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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*

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Abstract
Crassostrea gigas is a commercially important species which is the mostly widely cultured in the world. However, most of the oyster broodstock in China remains unselected. Through a 7-generation of selection on the shell color and growth traits of adult oysters, an excellent strain of *C. gigas* with black shell coloration and mantle has been developed. In order to facilitate the industrialized breeding, it is necessary to explore the growth performance of the black shell strain in larval stage and find out the optimal conditions for larval development. In this study, the accumulated growth rate and survival rate of the black shell strain and wild population of *C. gigas* larvae were measured respectively, and a central composite design as well as a response surface method was used to investigate the combined effect of temperature, salinity, and rearing density on the growth of both the two populations. No significant differences were found in survival rate between the two populations, and two model equations for the growth of the two populations were established. The optimizations of accumulated growth rate for two populations were explored. When the temperature, salinity, and rearing density was 25.14 °C, 30.28 psu, and 1.00 ind. ml^{-1}, respectively, the accumulated growth rate for the black shell strain maximized 15.40 μm day^{-1}. With a combination of temperature of 25.06 °C, salinity of 29.27 psu and rearing density of 1.00 ind. ml^{-1}, the maximum value of accumulated growth rate for wild population was 13.20 μm day^{-1}. Furthermore, the larval growth rates were compared between the black shell strain and wild population, and the larvae of the black shell strain significantly grew faster than those of wild population when temperature, salinity and rearing density in a suitable range.

Keywords *Crassostrea gigas* · Larvae · Black-shell color strain · Growth · Central composite design · Response surface methodology

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Introduction

The Pacific oyster, *Crassostrea gigas*, is a major global aquaculture species because of its potential high growth rates and wide tolerance to different environmental conditions, ranking first in production among all aquaculture fish and shellfish species. However, worldwide production is mainly based on unimproved populations of stocks (Gjedrem et al. 2012), likely limiting profitability and expanded production of this species due to the unrealized potential for genetic improvement (de Melo et al. 2013). Consequently, numerous selective breeding programs for improving desirable traits of *C. gigas* have been initiated in many countries, such as the selection on body growth in USA (Evans and Langdon 2006), resistance to mortality in France (Dégremont et al. 2015), and shell coloration in China (Wan et al. 2017; Wang et al. 2016).

The shell colorations of mollusks present high diversity and have attracted the interest of many naturalists and collectors. Moreover, the visual perception of food products can affect consumer preference and product value (Kahn and Wansink 2004). For instance, consumers' predilection for salmon flesh and their willingness to pay a premium price depend on the level of red pigmentation, which endows the additive value to coloration in seafood (Alfnes et al. 2006). Similarly, the shell pigmentation of *C. gigas* continuously varies from near-white, pigmentation free shells to near-black, fully pigmented shells, and the individuals with black pigmentation shell could have a higher price than other common oysters in Korea (Kang et al. 2013). Due to the additive commercial value of shell coloration, a selective breeding program for *C. gigas* was initiated in 2010 in China. After a 7-generation of selection on the shell color and shell height of adult oysters, an excellent strain of *C. gigas* with black shell coloration and mantle and impressive improvement on growth rate has been obtained (Wang et al. 2016; Xu et al. 2017).

Larvae of *C. gigas* live a planktonic life which are sensitive to seawater environmental changes. The temperature, salinity, and rearing density are important environmental factors that influence the ecology and biology of aquatic species. Temperature and salinity have been observed to influence many aspects of mollusks, such as food absorption and conversion ability (Schulte 1975; Walne 1972; Widdows and Bayne 1971), metabolic activity of enzymes (Bayne 1976), osmotic adjustment ability (Deaton et al. 1989), immune response (Bussell et al. 2008), and reproduction and larval development (Honkoop and Van der Meer 1998; Legat et al. 2017; Nicolini and Penry 2000; Sastry 1963). Rearing density, which is widely recognized as a critical factor in intensive aquaculture, represents a potential source of chromic stress, affecting the physiology and behavior of farmed species (Li and Li 2010; Li et al. 2007; Velasco and Barros 2008). To date, there have been many studies of single environmental factor on mollusks (Jiang et al. 2016; Laing 2002; Saucedo et al. 2004; Tan and Wong 1996). Recently, many researchers focus on the combined effect of temperature and salinity on the development of marine bivalve species (Kim et al. 2017; Nie et al. 2017). And for tripartite combination, His et al. (1989) investigated the combined effects of temperature, salinity, and nutrition on survival and growth of larvae of *C. gigas*. Nevertheless, there is no document on the combined effects of temperature, salinity, and rearing density on the larval growth of *C. gigas*.

In present study, we investigated the combined effects of temperature, salinity, and rearing density on the larval growth and survival of the black shell strain and wild population of *C. gigas*. The aim of present study was to determine the optimal combination of temperature, salinity, and rearing density for the larval growth of black shell strain, and to provide theoretical basis for industrialized seeding production of black of shell strain of *C. gigas*.

Materials and methods

Biological materials

The larvae used for the experiment were produced by the black shell strain and the wild population of *C. gigas* respectively. Black shell individuals were found in the cultured population of *C. gigas* in 2010. Using those black shell individuals as parents, we had bred genetically stable strain of *C. gigas* with black left and right shell color, combining four generations family selection for fixing black shell color and three generations mass selection for promoting growth (Wang et al. 2016; Xu et al. 2017). The broodstock of both populations were collected in May 2017 from Rushan, China, and were cultivated at Haiyi Hatchery in Laizhou, China. A pool with 24 m^3 of seawater (temperature 24 ± 1 °C; salinity 32 ± 1 psu) was used for 1 month of conditioning. Twenty mature *C. gigas* from each population were induced to spawn. Twenty hours after fertilization, all embryos developed into the D-larvae, and the density of each population was calculated under an optical microscope.

Measurement of accumulated growth rate and survival rate

After 2 weeks of artificial cultivation, the larval growth and survival of different groups were measured. The larval shell height of a random sample of 30 larvae from each group was recorded by an optical microscope. The final volume of rearing condition from each group was measured by a graduated cylinder. The accumulated growth rate (AGR) was the ratio of the difference of the measured shell height and initial shell height divided by the number of days. The survival rate (SR) was the ratio of the final volume divided by the initial volume. The equations of AGR and SR was as follows:

$$\mathrm{AGR}\left(\mu\mathrm{m\ day}^{-1}\right) = \frac{H_t - H_0}{t - t_0}$$

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

In these equations, t_0 and t were the beginning time and end time of the experiment respectively; H_0 and H_t were the shell height of the same group at the beginning time and end time of the experiment respectively; V_0 and V_t were the volume of rearing condition in the same group at the beginning time and end time of the experiment respectively.

Experimental protocol

The maximum and minimum temperature for experiment were 33 °C and 15 °C, respectively, and the maximum and minimum salinity were 40 psu and 10 psu, respectively, and the maximum and minimum rearing density were 11 ind.mL^{-1} and 1 ind. ml^{-1} respectively. Electrical heaters were used to maintain the high temperature, and an electrical thermometer was used to control the temperature with the precision of ± 0.2 °C. The low temperature was regulated and controlled by using a low-temperature refrigerator with the precision of ± 0.2 °C. Low salinity was obtained by diluting seawater with dechlorinated freshwater and high salinity by adding sea salt to seawater. A refractometer (ATAGO) was used to monitor the salinity with a precision of ± 0.1%. According the precalculated density, appropriate amount of the energetic and healthy D-larvae were delivered into plastic buckets with a volume of 25 l and acclimated

to the experiment temperature and salinity combinations for 1 day by adjusting 0.4 °C/h and 1 psu/h. Each plastic bucket was continuously aerated by an air stone to improve dissolved oxygen. After a temporarily adaptive process of the D-larvae, the experiment was carried out, and the shell height of D-larvae were recorded as the initial values without any significant difference. During the experiment, we calculated the density of each group and adjusted the density to preset value by reducing the volume of seawater. The D-larvae of both populations were fed to excess with the mixture by *Isochrysis galbana* and *Nitzschiaclosterium* every 8 h (make sure the algal density ≥ 20 cells μl^{-1}), and the seawater of each container was exchanged a half every 12 h. The replaced seawater was filtered through sand filters and nonwovens polypropylene fabric and adjusted to the experimental condition.

Experiment design

Design Expert software version 8.06 (Minneapolis, USA) was used to make the central composite design (CCD) (Table 1). The range of all three factors was determined by reference to previous research and production practical experience. The design contained one experiment factorial points, one axial point, and six center points. The code value for the central composite was 0, the upper limit and lower limit of the code value were 1 and − 1, respectively, and the asterisk arm was |1.682|. And the factor ranges were in terms of the alphas, so the temperature ranged from 15 to 33 °C, the salinity ranged from 10 to 40 psu, and the rearing density ranged from 1 to 11 ind.

Table 1 Central composite design used in response surface method studies and calculated values of AGR and SR

Run	Code			Actual			*AGR* (μm day^{-1})		SR (%)	
	T	*S*	*D*	*T* (°C)	*S* (ppt)	*D* ($ind.ml^{-1}$)	Black	Wild	Black	Wild
1	0	0	− α	24	25	1	14.69 ± 0.45*	12.81 ± 0.35	80.22 ± 11.01	81.69 ± 4.55
2	α	0	0	33	25	6	1.82 ± 0.07	1.76 ± 0.10	30.62 ± 2.37	34.46 ± 2.16
3	− 1	1	− 1	18.65	33.92	3.03	6.14 ± 0.32	4.97 ± 0.60	51.38 ± 9.76	52.2 ± 7.18
4	0	0	0	24	25	6	7.52 ± 0.11	6.98 ± 0.28	55.96 ± 6.89	55.94 ± 4.38
5	− 1	− 1	− 1	18.65	16.08	3.03	4.50 ± 0.27*	3.50 ± 0.04	42.18 ± 4.09	44.34 ± 3.56
6	1	− 1	1	29.35	16.08	8.97	2.98 ± 0.16*	3.76 ± 0.35	33.72 ± 2.88	34.91 ± 3.06
7	0	0	0	24	25	6	8.43 ± 0.25*	6.58 ± 0.72	59.07 ± 3.58	60.97 ± 7.19
8	0	0	0	24	25	6	7.72 ± 0.09	7.63 ± 1.13	58.74 ± 11.25	60.9 ± 8.23
9	− 1	− 1	1	18.65	16.08	8.97	3.14 ± 0.05*	2.12 ± 0.05	40.95 ± 5.83	40.87 ± 3.83
10	1	1	− 1	29.35	33.92	3.03	9.32 ± 0.41	8.35 ± 0.59	66.31 ± 4.27	66.91 ± 7.9
11	1	− 1	− 1	29.35	16.08	3.03	5.75 ± 0.50*	4.43 ± 0.11	49.44 ± 4.39	53.4 ± 5.38
12	0	0	0	24	25	6	7.65 ± 0.29	7.28 ± 0.30	62.21 ± 9.01	64.66 ± 4.8
13	1	1	1	29.35	33.92	8.97	3.73 ± 0.11	4.06 ± 0.23	37.53 ± 5.77	43.55 ± 2.52
14	0	0	0	24	25	6	8.22 ± 0.13*	7.54 ± 0.11	59.45 ± 4.8	62.27 ± 6.33
15	0	α	0	24	40	6	5.13 ± 0.07*	3.73 ± 0.47	45.62 ± 3.89	45.78 ± 7.67
16	− 1	1	1	18.65	33.92	8.97	4.43 ± 0.14	4.18 ± 0.15	39.4 ± 4.55	43.78 ± 2.93
17	− α	0	0	15	25	6	1.04 ± 0.09*	0.76 ± 0.04	28.2 ± 2.33	31.22 ± 2.17
18	0	− α	0	24	10	6	1.98 ± 0.18	1.84 ± 0.17	30.06 ± 7.29	37.76 ± 4.73
19	0	0	0	24	25	6	7.08 ± 0.22	7.37 ± 0.21	54.54 ± 9.84	57.17 ± 2.75
20	0	0	α	24	25	11	5.44 ± 0.08*	6.24 ± 0.12	47.72 ± 6.92	50.93 ± 4.45

T, *S*, and *D* represented the temperature, salinity, and rearing density respectively; *AGR* represented the accumulated growth rate; *SR* represented the survival rate; black and wild represented the black shell strain and wild population of *C. gigas*; |α|was asterisk arm; *represented the significant difference between the black shell strain and wild population

ml^{-1}. In this design, each population has 24 experimental points. To minimize errors, two replicates were set up, and 120 group points in total were tested. The average and standard deviation of the three replications were to be calculated.

Data analysis

The data were analyzed using Design Expert 8.06 for the selection of a response surface model with temperature, salinity, and rearing density, and a back stepwise regression method (Alpha out was 0.1) was used to correct the hierarchy automatically. The model general formula was:

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_{\mathrm{i}} \chi_{\mathrm{i}} + \sum_{j=1}^{n} \beta_{\mathrm{jj}} \chi_{\mathrm{j}}^2 + \sum_{l=1}^{n} \beta_{\mathrm{lll}} \chi_{\mathrm{l}}^3 + \sum_{ij}^{n} \beta_{\mathrm{ij}} \chi_{\mathrm{ij}} + \sum_{il}^{n} \beta_{\mathrm{il}} \chi_{\mathrm{il}} + \sum_{jl}^{n} \beta_{\mathrm{jl}} \chi_{\mathrm{jl}} + \sum_{ijl}^{n} \beta_{\mathrm{ijl}} \chi_{\mathrm{ijl}} + e$$

where Y was the response (AGR); β_0 was the intercept of regression equation; β_i, β_j, β_l,⋯ β_{ijl} were linear, quadratic, and cubic and interactive effects of temperature, salinity, and rearing density on AGR; χ_i, χ_j, and χ_l were coding variables of temperature (T), salinity (S), and rearing density (D), respectively, and e is random error, with the assumption that it has a normal distribution with a mean of zero.

Fisher's t test was used to analyze the statistical significance. Variance analysis was used to select the significant experimental factor and confirm the regression equation model. The coefficient of the determination (R^2), adjusted coefficient (Adj-R^2), and predicted coefficient (Pred-R^2) were used to assess the feasibility of the model. We used the Origin 9.1 (Northampton, USA) to prepare the three-dimensional response surface diagram and the corresponding contour map to analyze the effects of temperature, salinity, and rearing density on growth of the larvae.

Results

Growth and survival of two populations

The SR and AGR of black shell strain and wild population at different combinations were calculated respectively (Table 1). And a significance test was analyzed between black shell train and wild population in same combinations of three factors. There were no significant differences in SR between wild population and black shell strain. In contrast, significant differences of AGR were found between black shell strain and wild population in some combinations. AGR of black shell strain was higher than that of wild population in most cases with normal ranges of three factors, whereas AGR of wild population was higher than that of black shell strain in some cases especially with a high rearing density.

Modeling and significance test

The results of measured AGR were used for model fitting. By means of statistics analysis, the coefficient was estimated (Table 2), and the regression equations were determined to be as follows:

Table 2 Regression coefficients, standard errors, and 95% confidence intervals for the predicted model of accumulated growth rate of the black shell strain and wild population

Factor	Coefficient	d.f.	SE	95% CI	
				Low	High
The black shell strain					
Intercept	7.77	1	0.17	7.37	8.16
T	0.36	1	0.11	0.09	0.62
S	0.92	1	0.11	0.66	1.18
D	−2.75	1	0.18	−3.16	−2.34
TS	0.17	1	0.15	−0.17	0.52
TD	−0.66	1	0.15	−1.01	−0.32
SD	−0.40	1	0.15	−0.74	−0.05
T^2	−2.21	1	0.11	−2.47	−1.95
S^2	−1.46	1	0.11	−1.72	−1.20
D^2	0.84	1	0.11	0.59	1.10
TSD	−0.31	1	0.15	−0.65	0.03
T^2D	1.32	1	0.23	0.79	1.86
The wild population					
Intercept	7.23	1	0.15	6.87	7.59
T	0.30	1	0.15	−0.07	0.67
S	0.56	1	0.15	0.19	0.93
D	−1.95	1	0.15	−2.32	−1.58
TS	0.09	1	0.13	−0.22	0.40
TD	−0.35	1	0.13	−0.66	−0.04
SD	−0.38	1	0.13	−0.69	−0.07
T^2	−2.10	1	0.09	−2.33	−1.87
S^2	−1.56	1	0.09	−1.79	−1.33
D^2	0.82	1	0.09	0.59	1.06
TSD	−0.53	1	0.13	−0.84	−0.22
T^2S	0.41	1	0.20	−0.08	0.89
T^2D	1.06	1	0.20	0.58	1.54
TS^2	0.43	1	0.20	−0.05	0.91

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, the final equations for the black shell strain and wild population obtained by the actual value were as follows:

$$Y_{\text{Black}} = -95.2979 + 8.0814\text{T} + 0.7094\text{S} + 6.9512\text{D} - 0.0167\text{TS} - 0.7335\text{TD} + 0.0373\text{SD} - 0.1705\text{T}^2 - 0.0183\text{S}^2 - 0.0954\text{D}^2 - 0.0022\text{TSD} + 0.0155\text{T}^2\text{D}$$

$$Y_{\text{Wild}} = -114.754 + 9.2250\text{T} + 2.6827\text{S} + 4.0424\text{D} - 0.1030\text{TS} - 0.5259\text{TD} + 0.0748\text{SD} - 0.1877\text{T}^2 - 0.0439\text{S}^2 + 0.0933\text{D}^2 - 0.0037\text{TSD} + 0.0016\text{T}^2\text{S} + 0.0124\text{T}^2\text{D} + 0.0010\text{TS}^2$$

$$Y_{\text{Wild}} = -95.2979 + 8.0814T + 0.7094S + 6.9512D - 0.0167TS - 0.7335TD + 0.0373SD - 0.1705T^2 - 0.0183S^2 - 0.0954D^2 - 0.0022TSD + 0.0155T^2D$$

$$Y_{\text{Black}} = -114.754 + 9.2250T + 2.6827S + 4.0424D - 0.1030TS - 0.5259TD + 0.0748SD - 0.1877T^2 + 0.0933D^2 - 0.0037TSD + 0.0016T^2S + 0.0124T^2D + 0.0010TS^2$$

Y_{Wild} and Y_{Black} represented the accumulated growth rate of the wild population and the black shell strain respectively.

Analysis of variance for the model of two groups was compared with each other (Table 3). The model equation of both two populations could fully fit the experimental data ($P < 0.0001$). The linear effect of salinity and rearing density were significantly contributed to the variation of growth for both two populations. However, the difference existed in the linear effect of temperature between the two populations. The linear effect of temperature is significant for the black shell strain. In contrast, effect of temperature is not significant for the wild population ($P > 0.05$). For the two populations, both the interactive effect between temperature and rearing density as well as the interactive effect between salinity and rearing density were significant ($P < 0.05$), but it was different between temperature and salinity. All quadratic effects of temperature, salinity, and rearing density were highly significant to the model equation of both two populations ($P < 0.0001$). For both two populations, the interaction between the

Table 3 Analysis of variance table for the cubic model of response, accumulated growth rates of the black shell strain and wild population

Source	SS	df.	MS	*F* value	*P* value
The black shell strain					
Model	191.11	11	17.37	97.83	< 0.0001
T	1.74	1	1.74	9.82	0.0139
S	11.51	1	11.51	64.84	< 0.0001
D	42.78	1	42.78	240.91	< 0.0001
TS	0.24	1	0.24	1.35	0.2795
TD	3.51	1	3.51	19.75	0.0022
SD	1.26	1	1.26	7.07	0.0288
T^2	70.39	1	70.39	396.35	< 0.0001
S^2	30.7	1	30.7	172.85	< 0.0001
D^2	10.26	1	10.26	57.76	< 0.0001
TSD	0.76	1	0.76	4.3	0.0718
T^2D	5.81	1	5.81	32.7	0.0004
Residual	1.42	8	0.18		
Lack of fit	0.23	3	0.08	0.32	0.8139
Pure error	1.19	5	0.24		
Total	192.53	19			
The wild population					
Model	155.83	13	11.99	93.3	< 0.0001
T	0.5	1	0.5	3.88	0.0963
S	1.78	1	1.78	13.89	0.0098
D	21.54	1	21.54	167.63	< 0.0001
TS	0.06	1	0.06	0.47	0.5187
TD	0.97	1	0.97	7.56	0.0334
SD	1.16	1	1.16	9.07	0.0237
T^2	63.41	1	63.41	493.52	< 0.0001
S^2	34.96	1	34.96	272.08	< 0.0001
D^2	9.8	1	9.8	76.3	0.0001
TSD	2.22	1	2.22	17.31	0.0059
T^2S	0.55	1	0.55	4.26	0.0845
T^2D	3.71	1	3.71	28.91	0.0017
TS^2	0.62	1	0.62	4.8	0.0709
Residual	0.77	6	0.13		
Lack of fit	0.01	1	0.01	0.07	0.7952
Pure error	0.76	5	0.15		
Total	156.6	19			

T, *S*, and *D* represented the temperature, salinity, and rearing density respectively; For black shell strain, $R^2 = 0.9926$, Adj-$R^2 = 0.9825$, and Pred-$R^2 = 0.9593$; For wild population, $R^2 = 0.9951$, Adj-$R^2 = 0.9844$, and Pred-$R^2 = 0.9770$

quadratic effect of temperature and linear effect of rearing density has significant difference ($P < 0.05$). However, the other terms were not significant for the equations of both two populations ($P > 0.05$).

For the two populations, both the lack of fit and pure error of the model equation were not significant ($P > 0.05$). The coefficients of determination (R^2) for the wild population and black shell strain were 0.9551 and 0.9926 respectively. Adjusted coefficients (Adj-R^2) for the wild population and black shell strain were 0.9844 and 0.9528 respectively. And predicted coefficients (Pred-R^2) for the wild population and black shell strain were 0.9770 and 0.9593 respectively.

Interactive effect of temperature and salinity on AGR for two populations

There was a spherical surface showing the effect of temperature and salinity on the larval growth for the black shell strain (Fig. 1a). Both the extreme temperature and salinity generated the low AGR. The gentle slope of the response surface indicated that when the temperature and salinity was within a certain range, there were no great fluctuations on the growth for larvae. The similar trend of response surface was found in wild population (Fig. 1b). However, the AGR for wild population was lower than that for the black shell strain when in the same combination of temperature and salinity.

Interactive effect of salinity and rearing density on AGR for two populations

A ridged shape of the response surface was shown in Fig. 2a, and the ridge of the plot was found when the salinity was ~ 27 psu. Too high or too low salinity could lead to the decline of AGR for the black shell strain. When the salinity was ~ 27 psu, AGR for the black shell strain was rising with declining rearing density. Similarly, a smoother ridged shape of the plot was found in wild population (Fig. 2b) which showed the effect of salinity and rearing density on AGR for wild population. No matter what the salinity was, the AGR of wild population was declined with the rearing density rising. Only with a combination of high rearing density and low salinity, the AGR of wild population was higher than that of black shell strain.

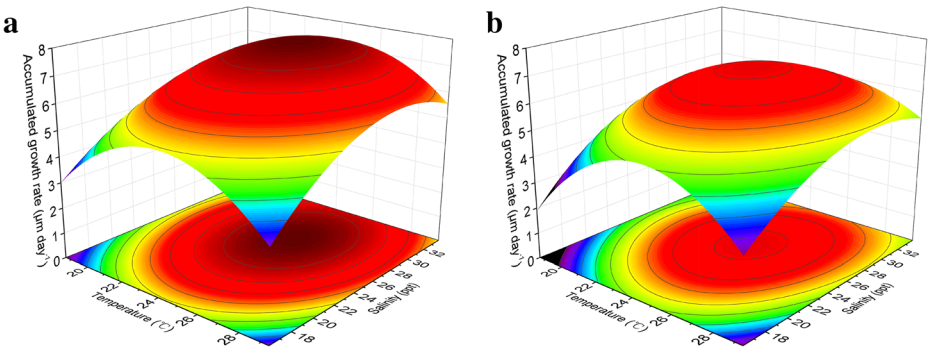

Fig. 1 Response surface plot of the effect of temperature and salinity on the accumulated growth rate for the black shell strain (**a**) and wild population (**b**) (rearing density = 6 ind. ml^{-1})

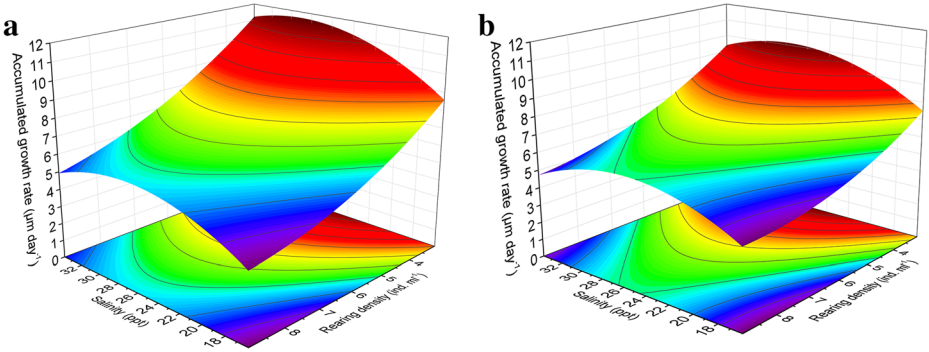

Fig. 2 Response surface plot of the effect of salinity and rearing density on the accumulated growth rate for the black shell strain (**a**) and wild population (**b**) (temperature = 25 °C)

Interactive effect of temperature and rearing density on AGR for two populations

The response surface plot was an oval, suggesting an interactive effect of temperature and rearing density (Fig. 3a). Under the same temperature, AGR of black shell strain was rising with the rearing density declining. Comparing the response surface plot for the black shell strain, the response surface for wild population (Fig. 3b) was with a smaller gradient. When the temperature in a certain range, the AGR for wild population in low rearing density was lower than that for the black shell strain. However, under the combination of high temperature and high rearing density, the AGR for wild population was higher than that for the black shell strain, which indicated that the tolerance of the black shell strain for high temperature was worse than that of wild population when under a high rearing density.

Optimization and comparison between the growth of two populations

In the range of experimental conditions for three factors, the optimized combinations for two populations were analyzed according the growth models. When the temperature, salinity and rearing density was 25.14 °C, 30.28 psu, and 1.00 ind. ml^{-1}, respectively, the AGR for the black shell strain maximized 15.40 μm day^{-1}. With a combination of temperature of 25.06 °C, salinity of 29.27 psu, and rearing density of 1.00 ind. ml^{-1}, the maximum value of AGR for

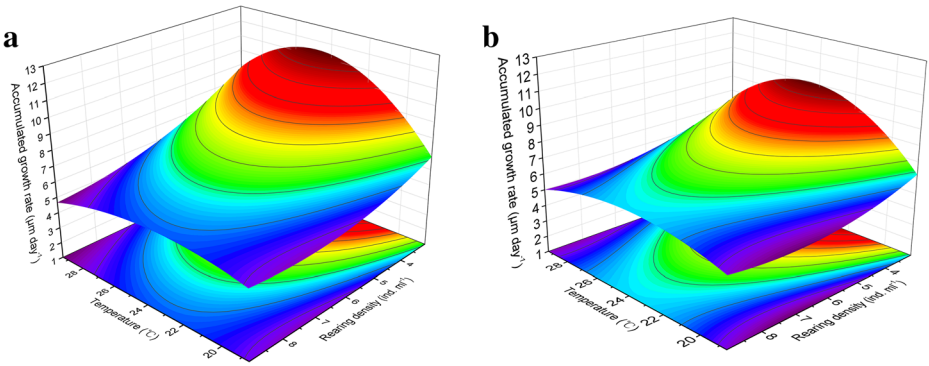

Fig. 3 Response surface plot of the effect of temperature and rearing density on the accumulated growth rate for the black shell strain (**a**) and wild population (**b**) (salinity = 25 ppt)

wild population was 13.20 μm day^{-1}, which is lower than that for the black shell strain. However, when in a high rearing density (≥ 8.97 ind. ml^{-1}) and a high temperature (≥ 29.35 °C), the AGR for wild population was higher than that for black shell strain no matter salinity.

Discussion

No significant differences were found in SR between black shell strain and wild population, and possible explanations were explored. Firstly, a combined selection method and a low selection pressure may lead to no decline on survivability of black shell strain even through a multi-generation selection. Secondly, 2 weeks of artificial cultivation may be no long enough to result in the death of larvae, because of the high tolerance to extreme conditions in *C. gigas*. According to results of measured SR and AGR for two populations, we considered that the extreme conditions had a deep influence on the larval growth of both populations, but not enough for the survivability.

In our study, the linear effects of temperature, salinity, and rearing density on AGR of both two populations were significant (except temperature for wild population). In previous studies, the effects of temperature on the growth, survival, and physiological activity of marine bivalves were investigated in many species (Cáceres-Puig et al. 2007; Kang et al. 2008; Shin et al. 2009), and temperature was also considered to be the most important modifier of energy flow and organism growth. The growth rate of mollusk was proportional to temperature within certain range. However, the growth rate had a negative correlation with the increase of temperature when temperature exceeded the normal range. Cáceres-Puig et al. (2007) studied the effect of temperature on the growth and survival of *Crassostrea corteziensis* spat and found that the growth rate of shell height rised first then declined with a threshold temperature about 29 °C. Nevertheless, temperature was not the most important factor for growth in our study, and the effect of temperature on AGR was different between the black shell strain and wild population. According the analysis of the models, the effect of temperature on the larval growth was significant for the black shell strain ($P < 0.05$), but not for wild population ($P > 0.05$). When the rearing density was over 10 ind. ml^{-1}, the larval growth rates of the black shell strain were much higher at the temperature regime of 15–24 °C, but lower at the temperature regime of 29–33 °C. This suggests that the 7 generations of artificial selection of fast growth might weaken adaptability to high temperature environment.

Salinity is another important abiotic factor that can influence larval development. To date, many studies on the effects of salinity on the performance of mollusks have been conducted (Carregosa et al. 2014; Ji et al. 2015; Kim et al. 2001). Different species have different suitable salinity ranges for growth in an otherwise equivalent environment. In our study, the effect of salinity on AGR was significant, and the larvae grew best at salinity at ~ 29 psu. The similar result was found in previous study. Nell and Holliday (1988) revealed that the equivalent optimum salinity range for the growth of *C. gigas* larvae was 19 to 27 psu.

An important finding of this study is that rearing density is the most important factor as suggested by the variance analysis ($P < 0.0001$). There was a negative correlation between growth and rearing density, and similar result was found in previous studies. Liu et al. (2006) reported that larvae of the clam *Meretrix meretrix* reared at the highest density had the smallest mean size, and shell length of settled larvae decreased as density increased. Yan et al. (2006) reported that larval growth decreased significantly with increasing rearing density, and a

density of 5–10 ind. ml^{-1} appeared to be optimal for normal growth of Manila clam (*Ruditapes philippinarum*) larvae. In the present study, high rearing density led to a low AGR in both populations, and possible explanation was that the excretory products of larvae at high density can reduce water quality, especially in a limited rearing condition. Excretory products also be considered to increase with the rearing density increasing, and it mostly composed of nitrogenous compounds, largely ammonia which is usually identified as a toxic metabolite beyond a certain threshold. Due to the limited volume of containers, it was difficult to remove the effect of excretory product. Thus, rearing density had achieved a very significant effect on both two populations in our study.

According to the results of the stepwise regression and variance analysis for both two model equations, the quadratic effects of temperature, salinity, and rearing density on AGR for both two populations were highly significant ($P < 0.01$). The cubic effect of these three factors was significant in the wild population ($P < 0.05$), but was not significance in the black shell strain ($P > 0.05$). This indicated that temperature, salinity, and rearing density may act on wild population synergistically, but on the black shell strain independently.

In summary, the larval growth of black shell strain and wild population are influenced by temperature, salinity, and rearing density, and rearing density is the dominant factor for both the two populations. Within suitable temperature, salinity, and rearing density range, the larvae of the black shell strain have obvious growth advantages compared with those of the wild population, demonstrating that the black strain of *C. gigas* has great improvement in larval growth after seven generations of artificial selection.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

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