Aquaculture 585 (2024) 740690

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Combined effects of temperature, salinity and rearing density on the larval growth and survival of the diploid, triploid and tetraploid of the Pacific oyster *Crassostrea gigas*

Xianchao Bai [a], Yuanxin Liang [a], Haining Zhang [a], Geng Cheng [a], Chengxun Xu [a], Qi Li [a,b,*]

[a] *Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao 266003, China*
[b] *Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, China*

ARTICLE INFO

Keywords:
Crassostrea gigas
Response surface methodology
Polyploid
Growth
Survival

ABSTRACT

The Pacific oyster, *Crassostrea gigas*, is a commercially important species, which is widely cultured in the world. In recent years, triploid cultivation of *C. gigas* has gradually emerged due to the advantages of poor fertility and rapid growth in China. However, the adaptation of triploid and tetraploid larvae to temperature, salinity and rearing density has not been studied. A central composite design and a response surface method were used to analyze the combined effects of temperature, salinity and rearing density on growth and survival of triploid and tetraploid larvae. Temperature, salinity and rearing density exerted significant effects on larvae growth and survival of triploid and tetraploid. Rearing density was identified as the most significant negative factor affecting the growth and survival of three populations. The reliable models on accumulated growth rate (AGR) and survival rate (SR) of three populations were obtained. The optimal conditions for larval development in the three populations were achieved by simultaneously maximizing growth and survival models. In diploids, the maximum AGR of 13.92 μm day^{-1} and the maximum SR of 65.73 were achieved at 23.66 °C / 28.47 psu / 2.23 ind.ml^{-1} with a value of desirability being 100%. In triploids, the maximum AGR of 15.82 μm day^{-1} and the maximum SR of 69.26 were achieved at 23.16 °C / 30.67 psu / 2.15 ind.ml^{-1} with a value of desirability being 100%. In tetraploids, the maximum AGR of 10.75 μm day^{-1} and the maximum SR of 46.29 were achieved at the 25.48 °C / 28.94 psu / 2.00 ind.ml^{-1} with a value of desirability being 95%. This study provides valuable new insights into refining the production efficiency of *C. gigas* larvae of different ploidy.

1. Introduction

The Pacific oyster (*Crassostrea gigas*), known for its high fecundity, adaptability to various environments, short breeding cycle, and high nutritional value, has established itself as one of the most economically important shellfish worldwide. For example, the yield of *C. gigas* has amounted to approximately 1.93 million tons in 2022 in China. This represents 31.18% of China's total oyster production, highlighting its significant economic value (China Fishery Statistical Yearbook, 2023). However, the efficiency of *C. gigas* production meets with some challenges.

During the intensive breeding season, the development of diploid gonads consumes substantial nutrients and energy, leading to slower growth and increased mortality, phenomena also related to gamete production. Nevertheless, triploid can compensate for these limitations due to their advantages of sterility, high survival rates, and rapid growth, and the performance levels of these advantages are affected by environmental conditions and region (Francesc et al., 2009; Qin et al., 2022; Zhang et al., 2022; Zhou et al., 2023). Triploid can be produced via two approaches, one of which involves induction using physical or chemical processes (Francesc et al., 2009; Guo and Allen, 1994a; Guo et al., 2009). However, the inconsistent triploid rate, poor survival rate, and low growth rate limit the large-scale application of these methods. As a result, the proven commercial application is the production of 100% triploids through the hybridization of tetraploid males and diploid females (Francesc et al., 2009; Guo and Allen, 1994a; Zhang et al., 2016;). Tetraploid oysters play an essential role in this method. These tetraploids, produced similarly to triploids through artificial induction (Guo and Allen, 1994b; Benabdelmouna and Ledu, 2015; Guo et al., 2009; Qin et al., 2021), can subsequently self-propagate, generating

* Corresponding author at: Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao 266003, China.
E-mail address: qili66@ouc.edu.cn (Q. Li).

https://doi.org/10.1016/j.aquaculture.2024.740690
Received 21 July 2023; Received in revised form 31 January 2024; Accepted 18 February 2024
Available online 20 February 2024

large numbers of tetraploids. However, not all polyploid oysters possess superior growth characteristics.

Ploidy increase does not always result in enhanced characteristics in polyploids. For *C. gigas* and *C. angulata*, the best traits were exhibited by triploids, followed by diploids and tetraploids under identical environmental conditions (Zhang et al., 2022; Qin et al., 2022). However, not all polyploid oysters possess superior growth characteristics and the growth and survival are related to environment factors (Zhou et al., 2023; Francesc et al., 2009). Environment factors significantly influenced the growth and survival of many aquatic animals (Liu et al., 2006; Ramos et al., 2021; Li and Li, 2009; Lü et al., 2017; Wang et al., 2018). Factors such as temperature and salinity have been observed to influence various aspects of bivalves, including feeding behavior (Rato et al., 2022), immune responses (Rahman et al., 2019), haemocyte activities (Gagnaire et al., 2006), endocrine responses (Joyce and Vogeler, 2018), molecular responses (Fu et al., 2011), and both reproduction and larval development (Wang et al., 2021; Legat et al., 2017). Rearing density, an important and easily manipulated cultivation parameter, can affect various aspects of growth. High rearing density can negatively impact feeding rates, oxygen consumption, and growth efficiency (MacDonald, 1988; Yan et al., 2006; Velasco and Barros, 2008). Conversely, low rearing density may not yield high economic returns. Therefore, determining the appropriate rearing density is crucial for maximizing economic benefits. Notably, the environment factors simultaneously acting on the performance of bivalves (Gosling, 2015), hence the combined effects of some factors should be investigated. While polyploidy in *C. gigas* was initially reported in the 1990s (Guo and Allen, 1994a; Guo and Allen, 1994b), no studies to date have examined the combined influences of temperature, salinity, and rearing density on the growth and survival of polyploid *C. gigas* larvae. These factors essentially govern the existence of triploid and tetraploid oysters and their market suitability. To utilize these different ploidy oysters better, we need to understand and optimize their rearing environment simultaneously.

In this study, we explore the combined effects of temperature, salinity, and rearing density on the growth and survival of diploid, triploid, and tetraploid populations of *C. gigas* larvae. This objective was to ascertain the optimal combination of temperature, salinity, and rearing density for the growth and survival of triploid and tetraploid populations, thereby advancing the development of hatchery techniques for seed production of triploid and tetraploid oysters.

2. Materials and methods

2.1. Biological materials

The sperm or eggs from three populations were collected separately and divided equally into two for artificial fertilization. The fertilization was carried out by (1) mixing the eggs of diploids with sperm from diploids or tetraploids to produce the DD (diploids ♀ × diploids ♂) group as diploid population and DT (diploids ♀ × tetraploids ♂) group as triploid population, respectively; and (2) mixing the eggs of tetraploids with sperm from tetraploids to produce the TT (tetraploids ♀ × tetraploids ♂) group as tetraploid population. As large-scale production of triploids was produced by crossbreeding between male tetraploids and female diploids. Thus, diploids and tetraploids were crossed in only one direction in this study - female diploids were crossed with male tetraploids to produce triploids (diploids ♀ × tetraploids ♂). The progenitors of these tetraploid Pacific oyster were obtained in May 2022 by inhibiting the first polar body of triploid eggs that were fertilized using diploid sperm. The progenitors for three populations were gathered in May 2023 from Jiaonan, China, and were cultivated in a Hatchery in Laizhou, China. The oysters were reared for a month in 24 m^3 tanks, maintaining temperature and salinity of 24 ± 1 °C and 30 ± 1 psu, respectively. From each population, twenty mature individuals were induced to spawn. The embryos were put into a 20 m^3 tank for hatching (temperature 24 °C; salinity 30 psu). A day post-fertilization, all embryos developed into D-larvae. The ploidy of larvae was tested by flow cytometry (Beckman CytoFLEX) to ensure the ploidy of experimental larvae (Fig. 1).

2.2. Experimental design

The experimental temperature (T) ranged between 18 and 30 °C, and the salinity (S) ranged between 15 and 35 psu. The rearing density (D) varied from 2 to 10 individuals per milliliter. To maintain the temperature, electrical heaters were utilized, and precision control was ensured with an electrical thermometer with an accuracy of ±0.2 °C. A cooling-water machine facilitated the regulation and control of low temperatures within the same precision range. Low salinity was achieved by diluting seawater with dechlorinated freshwater, while high salinity was obtained by adding sea salt to seawater. Salinity was monitored using an ATAGO refractometer with a precision of ±0.1%. Before the experiment commenced, the larvae began acclimation at 24 °C and 30 psu. The adjustments in experimental temperature and salinity were maintained at 0.4 °C/h and 1 psu/h, respectively. Following a temporary acclimation period (24 h), the healthy larvae were transferred into 25 L plastic buckets according to the density design for the experiment (Table 1). The experiment was begun at the same time. Continuous aeration was provided using an air stone to ensure dissolved oxygen. The temperature of larval containers was maintained using a way of water bath within the 300 L plastic buckets. The larvae of three populations were fed with the mixture by *Isochrysis galbana* and *Nitzschiaclosterium* every 8 h (make sure the algal density ≥ 20 cells μl^{-1}), and the 1/3–1/2 of the seawater in each container was exchanged daily. Seawater at all temperatures and salinity levels was prepared in advance. The replaced seawater was filtered through sand filters and nonwovens polypropylene fabric and adjusted to the experimental condition. Design Expert software (version 13.0, Minneapolis, USA) was employed to establish the rotatable central composite design (Table 1). The ranges of all three factors were defined based on previous research and production experience. The design incorporated eight factorial point, six axial point, and six center points. For the central composite, the coded value was set at 0, with the upper and lower coded value limits being α and -α, respectively. The asterisk arm was established at |1.682|. The factor ranges were represented in terms of alphas (α). In the present study, 20 experimental points in total were designed for analysis. The temperature at 18 °C, 20.4 °C, 24 °C, 27.6 °C, 30 °C represented the code value of -α, −1, 0, 1, α respectively. The salinity at 15 psu, 19.1 psu, 25 psu, 31 psu, 35 psu represented the code value of -α, −1, 0, 1, α respectively. The rearing density at 2 ind. ml^{-1}, 3.6 ind.ml^{-1}, 6 ind.ml^{-1}, 8.4 ind.ml^{-1}, 10 ind.ml^{-1} represented the code value of -α, −1, 0, 1, α respectively. Each experiment point was set to be replicated twice, and 60 experimental units were used for each population (Fig. S1). The rearing density and water quality was maintained the consistent for the same experiment point of three groups (DD, DT and TT).

2.3. Measurement of accumulated growth rate and survival rate

A 100 ml sample was collected randomly and fixed by the addition of 1% Lugol's solution. The larvae density was determined by the larvae number of each sample. A random selection of 30 larvae from each sample was used to determine shell height with an optical microscope. The density of each experiment unit was calculated and adjusted to the pre-set value by modulating the volume of seawater. The water volume of the rearing environment for each experiment unit was evaluated using a graduated cylinder. After 15 days of artificial cultivation, the growth and survival of each population were assessed. The Accumulated Growth Rate (AGR) was calculated as the ratio of the difference between the final and initial shell heights to the number of days. Similarly, the Survival Rate (SR) was determined by the ratio of the final to the initial water volume. The mathematical representation for both AGR and SR was as follows (Xu et al., 2019):

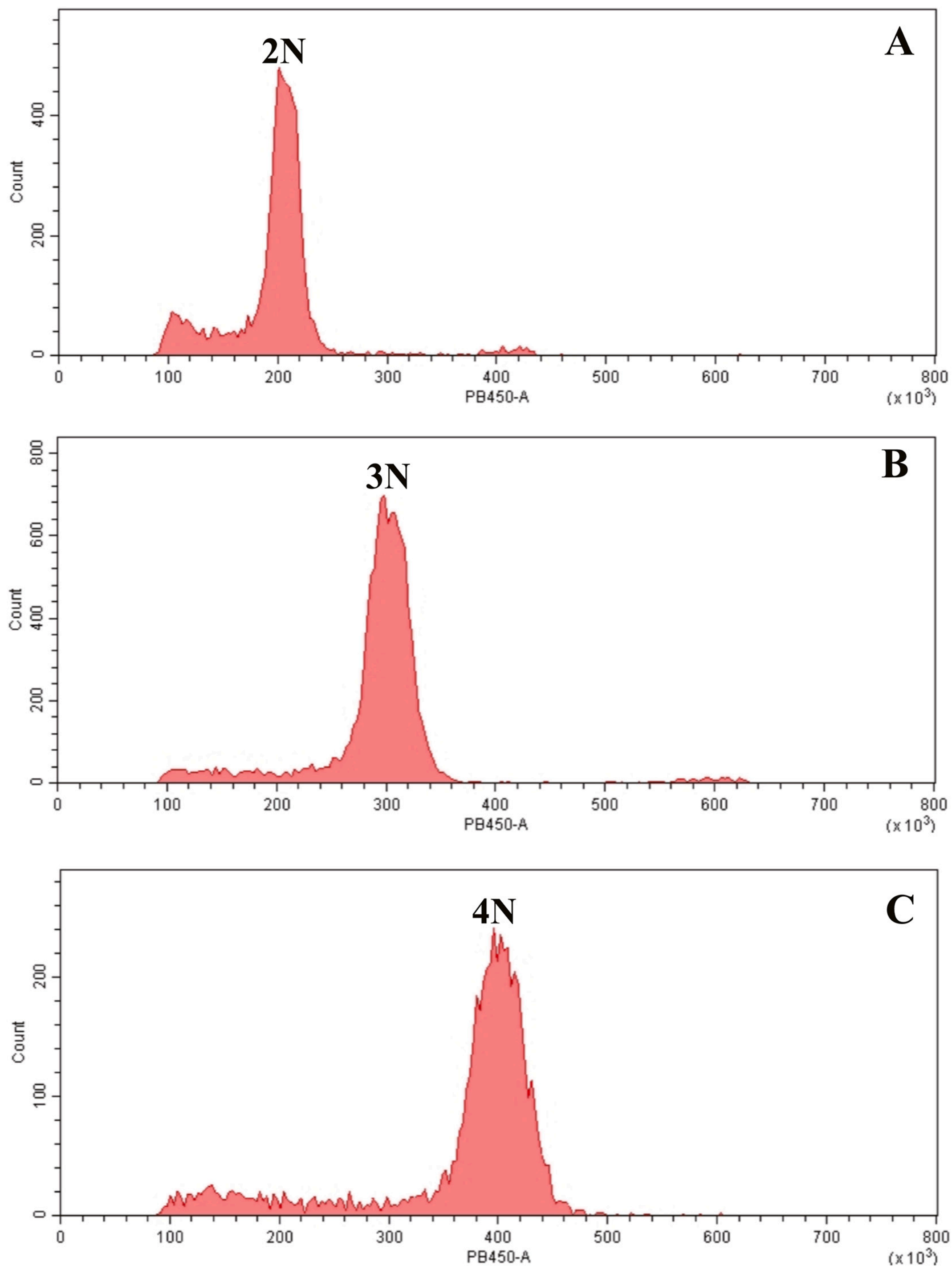

Fig. 1. Relative DNA content and ploidy level of three populations detected by flow cytometry. Note: 2 N represents diploid; 3 N represents triploid; 4 N represents tetraploid.

$$\text{AGR}\left(\mu\text{m day}^{-1}\right) = \frac{H_t - H_0}{t - t_{0.}}$$

$$\text{SR}(\%) = \frac{V_t}{V_0} \times 100$$

In these equations, t_0 and t represent the beginning and end times of the experiment, respectively; H_0 and H_t denote the shell heights of the same experiment unit at the start and end of the experiment, respectively; V_0 and V_t signify the water volumes of the rearing environments for the same experiment unit at the beginning and end of the experiment, respectively.

2.4. Data analysis

The data were processed using Design Expert 13.0 to select a response surface model incorporating temperature, salinity, and rearing density. A stepwise backward regression method was employed for automatic hierarchy correction (with Alpha set at 0.1). The general form for the model was as follows:

$$Y = \beta_0 + \beta_1 T + \beta_2 S + \beta_3 D + \beta_{12} T \times S + \beta_{13} T \times D + \beta_{23} S \times D + \beta_{11} T^2 + \beta_{22} S^2 + \beta_{33} D^2 + \beta_{123} T \times S \times D + \beta_{112} T^2 \times S + \beta_{113} T^2 \times D + \beta_{122} T \times S^2 + e$$

Table 1
Experimental runs of the rotatable central composite design and corresponding results.

Run	Actual			*AGR* (μm day^{-1})			*SR* (%)		
	T (°C)	*S* (psu)	*D* (ind.ml^{-1})	Diploid	Triploid	Tetraploid	Diploid	Triploid	Tetraploid
1	24	25	6	9.26 ± 0.50	11.65 ± 0.11	5.42 ± 0.10	40.52 ± 2.98	39.47 ± 4.02	41.18 ± 2.73
2	24	15	6	1.74 ± 0.45	1.96 ± 0.15	2.61 ± 0.19	11.09 ± 0.26	6.81 ± 0.82	19.65 ± 1.69
3	30	25	6	1.19 ± 0.29	4.08 ± 0.09	1.06 ± 0.35	3.17 ± 0.38	3.65 ± 0.62	2.17 ± 0.22
4	20.4	19.1	3.6	4.12 ± 0.46	2.67 ± 0.26	1.76 ± 0.18	5.75 ± 0.97	5.54 ± 0.39	8.75 ± 2.73
5	24	25	2	13.79 ± 0.13	15.21 ± 0.08	8.22 ± 0.14	65.05 ± 0.75	64.29 ± 0.85	51.13 ± 3.74
6	27.6	31	3.6	8.64 ± 0.30	13.88 ± 0.47	10.75 ± 0.08	12.93 ± 1.33	9.35 ± 1.11	35.64 ± 2.24
7	24	25	6	9.82 ± 0.16	11.51 ± 0.19	5.28 ± 0.22	41.67 ± 1.55	40.81 ± 0.72	40.25 ± 3.23
8	24	25	6	9.11 ± 0.25	11.29 ± 0.16	5.49 ± 0.33	42.81 ± 4.03	39.12 ± 2.99	40.97 ± 0.77
9	20.4	19.1	8.4	2.41 ± 0.10	2.35 ± 0.30	1.17 ± 0.21	7.35 ± 1.64	6.87 ± 1.89	4.15 ± 0.51
10	24	25	6	9.31 ± 0.05	10.97 ± 0.13	5.31 ± 0.21	39.85 ± 3.96	40.06 ± 2.94	40.05 ± 0.64
11	20.4	31	8.4	3.96 ± 0.24	1.32 ± 0.03	2.29 ± 0.04	21.79 ± 2.71	25.45 ± 0.97	6.19 ± 0.55
12	24	25	6	9.06 ± 0.18	11.27 ± 0.25	5.23 ± 0.15	40.28 ± 2.48	41.27 ± 0.29	40.76 ± 2.64
13	24	35	6	6.67 ± 0.22	7.55 ± 0.29	4.39 ± 0.11	9.75 ± 1.42	15.81 ± 1.71	1.15 ± 0.06
14	24	25	10	4.99 ± 0.15	2.46 ± 0.04	1.53 ± 0.06	3.29 ± 0.71	5.69 ± 1.23	15.68 ± 0.90
15	27.6	19.1	3.6	6.43 ± 0.26	4.68 ± 0.05	2.44 ± 0.16	5.56 ± 1.03	2.85 ± 0.29	18.34 ± 2.15
16	20.4	31	3.6	6.93 ± 0.20	8.27 ± 0.44	1.99 ± 0.35	58.25 ± 1.99	61.78 ± 3.80	16.72 ± 1.27
17	27.6	19.1	8.4	1.95 ± 0.08	1.13 ± 0.13	2.02 ± 0.12	1.98 ± 0.21	3.85 ± 0.20	12.58 ± 0.28
18	27.6	31	8.4	3.16 ± 0.24	5.51 ± 0.24	1.31 ± 0.03	10.54 ± 0.35	11.79 ± 0.12	12.29 ± 1.24
19	18	25	6	1.37 ± 0.11	1.58 ± 0.14	1.18 ± 0.12	11.63 ± 1.27	10.24 ± 0.73	5.85 ± 1.59
20	24	25	6	9.54 ± 0.25	11.01 ± 0.33	5.38 ± 0.29	42.27 ± 5.70	40.33 ± 3.06	39.95 ± 0.93

T, S and D represented the temperature, salinity, and rearing density respectively; AGR represented the accumulated growth rate; SR represented the survival rate; Errors were found to be normally distributed and homoscedastic based upon the normal probability plot and externally Studentized residuals plot, respectively. Run 1, 7, 8, 10, 12, 20 represented the center points; Run 2, 3, 5, 13, 14, 19 represented the axial points; Run 4, 6, 9, 11, 15, 16, 17, 18 represented the factorial points.

In the model, Y represented the response (AGR); β_0 denoted the intercept of the regression equation; β_1, β_2, β_3 are the linear effects of temperature, salinity and rearing density, respectively; β_{12}, β_{13}, β_{23} are the interactive effects of temperature and salinity, temperature and rearing density, and salinity and rearing density, respectively; β_{11}, β_{22}, β_{33} are the quadratic effects of temperature, salinity and rearing density, respectively; β_{123} are the cubic effects of temperature, salinity and rearing density. β_{112}, β_{113}, β_{122} are the cubic effects of temperature and salinity, temperature and rearing density, respectively; The random error, denoted by e, was assumed to follow a normal distribution with a mean of zero. The variance analysis was generated to demonstrate the significance and adequacy of the regression equation model. The model's fitting was evaluated based on the coefficient of determination (R^2), adjusted coefficient (Adj-R^2). The model's predictive ability was evaluated based on the coefficient of determination ($Pred - R^2$). The Origin 2022 software (Northampton, USA) was utilized to generate the three-dimensional response surface diagram and the corresponding contour map, assisting in the analysis of temperature, salinity, and rearing density effects on growth and survival. With the models of AGR and SR established, they were optimized simultaneously using the approach of Montgomery (2013). Reliability of the optimization was measured by the desirability value, which ranges between 0 and 100%. To further test the accuracy and effectiveness of the response surface model, the prediction ability of the model was verified by comparing the error between the predicted value and the measured value of the model. The statistical significance between the predicted value and the measured value were calculated using the SPSS 23.0 software.

3. Results

3.1. Growth and survival of three populations

The AGR and SR of different ploidy at different combinations were given respectively (Table 1). The highest AGR and SR for diploid larvae were 13.79 ± 0.13 μm day^{-1} and 65.05 ± 0.75%, respectively. The highest AGR and SR for triploid larvae were 15.21 ± 0.08 μm day^{-1} and 64.29 ± 0.85%, respectively. The highest AGR and SR for tetraploid larvae were 10.75 ± 0.08 μm day^{-1} and 51.13 ± 3.74%, respectively. The lowest AGR were lower than 2 μm day^{-1} for three populations. The AGR for triploid larvae were higher than diploid and tetraploid larvae under the experimental center point. Similarly, the lowest SR were lower than 3% for three populations. The SR for diploid larvae were higher than triploid and tetraploid larvae under the experimental center point.

3.2. Modeling and significance test

The final regression equations for the different ploidy population obtained from the actual values are provided in supplementary file 1. The model equations were significant for all three populations ($P < 0.001$). The coefficients of determination for each model were >0.98, indicating that the model equations could explained at least 98% of the variability in two responses (Table 3). Based on these $Pred - R^2$ values, the models exhibit higher predictability.

For both diploid and tetraploid larvae, the linear effects of salinity and rearing density were highly significantly contributed to the variation of AGR ($P < 0.001$). In the case of triploid larvae, all three linear effects significantly and highly contributed to AGR ($P < 0.001$). The interactive effects between temperature and rearing density, as well as between salinity and rearing density, were significant for the AGR of diploid larvae ($P < 0.05$). All three interactive effects were significant for the AGR of triploid and tetraploid larvae ($P < 0.001$). The quadratic effects of temperature and salinity were highly significant in the model equation for the AGR of all populations ($P < 0.001$).

The linear effect of temperature and rearing density significantly and contributed to the variation of SR for diploid larvae ($P < 0.05$), while the linear effect of temperature, salinity, rearing density significantly influenced the variation in SR for triploid and tetraploid larvae ($P < 0.05$). All three interactive effects were significant for SR of three populations ($P < 0.05$). The quadratic effect of temperature and salinity were highly significant for three populations ($P < 0.001$).

3.3. Influence of temperature, salinity and rearing density on AGR for three populations

For diploid and triploid population (Fig. 2a, b), both extreme temperature and salinity contributed to low AGR. When the salinity ranged between 22 and 30 psu, the AGR initially increase followed by a decrease with rising temperature. For the tetraploid population

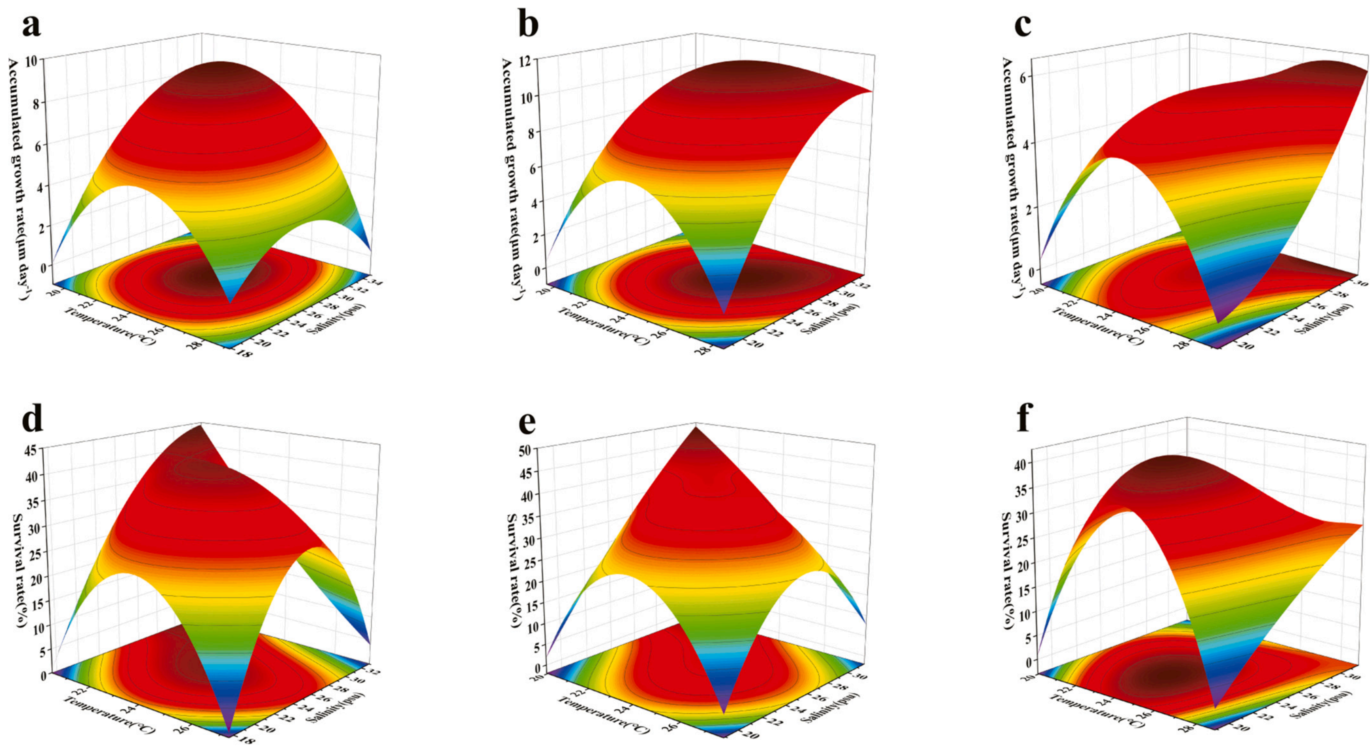

Fig. 2. Response surface plot of the effect of temperature and salinity on the accumulated growth rate (AGR) and survival rate (SR) for the three populations (rearing density = 6 ind. ml^{-1}). Note: diploid population (a, d); triploid population (b, e); tetraploid population (c, f).

(Fig. 2c), when temperature was 26.42 °C, the AGR tended to decrease as salinity dropped from its extreme levels. The combined effects of temperature and rearing density on the AGR for three populations were consistent (Fig. 3a, b, c). Both diploid and triploid populations exhibited optimal AGR at 24.48 ± 0.09 °C and a rearing density of 2 ind.ml^{-1}, with a decline as rearing density increased within the 20–28 °C range (Fig. 3a, b). For the tetraploid population (Fig. 3c). The surface featured peaks when the temperature was 25.19 °C and the rearing density was 2 ind.ml^{-1}. Both populations exhibit peak AGR at 28.93 ± 0.92 psu salinity and a rearing density of 2 ind.ml^{-1} (Fig. 4a, b). However, a

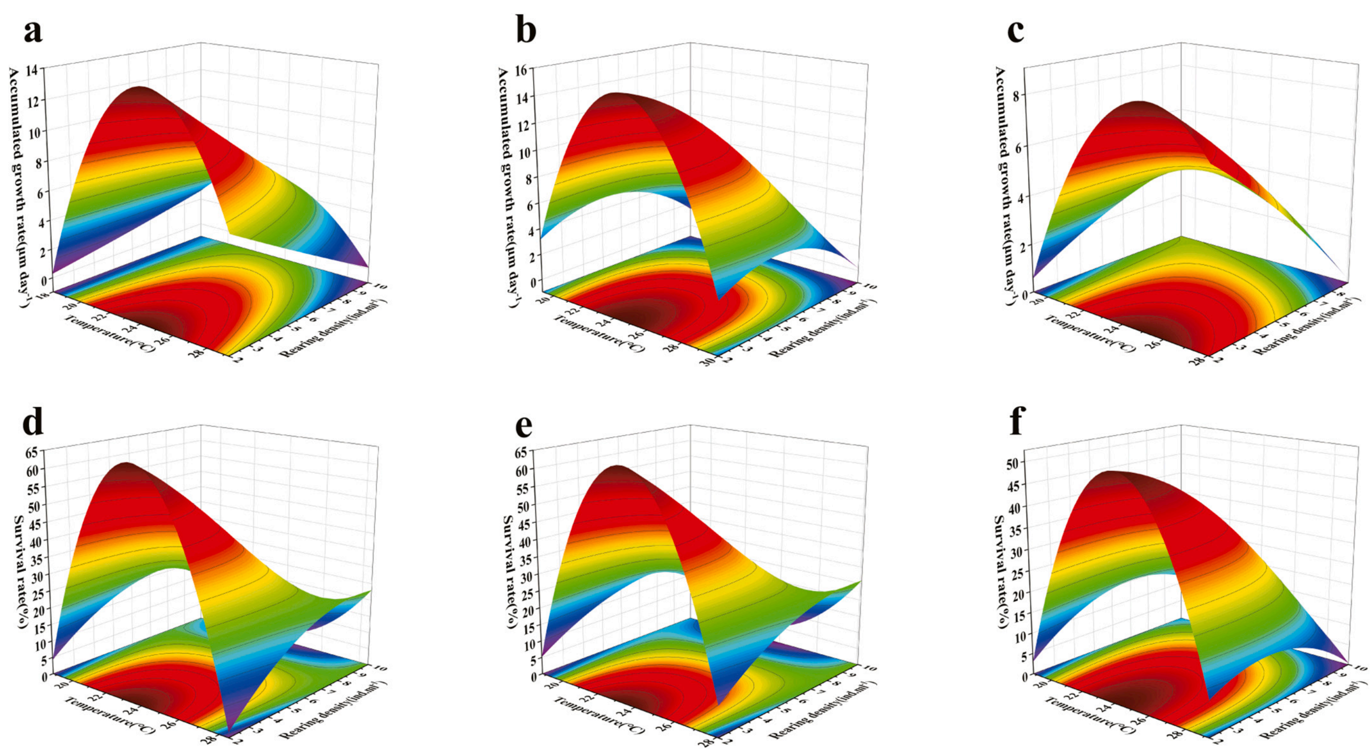

Fig. 3. Response surface plot of the effect of temperature and rearing density on the accumulated growth rate (AGR) and survival rate (SR) for the three populations (salinity = 25 psu). Note: diploid population (a, d); triploid population (b, e); tetraploid population (c, f).

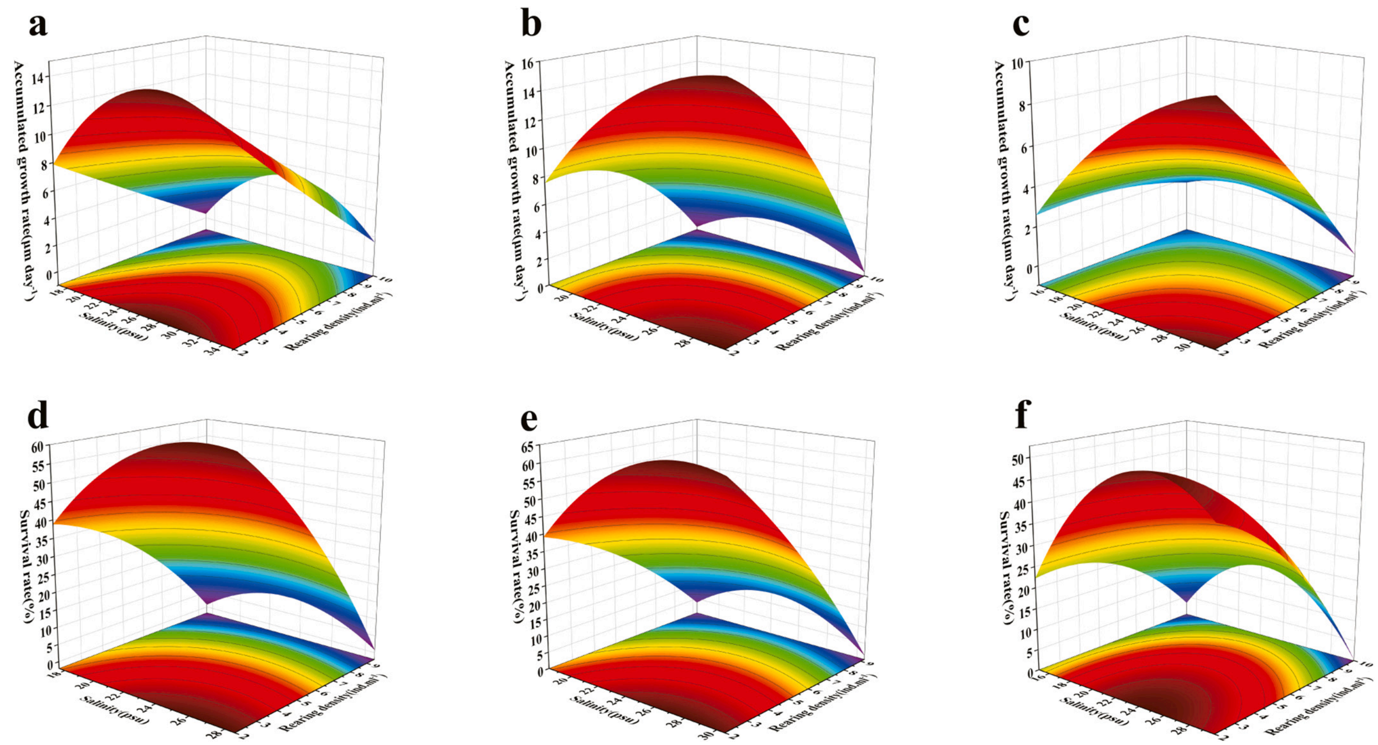

Fig. 4. Response surface plot of the effect of salinity and rearing density on the accumulated growth rate (AGR) and survival rate (SR) for the three populations (temperature = 24 °C). Note: diploid population (a, d); triploid population (b, e); tetraploid population (c, f).

noticeable decrease in AGR is observed as the rearing density increases within the salinity range of 20–30 psu. For tetraploid population, the AGR reaches its lowest value at the lowest salinity and highest rearing density. Conversely, the AGR attains its highest value at the highest salinity and lowest rearing density (Fig. 4c).

3.4. Influence of temperature, salinity and rearing density on SR for three populations

The response surface of SR for diploid population featuring two distinct peaks in response values (Fig. 2d). First, at a salinity of 25.07 psu, the SR began to decline as temperature rose above 24 °C. Second, when the temperature was at its lowest extreme and salinity at its highest extreme, the SR reached its maximum value. The peak appearing when the temperature was at its lowest extreme and salinity at its highest extreme for triploid population (Fig. 2e), after which it decreased with changes in temperature or salinity. For the tetraploid population, the peak appearing when temperature was 24.53 °C (Fig. 2f). The SR tended to decline with temperature decreased.

Comparable patterns were observed for the SR of three populations (Fig. 3d, e, f), the SR surface of three population suggested that the peak value could be achieved when the temperature was 24.04 ± 0.34 °C and the rearing density was 2 ind.ml^{-1} (Fig. 3d, e, f). Similar trends were noted in the SR of the three populations (Fig. 4d, e, f). The SR reaches its peak value when the salinity was 26.89 ± 1.54 psu and the rearing density is at the lowest extreme.

3.5. Optimization for the AGR and SR among the three populations

Under various experimental conditions, optimal combinations were analyzed for diploid, triploid, and tetraploid populations using response models. For diploid populations, a temperature of 23.66 °C, salinity of 28.47 psu, and rearing density of 2.23 ind.ml^{-1} resulted in a maximum AGR of 13.92 μm day^{-1} and a maximum SR of 65.73 with 100% desirability. For triploid populations, the maximum AGR of 15.82 μm day^{-1} and the maximum SR of 69.26% with 100% desirability occurred at 23.16 °C, 30.67 psu, and 2.15 ind.ml^{-1}. For tetraploids, the maximum AGR of 10.75 μm day^{-1} and the maximum SR of 46.29% with 95% desirability was achieved at 25.48 °C, 28.94 psu, and 2.00 ind.ml^{-1}. The error between the measured values and predicted values for the three populations is <5% (Table 4).

4. Discussion

The larvae of bivalve mollusks lead a planktonic life, making them sensitive to changes in the environment. Larval cultivation is a critical process in the oyster industry. In recent years, triploid oysters have gained global popularity. Previous research has evaluated the impact of temperature and salinity on the larvae of diploid and tetraploid oysters (Wang et al., 2018; Legat et al., 2017; Xu et al., 2019; Li et al., 2022). Despite being a central aspect of the triploid industry, there is a lack of studies on the biological traits of triploid larvae. To support industrial-scale breeding, there is a need to investigate the performance of diploid, triploid, and tetraploid *C. gigas* larvae and determine the optimal breeding conditions for their development.

In this study, the significances of the linear effects of temperature, salinity and rearing density for the growth and survival of three populations were different. Temperature, salinity and rearing density play different role in the growth and/or survival of the three populations. Based on the code effects of the three factors (Table 2), rearing density was found to be the most important factor affecting growth and survival of three populations. Both the AGR and SR of all populations were negatively influenced by rearing density, a trend consistent with earlier research findings. For instance, the Iwagaki oyster (*C. nippona*) larvae reared at the highest density (12 ind.ml^{-1}) exhibited the smallest shell length and survival rate, while those at the lowest density (0.5 ind.ml^{-1}) achieved the highest (Wang et al., 2018). Likewise, the clam (*Meretrix meretrix*) manifested a lower growth rate when its larvae were raised in high-density conditions (Liu et al., 2006). The high rearing density may facilitate bacterial proliferation which worsens water quality and limits

Table 2
Regression coefficients, standard errors (SE), significance and 95% confidence intervals (CI) for the predicted model of AGR and SR of the different ploidy population.

Factor	Coefficient(±SE)		*P* value		95% CI			
					Low		High	
	AGR	*SR*	*AGR*	*SR*	*AGR*	*SR*	*AGR*	*SR*
Diploid								
Intercept	9.35(±0.11)	41.24(±0.45)			9.08	40.15	9.61	42.33
T	−0.05 (±0.11)	−2.52(±0.46)	0.6466	0.0016	−0.32	−3.64	0.22	−1.39
S	1.47(±0.11)	−0.4(±0.46)	< 0.0001	0.4195	1.19	−1.52	1.74	0.73
D	−2.62(±0.11)	−18.36(±0.46)	< 0.0001	< 0.0001	−2.89	−19.49	−2.34	−17.24
TS	−0.12(±0.09)	−6.23(±0.38)	0.2315	< 0.0001	−0.35	−7.17	0.1	−5.29
TD	−0.65(±0.09)	3.51(±0.38)	0.0004	< 0.0001	−0.87	2.58	−0.42	4.44
SD	−0.28(±0.09)	−4.56(±0.38)	0.0231	< 0.0001	−0.51	−5.5	−0.05	−3.63
T^2	−2.84(±0.07)	−12(±0.29)	< 0.0001	< 0.0001	−3.01	−12.7	−2.67	−11.3
S^2	−1.81(±0.07)	−10.93(±0.29)	< 0.0001	< 0.0001	−1.98	−11.63	−1.64	−10.23
D^2	0.03(±0.07)	−2.53(±0.29)	0.7234	0.0001	−0.14	−3.24	0.2	−1.83
TSD	0.03(±0.09)	4.82(±0.38)	0.7394	< 0.0001	−0.19	3.89	0.26	5.74
T^2S	−0.46(±0.14)	10.74(±0.59)	0.0187	< 0.0001	−0.8	9.29	−0.11	12.18
T^2D	0.79(±0.14)	13.1(±0.59)	0.0014	< 0.0001	0.44	11.66	1.14	14.54
TS^2	0.4(±0.14)	−5.12(±0.6)	0.0335	0.0001	0.04	−6.58	0.75	−3.66
Triploid								
Intercept	11.29(±0.1)	40.19(±0.31)			11.03	39.43	11.54	40.95
T	0.74(±0.11)	−1.96(±0.32)	0.0004	0.0009	0.48	−2.75	1	−1.17
S	1.66(±0.11)	2.68(±0.32)	< 0.0001	0.0002	1.4	1.89	1.92	3.46
D	−3.79(±0.11)	−17.42(±0.32)	< 0.0001	< 0.0001	−4.05	−18.21	−3.53	−16.64
TS	1.11(±0.09)	−7.36(±0.27)	< 0.0001	< 0.0001	0.89	−8.01	1.32	−6.7
TD	−0.57(±0.09)	4.68(±0.27)	0.0006	< 0.0001	−0.79	4.03	−0.36	5.33
SD	−1.42(±0.09)	−4.48(±0.27)	< 0.0001	< 0.0001	−1.63	−5.14	−1.2	−3.83
T^2	−3.01(±0.07)	−11.81(±0.2)	< 0.0001	< 0.0001	−3.17	−12.3	−2.85	−11.32
S^2	−2.33(±0.07)	−10.27(±0.2)	< 0.0001	< 0.0001	−2.49	−10.76	−2.17	−9.77
D^2	−0.89(±0.07)	−1.89(±0.2)	< 0.0001	< 0.0001	−1.05	−2.39	−0.72	−1.4
TSD	0.22(±0.09)	4.8(±0.27)	0.0448	< 0.0001	0.01	4.15	0.44	5.45
T^2S	0.63(±0.14)	8.49(±0.41)	0.0036	< 0.0001	0.3	7.48	0.97	9.5
T^2D	1.4(±0.14)	13.31(±0.41)	< 0.0001	< 0.0001	1.07	12.3	1.73	14.31
TS^2	0.56(±0.14)	−6.86(±0.42)	0.0068	< 0.0001	0.22	−7.89	0.9	−5.84
Tetraploid								
Intercept	5.35(±0.04)	40.52(±0.20)			5.26	40.04	5.45	41
T	−0.04(±0.04)	−1.09(±0.20)	0.3991	0.0016	−0.13	−1.59	0.06	−0.6
S	0.53(±0.04)	−5.5(±0.20)	< 0.0001	< 0.0001	0.43	−5.99	0.63	−5.01
D	−1.99(±0.04)	−10.54(±0.20)	< 0.0001	< 0.0001	−2.09	−11.03	−1.89	−10.04
TS	0.75(±0.03)	0.76(±0.17)	< 0.0001	0.0041	0.67	0.35	0.83	1.17
TD	−1.16(±0.03)	−1.7(±0.17)	< 0.0001	< 0.0001	−1.24	−2.11	−1.09	−1.3
SD	−1.01(±0.03)	−2.91(±0.17)	< 0.0001	< 0.0001	−1.09	−3.32	−0.93	−2.5
T^2	−1.51(±0.02)	−12.88(±0.13)	< 0.0001	< 0.0001	−1.57	−13.19	−1.45	−12.57
S^2	−0.66(±0.02)	−10.62(±0.13)	< 0.0001	< 0.0001	−0.72	−10.93	−0.6	−10.31
D^2	−0.18(±0.02)	−2.49(±0.13)	0.0003	< 0.0001	−0.24	−2.79	−0.12	−2.18
TSD	−1.22(±0.03)	−1.43(±0.17)	< 0.0001	0.0001	−1.30	−1.84	−1.14	−1.02
T^2S	0.59(±0.05)	8.89(±0.26)	< 0.0001	< 0.0001	0.47	8.26	0.71	9.53
T^2D	0.73(±0.05)	4.99(±0.26)	< 0.0001	< 0.0001	0.6	4.36	0.85	5.62
TS^2	1.18(±0.05)	6.41(±0.26)	< 0.0001	< 0.0001	1.06	5.77	1.31	7.05

T, S and D represented the temperature, salinity, and rearing density respectively; The values in the table were all coded values, and the coefficient was estimated according to the coded value.

larval growth (Andersen et al., 2000; Sarkis et al., 2006; Marshall et al., 2014). High larval densities could also escalate the chance of collision among individuals and the risk of shell and tissue damage, a rise in metabolic wastes accumulating in the water, leading to lower oxygen levels and higher ammonia content, which could adversely affect larval growth and survival (Cragg, 1980; Avila et al., 1997; Deng et al., 2013; Marshall et al., 2014). This explains the low growth and survival performance of three populations under high rearing density. The linear effects of salinity on the AGR of all three populations were significant (Table 2). Earlier research has suggested that the mechanisms behind salinity's influence include the modification of the osmotic stress effector (comprising ion channels, aquaporins, and free amino acids) and the regulation of protein expression and key genes involved in free amino acid metabolism (Meng et al., 2013). Salinity stress impacts the energy acquisition and utilization of bivalves, affecting factors such as clearance rate, absorption, oxygen uptake, and excretion (Peteiro et al., 2018). These attributes decrease under unsuitable salinity conditions, offering a concurrent growth advantage (Navarro and Gonzalez, 1998). Therefore, it is necessary to control the appropriate salinity condition in the breeding process. In our study, diploid larvae exhibited optimal growth at a salinity of approximately 27 psu. This aligns with previous research indicating that *C. gigas* larvae thrive best in salinity levels ranging from 25 to 30 psu (Xu et al., 2019; Li et al., 2022). Interestingly, the optimal salinity for triploid and tetraploid larvae exceeded that of diploid larvae (Table 4), suggesting a polyploid preference for higher salinity environments. Such differential adaptability may be attributed to the potential for duplicated gene copies to evolve new or marginally altered functions, enabling niche expansion or bolstering an organism's adaptability in response to environmental changes (Adams and Wendel, 2005; Moore and Purugganan, 2005). The linear effects of salinity on the SR of triploid and tetraploid larvae were significant, while those of diploid larvae were not which indicated that polyploids displayed a higher sensitivity to environmental changes compared to diploids. In addition, the low salinity could cause a decline in the SR of all three populations, potentially due to the detrimental impact of low salinity on hemolymph function (Gajbhiye and Khandeparker, 2017).

In this study, the interactions among temperature, salinity, and rearing density were constituted significant portions in the variability of

Table 3
Coefficient of determination for the predicted model of accumulated growth and survival rate of the different ploidy population.

Model	R^2	Adj-R^2	Pred-R^2
Diploid			
AGR	0.9983	0.9946	0.9893
SR	0.9991	0.9971	0.9961
Triploid			
AGR	0.9991	0.9971	0.9853
SR	0.9995	0.9986	0.9920
Tetraploid			
AGR	0.9996	0.9987	0.9890
SR	0.9997	0.9992	0.9965

growth and survival for triploid and tetraploid population (Table 2). The interactions effect between temperature and salinity were positive, showing that they were accelerative to the growth of triploid and tetraploid populations. Furthermore, the interactions effects between temperature and rearing density, salinity and rearing density were negative, showing that they were inhibitory to the growth of triploid and tetraploid populations. In general, the interactions effects among experimental factors varies with species, response, and ontogenetic stages (Wang et al., 2021). In our study, the interactions effects vary with ploidy and genetic background. For instance, the interaction effect between temperature and salinity on survival for diploid and tetraploid populations were opposite (Table 2). When interactions occur, factor effects should be checked in a combined manner (Montgomery, 2013). It can be seen that at low salinity / low rearing density conditions of 19.1 psu / 3.6 ind.ml^{-1}, growth increased with the temperature from 20.4 to 27.6 °C for three populations. The extent of growth for three populations varied from 37.78% to 72.36% (Fig. 5a, b, c). Notably, the survival also increased from 8.95% to 18.61% for tetraploid population while the survival of diploid and triploid populations decreased under the same conditions. This indicated that the tetraploid larvae were better adapted to high temperatures (Fig. 5f).

In this study, the presence of significant interactive and quadratic effects within the ranges of the three factors indicated that there is a pinnacle in the growth and survival of the three populations (Figs. 2, 3,4). For instance, compared with the optimal AGR and SR for triploid larvae in this study, the AGR decreased by 82.62% and 64.54%, respectively, under unfavorable three-factor environment conditions such as 20.4 °C / 19.1 psu / 3.6 ind.ml^{-1} and 27.6 °C / 31 psu / 8.4 ind. ml^{-1}. The SR decreased by 91.29% and 94.46%, respectively, under the same environment conditions (Fig. 5b, e). It indicates that hypo- and hyper-optimal environment conditions are all adverse to growth and survival of three populations. The cubic effect of temperature, salinity, and rearing density was highly significant for triploid and tetraploid populations (Table 2), indicating that these factors may synergistically influence the triploid and tetraploid populations and these influence

Table 4
The verification results for the optimal combinations for different ploidy population.

	T	S	D	Predicted value		Measured value	
				AGR (μm day^{-1})	SR (%)	AGR (μm day^{-1})	SR (%)
Diploid	23.66	28.47	2.23	13.92	65.728	14.41 ± 0.18*	67.37 ± 3.06*
Triploid	23.16	30.67	2.15	15.82	69.255	15.13 ± 0.10*	66.97 ± 0.99*
Tetraploid	25.48	28.94	2	10.75	46.293	10.98 ± 0.14*	48.35 ± 4.98*

T, S and D represented the temperature, salinity, and rearing density respectively; *represented the error between the measured and predicted values is <5%.

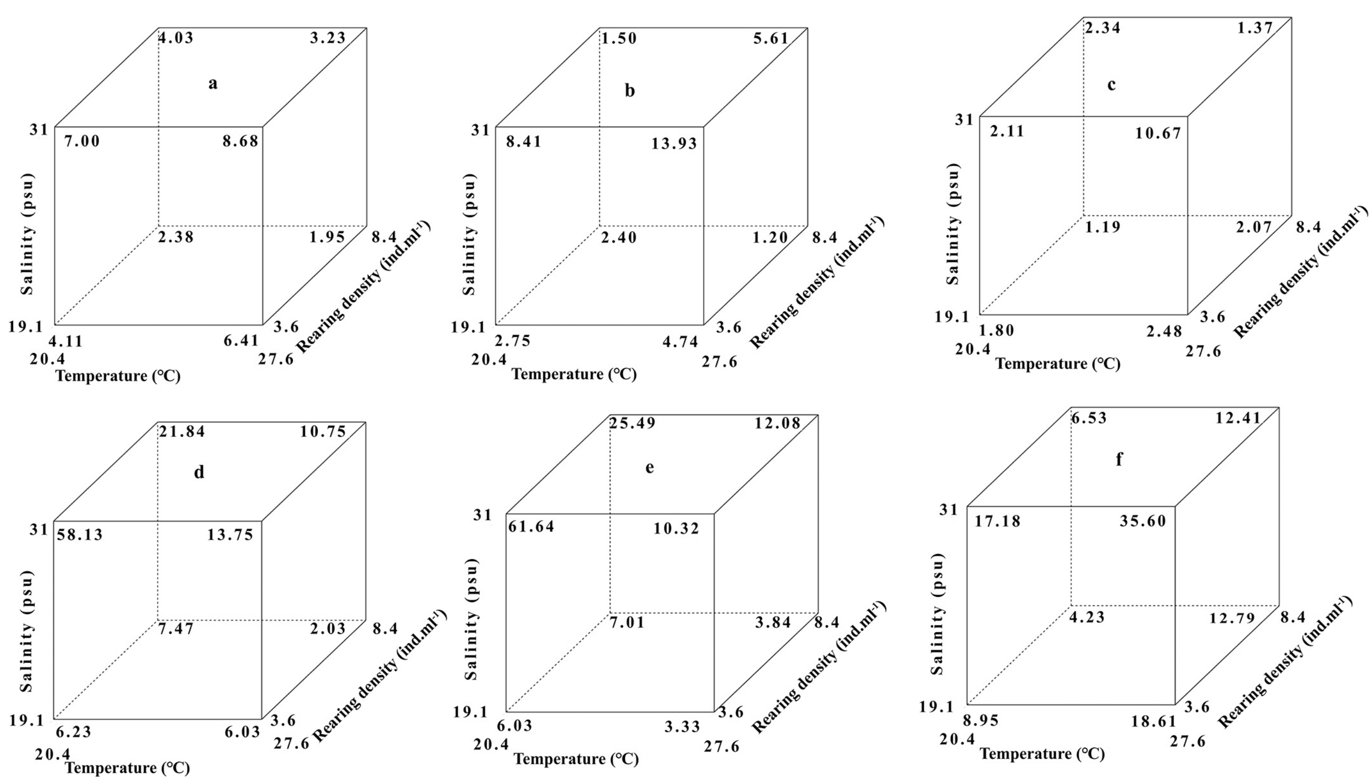

Fig. 5. Cube plot for the changes on the accumulated growth rate (AGR) and survival rate (SR) at different combinations of temperature, salinity and rearing density for the three populations. Note: diploid population (a, d); triploid population (b, e); tetraploid population (c, f).

about triploid and tetraploid populations were inconsistent.

In the oyster breeding industry, the key to production largely depends on the success of growth and survival success during the larvae stage. In this study, the optimal combination conditions for the three populations were achieved through simultaneous optimization based on the response models (Table 4). Li et al. (2022) reported that the salinity range of 25–30 psu and a temperature range of 23–28 °C are suitable culture conditions for tetraploid larvae, which is consistent with the results of this study. Notably, the respective optimal conditions combinations on AGR and SR for three populations were almost overlapping. It indicated that optimal temperature-salinity-rearing density conditions for growth are roughly identical to those for the survival of three populations. The optimal conditions are within the range of environmental condition of the oyster farming area in Northern China (Chu et al., 2005). Therefore, they have practical operation intervals that can being used for the seed production of three populations. For further applications, the optimal culture conditions determined in this study were verified (Table 4). The measured value was consistent with the predicted value. For instance, the survival of tetraploid larvae was 48.35 ± 4.98%, compared with 19.67% reported by Li et al. (2022). This demonstrates the importance of optimization of culture conditions to promote the hatchery production. In reality, the growth and survival of *C. gigas* larvae of different ploidies can be influenced by many factors, beyond the three considered in this paper. For instance, the experiment water volume in this study is smaller than that of the open circulation system which may cause the spatial restrictions on the growth and survival of larvae. Within a certain range, a significant interaction may exist among these factors. Given the complexity of these relationships and the difficulty of experimentally manipulation of some factors, future studies should explore additional factors relevant to the growth and survival of *C. gigas* larvae of varying ploidies.

5. Conclusions

This study underscores the intricate impacts of temperature, salinity, and rearing density - three crucial factors on the growth and survival of *C. gigas* larvae with varying ploidies, providing an in-depth examination of the interactions among these factors. Salinity and rearing density emerged as dominant factors influencing the performance of all three populations. We established robust growth and survival models relative to temperature, salinity, and rearing density. The optimal trifactor culture conditions were attained by simultaneously maximizing growth and survival models. The insights derived from this study furnish valuable new information about the growth and survival of *C. gigas* larvae with different ploidies. Consequently, these findings bear significant potential for application in commercial-scale cultivation of *C. gigas* larvae with different ploidies, with the ultimate goal of maximizing seed production for *C. gigas*.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2024.740690.

Credit author statement

Xianchao Bai: Completion of the experiment, data analysis, and manuscript drafting. **Yuanxin Liang**: Experimental coordination and Oyster farming. **Geng Cheng**: Experimental coordination and Oyster farming. **Haining Zhang**: Experimental coordination and Oyster farming. **Chengxun Xu**: Oyster farming and data analysis. **Qi Li**: Experimental design and coordination and manuscript revision.

CRediT authorship contribution statement

Xianchao Bai: Writing – original draft, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yuanxin Liang:** Validation, Methodology, Investigation, Formal analysis, Data curation. **Haining Zhang:** Visualization, Validation, Investigation. **Geng Cheng:** Validation, Supervision, Investigation. **Chengxun Xu:** Validation, Supervision, Methodology, Data curation, Conceptualization. **Qi Li:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by grants from the National Key R&D Program of China (2022YFD2400305), Shandong Province (2021ZLGX03, 2022LZGCQY010 and 2021LZGC027), and China Agriculture Research System Project (CARS-49).

References

Adams, K.L., Wendel, J.F., 2005. Polyploidy and genome evolution in plants. Curr. Opin. Plant Biol. 8, 135–141. https://doi.org/10.1016/j.pbi.2005.01.001.

Andersen, S., Burnell, G., Bergh, Ø., 2000. Flow-through systems for culturing great scallop larvae. Aquac. Int. 8, 249–257. https://doi.org/10.1023/A:1009271220868.

Avila, C., Grenier, S., Tamse, C.T., Kuzirian, A.M., 1997. Biological factors affecting larval growth in the nudibranch mollusc *Hermissenda crassicornis* (Eschscholtz, 1831). J. Exp. Mar. Biol. Ecol. 218, 243–262. https://doi.org/10.1016/S0022-0981(97)00077-4.

Benabdelmouna, A., Ledu, C., 2015. Autotetraploid Pacific oysters (*Crassostrea gigas*) obtained using normal diploid eggs: induction and impact on cytogenetic stability. Genome 58, 333–348. https://doi.org/10.1139/gen-2015-0014.

Chu, P., Chen, Y.C., Kuninaka, A., 2005. Seasonal variability of the Yellow Sea/East China Sea surface fluxes and thermohaline structure. Adv. Atmos. Sci. 22, 1–20. https://doi.org/10.1007/BF02930865.

Cragg, S.M., 1980. Swimming behaviour of the larvae of *Pecten maximus* (L.) (Bivalvia). J. Mar. Biol. Assoc. U. K. 60, 551–564. https://doi.org/10.1017/S002531540004025X.

Deng, Y., Fu, S., Liang, F., Xie, S., 2013. Effects of stocking density, diet, and water exchange on growth and survival of pearl oyster *Pinctada maxima* larvae. Aquac. Int. 21, 1185–1194. https://doi.org/10.1007/s10499-013-9622-0.

Francesc, P., Andy, B., Jeanclaude, F.R., Martin, F.H., Pierrick, H., Lorenzo, C., 2009. Polyploid fish and shellfish: production, biology and applications to aquaculture for performance improvement and genetic containment. Aquaculture. 293, 125–156. https://doi.org/10.1016/j.aquaculture.2009.04.036.

Fu, D., Chen, J., Zhang, Y., Yu, Z., 2011. Cloning and expression of a heat shock protein (HSP) 90 gene in the haemocytes of *Crassostrea hongkongensis* under osmotic stress and bacterial challenge. Fish Shellfish Immunol. 31, 118–125. https://doi.org/10.1016/j.fsi.2011.04.011.

Gagnaire, B., Frouin, H., Moreau, K., Thomas-Guyon, H., Renault, T., 2006. Effects of temperature and salinity on haemocyte activities of the Pacific oyster, *Crassostrea gigas* (Thunberg). Fish Shellfish Immunol. 20, 536–547. https://doi.org/10.1016/j.fsi.2005.07.003.

Gajbhiye, D.S., Khandeparker, L., 2017. Immune response of the short neck clam *Paphia malabarica* to salinity stress using flow cytometry. Mar. Environ. Res. 129, 14–23. https://doi.org/10.1016/j.marenvres.2017.04.009.

Gosling, E., 2015. Marine Bivalve Molluscs, 2nd ed. John Wiley & Sons, Ltd, The Atrium, Southern Gate, Chichester, West Sussex, UK. https://doi.org/10.1002/9781119045212.

Guo, X., Allen, S., 1994a. Reproductive potential and genetics of triploid Pacific oysters, *Crassostrea gigas* (Thunberg). Biol. Bull. 187, 309–318. https://doi.org/10.2307/1542288.

Guo, X., Allen, S., 1994b. Viable tetraploid Pacific oyster (*Crassostrea gigas* Thunburg) produced by inhibiting polar body I in eggs of triploids. Mol. Mar. Biol. Biotechnol. 3, 42–50.

Guo, X., Wang, Y., Xu, Z., Yang, H., 2009. Chromosome set manipulation in shellfish. New Technol. Aquacul. 165-194 https://doi.org/10.1533/9781845696474.1.165.

Joyce, A., Vogeler, S., 2018. Molluscan bivalve settlement and metamorphosis: neuroendocrine inducers and morphogenetic responses. Aquaculture 487, 64–82. https://doi.org/10.1016/j.aquaculture.2018.01.002.

Legat, J.F.A., Puchnick-Legat, A., de Miranda, Gomes C.H.A., Sühnel, S., de Melo, C.M.R., 2017. Effects of salinity on fertilization and larviculture of the mangrove oyster, *Crassostrea gasar* in the laboratory. Aquaculture. 468, 545–548. https://doi.org/10.1016/j.aquaculture.2016.11.016.

Li, L., Li, Q., 2009. Effects of stocking density, temperature, and salinity on larval survival and growth of the red race of the sea cucumber *Apostichopus japonicus* (Selenka). Aquac. Int. 18, 447–460. https://doi.org/10.1007/s10499-009-9256-4.

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. https://doi.org/10.1016/j.aquaculture.2021.737809.

Liu, B., Dong, B., Tang, B., Zhang, T., Xiang, J., 2006. Effect of stocking density on growth, settlement and survival of clam larvae, *Meretrix meretrix*. Aquaculture. 258, 344–349. https://doi.org/10.1016/j.aquaculture.2006.03.047.

Lü, W., Shen, M., Fu, J., Li, W., You, W., Ke, C., 2017. Combined effects of temperature, salinity and rearing density on growth and survival of juvenile ivory shell, *Babylonia areolate* (link 1807) population in Thailand. Aquac. Res. 48, 1648–1665. https://doi.org/10.1111/are.13000.

MacDonald, B.A., 1988. Physiological energetics of Japanese scallop *Patinopecten yessoensis* larvae. J. Exp. Mar. Biol. Ecol. 120, 155–170. https://doi.org/10.1016/0022-0981(88)90086-X.

Marshall, R., Pearce, C.M., McKinley, R.S., 2014. Interactive effects of stocking density and algal feed ration on growth, survival, and ingestion rate of larval geoduck clams. N. Am. J. Aquac. 76, 265–274. https://doi.org/10.1080/15222055.2014.886645.

Meng, J., Zhu, Q., Zhang, L., Li, C., Li, L., She, Z., Huang, B., Zhang, G., 2013. Genome and transcriptome analyses provide insight into the Euryhaline adaptation mechanism of *Crassostrea gigas*. PLoS One 8, e58563. https://doi.org/10.1371/journal.pone.0058563.

Montgomery, D.C., 2013. Design and Analysis of Experiments, 8th ed. John Wiley & Sons, Inc. https://doi.org/10.1002/ep.11743

Moore, R.C., Purugganan, M.D., 2005. The evolutionary dynamics of plant duplicate genes. Curr. Opin. Plant Biol. 8, 122–128. https://doi.org/10.1016/j.pbi.2004.12.001.

Navarro, J.M., Gonzalez, C.M., 1998. Physiological responses of the Chilean scallop *Argopecten purpuratus* to decreasing salinities. Aquaculture 167, 315–327. https://doi.org/10.1016/S0044-8486(98)00310-X.

Peteiro, L.G., Woodin, S.A., Wethey, D.S., Costas-Costas, D., Martínez-Casal, A., Olabarria, C., Vázquez, E., 2018. Responses to salinity stress in bivalves: evidence of ontogenetic changes in energetic physiology on *Cerastoderma edule*. Sci. Rep. 8, 8329. https://doi.org/10.1038/s41598-018-26706-9.

Qin, Y., Noor, Z., Li, X., Ma, H., Li, J., Zhou, Y., Yu, Z., 2021. Tetraploid induction of *Crassostrea hongkongensis* and *C. sikamea* by inhibiting the polar body 1 release in diploid fertilized eggs. Mar. Life Sci. Technol. 3, 463–473. https://doi.org/10.1007/s42995-021-00107-w.

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 https://doi.org/10.1016/j.aquaculture.2022.738044.

Rahman, M.A., Henderson, S., Miller-Ezzy, P., Li, X., Qin, J., 2019. Immune response to temperature stress in three bivalve species: Pacific oyster *Crassostrea gigas*, Mediterranean mussel *Mytilus galloprovincialis* and mud cockle *Katelysia rhytiphora*. Fish Shellfish Immunol. 86, 868–874. https://doi.org/10.1016/j.fsi.2018.12.017.

Ramos, C.D.O., da Silva, F.C., Gomes, C.H.A.D.M., Langdon, C., Takano, P., Gray, M.W., de Melo, C.M.R., 2021. Effect of larval density on growth and survival of the Pacific oyster *Crassostrea gigas* in a recirculation aquaculture system. Aquaculture 540. https://doi.org/10.1016/j.aquaculture.2021.736667.

Rato, A., Joaquim, S., Matias, A.M., Roque, C., Marques, A., Matias, D., 2022. The impact of climate change on bivalve farming: combined effect of temperature and salinity on survival and feeding behavior of clams *Ruditapes decussatus*. Front. Mar. Sci. 9 https://doi.org/10.3389/fmars.2022.932310.

Sarkis, S., Helm, M., Hohn, C., 2006. Larval rearing of calico scallops, *Argopecten gibbus*, in a flow-through system. Aquac. Int. 14, 527–538. https://doi.org/10.1007/s10499-006-9052-3.

Velasco, L.A., Barros, J., 2008. Experimental larval culture of the Caribbean scallops *Argopecten nucleus* and *Nodipecten nodosus*. Aquac. Res. 39, 603–618. https://doi.org/10.1111/j.1365-2109.2008.01917.x.

Wang, H., Zhang, Y., Wang, L., Zhu, C., Zhang, J., Wu, N., 2021. Growth and survival of *Pinctada martensii* (dunker) postlarvae under concurrent variation in temperature, algal ration and stocking density. Aquac. Rep. 20 https://doi.org/10.1016/j.aqrep.2021.100668.

Wang, T., Li, Q., Zhang, J., Yu, R., 2018. Effects of salinity, stocking density, and algal density on growth and survival of Iwagaki oyster *Crassostrea nippona* larvae. Aquac. Int. 26, 947–958. https://doi.org/10.1007/s10499-018-0261-3.

Xu, C., Li, Q., Chong, J., 2019. Combined effect of temperature, salinity, and rearing density on the larval growth of the black shell strain and wild population of the Pacific oyster *Crassostrea gigas*. Aquac. Int. 28, 335–347. https://doi.org/10.1007/s10499-019-00465-4.

Yan, X., Zhang, G., Yang, F., 2006. Effects of diet, stocking density, and environmental factors on growth, survival, and metamorphosis of Manila clam *Ruditapes philippinarum* larvae. Aquaculture 253, 350–358. https://doi.org/10.1016/j.aquaculture.2005.07.030.

Zhang, Y., Li, J., Qin, Y., Zhou, Y., Zhang, Y., Yu, Z., 2016. A comparative study of the survival, growth and gonad development of the diploid and triploid Hong Kong oyster, *Crassostrea hongkongensis* (Lam & Morton 2003). Aquac. Res. 48, 2453–2462. https://doi.org/10.1111/are.13081.

Zhang, Y., Qin, Y., Yu, Z., 2022. Comparative study of tetraploid-based reciprocal triploid Portuguese oysters, *Crassostrea angulata*, from seed to marketsize. Aquaculture 547. https://doi.org/10.1016/j.aquaculture.2021.737523.

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. https://doi.org/10.1016/j.aquaculture.2023.739472.