

The combined effects of temperature and salinity on hatching success and survival of early life stages in the economically candidate marine mollusks : Spotted babylon
Babylonia areolata

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ABSTRACT:

The combined effects of temperature and salinity on the hatching success and larval and juvenile survival of the spotted babylon *Babylonia areolata* were investigated in a 3x3 complete factorial experimental design employing three temperatures (25, 30 and 35°C) and three salinities (27, 30 and 33‰). The results showed that hatching of egg capsules *B. areolata* was only significantly affected by salinity but not for temperature. No significant interaction between both factors occurred. However, survival of larvae and early juvenile were significantly affected by temperature and salinity. A significant interaction between both factors occurred in this experiment. The highest survival of early juveniles was founded at the lowest temperature (25°C) with the highest salinity (33‰) and the highest temperature (25°C) with the highest salinity (33‰). From an aquaculture point of view, high hatching of egg capsules *B. areolata* was obtained at temperature of 29 -35°C and salinity above 32‰ to 33‰. As well as the high survival of larvae was obtained at temperature of 29 -30.5°C and salinity above 32‰ to 33‰ and 29 -35°C and 32 - 33‰ for early juveniles.

Keywords:

Spotted babylon, *Babylonia areolata*, temperature, salinity, hatching, survival.

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INTRODUCTION:

Temperature and salinity are considered to be the most important physical factors influencing marine organisms, and the biological effects of these factors are complex and wide ranging. Temperature is one of the most critical external factors of ontogeny in the early life stages of fish. There are two ways in which temperature affects ontogeny. Firstly, temperature, if within a viable range, strongly affects the rate of ontogeny. A temperature beyond this range is lethal for the species. Secondly, temperature affects the hatch rate, incubation period, the size of the newly hatched larvae, larval yolk absorption and utilization, larval feeding behavior, larval survival and larval growth (Shi et al. 2010). Tolerance and resistance tests can be used to determine the survival capacity of various life stages of a species in relation to certain factors. The combined effects of temperature and salinity on the survival of marine animals have been demonstrated in many marine organisms, mainly fish (Shi et al. 2010, Berlinsky et al. 2004, Cook et al. 2005), crustaceans (Dawirs 1979, Ponce-Palafox et al. 1997, Mene et al. 1991, Nagaraj 1988, Albuquerque et al. 2009), echinoderms (Asha & Muthiah 2005, Dong & Dong 2006, Ji et al. 2008, Lavitra et al. 2010), bivalves (Taylor et al. 2004, Robert et al. 1988, Tettelbach et al. 1981), and gastropods (Lu et al. 2004, Davis 2000, Chen & Chen 2004, Zheng et al. 2000). However, there is scant information on the effects of environmental fluctuation, such as temperature, salinity on growth and survival of *Babylonia areolata* particularly the combined effects of temperature and salinity. This is the case for early life stage of *Babylonia areolata* that may be more sensitive to environmental change. Albuquerque et al. (2009) stated that salinity could modify the effects of temperature and alter the temperature range of many biological processes. In turn, temperature can also modify the effects of salinity. The objective of this study is to investigate the combined effect of temperature and salinity on the eggs, larvae and early juvenile of spotted Babylon *Babylonia areolata* to determine the optimal temperature and salinity for incubation and larval culture of this species. The results of this study will be useful in increasing the production of this species through incubation and larval culture.

MATERIALS AND METHODS

Experimental design

The study was conducted from February to

April 2011 in indoor air condition room at Sichang Marine Science Research and Training Station, Chulalongkorn University, located on Sichang Island Cholburi province, Thailand. To verify the combined effects of temperature and salinity on various life stages of the spotted babylon resistance, a 3x3 complete factorial design was used for the experiments including three temperatures (25, 30 and 35°C) and three salinities (27, 30 and 33‰). Each of the nine different temperature–salinity combinations was conducted in three replicates. A total of 27 experimental units were set up initially. The experiment followed a completely randomized design, using the egg capsule, larvae and juveniles from a single spawn to minimize variability between experimental units. This experiment was conducted in the air condition room with normal temperature control at 25°C. Seawater used in all treatments was filtered (10 µm) and dechlorinated for two days before use to ensure no chlorine residue. Seawater with low salinities was made up daily by diluting a source of natural seawater with de-chlorinated freshwater and high salinities water was made by evaporating seawater. Temperature were maintained within +0.2°C using 3 identical 22.0 L thermostatically controlled water baths (L x W x H = 50 x 30 x 15 cm). Each water bath contained nine experimental units (1000 mL glass beaker). Gently aeration was provided during larval culture, a normal photoperiod of 12L:12D was adopted throughout the experiment. Water exchange was not done during the experiment for all aquaria. Salinity and temperature in all experimental units were measured every six hours using a portable refractosalinometer and a mercury thermometer, respectively. All experimental units were covered with aluminum foil to prevent evaporation. The experiments lasted after 96 h.

Experiment 1: Effect of temperature and salinity on the hatching success

After spawning, the egg capsules were transferred to experimental units maintained at different temperature – salinity combinations. The mean size (+SD) of egg capsules at the start of the experiment was 1.2+0.03 cm in length. For each temperature and salinity combination, each experimental unit consisted of 100 egg capsules acclimated in a 1000 mL glass beaker filled with 700 mL seawater. Each unit was stocked with low stocking density of 10 egg capsules per unit so that stocking density was not a limiting factor for survival. Hatching –out of veliger larvae from egg capsule usually was observed by naked eyes and



considered as hatched or unhatched egg capsule. Hatched egg capsules expressed by no fertilized eggs inside or partial retain of fertilized eggs. The number of hatched capsules and duration of incubating period in each experimental unit were recorded. The mean percentage of hatching rate was calculated by combining the data from three replicates at the end of the experiment.

Experiment 2: Effect of temperature and salinity on the survival rate of veliger larvae

Within 6 h after hatching, aeration was stopped, and the veliger larvae were allowed to concentrate at the water surface; they were then collected from the hatching tanks for use in the experiments. The mean size (+SD) of larvae at the start of the experiment was $428 \pm 0.01 \mu\text{m}$ in shell length. For each temperature and salinity combination, each experimental unit consisted of 100 larvae reared in a 1000 mL glass beaker filled with 700 mL seawater. Each unit was stocked with low stocking density of 100 larvae per unit so that stocking density was not a limiting factor for survival. Larvae were fed with a single algal diet (*Chaetoceros calcitrans*) once daily during the experiment. Optimal concentrations of algal cells ($6 \times 10^4 \text{ cell mL}^{-1}$) were fed so that food availability was not a limiting factor for survival and was adjusted for each experiment according to the size of larvae (Chaitanawisuti and Kritsanapuntu 1997). Each experimental unit was initially examined the dead larvae after 6 h, and every 12 h thereafter. The number of larvae which sink down to bottom of the aquaria / empty shell or no movement of velum were observed microscopically and considered as dead. The mean percentage of survival rate was calculated by combining the data from three replicates at the end of the experiment.

Experiment 3: Effect of temperature and salinity on the survival rate of early juveniles

Within 48 h after settlement, the mean size (+SD) of juveniles was $1393.33 \pm 8.18 \mu\text{m}$ in shell length and are used at the start of the experiment. For each temperature and salinity combination,

each experimental unit (1000 mL glass beaker filled with 700 mL seawater) was stocked with low stocking density of 50 larvae per unit so that stocking density was not a limiting factor for survival. Juveniles were fed with fresh meat of trash fish at once daily during the experiment. Uneaten food was removed immediately after they stopped eating to prevent water degradation. Then tested juveniles were fed so that food availability was not a limiting factor for survival. Each experimental unit was initially examined for the dead juveniles after 6 h, and every 12 h thereafter. The number of juveniles which did not respond to the touch of a needle were observed by naked eyes and considered as dead. The mean percentage of survival rate was calculated by combining the data from three replicates at the end of the experiment.

Data analysis

To investigate the effect of temperature and salinity on hatching of egg capsule, and survival of larvae and juveniles, a two-way analysis of variance (ANOVA) (fixed factors: temperature and salinity) with a 95% confidence interval was used. All data were tested for normality and homoscedasticity. On significant difference indication, Tukey test was used to verify the difference among the treatments. The correlation between the survival and the temperature and salinity was estimated by multiple regression analysis.

RESULTS

Experiment 1: Effect of temperature and salinity on the hatching success

Hatching of egg capsules *B. areolata* over the three temperatures and three salinity combinations for 96 h was presented in **Table 1**. The highest hatching of 86.67% and 100% were founded at the lowest temperature (25°C) with the highest salinity (33‰) and the highest temperature (35°C) with the highest salinity (33‰), respectively, while the lowest hatching 63.33% was founded at the lowest temperature (25°C) with the lowest salinity (27‰).

Table 1. Percentage hatching of egg capsules *B. areolata* through 96 h under 9 different combinations of temperature and salinity.

| Temperature ($^\circ\text{C}$) | Salinity (‰) | | |
|----------------------------------|--------------|-------------|-------------|
| | 27 | 30 | 33 |
| 25 | 63.33+25.20 | 83.33+15.30 | 86.67+5.70 |
| 30 | 76.67+11.50 | 86.67+15.30 | 96.67+20.80 |
| 35 | 76.67+20.80 | 83.33+15.30 | 100 |

Mean values are calculated from a total of 3 replicate experiments

Values with different superscript letters are significantly different ($P < 0.05$)

Two-way ANOVA showed that hatching of egg capsules *B. areolata* was only significantly affected by salinity ($F = 5.102$; $P = 0.018$) but not for temperature ($F = 1.085$; $P = 0.359$). No significant interaction between both factors were occurred in this experiment ($F = 0.220$; $P = 0.924$). Tukey test showed that hatching of egg capsules were significantly different between the salinity of 27‰ and 33‰ (**Table 2**).

Multiple regression analysis showed that only salinity ($P = 0.002$) were statistically significant, showing a negative correlation with the percentage hatching of egg capsules *B. areolata* (**Table 3**). In additional, standard coefficient (Beta) of salinity (0.570) was higher than that of temperature (0.228), indicating a higher correlation between salinity and hatching. The higher salinity provided the higher hatching. The multiple regression equation on hatching of egg capsules *B. areolata* over the combined effects of temperatures and salinity were estimated as follows:

$$\text{Hatching} = -54.074 + 0.889 \text{ Temperature} + 3.704 \text{ Salinity}$$

The response surface plots summarizing percentage hatching under nine different combinations of temperature and salinity over 96 h showed that high hatching (95%) was obtained at temperature of 29 -35°C and salinity above 32‰ to 33‰. Lower temperatures and salinities reduced hatching markedly and higher salinities had a marked positive effect at lower temperatures (**Fig 1**).

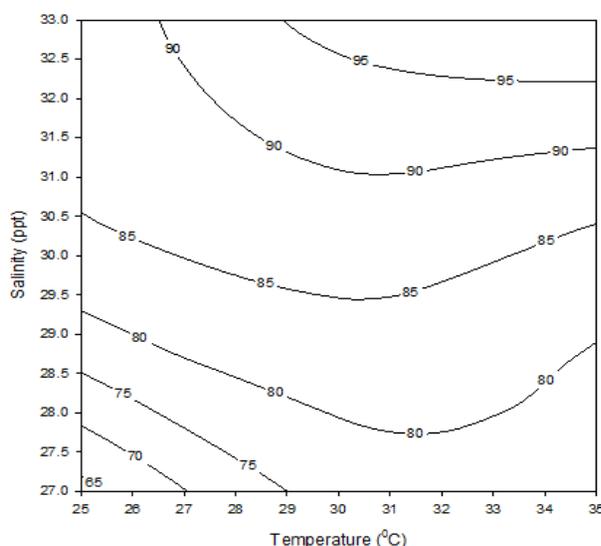


Fig 1. Response surface plots with estimating the mean percentage hatching of egg capsules *B. babylonica* after 96 h under 9 different combinations of temperature and salinity.

Experiment 2: Effect of temperature and salinity on the survival rate of veliger larvae

Survival of veliger larvae *B. areolata* over the three temperatures and three salinity combinations for 96 h was presented in **Table 4**. The highest survival of veliger larvae of 78.67% and 47.00% were founded at the lowest temperature (25°C) with the highest salinity (33‰), and the highest temperature (35°C) with the highest salinity (33‰), respectively, while the lowest survival of veliger larvae of 23.33% and 17.67% were founded at the lowest temperature (25°C) with the lowest salinity (27‰), and the highest temperature (35°C) with the lowest salinity (27‰), respectively.

Two-way ANOVA showed that survival of veliger larvae *B. areolata* was significantly affected by temperature ($F = 1662.01$; $P = 0.000$) and salinity ($F = 488.96$; $P = 0.000$). A significant interaction between both factors were occurred in this experiment are observed ($F = 152.69$; $P = 0.000$). Tukey test showed that survival of veliger larvae were significantly different one another among temperature and salinity treatments (**Table 5**).

Multiple regression analysis showed that both temperature ($P = 0.020$) and salinity ($P = 0.012$) were statistically significant, showing a negative correlation with the percentage survival of veliger larvae *B. areolata* (**Table 6**). In additional, standard coefficient (Beta) of salinity (0.445) was higher than that of temperature (0.406), indicating a higher correlation between salinity and survival. It also indicated that the higher salinity provided the higher survival. The multiple regression equation on survival of veliger larvae *B. areolata* over the combined effects of temperatures and salinity was estimated as follows:

$$\text{Survival} = -13.037 - 2.789 \text{ Temperature} + 5.093 \text{ Salinity}$$

The response surface plots summarizing percentage hatching under nine different combinations of temperature and salinity over 96 h showed that high hatching (90%) was obtained at temperature of 29 -30.5°C and salinity above 32‰ to 33‰. Lower temperatures and salinity reduced hatching markedly but higher temperature and salinities had a marked positive effect (**Fig 2**).

Experiment 3: Effect of temperature and salinity on the survival rate of early juveniles

Survival of early juvenile *B. areolata* over the three temperatures and three salinity combinations for 96 h was presented in **Table 7**.

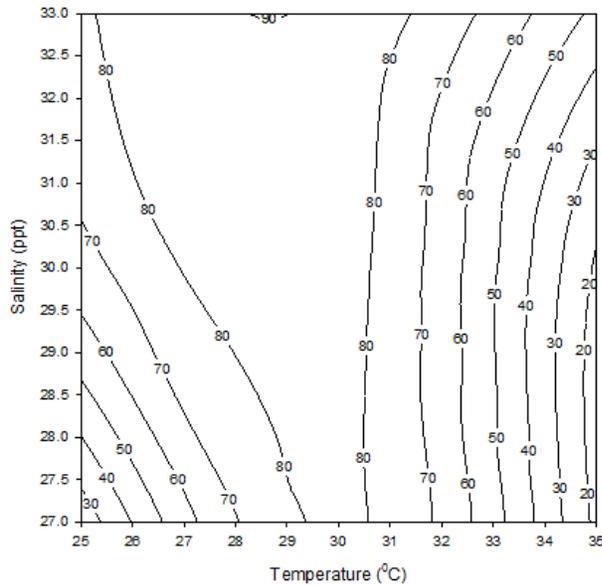


Fig 2. Response surface plots with estimating the mean percentage survival of veliger larvae *B. babylonica* after 96 h under 9 different combinations of temperature and salinity.

The highest survival of early juveniles of 100% and 65.56% was founded at the lowest temperature (25°C) with the highest salinity (33‰), and the highest temperature (25°C) with the highest salinity (33 ‰), respectively, while the lowest survival of early juveniles (15.56%) was founded at the highest temperature (35°C) with the lowest salinity (27‰).

Two-way ANOVA showed that survival of early juvenile *B. areolata* was significantly affected by temperature ($F = 605.192$; $P = 0.000$) and

salinity ($F = 49.060$; $P = 0.000$). A significant interaction between both factors occurred in this experiment are observed ($F = 36.376$; $P = 0.000$). Tukey test showed that survival of early juveniles were significantly different one another among temperature and salinity treatments (**Table 8**).

Multiple regression analysis showed that both temperature ($P = 0.002$) and salinity ($P = 0.026$) were statistically significant, showing a negative correlation with the percentage survival of early juvenile *B. areolata* (**Table 9**). Standard coefficient (Beta) of temperature (0.818) was higher than that of salinity (0.251), indicating a higher correlation between temperature and survival. The multiple regression equation on survival of early juvenile *B. areolata* over the combined effects of temperatures and salinity was estimated as follows:
Survival = 165.176 – 6.148 Temperature + 3.148 Salinity

The response surface plots summarizing percentage survival under nine different combinations of temperature and salinity over 96 h showed that high survival (100%) was obtained at 29 -35°C and 32 - 33‰. Higher temperatures at all salinities reduced hatching markedly (**Fig 3**).

DISCUSSION

Our results showed that hatching of egg capsules *B. areolata* was only significantly affected by salinity but not for temperature. No significant interaction between both factors occurred in this experiment. The highest hatching was founded at the lowest temperature (25°C) with the highest

Table 2. ANOVA comparing the effects of temperature and salinity on percentage hatching of egg capsules *B. areolata* (95% confidence interval).

| Parameters | Sum of square | df | Mean square | F-value | P-value |
|------------------------|---------------|----|-------------|---------|---------|
| Intercept | 189170.37 | 1 | 189170.37 | 865.69 | 0.000 |
| Temperature | 474.07 | 2 | 237.03 | 1.08 | 0.359 |
| Salinity | 2229.63 | 2 | 1114.81 | 5.10 | 0.018 |
| Temperature x salinity | 192.59 | 4 | 48.14 | 0.22 | 0.924 |
| Error | 3933.33 | 18 | 218.51 | | |

Table 3. Multiple regression analysis of data on hatching of egg capsules *B. areolata* through 96 h under 9 different combinations of temperature and salinity on (95% confidence interval).

| Parameters | B | Standard error | Beta | t-value | p-value |
|-------------|---------|----------------|-------|---------|---------|
| Intercept | -54.074 | 36.676 | | -1.474 | 0.153 |
| Temperature | 0.889 | 0.627 | 0.228 | 1.417 | 0.169 |
| Salinity | 3.704 | 1.046 | 0.570 | 3.542 | 0.002 |

Standard error of estimation = 13.310

R = 0.614

F = 7.275

R² = 0.377

P = 0.003

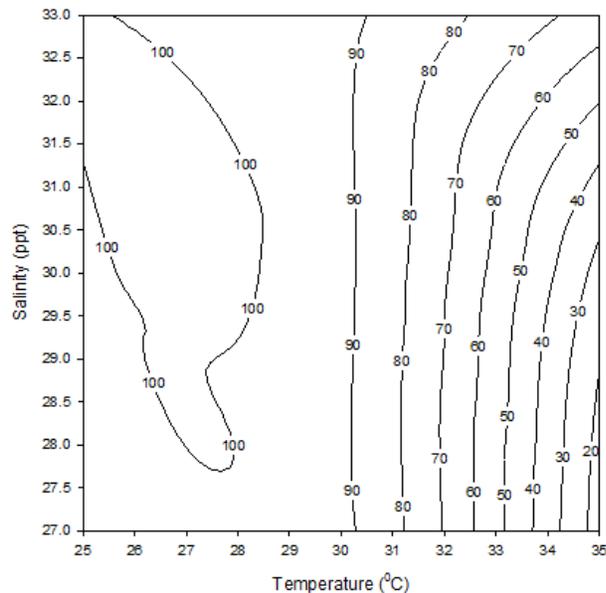
Adjusted R² = 0.326

Table 4. Percentage survival rate of veliger larvae *B. areolata* through 96 h under 9 different combinations of temperature and salinity.

| Temperature (°C) | Salinity (‰) | | |
|------------------|--------------|------------|------------|
| | 27 | 30 | 33 |
| 25 | 23.33+1.53 | 65.67+2.08 | 78.67+1.53 |
| 30 | 81.33+3.51 | 84.00+3.00 | 87.67+1.53 |
| 35 | 17.67+1.15 | 18.67+1.53 | 47.00+2.65 |

Mean values are calculated from a total of 3 replicate experiments

Values with different superscript letters are significantly different (P<0.05)


Fig 3. Response surface plots with estimating the mean percentage survival of new settled juvenile *B. babylonia* after 96 h under 9 different combinations of temperature and salinity.

salinity (33‰) and the highest temperature (35°C) with the highest salinity (33‰). Multiple regressions showed a higher correlation between salinity and hatching. High hatching was obtained at temperature of 29 -35°C and salinity above 32‰ to 33‰. Survival of larvae and early juvenile *B. areolata* were significantly affected by temperature and salinity. A significant interaction between both factors occurred in this experiment was observed. The highest survival of early juveniles was founded at the lowest temperature (25°C) with the highest

salinity (33‰) and the highest temperature (25°C) with the highest salinity (33 ‰). Multiple regression analysis showed a negative correlation with the percentage survival of early juvenile *B. areolata* as well as indicating a higher correlation between temperature and larval survival, as well as salinity and early juvenile survival. The response surface plots showed that high survival of larvae was obtained at temperature of 29 -30.5°C and salinity above 32‰ to 33‰ and 29 -35°C and 32 -33‰ for early juveniles. The results of this study agreed with various studies that salinity had strongly effect on larvae of various mollusks (Doroudi et al. 1999, Tettelbach & Rhode 1981, Davis 2000 and Dove & O’conner 2007. Doroudi et al. (1999) reported that optimal conditions for maximum larval survival of the black-lip pearl oyster *Pinctada margaritifera* were 26-29°C and 28 -32‰. Temperature of 35°C or greater were lethal for larvae and at all temperature tested, larval survival were lowest at a salinity of 40‰. Tettelbach & Rhode (1981) reported that optimum combination of temperature and salinity for survival of the Northern Bay scallop *Argopecten irradians* larvae from 2 to 5 day after fertilization, as estimated from the response surface plot, was 18.7°C and 28.1‰. Temperatures of 35°C or greater and / or salinities of 10‰ or less were lethal for all life stages of this species. Davis (2000) showed that at the end of 0 to 7 day interval, percent mortality of tropical gastropod veligers *Strombus gigas* was highest for veligers grown at 20 and 24°C and at salinity of 45‰, while percent mortality was low and not different for veligers

Table 5. ANOVA comparing the effects of temperature and salinity on percentage survival of veliger larvae *B. areolata* (95% confidence interval).

| Parameters | Sum of square | df | Mean square | F-value | P-value |
|------------------------|---------------|----|-------------|----------|---------|
| Intercept | 84896.14 | 1 | 84896.14 | 19760.31 | 0.000 |
| Temperature | 14280.96 | 2 | 7140.48 | 1662.00 | 0.000 |
| Salinity | 4201.40 | 2 | 2100.70 | 488.95 | 0.000 |
| Temperature x salinity | 2624.14 | 4 | 656.03 | 152.69 | 0.000 |
| Error | 77.33 | 18 | 4.29 | | |



Table 6. Multiple regression analysis of data on survival of veliger larvae *B. areolata* through 96 h under 9 different combinations of temperature and salinity on (95% confidence interval).

| Parameters | B | Standard error | Beta | t-value | p-value |
|-------------|---------|----------------|--------|---------|---------|
| Intercept | -13.037 | 65.309 | | -.0200 | 0.843 |
| Temperature | -2.789 | 1.117 | -0.406 | -2.496 | 0.020 |
| Salinity | 5.093 | 4.862 | 0.445 | 2.735 | 0.012 |

Standard error of estimation = 23.701

R = 0.603

R² = 0.364

Adjusted R² = 0.311

F = 6.855

P = 0.004

Table 7. Percentage survival rate of early juvenile *B. areolata* through 96 h under 9 different combinations of temperature and salinity.

| Temperature (°C) | Salinity (‰) | | |
|------------------|--------------|------------|------------|
| | 27 | 30 | 33 |
| 25 | 93.33+1 | 98.89+1.92 | 100 |
| 30 | 92.23+1.93 | 92.23+3.05 | 92.23+1.93 |
| 35 | 15.56+5.09 | 26.67+5.77 | 65.56+7.70 |

Mean values are calculated from a total of 3 replicate experiments

Values with different superscript letters are significantly different ($P < 0.05$)

grown at 24°C and at salinity of 30, 35 and 40‰. Dove & O’Conner (2007) showed that salinity had a significant effect on D-veliger larval survival of Sydney rock oysters *Saccostrea glomerata* whereas temperature significantly affected survival of both D-veliger and pediveliger larvae. There was an interaction between salinity and temperature for D-veliger larval survival. While spat survival was significantly affected by salinity only and no interaction was detected between salinity and temperature for spat survival.

In addition, results of this study also agreed with the combined effects of temperature and salinity on larvae and juveniles of various organisms; crab (Paula et al. 2003, Nurdiani & Zeng 2007) and shrimp (Palafox et al. 1997, Jackson & Burford 2003 and Zacharia & Kakati 2004). Palafox et al. (1997) indicated that good survival (80 to 90%) of *Penaeus vannamei* postlarvae was obtained below 30°C and below 40‰. Higher temperatures reduced survival markedly. Higher salinities only had a marked negative effect at higher temperatures. The

optimum temperature and salinity conditions for survival of *P. vannamei* postlarvae coincides the best at 28 to 30°C and 33 to 40‰. Jackson & Burford (2003) showed that salinity did not have a significant effect on survival of larval shrimp *Penaeus semisulcatus* above 28‰. At 28‰, survival rate decreased, while temperature had a substantial and regular influence on growth rate. Zacharia & Kakati (2004) showed that salinity exerted a greater influence than temperature on the survival and development of larvae *Penaeus merguensis*. The best temperature – salinity combination for hatching and larval survival was obtained at 33°C and 35‰, and a salinity range of 30-35‰ is ideal for larval development. Paula et al. (2003) indicated that for all zoeal stages of mangrove crab *Parasesarma catenata*, the highest survival was obtained at temperature of 25°C and salinity of 35‰, but survival decreased towards the lower and higher salinity. Nurdiani & Zeng (2007) showed that temperature and salinity as well as the interaction of the two parameters significantly affected the survival of zoeal larvae of mud crab

Table 8. ANOVA comparing the effects of temperature and salinity on percentage survival of early juvenile *B. areolata* (95% confidence interval).

| Parameters | Sum of square | df | Mean square | F-value | P-value |
|------------------------|---------------|----|-------------|---------|---------|
| Intercept | 152616.90 | 1 | 152616.90 | 8826.82 | 0.000 |
| Temperature | 20927.67 | 2 | 10463.84 | 605.19 | 0.000 |
| Salinity | 1696.51 | 2 | 848.25 | 49.06 | 0.000 |
| Temperature x salinity | 2515.77 | 4 | 628.94 | 36.37 | 0.000 |
| Error | 311.22 | 18 | 17.29 | | |

Table 9. Multiple regression analysis of data on survival of early juvenile *B. areolata* through 96 h under 9 different combinations of temperature and salinity (95% confidence interval).

| Parameters | B | Standard error | Beta | t-value | p-value |
|-------------|---------|----------------|--------|---------|---------|
| Intercept | 165.176 | 46.503 | | 3.552 | 0.002 |
| Temperature | -6.148 | 0.796 | -0.818 | -7.728 | 0.000 |
| Salinity | 3.148 | 1.326 | 0.251 | 2.347 | 0.026 |

Standard error of estimation = 16.876

R = 0.855

R² = 0.731

Adjusted R² = 0.709

F = 32.679

P = 0.000

Scylla serrate. At low salinity, both high and low temperature led to mass mortality of newly hatched larvae, while the low temperature and high salinity combination of 25°C and 35 gL⁻¹ resulted in the highest survival to the megalopal stage.

This study is the first study to investigate salinity and temperature effects on hatching success, and survival of larvae and early juvenile *B. areolata*. Although the results suggest the possibility that the culture and natural population of this species in Gulf of Thailand may suffer mortality from higher water temperature due to climate change and lower salinity from heavy rainfall.

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