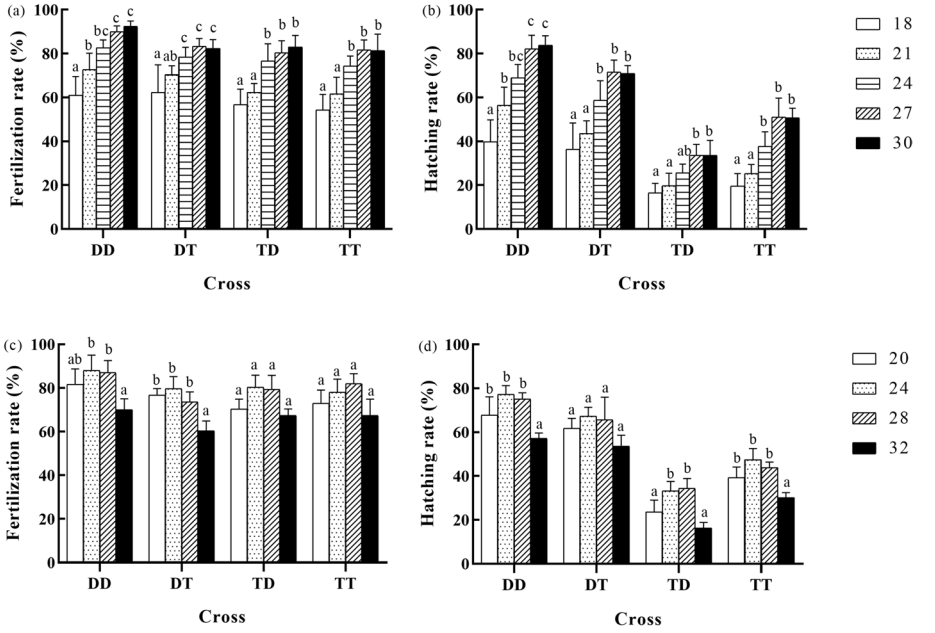

Fig. 2 Fertilization rate and hatching rate of each cross under different salinities (**a**, **b**) and temperatures (**c**, **d**). Different letters indicate significant difference ($P < 0.05$)

temperature (32 ℃) was apparently unfavorable for embryonic development as the lower frequency of embryo at stage ξ than that at 24 and 28 ℃. Under an appropriate range of salinity and temperature (24–30 psu and 24–28 ℃), the order of the rate of embryonic development in the 12 h of fertilization among the four crosses was mainly as follows: TD = TT > DD > DT. Though TD and TT group showed quicker development than the other two groups, the frequency of embryo at stage ξ was lower than DD and DT crosses at 20 h.

Effects of salinity and temperature on larval rearing

Low salinity not only slowed down the growth rate but also increased larval mortality (Fig. 4). RGRs were 270.70%, 236.00%, and 95.78% on day 18 under the salinity of 18 psu while the ASRs were only 18.33%, 13.00%, and 3.67% for DD, DT, and TT crosses, respectively. No significant differences in shell height, RGRs, and ASRs were observed between DD and DT groups, but the value of the three indexes for DD group was significantly higher than those for TT group on day 18 ($P < 0.05$).

Although low temperature (20 ℃) slowed down the growth rate of the three crosses, ASRs had the highest value with 48.67%, 41.67%, and 19.33% (Fig. 5). Obviously, elevated temperature favored RGRs as more than 50% of larvae were eyed on day 14 for DD and DT group under the temperature of 28 and 32 ℃, while no larvae were eyed at the same time under the temperature of 20 and 24 ℃. In addition, significant differences in RGRs and ASRs were observed only between DD and TT group on day 14 or day 18 ($P < 0.05$).

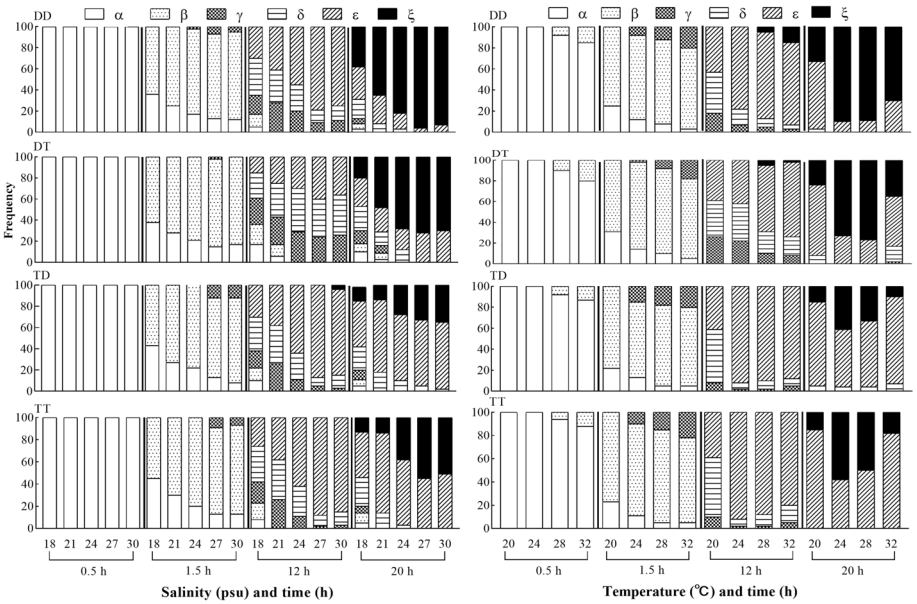

Fig. 3 Frequency (mean, $n = 3$ replicates) of embryonic development stages of DD, DT, TD, and TT crosses under different salinities (18, 21, 24, 27, and 30 psu, 24 ℃) and temperatures (20, 24, 28, and 30 ℃, 27 psu) in different times (0.5, 1, 12, and 20 h). The embryonic development stage is divided into six parts: stage "*α*" represents the embryo with clearly visible polar body but with no division; stage "*β*" represents the stage when the embryo is undergoing cell division; stage "*γ*" represents the blastula stage; at this stage, the embryo is densely surrounded by short cilia and the embryo begins to rotate; stage "*δ*" represents primal period; at this stage, the cells of the vegetal pole portion of the embryo invaginate to form the original intestine, and the invaginated notch forms the endoderm; "*ε*" represents trochophore; at this stage, the invaginated shell glands turn out and begin to secrete shells; "*ξ*" represents D-shaped larvae

Effects of salinity and temperature on settlement

As salinity increased, so did the settlement rate (Table 2). However, there were no significant differences in the settlement rate among DD, DT, and TT crosses under all the salinity levels, except for the TT group which had the lowest rate of settlement (48.00%) under the salinity of 18 psu. The rate of settlement was temperature-dependent. High rate of settlement linked with high temperature regardless of ploidy. However, high temperature exceeding 28 ℃ did not have a better rate of settlement.

Discussion

Effects of temperature and salinity on sperm movement and GVBD ratio of diploids and tetraploids

Sperm of many marine bivalve which are in vitro fertilization organisms are able to survive and become capable for fertilization for a considerable period of time after exclusion and contact with seawater. Changes in environmental conditions, such as 5-hydroxytryptamine

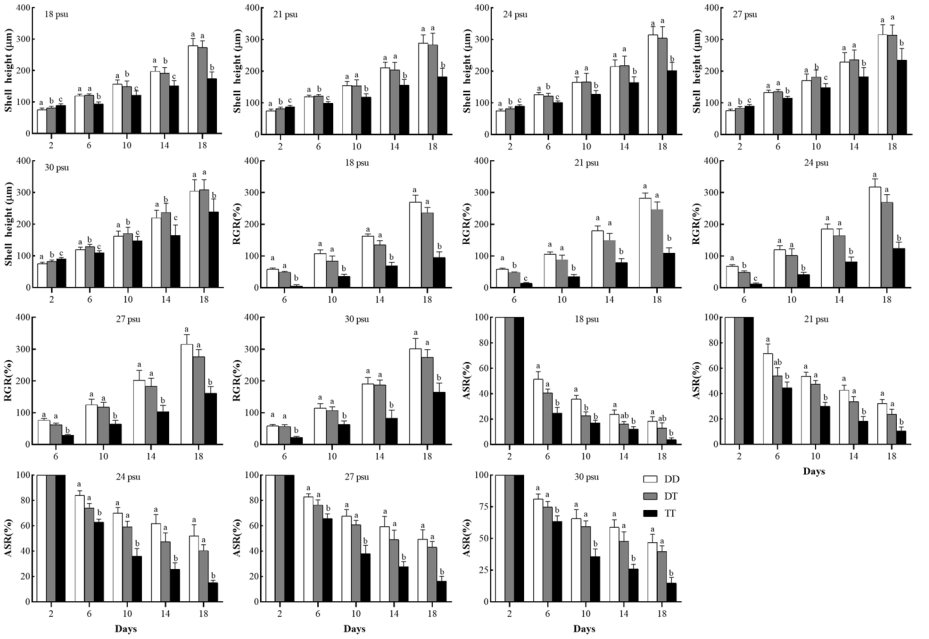

Fig. 4 Comparison of shell height, relative growth rate (RGR), and accumulative survival rate (ASR) among DD, DT, and TT crosses of *C. gigas* under different salinities (18, 21, 24, 27, and 30 psu, 24 ℃). Different letters indicate significant difference ($P < 0.05$)

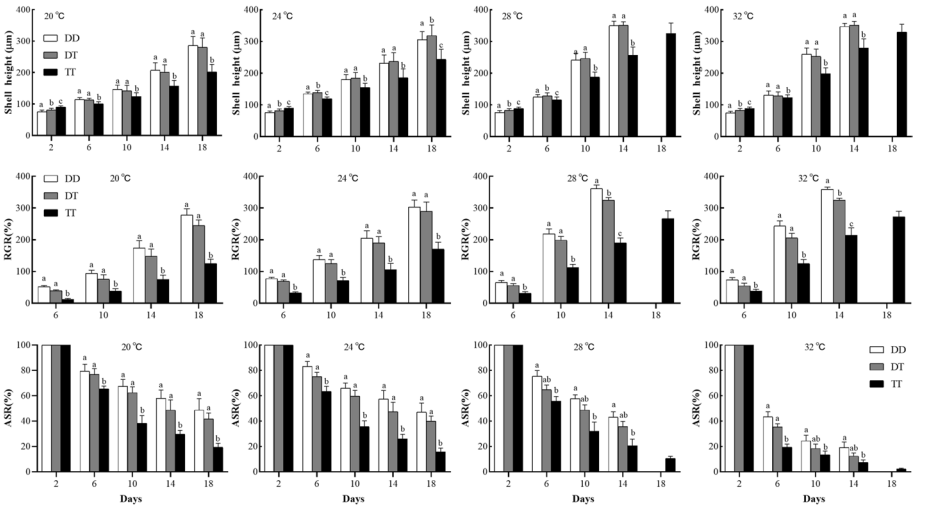

Fig. 5 Comparison of shell height, relative growth rate (RGR), and accumulative survival rate (ASR) among DD, DT, and TT crosses of *C. gigas* under different temperatures (20, 24, 28, and 30 ℃, 27 psu). Different letters indicate significant difference ($P < 0.05$)

Table 2 The comparison of settlement rate (SR) among DD, DT, and TT crosses (mean ± SD, $n = 3$ replicates)

Temperature (℃)	Cross	SR (%)	Salinity (psu)	Cross	SR (%)
20	DD	36.67 ± 6.11^{a}	18	DD	53.33 ± 8.50^{ab}
	DT	36.00 ± 6.00^{a}		DT	54.33 ± 8.74^{ab}
	TT	33.33 ± 5.51^{a}		TT	48.00 ± 7.00^{a}
24	DD	66.00 ± 5.00^{b}	21	DD	60.00 ± 9.00^{ab}
	DT	65.33 ± 5.51^{b}		DT	59.33 ± 10.26^{ab}
	TT	62.33 ± 8.08^{b}		TT	54.67 ± 8.50^{ab}
28	DD	86.33 ± 10.97^{c}	24	DD	64.67 ± 7.51^{ab}
	DT	84.67 ± 9.61^{c}		DT	66.33 ± 8.62^{ab}
	TT	82.67 ± 9.02^{c}		TT	63.00 ± 9.54^{ab}
32	DD	85.67 ± 6.66^{c}	27	DD	69.00 ± 3.61^{ab}
	DT	85.00 ± 7.55^{c}		DT	69.00 ± 5.20^{ab}
	TT	82.00 ± 6.00^{c}		TT	70.33 ± 4.73^{b}
			30	DD	70.33 ± 5.51^{b}
				DT	69.67 ± 5.03^{ab}
				TT	68.67 ± 5.51^{ab}

Different letters in each column indicate significant differences ($P < 0.05$). The temperature was at 24 ℃ in the salinity treatments, and the salinity was at 27 psu in the temperature treatments

creatinine sulfate, pH, temperature, and salinity, could significantly affect sperm viability (Alavi et al. 2014; Eads et al. 2016; Nichols et al. 2021). Temperature and salinity are two of the most common dynamically varied environmental factors in the ocean. Sperm of tetraploid and diploid *C. gigas* were extremely sensitive to changes in seawater temperature, and their viability decreased with increasing temperature in our study. ATP level is a key factor in spermatozoa viability and ATP level in spermatozoa is produced by oxidative phosphorylation (OXPHOS) (Boulais et al. 2017). Sperm motility requires the consumption of ATP for energy supply. It has been observed that ATP levels decreased and sperm motility was subsequently terminated in sea urchins (Mita et al. 1994) and the king scallop, *Pecten maximus* (Suquet et al. 2013). OXPHOS requires the participation of various enzymes, and enzyme activity is temperature-dependent. Enzyme activity could be weakened and the energy consumption became retarded under low temperature, resulting in a longer sperm motility time. The enzyme activity, however, was more vigorous and energy was burned up faster when the temperature was higher, contributing to a shorter sperm motility time. Total movement time (TT) of diploid and tetraploid *C. gigas* spermatozoa significantly decreased under hypotonic conditions in this study. Previous studies have shown that sperm of marine bivalves performed worse in hypotonic environments (Dong et al. 2002; Nichols et al. 2021). Kladchenko et al. (2024) found a significant increase of reactive oxygen species (ROS) levels in the hemocytes of *Magallana gigas* after being exposed to a hypotonic environment. However, overwhelming ROS level could cause DNA damage, oxidation of protein, cellular damage, and death (Choi et al. 2008; Kalogeris et al. 2012; Lesnefsky et al. 2017), which could help explain the worse performance of sperm in a hypotonic environment. It is noteworthy that sperm viability of diploids presented superior performance compared to that of tetraploids under the same condition. Similarly, Suquet et al. (2010) found that the percentage of motile spermatozoa was higher in diploid

males than in tetraploid males, and the cessation of sperm movement was attributed to the drastic changes in morphology of spermatozoa due to deeper damages in chromatin and plasma membrane were observed in spermatozoa of *C. gigas* tetraploid males in comparison to that of diploid males.

Just like many other marine mollusks, the spawned oocytes of the Pacific oyster still remain immature and require undergo GVBD to reach the stage of meiotic metaphase I (Guo et al. 1992). Soaking in seawater was a very effective way to initiate maturation. However, GVBD ratio was influenced by the temperature and salinity (Qin et al. 2018; Li et al. 2021). Our research has shown that both high temperature and high salinity favor the breakdown of the germinal vesicles of eggs. Notably, the GVBD rate of eggs of diploids was higher than that of tetraploids under the same temperature and salinity, which might due to the larger size of eggs of tetraploids. The similar performance was also observed in triploid Hong Kong oyster, *C. hongkongensis* (Qin et al. 2018).

Effects of temperature and salinity on fertilization rate, hatching rate, and embryonic development of DD, DT, TD, and TT crosses

Fertilization rate and hatching rate of the four crosses under high temperature and hypotonic environment were the lowest, which illustrated that the damage to gametes from environmental stress could be directly manifested in fertilization rate and hatching rate. In addition, DT, TD, and TT crosses exhibited lower hatching rate compared with DD crosses, which was largely consistent with previous reports (Guo et al. 1996; Li and Li 2022; Zhang et al. 2022). Li et al. (2022) found high percentage of malformed larvae in DT and TT crosses whereas few malformed larvae appeared in DD group, and this was attributed to the increase of intracellular chromosomes. The negative effects of increased intracellular chromosomes may be reflected in mitotic abnormalities, as pairing and segregation interactions are much more complex (Comai 2005).

The embryonic development is highly temperature-dependent. Our result showed the embryo of the four crosses developed faster at 24 and 28 ℃ than at 20 ℃. In several cephalopods, temperature also exhibited a facilitative effect on embryonic development (Caverivière et al. 1999; Uriarte et al. 2012; Repolho et al. 2014). Caamal-Monsreal et al. (2016) found embryos of *Octopus maya* had a higher metabolic rate at 26 ℃ than at 18 and 22 ℃, which might explain the correlation between temperature and embryonic development. However, when the incubation temperature exceeded the suitable interval for embryonic development, increased malondialdehyde level could cause cellular injury (Repolho et al. 2014). Exposure to low salinity seawater is detrimental to embryonic development. The hatching rate and embryonic development rate of the four crosses were lower at 18 and 21 pus than at other salinity. Similar results were also observed in fish (Hart and Purser 1995; Berlinsky et al. 2004; Shi et al. 2008), which might stem from the fact that salinity stress affected the energy required for osmoregulation in the organism (Howell et al. 1998). Besides, the rate of embryonic development of the four crosses had different performances even under the same temperature and salinity in this study. Under the appropriate temperature and salinity conditions (24–28 ℃, 24–30 psu), the speed of embryonic development was TD = TT > DD > DT within 12 h after fertilization. Ploidy and egg size might be responsible for the result. Beatty and Fischberg (1951) described a decrease in cell number with the increase of ploidy in mice, while the ploidy had little influence on the rate of mitotic division (Eglitis and Wiley 1981), so that the quicker development in TD and TT crosses was observed. Though TD and DT crosses were all triploids, and the ploidy results

were same when tested by flow cytometry (Beckman CytoFLEX), the egg diameter of the maternal parent in the TD group was significantly larger than that in the DT group (Guo and Allen 1997; Li and Li 2022). The different embryonic development of the two crosses might connect with egg size because larger eggs mean more yolk, and oysters are endogenously nourished during embryonic development, with yolk as their nutrient source. Also, Fu et al. (2013) found accurate zygote cleavage required more energy. In addition, the proportion of D-larvae was much lower in the TD and TT crosses, despite the fact that the TD and TT crosses had a higher rate of embryonic development in the first 12 h than the other two crosses. Zhang et al. (2022) used "zygote sterility" to explain the different performance among the four crosses, which mean the embryo developed abnormally though the gametes were successfully fertilized (Chevassus 1983). Zhang et al. (2022) found a lower D-larvae rate and a higher percentage of abnormal individuals in TD and TT crosses in *C. angulata*. Likewise, similar results were observed in *C. virginica* (Matt and Allen Jr 2014). Based on these facts, the lower proportion of D-larvae possible in the TD and TT crosses may due to "zygotic sterility" in the tetraploid "Haida No. 2" line of *C. gigas* in this study.

Effects of temperature and salinity on ASR and RGR of DD, DT, and TT crosses

Our results showed the performance of tetraploid larvae was worse than diploids and triploids under all conditions by comparing the three metrics (shell height, ASR, and RGR), suggesting that tetraploid larvae were of low temperature and salt tolerance. The defects of tetraploids may be associated with chromosome doubling. The extra two sets of chromosomes complicated chromosome pairing and division and interfered with normal mitosis (Comai 2005). However, triploids showed similar performance to diploids. Despite the negative impact of extra chromosomes on normal mitosis, the positive effect of increased triploid heterozygosity may also play a large role in growth and survival performance (Wang et al. 2002).

RGR increased with increasing temperature, while the opposite was true for ASR for the three crosses in this study, indicating the increase in temperature stimulated the growth rate of the larvae on the one hand, but on the other hand, caused massive mortality of the larvae. Rico-Villa et al. (2009) found that larval feeding activity was deeply influenced by temperature. Also, the metabolic rates of oyster larvae were associated with temperature (Lemos et al. 1994; Han and Li 2018). Both of these favored the larval growth. Nevertheless, the level of dissolved oxygen in seawater decreased with increasing temperature, while the organism's demand for dissolved oxygen increased further, creating a conflict between the two that was detrimental to the survival of the larvae (Nie et al. 2017; Gao et al. 2020). The influence of salinity on RGR and ASR mainly focused on the part of low salinity (18 and 21 psu), while the larvae of the three crosses showed superior performance in natural seawater (27 and 30 psu). Madrones-Ladja (2002) found hypotonic conditions resulted in increased ammonia excretion and loss of certain amino acids in bivalves, which would interfere with the normal conduct of their physiological activities and their survival.

Li and Li (2022) attributed the poor performance of tetraploid larvae to the first generation of tetraploid self-breeding populations and suggested that tetraploid adaptability should be improved through selective breeding. Wan et al. (2023) also reported significant improvement in growth traits (D larval rate, survival rate, and shell height) of tetraploids *C. gigas* through mass selection for four consecutive generations. Therefore, in order to improve the yield of tetraploid "Haida No. 2" line of *C. gigas*, what we should do in the

next stage is to use shell height as a target trait for consecutive generations of selective breeding of tetraploids to better meet the needs of large-scale production of triploids.

Effects of temperature and salinity on the settlement of DD, DT, and TT crosses

As for the settlement rate, we found the settlement rate was positively correlated with temperature, regardless of ploidy. This result parallels previous findings on *Ostrea edulis* and *C. gigas* (Robert et al. 2017; Matsubara et al. 2023). Rico-Villa et al. (2009) found that the ingestion rate of *C. gigas* larvae increased when the temperature rose from umbonate larvae stage to eyed larvae stage, but then, the ingestion rate decreased when approaching metamorphosis, which was due to the replacement of villi by gills during metamorphosis, resulting in limited feeding activity of larvae (Cannuel and Beninger 2006). Therefore, elevated temperatures increased larval feeding rates and favored energy reserves to meet the energy demands of metamorphosis (Haws et al. 1993). Salinity has a limited effect on the rate of settlement compared to temperature. Similarly, Jeon et al. (2012) found salinity had no significant effect on settlement of *C. gigas*. When Matsubara et al. (2023) explored the factors affecting the settlement of *C. gigas* in Hiroshima Bay, Japan, they found the settlement index was low under high salinity. However, it was subsequently found that its water temperature and food density were low during periods of high salinity, and ultimately its settlement index was influenced by temperature and food density. Focusing on ploidy, the rate of settlement had no significant difference among diploids, triploids, and tetraploids. This may be due to the fact that as some of the larvae died during the pre-incubation process, the remaining individuals adapted to the corresponding environment and were able to complete the metamorphosis process normally.

Conclusions

In order to better understand the early developmental characteristics of larvae of *C. gigas* with different ploidy, a comprehensive comparison of the effects of temperature and salinity on diploid, triploid, and tetraploid larvae from fertilization to settlement was carried out. Our results showed that diploid and triploid larvae had similar tolerance to temperature and salinity, but both performed better than tetraploids. Therefore, in order to expand the number of tetraploids and improve the performance of tetraploids, it is extremely urgent to carry out consecutive generations of selective breeding of tetraploids.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10499-024-01457-9.

Author contribution Geng Cheng: conceptualization, supervision, resources, project admi. Yuanxin Liang: resources. Haining Zhang: resources. Chengxun Xu: resources. Qi Li: conceptualization, supervision, resources, project admi.

Funding This work was supported by grants from the National Key R&D Program of Shandong Province (2022LZGCQY010, 2021ZLGX03, and 2021TSGC1240), and China Agriculture Research System Project (CARS-49).

Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval 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.

Competing interests The authors declare no competing interests.

References

Alavi SMH, Matsumura N, Shiba K, Itoh N, Takahashi KG, Inaba K, Osada M (2014) Roles of extracellular ions and pH in 5-HT-induced sperm motility in marine bivalve. Reproduction 147:331–345

Beatty RA, Fischberg M (1951) Cell number in haploid, diploid and polyploid mouse embryos. J Exp Biol 28:541–552

Berlinsky DL, Taylor JC, Howell RA, Bradley TM, Smith TI (2004) The effects of temperature and salinity on early life stages of black sea bass *Centropristis striata*. J World Aquac Soc 35:335–344

Bodenstein S, Walton WC, Steury TD (2021) Effect of farming practices on growth and mortality rates in triploid and diploid eastern oysters *Crassostrea virginica*. Aquac Environ Interact 13:33–40

Boulais M, Soudant P, Le Goïc N, Quéré C, Boudry P, Suquet M (2017) ATP content and viability of spermatozoa drive variability of fertilization success in the Pacific oyster (*Crassostrea gigas*). Aquaculture 479:114–119

Caamal-Monsreal C, Uriarte I, Farias A, Díaz F, Sánchez A, Re D, Rosas C (2016) Effects of temperature on embryo development and metabolism of *O. maya*. Aquaculture 451:156–162

Cannuel R, Beninger PG (2006) Gill development, functional and evolutionary implications in the Pacific oyster *Crassostrea gigas* (Bivalvia: Ostreidae). Mar Biol 149:547–563

Caverivière A, Domain F, Diallo A (1999) Observations on the influence of temperature on the length of embryonic development in *Octopus vulgaris* (Senegal). Aquat Living Resour 12:151–154

Chevassus B (1983) Hybridization in fish. Aquaculture 33:245–262

Choi CY, An KW, An MI (2008) Molecular characterization and mRNA expression of glutathione peroxidase and glutathione S-transferase during osmotic stress in olive flounder (*Paralichthys olivaceus*). Comp Biochem Physiol Part A Mol Integr Physiol 149:330–337

Chu P, Yuchun C, Kuninaka A (2005) Seasonal variability of the Yellow Sea/East China Sea surface fluxes and thermohaline structure. Adv Atmos Sci 22:1–20

Comai L (2005) The advantages and disadvantages of being polyploid. Nat Rev Genet 6:836–846

Cotter E, Malham SK, O'Keeffe S, Lynch SA, Latchford JW, King JW, Beaumont AR, Culloty SC (2010) Summer mortality of the Pacific oyster, *Crassostrea gigas*, in the Irish Sea: the influence of growth, biochemistry and gametogenesis. Aquaculture 303:8–21

Dong QX, Eudeline B, Allen SK Jr, Tiersch TR (2002) Factors affecting sperm motility of tetraploid Pacific oysters. J Shellfish Res 21:719

Eads AR, Kennington WJ, Evans JP (2016) Interactive effects of ocean warming and acidification on sperm motility and fertilization in the mussel Mytilus galloprovincialis. Mar Ecol Prog Ser 562:101–111

Eglitis MA, Wiley LM (1981) Tetraploidy and early development: effects on developmental timing and embryonic metabolism. Development 66:91–108

Eudeline B, Allen SK Jr, Guo X (2000) Optimization of tetraploid induction in Pacific oysters, *Crassostrea gigas*, using first polar body as a natural indicator. Aquaculture 187:73–84

Fraser TW, Lerøy H, Hansen TJ, Skjæraasen JE, Tronci V, Pedrosa CP, Fjelldal PG, Nilsen TO (2021) Triploid Atlantic salmon and triploid Atlantic salmon× brown trout hybrids have better freshwater and early seawater growth than diploid counterparts. Aquaculture 540:736698

Fu D, Mitra K, Sengupta P, Jarnik M, Lippincott-Schwartz J, Arias IM (2013) Coordinated elevation of mitochondrial oxidative phosphorylation and autophagy help drive hepatocyte polarization. Proc Natl Acad Sci 110:7288–7293

Gao X, Pang G, Luo X, You W, Ke C (2020) Effects of stocking density on the survival and growth of Haliotis discus hannai♀× H. fulgens♂ hybrids. Aquaculture 529:735693

Ge J, Li Q, Yu H, Kong L (2016) Selection response in mass selection of golden shell Pacific oyster (*Crassostrea gigas*). J Ocean Univ China 40:612–617 (**(in Chinese)**)

Guo X, Allen SK Jr (1997) Sex and meiosis in autotetraploid Pacific oyster, *Crassostrea gigas* (Thunberg). Genome 40:397–405

Guo X, Cooper K, Hershberger WK, Chew KK (1992) Genetic consequences of blocking polar body I with cytochalasin B in fertilized eggs of the Pacific oyster, *Crassostrea gigas*: I. Ploidy of resultant embryos. Biol Bull 183:381–386

Guo X, DeBrosse GA, Allen SK Jr (1996) All-triploid Pacific oysters (*Crassostrea gigas* Thunberg) produced by mating tetraploids and diploids. Aquaculture 142:149–161

Han Z, Li Q (2018) Different responses between orange variant and cultured population of the Pacific oyster *Crassostrea gigas* at early life stage to temperature-salinity combinations. Aquac Res 49:2233–2239

Hart PR, Purser GJ (1995) Effects of salinity and temperature on eggs and yolk sac larvae of the greenback flounder (*Rhombosolea tapirina* Günther, 1862). Aquaculture 136:221–230

Haws MC, DiMichele L, Hand SC (1993) Biochemical changes and mortality during metamorphosis of the Eastern oyster, *Crassostrea virginica*, and the Pacific oyster, *Crassostrea gigas*. Mol Mar Biol Biotechnol 2:207

Howell BR, Day OJ, Ellis T, Baynes SM (1998) Early life stages of farmed fish. In: Black KD, Pickering AD (eds) Biology of farmed fish. Sheffield, England: Sheffield Academic Press, pp 27–66

Jeon CY, Hur YB, Cho KC (2012) The effect of water temperature and salinity on settlement of Pacific oyster, *Crassostrea gigas* pediveliger larvae. The Korean J Malacol 28:21–28

Jouaux A, Blin JL, Adeline B, Heude-Berthelin C, Sourdaine P, Mathieu M, Kellner K (2013) Impact of energy storage strategies on gametogenesis and reproductive effort in diploid and triploid Pacific oysters *Crassostrea gigas*—involvement of insulin signaling. Aquaculture 388:173–181

Kalogeris T, Baines CP, Krenz M, Korthuis RJ (2012) Cell biology of ischemia/reperfusion injury. Int Rev Cell Mol Biol 298:229–317

Kladchenko ES, Tkachuk AA, Podolskaya MS, Andreyeva AY (2024) ROS production and mitochondrial membrane potential in hemocytes of marine bivalves, *Mytilus galloprovincialis* and *Magallana gigas*, under hypoosmotic stress. Comp Biochem Physiol Part B Biochem Mol Biol 269:110901

Lemos MBN, Nascimento IA, De Araujo MMS, Pereira SA, Bahia I, Smith DH (1994) The combined effects of salinity, temperature, antibiotic and aeration on larval growth and survival of the mangrove oyster, *Crassostrea rhizophorae*. J Shellfish Res 13:187–192

Lesnefsky EJ, Chen Q, Tandler B, Hoppel CL (2017) Mitochondrial dysfunction and myocardial ischemia-reperfusion: implications for novel therapies. Annu Rev Pharmacol Toxicol 57:535–565

Li Y, Li Q (2022) The growth, survival and ploidy of diploid, triploid and tetraploid of the Pacific oyster (*Crassostrea gigas*) in larval and juvenile stages. Aquaculture 553:738083

Li Y, Qin JG, Abbott CA, Li X, Benkendorff K (2007) Synergistic impacts of heat shock and spawning on the physiology and immune health of *Crassostrea gigas*: an explanation for summer mortality in Pacific oysters. Am J Physiol Regul Integr Comp Physiol 293:R2353–R2362

Li H, Yu R, Li C, Ma P (2021) Effects of salinity on early activities of artificial hybridization between *Crassostrea ariakensis* and *C. gigas*. Aquac Res 52:2540–2549

Li Y, Xu C, Li Q (2022) Effects of salinity and temperature on growth and survival of diploid and tetraploid larvae of the Pacific oyster, *Crassostrea gigas*. Aquaculture 550:737809

Liu Z, Hu D, Tang X (2008) Tidal current observation in the southern Yellow Sea in the summers of 2001 and 2003. Chin J Oceanol Limnol 26:121–129

Madrones-Ladja JA (2002) Salinity effect on the embryonic development, larval growth and survival at metamorphosis of *Placuna placenta* linnaeus (1758). Aquaculture 214:411–418

Martínez-García MF, Ruesink JL, Grijalva-Chon JM, Lodeiros C, Arreola-Lizárraga JA, de la Re-Vega E, Varela-Romero A, Chávez-Villalba J (2022) Socioecological factors related to aquaculture introductions and production of Pacific oysters (*Crassostrea gigas*) worldwide. Rev Aquac 14:613–629

Matsubara T, Yamaguchi M, Abe K, Onitsuka G, Abo K, Okamura T, Sato T, Mizuno K, Lagarde F, Hamaguchi M (2023) Factors driving the settlement of Pacific oyster *Crassostrea gigas* larvae in Hiroshima Bay, Japan. Aquaculture 563:738911

Matt JL, Allen SK Jr (2014) Heteroploid mosaic tetraploids of *Crassostrea virginica* produce normal triploid larvae and juveniles as revealed by flow cytometry. Aquaculture 432:336–345

McCombie H, Lapègue S, Cornette F, Ledu C, Boudry P (2005) Chromosome loss in bi-parental progenies of tetraploid Pacific oyster *Crassostrea gigas*. Aquaculture 247:97–105

Miller PA, Elliott NG, Vaillancourt RE, Kube PD, Koutoulis A (2014) Genetic diversity and pedigree assignment in tetraploid Pacific oysters (*Crassostrea gigas*). Aquaculture 433:318–324

Mita M, Fujiwara A, De Santis R, Yasumasu I (1994) High-energy phosphate compounds in spermatozoa of the sea urchins *Arbacia lixula* and *Paracentrotus lividus*. Comp Biochem Physiol Part A Mol Integr Physiol 109:269–275

Nichols ZG, Rikard S, Alavi SMH, Walton WC, Butts IA (2021) Regulation of sperm motility in Eastern oyster (*Crassostrea virginica*) spawning naturally in seawater with low salinity. PLoS One 16:e0243569

Nie H, Chen P, Huo Z, Chen Y, Hou X, Yang F, Yan X (2017) Effects of temperature and salinity on oxygen consumption and ammonia excretion in different colour strains of the Manila clam, *Ruditapes philippinarum*. Aquac Res 48:2778–2786

Osanai K (1985) In vitro induction of germinal vesicle breakdown in oyster oocytes. Bull Mar Biol Stn Asamushi, Tohoku Univ 18:1–9

Pongtippatee P, Laburee K, Thaweethamsewee P, Hiranphan R, Asuvapongpatana S, Weerachatyanukul W, Srisawat T, Withyachumnarnkul B (2012) Triploid *Penaeus monodon*: sex ratio and growth rate. Aquaculture 356:7–13

Qin JG, Fast AW, Ako H (1998) Growout performance of diploid and triploid Chinese catfish *Clarias fuscus*. Aquaculture 166:247–258

Qin Y, Xiao S, Ma H, Mo R, Zhou Z, Wu X, Zhang Y, Yu Z (2018) Effects of salinity and temperature on the timing of germinal vesicle breakdown and polar body release in diploid and triploid Hong Kong oysters, *Crassostrea hongkongensis*, in relation to tetraploid induction. Aquac Res 49:3647–3657

Qin Y, Zhang Y, Yu Z (2022) Aquaculture performance comparison of reciprocal triploid C. *gigas* produced by mating tetraploids and diploids in China. Aquaculture 552:738044

Repolho T, Baptista M, Pimentel MS, Dionísio G, Trübenbach K, Lopes VM, Rita Lopes AR, Calado R, Diniz M, Rosa R (2014) Developmental and physiological challenges of octopus (*Octopus vulgaris*) early life stages under ocean warming. J Comp Physiol B 184:55–64

Rico-Villa B, Pouvreau S, Robert R (2009) Influence of food density and temperature on ingestion, growth and settlement of Pacific oyster larvae, *Crassostrea gigas*. Aquaculture 287:395–401

Robert R, Vignier J, Petton B (2017) Influence of feeding regime and temperature on development and settlement of oyster *Ostrea edulis* (Linnaeus, 1758) larvae. Aquac Res 48:4756–4773

Samain JF (2011) Review and perspectives of physiological mechanisms underlying genetically-based resistance of the Pacific oyster *Crassostrea gigas* to summer mortality. Aquat Living Resour 24:227–236

Shi Z, Huang X, Fu R, Wang H, Luo H, Chen B, Liu M, Zhang D (2008) Salinity stress on embryos and early larval stages of the pomfret *Pampus punctatissimus*. Aquaculture 275:306–310

Stanley JG, Allen SK, Hidu H (1981) Polyploidy induced in the American oyster, *Crassostrea virginica*, with cytochalasin B. Aquaculture 23:1–10

Suquet M, Labbé C, Brizard R, Donval A, Le Coz JR, Quere C, Haffray P (2010) Changes in motility, ATP content, morphology and fertilisation capacity during the movement phase of tetraploid Pacific oyster (*Crassostrea gigas*) sperm. Theriogenology 74:111–117

Suquet M, Quéré C, Mingant C, Lebrun L, Ratiskol D, Miner P, Cosson J (2013) Effect of sampling location, release technique and time after activation on the movement characteristics of scallop (*Pecten maximus*) sperm. Aquat Living Resour 26:215–220

Uriarte I, Espinoza V, Herrera M, Zúñiga O, Olivares A, Carbonell P, Pino S, Farías A, Rosas C (2012) Effect of temperature on embryonic development of *Octopus mimus* under controlled conditions. J Exp Mar Biol Ecol 416:168–175

Wan W, Qin Y, Shi G, Li S, Liao Q, Ma H, Li J, Suo A, Ding D, Yu Z, Zhang Y (2023) Genetic improvement of aquaculture performance for tetraploid Pacific oysters, *Crassostrea gigas*: a case study of four consecutive generations of selective breeding. Aquaculture 563:738910

Wang Z, Guo X, Allen SK, Wang R (2002) Heterozygosity and body size in triploid Pacific oysters, *Crassostrea gigas* Thunberg, produced from meiosis II inhibition and tetraploids. Aquaculture 204:337–348

Wang Q, Li Q, Kong L, Yu R (2012) Response to selection for fast growth in the second generation of Pacific oyster (*Crassostrea gigas*). J Ocean Univ China 11:413–418

Xie J, Sun Y, Li Y, Zhang X, Hao P, Han L, Cao Y, Ding B, Chang Y, Yin D, Ding J (2023) TMT-based proteomics analysis of growth advantage of triploid *Apostichopus japonicus*. Comp Biochem Physiol Part D Genomics Proteomics 5:101043

Yang Q, Yu H, Li Q (2022) Refinement of a classification system for gonad development in the triploid oyster *Crassostrea gigas*. Aquaculture 549:737814

Zhang Z, Wang X, Zhang Q, Allen S (2014) Cytogenetic mechanism for the aneuploidy and mosaicism found in tetraploid Pacific oyster *Crassostrea gigas* (Thunberg). J Ocean Univ China 13:125–131

Zhang Y, Qin Y, Yu Z (2022) Comparative study of tetraploid-based reciprocal triploid Portuguese oysters, *Crassostrea angulata*, from seed to marketsize. Aquaculture 547:737523

Zhang E, Li Z, Lv T, Fu J, Dong L, Feng Y, Sun G, Xu X, Cui C, Wang W, Yang J (2023) Transcriptome profiling explores the immune defence mechanism of triploid Pacific oyster (*Crassostrea gigas*) blood against *Vibrio alginolyticus* based on protein interaction networks. Dev Comp Immunol 143:104677

Zhou J, Jiang G, Xu C, Bai X, Li Q (2023) Growth, survival and gonad development of diploids, triploids and tetraploids of 'Haida No. 3'line of the Pacific oyster *Crassostrea gigas*. Aquaculture 571:739472