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# Combined effects of temperature, salinity and rearing density on growth and survival of juvenile ivory shell, *Babylonia areolata* (Link 1807) population in Thailand

**Wengang Lü[1,2], Minghui Shen[3], Jingqiang Fu[2], Weidong Li[3], Weiwei You[1,2] & Caihuan Ke[1,2]**

[1]State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen, China

[2]College of Ocean and Earth Sciences, Xiamen University, Xiamen, China

[3]Tropical Marine Products Fine Breed Center, Hainan Provincial Fisheries Research Institute, Hainan, China

**Correspondence**: C Ke, State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen, Fujian Province 361102, China. E-mail: chke@xmu.edu.cn

## Abstract

The ivory shell, *Babylonia areolata* (Link 1807), has been exploited as an important aquaculture organism along the southern China coast. In order to obtain optimal culture conditions for ivory shell juvenile, the central composite rotatable design was used to estimate the combined effects of temperature, salinity and rearing density on accumulated growth rate (*AGR*) and survival rate (*SR*). The results showed that the linear effects of temperature and rearing density on both growth and survival were highly significant ($P < 0.01$), but there was no significant effect on salinity ($P > 0.05$). The quadratic effects of temperature, salinity and rearing density influenced growth significantly ($P < 0.01$). The quadratic effects of temperature and salinity on survival of juvenile snail were significant ($P < 0.01$), the combined effects between the quadratic effect of temperature and the linear effect of rearing density influenced survival significantly ($P < 0.01$); the interactive effects of temperature, salinity and rearing density played a significant role in survival ($P < 0.01$). As can be seen from the above experimental results, the effects of temperature and salinity on growth and survival of *B. areolata* were strengthened with enhanced rearing density in a certain range and vice versa. By optimization using the response surface method, the optimal point was found at a temperature of 26.81°C, a salinity of 28.76 ppt and a rearing density of 527.07 ind $m^{-2}$. Under these conditions, the optimal *AGR* and *SR* were 36.84 mg $day^{-1}$ and 99.99%, respectively, with a satisfaction function value of 99.71%.

**Keywords:** *Babylonia areolata*, accumulated growth rate, survival rate, response surface method, optimization

## Introduction

*Babylonia areolata*, in the phylum Mollusca, class Gastropoda, subclass Prosobranchia, order Neogastropoda and family Buccinidae, inhabits the sandy subtidal zone at depths of 4–20 m in the summer and 40–60 m in the winter (Zheng, Ke, Zhou & Li 2005), and is a very important marine economic benthic organism. In the last decade, because of its fairly high economic value, this ivory shell is recommended as an excellent candidate species for aquaculture and has recently become more heavily cultured. Due to intensive cultivation, uncertain ecological conditions and vibrio diseases, further development of the aquaculture of this species has been delayed in some provinces such as Hainan and Fujian in China and Chiengmai in Thailand.

In order to culture *B. areolata* in additional locations in China and elsewhere, it is necessary to establish technical procedures to produce sufficient juveniles in a hatchery, and to investigate the effects of exogenous factors, especially temperature, salinity and rearing density, on growth and survival. However, the little information available on ivory snail is not always consistent with field observations. Research frequently focuses on culturing technique and seed breeding (Feng, Zhou & Li 2009). For practical considerations, it is very important to establish a system that provides the

snail with the most suitable environment for optimal development and growth.

The temperature, salinity and rearing density are important environmental factors that influence growth and survival of shellfish. Wang, Liu and Yang (2014), Wang, Zhu, Wang, Qiang, Xu and Li (2014) indicated that temperature and salinity were two important factors, not only because temperature and salinity were significant factors that influenced growth and survival of many aquatic organisms but also because the two factors can be controlled more easily than other environmental factors in the laboratory. Temperature and salinity influence organisms in various ways, such as food absorption and conversion ability (Hutchinson & Hawkins 1992; Navarro & Gonzalez 1998; Imsland, Foss, Gunnarsson, Berntssen, FitzGerald, Bonga, Von Ham, Naevdal & Stefansson 2001; Silva, Calazans, Soares, Soares & Peixoto 2010), biological energy balance (Bricelj & Shumway 1991; Gardner & Thompson 2001; Imsland *et al.* 2001) and immune response (Gagnaire, Frouin, Moreau, Thomas-Guyon & Renault 2006; Chen, Yang, Delaporte & Zhao 2007; Munari, Chinellato, Matozzo, Bressan & Marin 2010). Rearing density is widely recognized as a critical factor in intensive aquaculture because it may affect physiology and behaviour of reared animals (Li, Dong, Lei & Li 2007; Velasco & Barros 2008; Li & Li 2010). In oceans or industrial aquaculture operations, when temperature and salinity remain constant, the stocking rearing density can be the key factor that influenced the growth of shellfish. High rearing density reduced the growth rate of shellfish and increased the death rate by influencing self-metabolism (Velasco & Barros 2008). In contrast, a low rearing density was unfavourable for producing high economic benefits; therefore, an appropriate rearing density is the key to maximize economic benefits.

Many studies of environmental factors (temperature, salinity and rearing density) on development and growth of molluscs exist (Laing 2002; Christophersen & Strand 2003; Rupp & Parsons 2004; Verween, Vincx & Degraer 2007; Rico-Villa, Pouvreau & Robert 2009). However, in these studies the effects of environmental factors of interest were only examined singly, namely one factor was manipulated at a time. Little is known about the effects of combined environmental factors on growth and survival of juvenile ivory snail. Xue, Ke, Wang, Wei and Xu (2010) did study the combined effects of temperature and salinity on growth and survival in *B. areolata*, but only these two factors were examined.

The combined effects of temperature, salinity and rearing density on growth, survival and development of marine economic organisms have been studied for a few organisms, such as *Dicentrarchus labrax* (Conides & Glamuzina 2001) and *Apostichopus japonicus* (Li & Li 2010). However, there are no studies on the combined effects of temperature, salinity and rearing density on growth and survival of *B. areolata*. In the present study, central composite rotatable design (CCRD) and the response surface method (RSM) were used to investigate growth and survival of juveniles of *B. areolata* under different temperatures, salinities and rearing densities and to establish model equations for growth and survival in relation to these three factors. The objective of the present research was to examine the synergistic effects of temperature, salinity and rearing density, and to determine the optimal combination of the three factors by using the resultant model equations.

## Materials and methods

### Biological materials

The snails used for the experiment were $F_1$-generation juveniles of *B. areolata* reproduced by wild population in Thailand and cultivated by Xiamen University in Hainan province in China. The shell height and the weight were 16.38 ± 1.04 mm and 0.87 ± 0.24 g respectively (Table 1). The juveniles were delivered to the seed-breeding facility of Aquatic Products Research Institute in Hainan Province (Qionghai, China) to be bred. The pool for temporary breeding (10 m × 1 m × 1.2 m) was lined with a 30-mm thick layer of sand (with particle size of 1 ± 0.02 mm). The water in the pool consisted of running water with a flow rate of 10 $m^3$ $day^{-1}$, and with continuous aeration. The water temperature and salinity were 23.5 ± 1°C and 26.9 ± 1 ppt respectively. The pH for the seawater was 8.1 ± 0.5. After a temporary breeding period of 2 days, oyster was fed to the juveniles once a day in an amount of 20% of the weight of the total juveniles. The temporary breeding occurred over 10 days and then the experiment commenced.

### Measurement of accumulated growth rate and survival rate

Growth and survival of the different groups of juveniles were measured every 15 days. A random sample of 30 juveniles was weighed on an

  

**Table 1** Selected individual differences in experiment

| Traits | Experimental group (mean ± SD) | | | | |
|---|---|---|---|---|---|
| | 1500 (ind $m^{-2}$) | 1256 (ind $m^{-2}$) | 900 (ind $m^{-2}$) | 543 (ind $m^{-2}$) | 300 (ind $m^{-2}$) |
| Shell height (mm) | 16.35 ± 1.03 | 16.40 ± 1.04 | 16.43 ± 1.12 | 16.1 ± 0.92 | 16.53 ± 1.09 |
| Body weight (g) | 0.80 ± 0.16 | 0.85 ± 0.19 | 0.96 ± 0.32 | 0.89 ± 0.23 | 0.87 ± 0.26 |
| | **SS** | **d.f.** | **MS** | ***F*-value** | ***P*-value** |
| ANOVA | | | | | |
| Shell height | 2.02 | 4 | 0.51 | 0.46 | 0.77 |
| Body weight | 0.44 | 4 | 0.11 | 1.94 | 0.12 |

Significance test ($P > 0.05$).

electronic balance with a precision of 0.01 g. The accumulated growth rate (AGR) was the ratio of the difference of the measured weight and initial weight divided by the number of days. Survival rate (SR) was the ratio of the measured survival and the initial stocking amount. Juveniles coming out of the shell but still alive were recorded as the being dead. The entire experiment lasted for 60 days. The equation of *AGR* and *SR* were as follows:

$$\mathrm{SR}\ (\%) = \frac{\text{survival amount}}{\text{total amount}} \times 100 \mathrm{AGR}\ (\mathrm{mg/d}) = \frac{\mathrm{gL}_t - \mathrm{gL}_0}{t - t_0} \times 100$$

In the equation, $t_0$ and $t$ were the beginning time and ending time of the experiment respectively.

## Experimental procedures

The maximum and minimum temperature were 40°C and 15°C, respectively, and the maximum and minimum salinity were 45 ppt and 10 ppt, respectively, and the maximum and minimum rearing density were 1500 ind $m^{-2}$ and 300 ind $m^{-2}$ respectively. The high temperature group was regulated and controlled by using a hard plastic cask with a volume of 3 $m^3$, with a 500 W stainless steel heating bar, electronic relay and electric contact thermometer. The regulation range was 10–50°C, and the precision of temperature control was ±0.1°C. The low temperature was regulated and controlled by using a small low-temperature refrigerator (autoMAN) with a regulation range of 10–25°C and a precision of temperature control of ±0.1°C. Water salinity was manipulated by dilution of normal sea water (<30 ppt) with dechlorinated freshwater or by the addition of small quantities of sea salt when salinity of >35 ppt was required. A salinity refractometer (ATAGO) was used to monitor salinity, with a precision of ±0.1%. Energetic, healthy and complete individuals from the temporarily breeding population were placed into the experimental container in appropriate experimental densities (the experimental container was 1 m × 1 m × 0.75 m, the paving particle size in the container was 0.5 mm and the thickness of the fine white sand was 30 mm). Individuals without any obvious difference in shell height and weight were selected and placed into each group ($P < 0.05$, shown in Table 1). The amount of dissolved oxygen, pH and light were controlled at more than 5 mg $L^{-1}$, 7.9–8.1, and using natural light respectively. Snails were fed oyster once every day. The sand was changed every 10 days.

Sea water was pumped from a three-level sand filter through a cotton filter bag and was then discharged into a salinity pool after being filtrated. Pool water with the same salinity was then supplied to the barrels with differing temperature designations. The seawater was discharged into the experimental containers automatically when the temperature rose to meet the requirement for the experiment. During the experimental period, all water flow was unidirectional. The operation process is shown in Fig. 1.

## Experiment design and data analysis

Central composite rotatable design (shown in Table 2) was implemented, and the range of temperature and salinity were determined by reference to previous research and preliminary

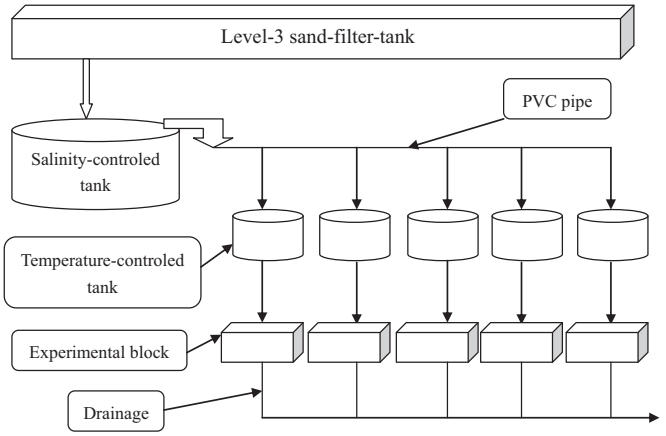


**Figure 1** Experimental operation process.

**Table 2** Central composite circumscribed design used in response surface method studies and experimental value

| | Cod | | | Actual | | | Experimental value | |
|---|---|---|---|---|---|---|---|---|
| Run | *T* | *S* | *D* | *T* (°C) | *S* (ppt) | *D* (ind $m^{-2}$) | *AGR* (mg $day^{-1}$) | *SR* (%) |
| 1 | 1 | −1 | 1 | 34.93 | 17.09 | 1256.76 | 3.34 ± 0.10 | 24.60 ± 1.78 |
| 2 | 0 | 0 | −α | 27.50 | 27.5 | 300 | 36.71 ± 3.21 | 99.40 ± 2.26 |
| 3 | −1 | 1 | 1 | 20.07 | 37.91 | 1256.76 | 2.03 ± 0.04 | 65.84 ± 2.12 |
| 4 | 0 | 0 | 0 | 27.5 | 27.5 | 900 | 32.77 ± 1.79 | 95.72 ± 3.45 |
| 5 | 0 | −α | 0 | 27.5 | 10 | 900 | 0.02 ± 0.00 | 0.00 ± 0.00 |
| 6 | α | 0 | 0 | 40 | 27.5 | 900 | 0.23 ± 0.00 | 0.00 ± 0.00 |
| 7 | 0 | −α | 0 | 27.5 | 10 | 900 | 0.04 ± 0.00 | 0.00 ± 0.00 |
| 8 | 0 | 0 | −α | 27.5 | 27.5 | 300 | 36.82 ± 2.63 | 99.70 ± 3.32 |
| 9 | α | 0 | 0 | 40.00 | 27.5 | 900 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 10 | 0 | 0 | α | 27.5 | 27.5 | 1500 | 11.73 ± 1.58 | 85.40 ± 4.27 |
| 11 | 0 | α | 0 | 27.5 | 45 | 900 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 12 | −1 | −1 | 1 | 20.07 | 17.09 | 1256.76 | 4.42 ± 0.93 | 46.24 ± 2.14 |
| 13 | 0 | α | 0 | 27.5 | 45 | 900 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 14 | 1 | −1 | −1 | 34.93 | 17.09 | 543.24 | 0.88 ± 0.05 | 42.6 ± 4.37 |
| 15 | 0 | 0 | 0 | 27.5 | 27.5 | 900 | 30.73 ± 3.18 | 96.71 ± 5.48 |
| 16 | −α | 0 | 0 | 15 | 27.5 | 900 | 0.42 ± 0.02 | 88.41 ± 4.79 |
| 17 | 1 | −1 | −1 | 34.93 | 17.09 | 543.24 | 9.98 ± 0.35 | 37.9 ± 2.41 |
| 18 | −1 | 1 | −1 | 20.07 | 37.91 | 543.24 | 27.12 ± 3.79 | 59.23 ± 4.17 |
| 19 | −α | 0 | 0 | 15 | 27.5 | 900 | 0.57 ± 0.07 | 85.72 ± 3.75 |
| 20 | 0 | 0 | α | 27.5 | 27.5 | 1500 | 13.55 ± 1.89 | 88.90 ± 3.32 |
| 21 | 0 | 0 | 0 | 27.5 | 27.5 | 900 | 30.20 ± 2.37 | 94.43 ± 9.28 |
| 22 | 0 | 0 | 0 | 27.5 | 27.5 | 900 | 31.40 ± 3.67 | 95.87 ± 4.84 |
| 23 | 1 | 1 | −1 | 34.93 | 37.91 | 543.24 | 1.25 ± 1.79 | 55.70 ± 2.45 |
| 24 | 1 | 1 | −1 | 34.93 | 37.91 | 543.24 | 2.06 ± 0.04 | 45.20 ± 1.94 |
| 25 | −1 | −1 | −1 | 20.07 | 17.09 | 543.24 | 22.22 ± 2.28 | 82.21 ± 3.38 |
| 26 | −1 | −1 | 1 | 20.07 | 17.09 | 1256.76 | 3.16 ± 1.02 | 44.63 ± 7.71 |
| 27 | −1 | 1 | 1 | 20.07 | 37.91 | 1256.76 | 1.65 ± 0.32 | 69.80 ± 2.49 |
| 28 | 1 | 1 | 1 | 34.93 | 37.91 | 1256.76 | 0.08 ± 0.00 | 7.50 ± 1.98 |
| 29 | −1 | 1 | −1 | 20.07 | 37.91 | 543.24 | 25.46 ± 1.06 | 62.42 ± 4.67 |
| 30 | −1 | −1 | −1 | 20.07 | 17.09 | 543.24 | 25.61 ± 3.27 | 84.80 ± 5.91 |
| 31 | 1 | 1 | 1 | 34.93 | 37.91 | 1256.76 | 0.79 ± 0.48 | 27.98 ± 1.26 |
| 32 | 1 | −1 | 1 | 34.93 | 17.09 | 1256.76 | 0.12 ± 0.07 | 20.35 ± 2.33 |
| 33 | 0 | 0 | 0 | 27.5 | 27.5 | 900 | 33.92 ± 7.18 | 98.80 ± 5.17 |
| 34 | 0 | 0 | 0 | 27.5 | 27.5 | 900 | 33.33 ± 2.45 | 98.11 ± 6.91 |

*T*, *S* and *D* represented the temperature, salinity and density respectively; *AGR* and *SR* represented the accumulated growth rate and survival rate respectively; |α| was asterisk arm.

  

experimentation. The rearing density factor was determined based on previous research and production practical experience, without any preliminary experimentation. The selected temperature ranged from 15 to 40°C, the salinity ranged from 10 to 45 ppt and the rearing density ranged from 300 to 1500 ind $m^{-2}$. The design contained three experimental factors, six axis points and six centre 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|. Thirty-four experimental points in total were designed for analysis. The experiment was set to be replicated once, so 68 group points in total were designated to be tested. The average and standard deviation of the two replications were to be calculated.

Analysis was performed using SPSS (V.16) software (SPSS, Chicago, IL, USA). A stepwise regression method (Backward stepwise, introducing level was 0.05; eliminating level was 0.1) was employed to create a response surface model with temperature, salinity and rearing density as independent variables and AGR and SR as dependent variables. The model general formula was:

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

where $Y$ was the response (*AGR*, *SR* or their transformations); $\beta_0$ was the intercept of regression equation; $\beta_i$, $\beta_j$, $\beta_l \cdots \beta_{ijl}$ were linear, quadratic and cubic and interactive effects of temperature, salinity and rearing density on *AGR* and *SR*; $\chi_i$, $\chi_j$ and $\chi_l$ 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.

A variance analysis was used to confirm the regression equation model and the significance of each experimental factor. The coefficient of determination ($R^2$), adjusted coefficient (Adj-$R^2$) and predicted coefficient (Pred-$R^2$) were used to assess the degree of fit of the model. Fisher's $F$-test was used to test for statistical significance. The three-dimensional response surface diagram and the corresponding contour map were used to analyse the effects of temperature, salinity and rearing density on growth and survival of the juvenile.

## Results

### Modelling and significance test

The experimental data for growth and SR are provided in Table 2. Results of statistical analysis are presented in Tables 3 and 4. The regression equations were determined to be as follows:

$$Y_{AGR} = -141.4974 + 8.9944T - 0.0352D + 5.8763S + 1.8668TD - 8.8409TS - 6.1283DS - 0.1981T^2 - 18270D^2 - 0.1020S^2$$

$$Y_{SR} = 357.2485 - 36.6528T - 0.4174D + 9.1223S + 0.0211TD + 0.2329TS + 7.7131DS + 1.1398T^2 - 0.3019S^2 - 2.3306TSD - 2.7642T^2D - 0.0146T^3$$

where $T$, $S$ and $D$ represented the temperature, salinity and density respectively.

Coefficient estimates, including significance and 95% confidence intervals, of the model equations generated for growth and survival are presented

**Table 3** Regression coefficients, standard errors and 95% confidence intervals for the predicted model of accumulated growth rate

| | | | | 95% CI | |
|---|---|---|---|---|---|
| **Term** | **Coefficient** | **d.f.** | **SE** | **Low** | **High** |
| Intercept | 31.95 | 1 | 1.47 | 28.91 | 34.99 |
| *T* | −3.45 | 1 | 0.69 | −4.88 | −2.03 |
| *D* | −6.59 | 1 | 0.69 | −8.02 | −5.17 |
| *S* | −0.35 | 1 | 0.69 | −1.77 | −1.08 |
| *TD* | 4.95 | 1 | 0.90 | 3.09 | 6.81 |
| *TS* | −0.68 | 1 | 0.90 | −2.55 | 1.18 |
| *DS* | −0.23 | 1 | 0.90 | −2.09 | 1.64 |
| $T^2$ | −10.94 | 1 | 0.76 | −12.51 | −9.37 |
| $D^2$ | −2.33 | 1 | 0.76 | −3.90 | −0.75 |
| $S^2$ | −11.05 | 1 | 0.76 | −12.62 | −9.48 |

*T*, *S* and *D* represented the temperature, salinity and density respectively; the values in the table were all coded values, and the coefficient was estimated according to the coded value, the final equation obtained by the actual value was as follows:

$$Y_{AGR} = -141.4974 + 8.9944T - 0.0352D + 5.8763S + 1.8668TD - 8.8409TS - 6.1283DS - 0.1981T^2 - 18270D^2 - 0.1020S^2$$

**Table 4** Regression coefficients, standard errors and 95% confidence intervals (CI) for the predicted model of survival rate

| | | | | 95% CI | |
|---|---|---|---|---|---|
| **Term** | **Coefficient** | **d.f.** | **SE** | **Low** | **High** |
| Intercept | 96.70 | 1 | 1.42 | 93.76 | 99.63 |
| *T* | 8.76 | 1 | 1.94 | 4.73 | 12.92 |
| *D* | −3.69 | 1 | 1.38 | −6.54 | −0.83 |
| *S* | −1.01 | 1 | 0.89 | −2.13 | −0.85 |
| *TD* | −1.36 | 1 | 1.16 | −3.76 | 1.05 |
| *TS* | 1.79 | 1 | 1.16 | −0.61 | 4.19 |
| *DS* | 4.84 | 1 | 1.16 | 2.44 | 7.24 |
| $T^2$ | −17.39 | 1 | 0.93 | −19.32 | −15.45 |
| $S^2$ | −32.69 | 1 | 0.93 | −34.62 | −30.75 |
| *TSD* | −6.43 | 1 | 1.16 | −8.83 | −4.03 |
| $T^2D$ | −5.45 | 1 | 1.80 | −9.18 | −1.72 |
| $T^3$ | −6.00 | 1 | 0.98 | −8.04 | −3.96 |

*T*, *S* and *D* represented the temperature, salinity and density respectively; the values in the table were all coded values, and the coefficient was estimated according to the coded value, the final equation obtained by the actual value was as follows:

$$Y_{SR} = 357.2485 - 36.6528T - 0.4174D + 9.1223S + 0.0211TD + 0.2329TS + 7.7131DS + 1.1398T^2 - 0.3019S^2 - 2.3306TSD - 2.7642T^2D - 0.0146T^3$$

in Tables 3–6. Model equations for both growth and survival adequately represented the experimental data ($P < 0.0001$). The linear and quadratic effects of temperature and rearing density, together with the quadratic effect of salinity and the interactive effect of temperature and rearing density, highly significantly contributed to the variation in growth data ($P < 0.0001$). The linear effect of salinity, the interactive effect of temperature and salinity, and the interactive effects between salinity and rearing density were not significant ($P > 0.05$).

The linear, quadratic and cubic effects of temperature as well as the linear effect of rearing density and the quadratic effect of salinity on SR statistically differed from zero ($P < 0.01$). The linear effect of salinity on the SR was not significant ($P > 0.05$). The interaction between rearing density and salinity was highly significant ($P < 0.01$), but the interactive effects of temperature and salinity, and of temperature and rearing density were not significant ($P > 0.05$). The interaction between the quadratic effect of temperature and the linear effect of rearing density was highly significant ($P < 0.01$). The interaction between the three factors of temperature, salinity and rearing density was significant ($P < 0.01$).

The test for lack-of-fit of the two models was significant ($P < 0.0001$). However, the square of the lack-of-fit and pure error of the model equation were not significant ($P > 0.05$). In addition, other conditions and factors as well as their interaction also had a slight influence. The coefficients of determination ($R^2$) of the model for growth and survival were 0.9527 and 0.9890 respectively. Adjusted coefficient (Adj-$R^2$) and predictive coefficient (Pred-$R^2$) were 0.9350 and 0.8986, respectively, for the growth model, and were 0.9836 and 0.9686 for the survival model, respectively, indicating that only a tiny portion of total variation could not be reflected accurately in the model.

### Influence of temperature, salinity and rearing density on the accumulated growth rate

The factors that influenced growth significantly were analysed by stepwise regression, which determined the growth model. A surface analysis was used to analyse the combined effects of temperature, salinity and rearing density (Figs 2–4).

As shown in Fig. 2a, the response surface plot was an obvious oval, which indicated that there was a very strong interaction between temperature and density within a certain range. When the temperature was 21.5–27.5°C and the rearing density was 300–780 ind $m^{-2}$, the AGR was 32.25–39.90 mg $day^{-1}$. When rearing density was 300–1500 ind $m^{-2}$, growth increased gradually with an increase in temperature. However, when temperature exceeded 27.5°C, growth tended to decline. Growth stopped at the highest temperature. When the temperature was 15–40°C, growth declined gradually from lower to higher rearing density. There was a gentle slope without a peak value for the response surface, indicating that when rearing density was within a certain range, temperature was the important factor influencing growth.

Figure 3a shows the effects of temperature and salinity on growth of juveniles. When temperature was 22.5–32.5°C and salinity was 24.5–32.5 ppt, AGR was ~30 mg $day^{-1}$, and the maximum growth rate was as much as 32.5 mg $day^{-1}$. Accumulated growth rate varied with temperature and salinity in a curvilinear fashion.

  

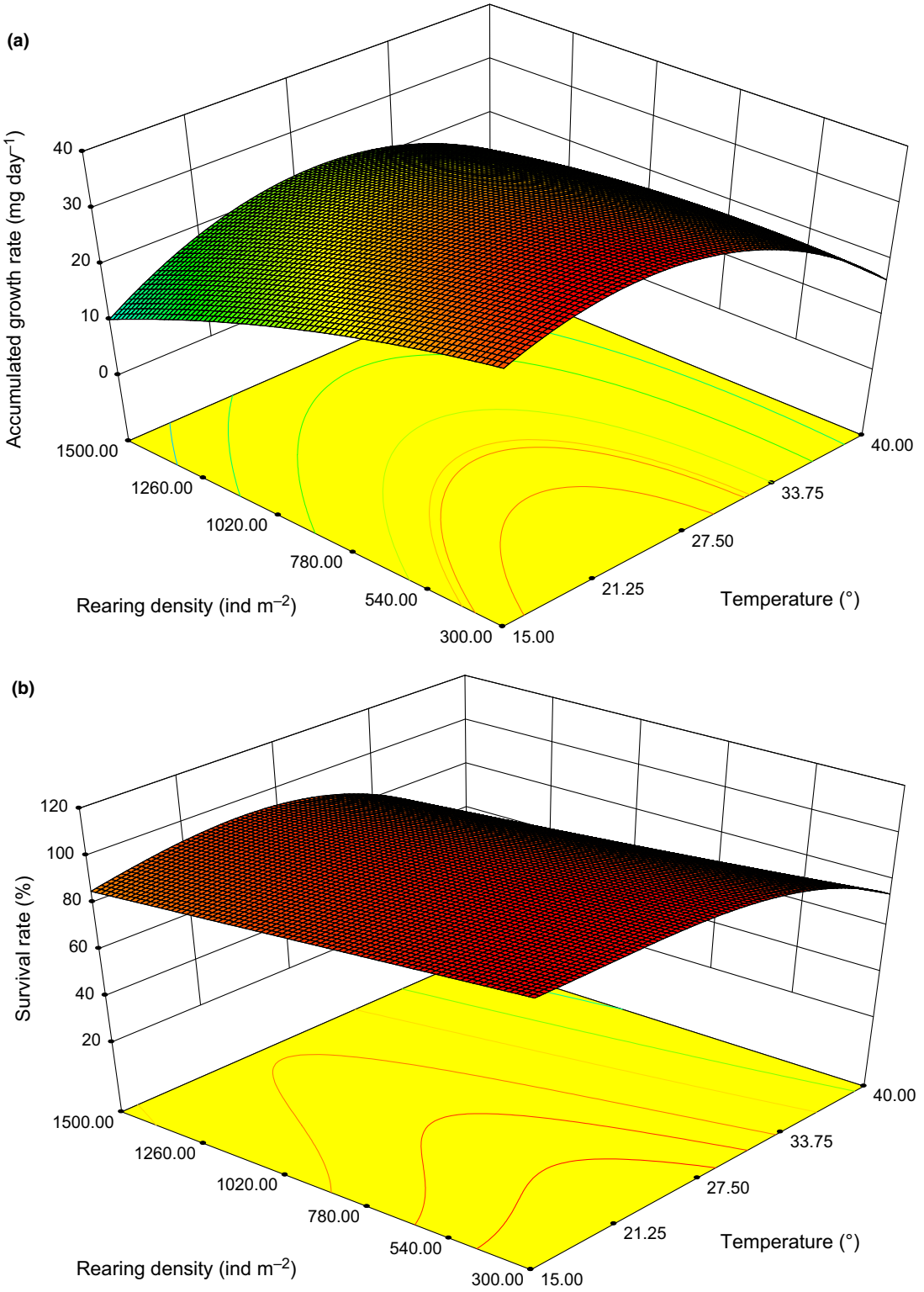


**Figure 2** Response surface plot of effects of rearing density and temperature on the accumulated growth rate (a) and survival rate (b) in *Babylonia areolata* (Link 1807) (salinity = 27.5 ppt).

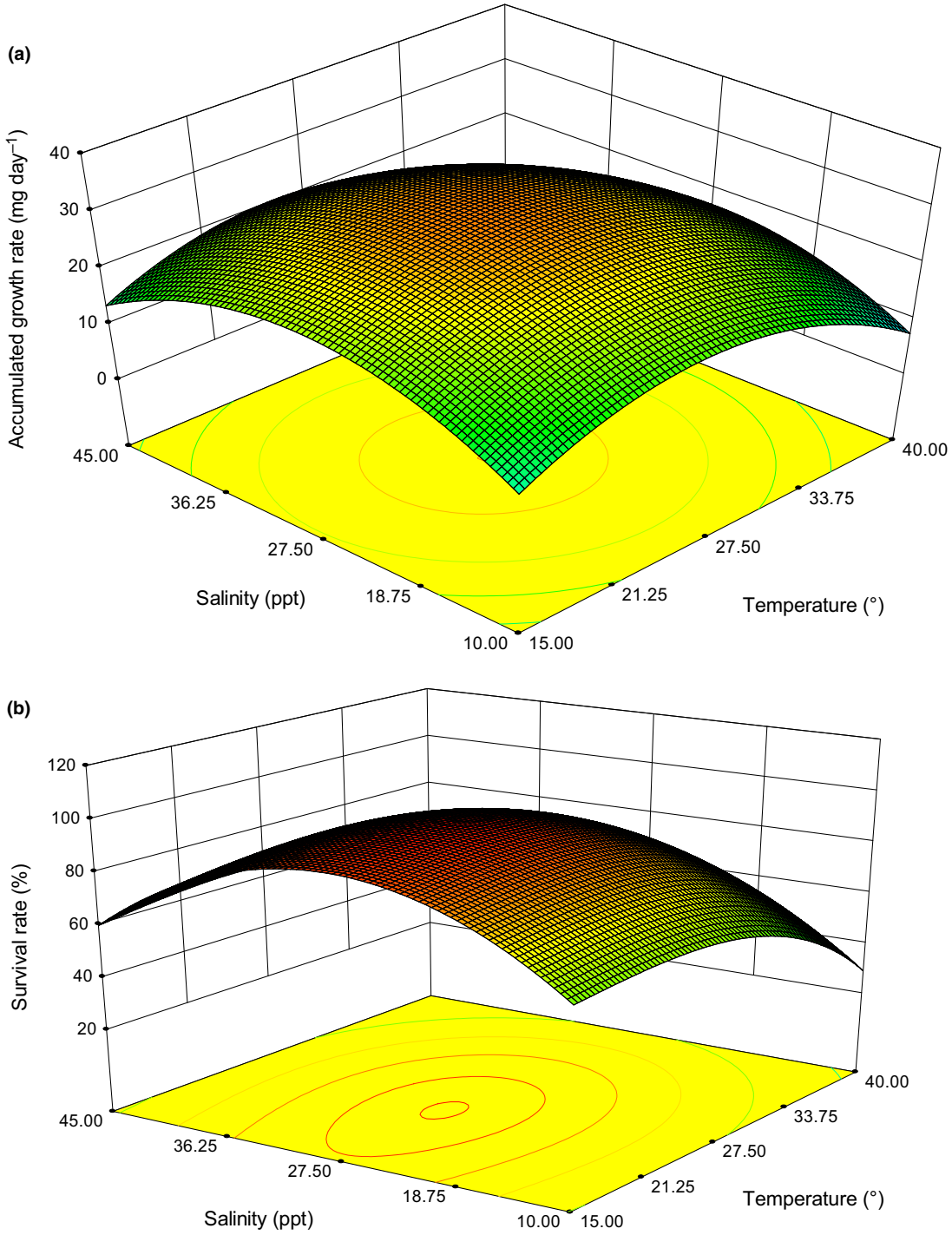


**Figure 3** Response surface plot of effects of salinity and temperature on the accumulated growth rate (a) and survival rate (b) in *Babylonia areolata* (Link 1807) (rearing density = 900 ind $m^{-2}$).

Under high salinities and high densities, growth of juveniles was very low, but at high salinities and low densities, growth was higher than under low salinities and low densities (Fig. 4a). The maximum value of AGR, 36.80 mg $day^{-1}$, occurred when salinity was 26.5–32 ppt and rearing density was 300–750 ind $m^{-2}$.

  

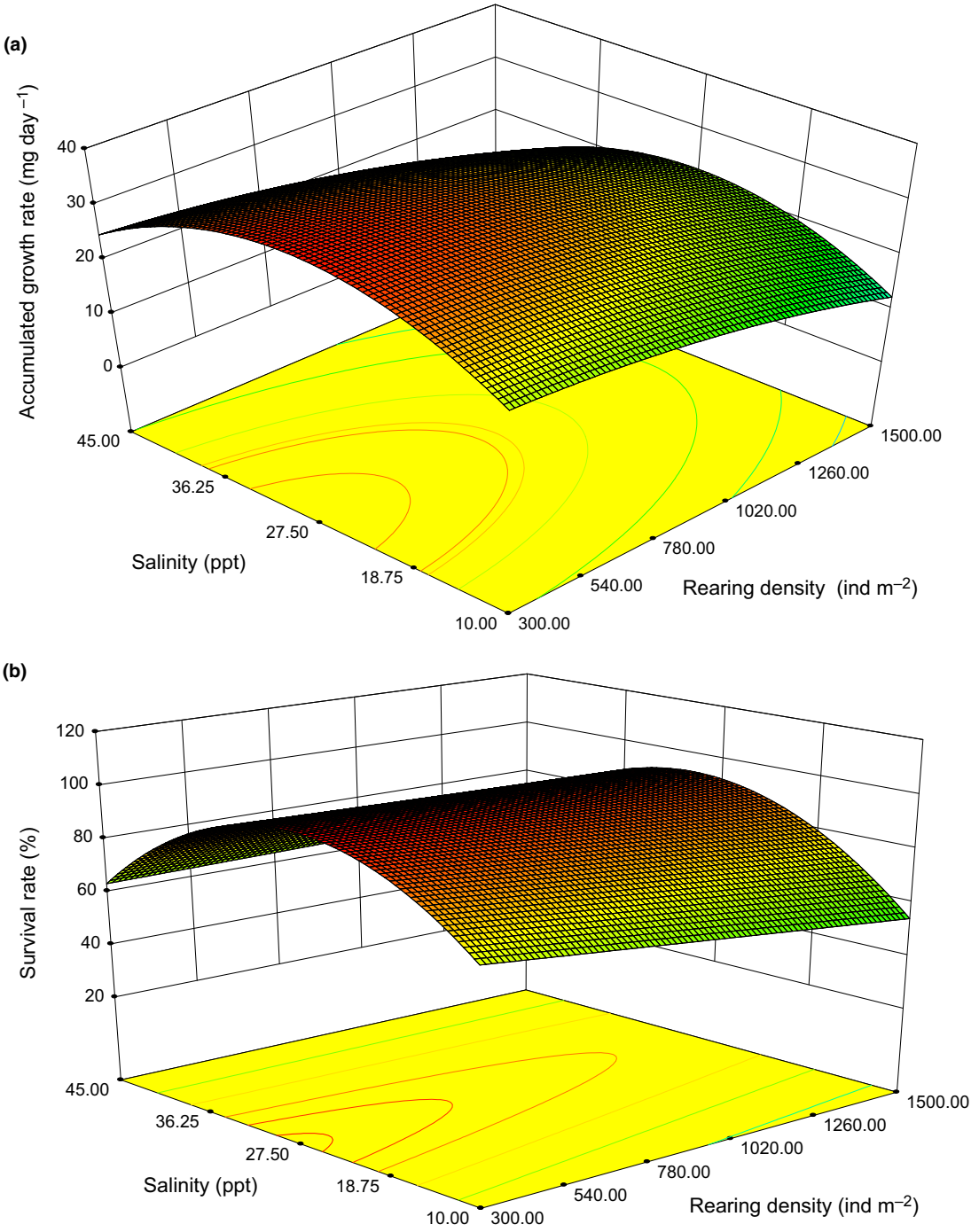


**Figure 4** Response surface plot of effects of salinity and rearing density on the accumulated growth rate (a) and survival rate (b) in *Babylonia areolata* (Link 1807) (temperature = 27.5°C).

### Influence of temperature, salinity and rearing density on survival rate

Graphical representations of response surface are shown in Figs 2–4b to illustrate the effects of temperature, salinity and rearing density on survival of juveniles.

The combined effects of temperature and rearing density on survival are shown in Fig. 2b. The plot had a ridged shape, and the ridge was found when

temperature was ~27.5°C, and the rearing density was 300–1000 ind $m^{-2}$, with the highest value being up to 99.6% or even 100%. When the temperature was 15–30°C and rearing density was 300–1500 ind $m^{-2}$, the shape of the survival surface was approximately planar, indicating *B. areolata* with different rearing densities could survive in this temperature range. However, when the temperature exceeded 30°C, no matter the rearing density, SR declined, indicating that temperature played a more important role on survival than rearing density.

Figure 3b illustrates the effects of salinity and temperature on SR. The plot was semi-circular, indicating that there was no interaction in the integrated effects of temperature and salinity on survival. For salinity ranges from 25 to 30 ppt, and temperature ranges from 24.5 to 29.5°C, the highest survival point reached 97.92%.

In Fig. 4b, the response surface plot was an oval, which indicated that the effect of the density and salinity on survival was obvious. In addition, there were interactive effects. When temperature was 25–30°C, and rearing density was 300–800 ind $m^{-2}$, SR was ~97.17%, and the maximum SR could be up to 99.99%. When the rearing density was in a certain range and the salinity extended from the lower point to the higher point, there was a peak value and the peak value was 25–30 ppt. However, when the salinity remained in a certain range, and rearing density increased gradually from the lower point to the higher point; the plot had as a gentle slope with no peak value. The change in SR was small, indicating that the effects of rearing density on survival varied with salinity.

### Optimization

According to the growth and survival models, the two factor conditions (where the central composite of one variable remained constant and the other two variables were optimized) and three factor conditions were optimized. The optimized results are found in Table 7.

The optimization theory of Montgomery (2005) was used to optimize experimental conditions, growth and survival models were simultaneously optimized. For the combination of a temperature of 26.89°C, a salinity of 28.27 ppt and a rearing density of 605.9 ind $m^{-2}$, the maximum value of the AGR was 37.21 mg $day^{-1}$ and the desirability function value was 98.43%. When the temperature, salinity and rearing density were 26.32°C, 28.14 ppt and 624.04 ind $m^{-2}$, respectively, the SR was to 99.79%, with a desirability function value of 99.20%. By optimizing the RSM, the optimal point was found at a temperature of 26.81°C, a salinity of 28.76 ppt and a rearing density of 527.07 ind $m^{-2}$. Under these conditions, the optimal AGR and survival were 36.84 mg $day^{-1}$ and 99.99%, respectively, with a desirability value of 99.71%.

## Discussion

### The linear effects of temperature, salinity and rearing density

From this study, it is clear that the linear and quadratic effects and even the cubic effect (for SR) of the temperature were significant, which indicated that temperature was the most important factor for growth and survival of juveniles (Tables 5 and 6). Meanwhile, the analysis of the models demonstrated that temperature, salinity and rearing density all in some extent affect the growth and survival of juveniles. Our experiment indicated that growth rate of juveniles was proportional to temperature within certain range. However, when temperature was more than some threshold, the AGR had an obvious negative correlation with temperature. These results are consistent with conclusions from another study on the

**Table 5** Analysis of variance table for the quadratic model of the response, accumulated growth rate

| Source | SS | d.f. | MS | *F*-value | *P*-value |
|---|---|---|---|---|---|
| Model | 6313.61 | 9 | 701.51 | 53.71 | <0.0001 |
| *T* | 325.83 | 1 | 325.83 | 24.95 | <0.0001 |
| *D* | 1187.01 | 1 | 1187.01 | 90.89 | <0.0001 |
| *S* | 3.26 | 1 | 3.26 | 0.25 | 0.6217 |
| *TD* | 392.04 | 1 | 392.04 | 30.02 | <0.0001 |
| *TS* | 7.48 | 1 | 7.48 | 0.57 | 0.4565 |
| *DS* | 0.83 | 1 | 0.83 | 0.063 | 0.8033 |
| $T^2$ | 2700.51 | 1 | 2700.51 | 206.78 | <0.0001 |
| $D^2$ | 121.92 | 1 | 121.92 | 9.34 | 0.0054 |
| $S^2$ | 2751.35 | 1 | 2751.35 | 210.67 | <0.0001 |
| Residual | 313.44 | 24 | 13.06 | | |
| Lack-of-fit | 245.50 | 5 | 49.10 | 13.73 | <0.0001 |
| Pure error | 67.94 | 19 | 3.58 | | |
| Total | 6627.06 | 33 | | | |

*T*, *S* and *D* represented the temperature, salinity and density respectively; $R^2 = 0.9527$, Adj-$R^2 = 0.9350$, Pred-$R^2 = 0.8986$.

  

**Table 6** Analysis of variance table for the quadratic model of the response, survival rate

| Source | SS | d.f. | MS | *F*-value | *P*-value |
|---|---|---|---|---|---|
| Model | 42 575.19 | 11 | 3870.47 | 180.38 | <0.0001 |
| *T* | 435.87 | 1 | 435.87 | 20.31 | 0.0002 |
| *D* | 153.76 | 1 | 1573.76 | 7.17 | 0.0138 |
| *S* | 27.71 | 1 | 27.71 | 1.29 | 0.2680 |
| *TD* | 29.40 | 1 | 29.40 | 1.37 | 0.2543 |
| *TS* | 51.30 | 1 | 51.30 | 2.39 | 0.1363 |
| *DS* | 374.91 | 1 | 374.91 | 17.47 | 0.0004 |
| $T^2$ | 7468.19 | 1 | 7468.19 | 348.06 | <0.0001 |
| $S^2$ | 26 394.03 | 1 | 26 394.03 | 1230.10 | <0.0001 |
| *TSD* | 661.65 | 1 | 661.65 | 30.84 | <0.0001 |
| $T^2D$ | 196.69 | 1 | 196.69 | 9.17 | 0.0062 |
| $T^3$ | 797.85 | 1 | 797.85 | 37.18 | <0.0001 |
| Residual | 472.05 | 24 | 21.46 | | |
| Lack-of-fit | 352.24 | 5 | 117.41 | 18.62 | <0.0001 |
| Pure error | 119.81 | 19 | 6.31 | | |
| Total | 43 047.24 | 33 | | | |

*T*, *S* and *D* represented the temperature, salinity and density respectively; $R^2 = 0.9890$, Adj-$R^2 = 0.9836$, Pred-$R^2 = 0.9686$.

effects of temperature and salinity on growth and survival of *B. areolata* (Xue *et al.* 2010). Similar results have been found in other studies of ontogenesis, growth and survival of other molluscs, including *Pecten maximus* (Chauvaud, Thouzeau & Paulet 1998; Laing 2000) and *Ruditapes philippinarum* (Munari, Matozzo & Marin 2011). Temperature had a significant impact on growth and survival of *B. areolata* in a curvilinear fashion, especially for the effect of high temperature on survival (Tables 3 and 4). Some researchers posited high temperature would cause larvae to consume more energy and decline in resistance to infection, resulting in a mass propagation of pathogenic bacteria. Le, Renault and Gérard (1996) studied the transmission of infection of viruses in the body of oyster larvae, and found that 80–90% of oyster larvae died when temperature was 25–26°C instead of 22–23°C, and that an increase in environmental temperature would cause the viral infection to become dominant. Some studies have reported that high temperature led to a decline of the immune capability of shellfish. Hegaret, Wikfors, Soudant, Delaporte, Alix, Smith, Dixon, Quere, Le Coz, Paillard, Moal and Samain (2004) found that an increase in temperature caused ecphysesis and a decline in the phagocytic capacity and polymerization ability of the haemocyte, resulting in a loss of immune capability in *Crassostrea virginica*. Chen *et al.* (2007) reported that *Chlamys farreri* had different immunological reactions to widely different temperatures. When temperature was 28°C, number of cells after 72 h with phagocytic function in the haemolymph in the body of the shellfish declined, and activity of acid phosphatase was reduced. No change occurred at 11°C, indicating that the immune

**Table 7** Model optimized the best combination of factors for two response

| | | | | Optimal point | | | 95% CI | | |
|---|---|---|---|---|---|---|---|---|---|
| Optimal type | Factor number | Response | Optimal value | *T* | *S* | *D* | Low | High | Desirability |
| Single optimization | 2 | AGR | 29.23 | 26.37 | 27.39 | 900 | 27.21 | 35.24 | 99.12 |
| | | SR | 97.92 | 25.29 | 27.56 | 900 | 94.68 | 100.63 | 93.60 |
| | | AGR | 30.80 | 27.20 | 27.5 | 637.90 | 32.35 | 38.57 | 98.03 |
| | | SR | 99.90 | 27.5 | 27.5 | 300 | 97.23 | 108.53 | 100 |
| | | AGR | 36.28 | 27.5 | 29.21 | 441.61 | 33.29 | 39.27 | 99.35 |
| | | SR | 99.90 | 27.5 | 27.5 | 300 | 94.99 | 104.40 | 100 |
| | 3 | AGR | 37.21 | 26.89 | 28.27 | 605.90 | 34.67 | 40.41 | 98.43 |
| | | SR | 99.79 | 26.32 | 28.14 | 624.04 | 95.96 | 104.90 | 99.20 |
| Simultaneous optimization | 2 | AGR | 30.21 | 26.03 | 27.50 | 900 | 29.19 | 35.22 | 95 |
| | | SR | 97.77 | | | | 94.79 | 100.78 | |
| | | AGR | 38.98 | 26.88 | 27.5 | 415.30 | 35.65 | 42.30 | 100 |
| | | SR | 99.80 | | | | 98.69 | 108.09 | |
| | | AGR | 36.63 | 27.5 | 27.49 | 394.55 | 33.37 | 39.88 | 99.8 |
| | | SR | 99.92 | | | | 96.92 | 106.90 | |
| | 3 | AGR | 36.84 | 26.81 | 28.76 | 527.07 | 33.91 | 39.71 | 99.71 |
| | | SR | 99.99 | | | | 96.47 | 105.24 | |

*T*, *S* and *D* represented the temperature, salinity and density respectively; *AGR* and *SR* represented the accumulated growth rate and survival rate respectively; Units in the table: *AGR* (mg $day^{-1}$), *SR* (%), *T* (°C), *S* (ppt), *D* (ind $m^{-2}$), Desirability (%).

capability of *C. farreri* declined at high temperature, while the shellfish displayed low temperature resistance.

Salinity is usually considered a 'masking factor' in aquaculture (Claireaux & Lagardere 1999; Conides & Glamuzina 2001), and it affects growth and survival of marine spat. Numerous studies have evaluated the effects of salinity on the performance of univalve spat (Cheung & Lam 1995; Nielsen & Gosselin 2011; Montory, Chaparro, Pechenik, Diederich & Cubillos 2014; Zhang, Cheung & Shin 2014). Different molluscs have different suitable salinity ranges for growth and survival in an otherwise equivalent environment. Best spat growth and maximum survival of *Saccostrea glomerata* were found at a salinity of 35 ppt and a temperature of 30°C, and a salinity of 30 ppt and a temperature of 23°C respectively (Dove & O'Connor 2007). Irrespective of temperature, high SR of juveniles of the pearl oyster, *Pinctada imbricate*, was found at salinities of 32 and 35 ppt (O'Connor & Lawler 2004). Condition index was not affected by salinity of 26–30 ppt at any of the temperatures tested in *P. maximus* (Ian Laing 2002). However, molluscs of the same species found in different populations can become acclimatized to different saline environments. The Little Point and Lowe's Cove are centres of oyster culture in the Damariscotta River estuary in Maine. Salinity was slightly higher at Lowe's Cove (32 ± 2 ppt) than at Little Point (30 ± 3 ppt), but the cumulative mortality of *Ostrea edulis* was greater (45.8%) at Little Point (where the salinity range was wider) than at Lowe's Cove (26.7%, where the salinity range was narrower) (Carnegie & Barber 2001). In our experiment, growth and survival of *B. areolata* juveniles increased with increased salinity when salinity ranged from 15 to 32 ppt and temperature was within a suitable range. Maximum growth and survival for *B. areolata* was obtained at salinities of 24–31 ppt; in contrast, optimal salinity for growth and survival of *B. areolata* (population in Hainan) was 26–30 ppt (Xue *et al.* 2010). Natural habitats of *B. areolata* (population in Thailand) may occasionally be subjected to lower salinity conditions due to increased freshwater input from the Mae Ping River and from land runoff following heavy rainfall. However, *B. areolata* is mainly distributed near Hainan Island in the South China sea and the salinity range is narrower than Chiengmai in Thailand South sea (Zhao, Liu & Fu 2012).

The mechanism for the effect of salinity is not entirely clear. There are two ways for animals to endure changing saline concentrations in external environments (Péqueux, Vallota & Gilles 1979; Péqueux, Bianchini & Gilles 1996). Animals can be osmoconformers. However, osmoconformers do not adjust osmotic pressure well, since the osmotic pressure of the bodily fluid is similar to that of the external environment (Péqueux *et al.* 1996). When ambient salinity increases, the weight of the animals will decrease due to dehydration; when the salinity decreases, their weight will increase due to osmosis. Lange (1970) demonstrated that isosmotic intracellular regulation was incomplete in the scallop *Pecten septemradiatus* Mueller, leading to an increase in volume of muscle tissue with a decrease in sea water salinity. In our study, growth and survival of juvenile *B. areolata* dramatically decreased with salinity when salinity was below ~20 ppt, and both temperature and rearing density were within a suitable range. However, juveniles can survive for a long time in a higher saline environment (~32–38 ppt). Carregosa, Figueira, Gil, Pereira, Pinto, Soares and Freitas (2014) found that *Venerupis philippinarum* had high mortality at lower salinities (0 and 7ppt), but tolerated high salinities (35 and 42 ppt). A decline in growth and survival at low salinities has been found in other molluscs including *Argopecten prupruatus* (Navarro & Gonzalez 1998), *Venerupis philippinarum* (Carregosa *et al.* 2014) and *P. maximus* (Ian Laing 2002). As most marine invertebrates have a changing osmotic pressure, gastropods have a slightly lower osmotic pressure in bodily fluids than that of the seawater (Robertson 1964). Some gastropods therefore can adjust to living in higher saline conditions. Osmoregulators, such as *Pleuronectiformes*, *Mugil*, *Gobius*, *Oryzias* and other hard-bone fish, are able to live varied salinities, while maintaining a constant bodily fluid concentration.

Rearing density factor, a husbandry parameter, plays an important role in ivory snail aquaculture. Rearing density had significant impacts on growth and survival of *B. areolata* in a curvilinear (nearly linear) fashion (Tables 5 and 6, $P < 0.01$). Growth and survival was relatively stable before reaching the optimal rearing density (~600 ind $m^{-2}$), but gradually declined with increasing rearing density when temperature and salinity were within a suitable range. The minimum values for growth and survival of juveniles were found at a rearing

density of 1500 ind $m^{-2}$ (maximum value in this experimental design). However, Chaitanawisuti and Kritsanapuntu (1998) reported that growth, in both shell length and body weight, and survival of juvenile *B. areolata* were not affected by stocking rearing density using component experimental design (50, 100, 150 and 200 ind $m^{-2}$). The differing results can be explained in two ways. First, there are differences between stocks and cultivation sites. Next, the range of rearing densities was very narrow in the experiment of Chaitanawisuti and Kritsanapuntu (1998). MacDonald (1988) reported that high larval densities led to decreases in ingestion rates, oxygen consumption and growth efficiency. Conides and Glamuzina (2001) found that overcrowding of hatched *Dicentrarchus labrax* larvae might cause a rapid decrease in available dissolved oxygen and subsequent increased larval mortality. Growth and survival larval *Apostichopus japonicus* were limited by higher rearing density in laboratory and field investigations (Li & Li 2010). The negative effects of high rearing density on growth and survival of economic aquaculture animals suggests there is rearing density-dependent intraspecific competition for space and food (Parsons & Dadswell 1992; Foster & Stiven 1996; Huchette, Koh & Day 2003; Yan, Zhang & Yang 2006; Raghavan & Gopinathan 2008; Velasco & Barros 2008). Water quality is important for growth and survival of *B. areolata*. Reduced growth and survival at higher rearing densities may be attributable to a deterioration of water quality (De Blok1972; Kinne1976; Raghavan & Gopinathan 2008). *B. areolata* is an opportunistic scavenger, and dead fresh or decomposed organisms can serve as food. Therefore, excretory products are mostly composed of nitrogenous compounds, largely ammonia, which is the major component of the protein catabolism (De Blok 1972; Colt & Armstrong 1981). Ammonia is usually identified as a toxic metabolite beyond a certain threshold, and increases with increased rearing density. Thus, higher rearing densities are adverse for *B. areolata* aquaculture. The phenomena of residual feeds, increased pathogenic bacteria and excessive energy expenditure for cultured marine animals may occur in conditions of high rearing density (Loosanoff & Davis 1963; Mgaya & Mercer 1995; Capinpin, Toledo, Encena & Doi 1999; Liu, Dong, Tang, Zhang & Xiang 2006; Liu, Gurney-Smith, Beerens & Pearce 2010).

### The quadratic effects of temperature, salinity and rearing density

The model equation for AGR established by CCRD and stepwise regression was used to perform a variance analysis of the various coefficients, indicating that the quadratic effects of the temperature, salinity and rearing density were significant (shown in Table 5, $P < 0.01$). There was, thus, a peak value that existed for the effects of these three factors on the AGR. The quadratic effects of temperature and salinity on the survival of juveniles were highly significant except for the effect of rearing density. The cubic effect of density and the interactive effect between the three factors (temperature, salinity and rearing density) were significant. This means that rearing density, salinity and temperature act on survival synergistically. Temperature and salinity may modify the effect of rearing density and change the suitable rearing density range for metabolism, energy budgets and oxygen expenditure of *B. areolata*. Likewise, rearing density can modify the effects of temperature and salinity (Lough & Gonor 1973).

### The synergistic effect of temperature, salinity and rearing density

The model of AGR and the response surface obtained using the equation indicated that when rearing density level was in the experimental range (300–900 ind $m^{-2}$), temperature and salinity affected the outcome of the experiment, and there was no obvious interaction between them in the experimental range. Xue *et al.* (2010) found that there was an interaction between temperature and salinity on growth of *B. areolata* under range of temperature from 26 to 30°C and salinity range from 26 to 30 ppt. Reasons for the contrasting results were follows: (1) This research studies the effects of temperature and salinity on the growth and survival of juvenile babylon under different rearing density. However, in the research of XUE, it studies the effects of temperature and salinity on the growth and survival of juvenile spotted babylon under the same rearing density (400 ind $m^{-2}$). (2) As to the shell length and weight, the juvenile used by this study (shell length: $16.38 \pm 1.04$ mm, body weight: $0.87 \pm 0.24$ g) is larger than that of Xue study (shell length: $9.77 \pm 0.73$ mm, body weight: $0.17 \pm 0.26$ g). (3) In this study, the parent of *B. areolata* was

introduced from Thailand. However, the objects of Xue study are aquaculture species of Xiamen, China. (4) The research cycles of the two studies are different, the experimental cycle of Xue study is 42 days and that of this research is 60 days. When considering the effect of different rearing densities and salinities on growth, we found that there was a strong interaction between temperature and rearing density, and that the interaction had covered the synergistic effect of temperature and salinity to a certain extent. Castagne and Chanley (1973) thought that in most cases, temperature mainly influenced the reaction speed of the organism to salinity, but could not change the tolerance limit to salinity. Only when temperature and/or salinity approached the limits of its range, the composite influence of the temperature and salinity would show an obvious correlation. When one of them was in the tolerable range, no obvious interaction occurred.

There was a strong interaction between temperature and rearing density in the model ($P < 0.01$). Within a certain temperature and rearing density range, when the temperature increased gradually and the rearing density declined gradually, the AGR increased gradually. But when the temperature and rearing density rose in the same direction, the AGR declined gradually. Yang, Zheng and Li (2008) reported that when the temperature was 34°C, growth of *B. areolata* was seriously affected, and when rearing density increased or the quantity of exchanged water declined, mass mortality occurred. The reasons why a decline in growth of the AGR was caused by high temperature and high rearing density might be as follows: (1) Generally, *B. areolata* perch in the sand layer when feeding, however, if rearing density was too high, there would not be enough space in the crowded sand layer, which increases the probability of physical collision and associated damage, and the healing process would slow growth rate (Foster & Stiven 1996; Huchette *et al.* 2003; Yan *et al.* 2006; Raghavan & Gopinathan 2008; Kritsanapuntu, Chaitanawisuti, Santhaweesuk & Natsukari 2009). (2) If temperature and rearing densities were high, a change in the physicochemical properties of water, and an associated decline in water quality, in the breeding tank would occur. Kritsanapuntu, Chaitanawisuti, Santhaweesuk and Natsukari (2006) stated that if breeding occurred at high rearing densities, metabolism would increase and ammonia and nitrite concentration would increase, leading to a decline in the water quality. Concentrations of $NH_3$ increase with rising temperatures and pH and decrease with elevated salinity (Downing & Merkens 1955). Huchette *et al.* (2003) examined rearing density and growth in *H. rubra*. They found differences in ammonia levels between stocking densities, and a decreased growth rate with variation in water quality in the bottom of tanks. However, no study has examined the effects of ammonia, temperature and rearing density on *B. areolata*, though some have examined the effects of ammonia, salinity, rearing density and temperature on gastropods (Patterson, Edward & Ayyakkannu 1996; Cheung 1997; Basuyaux & Mathieu 1999; Huchette *et al.* 2003; Chaparro, Montory, Pechenik, Cubillos, Navarro & Osores 2011; Chaparro, Segura, Osores, Pechenik, Pardo & Cubillos 2014). (3) Higher rearing density can change the physical and chemical composition of the organisms. Tolussi, Hilsdorf, Caneppele and Moreira (2010) reported that rearing density would influence the lipid metabolism of fish, and content of saturated fatty acids and fats would decrease with high rearing density. High temperatures and rearing densities would promote the activity of proteins in the body and generate immune responses; thus growth would decrease.

In the model for SR, the interactive effects of salinity and rearing density were significant ($P < 0.01$). When salinity and rearing density increased in the same direction, SR would tend to decrease. When salinity was near its maximum value, SR decreased more dramatically, even trending to zero. With an increase in salinity, specific alkalinity would decline, resulting in an imbalance of the carbonate system, and causing an increase in calcium carbonate precipitation in the seawater and a lack in the free calcium ion (Jiang, Tyrrell, Hydes, Dai & Hartman 2014). Calcium is a critical element in shelled molluscs. Calcium is also important in muscle contraction, neural signal conduction, hormone secretion and osmotic regulation (Coote, Hone, Kenyon & Maguire 1996; Chaitanawisuti, Sungsirin & Piyatiratitivorakul 2010; Ding, Chen, Sui & Wang 2010). Rearing density can influence the shell shape of molluscs. In our study, there was a significant interaction between temperature, salinity and rearing density on survival. When temperature was within a suitable range, the maximum SR that varied with different salinities depended on

  

rearing density. In the same way, when salinity was fixed at ~29 ppt, the maximum SR that varied with different temperature depended upon rearing density and vice versa. The synergistic effects of three factors (temperature, salinity and rearing density) on survival of *B. areolata* may be caused by the characteristics of the species and should be studied in further research.

### Model establishment and optimization

Central composite rotatable design was used in this study. The continuous variable surface growth and survival models of *B. areolata* were established using the RSM (Montgomery 2005; Wang, Liu *et al*. 2014; Wang, Zhu *et al*. 2014). The goodness of fit for model equations of AGR ($R^2$ = 0.9527; Adj-$R^2$ = 0.9350; *Pre*-$R^2$ = 0.8986) and SR ($R^2$ = 0.9890; Adj-$R^2$ = 0.9836; *Pre*-$R^2$ = 0.9686) illustrates the adequacy of two models. By optimization of the *RSM*, the optimal point was found at a temperature of 26.81°C, a salinity of 28.76 ppt and a rearing density of 527.07 ind $m^{-2}$. Under these conditions, the optimal AGR and SR were 36.84 mg $day^{-1}$ and 99.99%, respectively, with a satisfaction function value 99.71%. In fact, the growth and survival of *B. areolata* might as well be affected by many other factors except for these three factors involved in this paper, and within a certain range, a strong interaction may exist among these factors. Because the relationship among the various factors was complex, and some factors are not easily manipulated experimentally, more factors related to the growth and survival of *B. areolata* should be studied in the future.

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