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The combined effects of temperature and salinity on survival of larvae and juveniles of tropical abalone *Haliotis asinina* under laboratory conditions

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ABSTRACT:

This paper reports on a 3 x 3 factorial design experiment conducted to examine the combined effects of temperature and salinity on survival of larvae and juveniles of tropical abalone *Haliotis asinina* under laboratory conditions for 96 h. The temperatures used were 25, 30 and 35°C and the salinities were 27, 30 and 33 ppt. Response surface contour diagrams were generated from the survival data to estimate optimal conditions. The highest survival of newly-hatched larvae, newly-settled juvenile and fully juveniles of *H. asinina* was obtained at the lowest temperature tested (25°C) with the highest salinity tested (33 ppt), while the lowest survival was obtained at the highest temperature tested (35°C) with the lowest salinity tested (27 ppt). Two - way ANOVA showed that survival of larvae, newly - settled juveniles and fully juveniles were significantly affected by temperature and salinity. A significant interaction between both factors occurred in newly - settled juveniles and fully juveniles but not for larvae. Multiple regression analysis indicated a higher correlation between salinity and survival of larvae *H. asinina* but a higher correlation between temperature and survival for newly - settled juveniles and fully juveniles. This study indicated that the optimal conditions for maximum survival of larval, newly - settled juvenile and fully juveniles were 27-30°C and 31-33 ppt, 26-29°C and 27-33 ppt, and 26-27°C and 31-33 ppt, respectively.

Keywords:
Tropical abalone, *H. asinina*, temperature, salinity, survival, early life stages.

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INTRODUCTION

The tropical abalone (*Haliotis asinina*) is distributed along the East coast of the upper Gulf of Thailand, and in the Andaman Sea. Tropical abalone culture in Thailand is presently the very early stages of basic and applied research conducted in small-scale operations. The economic viability of commercial tropical abalone farming depends on the system design and techniques used for grow-out culture. *H. asinina* has fast growth rates and a relatively high salinity tolerance in comparison to other species (Jarayabhand and Paphavasit 1996). In general, major factors affecting growth and survival rates of the early life stage and mature of various aquatic animals are temperature and salinity. 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 development in the early life stages of shellfish. 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). In addition, 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 (Albuquerque *et al*., 2009). Temperature and salinity affect larval and juvenile growth and survival of many marine invertebrates. The combined effects of temperature and salinity on the survival of marine animals have been demonstrated in many marine mollusks such as bivalves (Taylor *et al*., 2004, Robert *et al*., 1988, Tettelbach and Rhode, 1981) and gastropods (Lu *et al*., 2004, Davis, 2000, Chen and Chen 2000, Zheng *et al*., 2000). However, there is no information on the effects of environmental parameters, such as temperature, salinity on survival of early life stages for tropical abalone *H. asinina* particularly the combined effects of temperature and salinity. Determination of the optimal salinity and temperature for larvae and juvenile culture of *Haliotis asinina* is an important step in developing more efficient large-scale hatchery culture techniques for this species. In addition, result of this study provides greater precision for assessing the interactions between different factors for survival of various life stages for this species under natural conditions particularly the effects of climate change. The objective of this study is to examine the combined effects of temperature and salinity on survival of larvae and juveniles of tropical abalone *Haliotis asinina* under laboratory conditions.

MATERIALS AND METHODS

This laboratory experiment was designed to test the combined effects of three temperatures (25, 30 and 35°C) and three salinities (27, 30 and 33 ppt) on survival for newly-hatched larvae, newly-settled juveniles and fully juveniles of *H. asinina*. The experiment was a 3x3 factorial design, with all nine temperatures and salinity combinations were tested. The study was conducted during summer season from February to April 2011 in which temperatures ranged 28-29°C and it was the spawning season for *H. asinina*. All temperature and salinity combination treatments for newly-hatched larvae, newly-settled juveniles and fully juveniles were not run simultaneously (newly-hatched larvae in February, newly-settled juveniles in March and fully juveniles in April).

Seawater brought from the shore, and filtered through a series of filter net down to 0.5 μm was used in all combination treatments. Ambient seawater was lowered to salinity of 27 ppt by dilution with de-ionised water, and it was increased salinity of 33 ppt by concentration using air evaporating. Treatment water was

made in the containers for 24 hrs in advance to allow thoroughly mixing of seawater and the temperatures to adjust to treatment conditions. Salinity and temperature were monitored every 6 hrs. Salinity was maintained within ±0.5 ppt and water temperature was maintained within ±0.2°C using thermostatically controlled water baths. Gently aeration was provided in the container during the experiment and a normal photoperiod of 12L:12D was adopted throughout the experiment. There were no water exchange during experiment for all treatments. Salinity and temperature in all experimental units were measured every six hours using a portable refracto-salinometer and a mercury thermometer, respectively. All experimental containers were covered with aluminum foil to prevent evaporation and consequent salinity increase.

This study was conducted at the hatchery of Research Unit for Abalone Cultivation, Aquatic Resources Research Institute, Chulalongkorn University. The newly-hatched larvae, newly-settled juveniles and fully juveniles *H. asinina* used for all combination treatments were obtained from the same batch of spawning. One male and female mature breeder was selected for induced spawning using dry method (Jarayabhand and Paphavasit 1996). The larvae were then incubated to reach the desired life stage for the experiment. The mean size (±SD) and age of newly-hatched larvae, newly-settled juveniles and fully juveniles *H. asinina* used in this experiment were 1200±0.04 µm in shell length (1 days after fertilization), 2.20±0.06 mm in shell length (45 days after fertilization) and 5.10±0.12 mm in shell length (50 days after settlement), respectively. The most active and healthy animals of each life stage were chosen for the combination treatments. A sample of 100 newly-hatched larvae, 50 newly-settled juveniles and 50 fully juveniles were initially stocked in each experimental units of 1000-mL transparent, glass container. This stocking density is lower than that used in standard aquaculture practice for this species so that stocking density was not a limiting factor for survival (Jarayabhand and Paphavasit 1996). In this study, the animals were suddenly exposed to each temperature and salinity combination. There were three replicate containers for each combination treatment. No food was served to the larvae during the experiment but the newly-settled juveniles and fully juveniles were fed at once daily with cultivated benthic diatom mainly (*Navicula sp.*) and commercial shrimp (*Penaeus monodon*) pellet, respectively so that food availability was not a limiting

Table 1. Percentage survival rate of larvae, newly- settled juveniles and fully juveniles *H. asinina* through 96 h under different temperature and salinity combinations

Temperature (°C)	Salinity (‰)	Larvae
Larvae		
25	27	29.94±3.33
	30	50.09±8.59
	33	62.20±2.90
30	27	30.41±9.09
	30	56.16±0.81
	33	59.30±1.97
35	27	18.81±1.40
	30	23.89±2.89
	33	41.94±3.12
Newly-settled juveniles		
25	27	94.67±1.15
	30	94.67±1.15
	33	97.33±1.15
30	27	94.67±1.15
	30	93.33±1.15
	33	93.33±3.06
35	27	10.00±5.29
	30	24.00±12.17
	33	48.67±4.16
Fully juveniles		
25	27	$83.34±3.34^{e}$
	30	$91.12±6.94^{c}$
	33	$98.89±1.92^{a}$
30	27	$58.89±7.70^{f}$
	30	$85.56±3.05^{d}$
	33	$93.56±3.67^{b}$
35	27	$1.11±1.93^{i}$
	30	$12.22±6.94^{h}$
	33	$34.45±5.09^{g}$

Mean in the same column with different superscript letters are significantly different ($P<0.05$)

factor for survival (Jarayabhand and Paphavasit 1996). Each experimental unit was initially examined for the dead larvae and juveniles after 6 h, and every 12 h thereafter. The experiments lasted for 96 h. The number of larvae which sank down to bottom of the aquaria / empty shell or no movement of velum were observed microscopically and considered as dead as well as the juveniles did not react to the touch of a needle were considered as dead. The mean percentage of survival was calculated by combining the data from three replicates at the end of the experiment.

To investigate the combined effect of temperature and salinity on survival of larvae, newly-settled juveniles and fully 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. If significant difference were indicated, then tukey test was used to verify the difference among the treatments. The correlation between the survival, temperature and salinity was estimated by multiple regression analysis. Response surface contour diagrams were generated from experimental data on survival rate using the SigmaPlot Version 7.0.

RESULTS

The highest survival of newly-hatched larvae (62.20±2.90%), newly-settled juvenile (97.33±1.15%) and fully juveniles (98.89±1.92%) of *H. asinina* was obtained at the lowest temperature tested (25°C) with the highest salinity tested (33 ppt), while the lowest survival (18.81±1.40, 10.00±5.29 and 1.11±1.93%, respectively), was obtained at the highest temperature tested (35°C) with the lowest salinity tested (27 ppt) (Table 1). Two-way ANOVA showed that survival of newly-hatched larvae, newly-settled juvenile and fully juvenile larvae of *H. asinina* were significantly affected by temperature ($P = 0.000$) and salinity ($P = 0.000$). Significant interaction between temperature and salinity was found in newly-settled juvenile and fully juveniles ($P = 0.000$) but not for the newly-hatched larvae. ($P = 0.087$) (Table 2). Results of tukey test showed that the survival of fully juveniles of *H. asinina* was significantly different among temperature treatments but survival at 25°C treatments and 30°C treatments of newly-hatched larvae and newly-settled juveniles were not significantly different. In addition, significant difference in survival among salinity treatments was found in newly-hatched larvae and fully juveniles of *H. asinina* but survival at 27 ppt treatments and 30 ppt treatments of newly-settled juveniles was not significantly different (Table 3).

Multiple regression analysis showed that both temperature and salinity were statistically significant, showing a negative correlation with the percentage survival of newly-hatched larvae, newly-settled juveniles and fully juveniles of *H. asinina*. Standard coefficient (Beta) of temperature was higher than that of salinity, indicating a higher correlation between temperature and survival of newly-settled juveniles and fully juveniles of *H. asinina* but not for those of newly-hatched larvae. The positive values of beta also indicated that the higher salinity provided the higher survival for newly-hatched larvae, newly-settled juveniles and fully juveniles *H. asinina* (Table 3). The multiple regression equation on survival of newly-hatched larvae, newly-settled juveniles and fully juveniles *H. asinina* over the combined effects of temperatures and salinity were estimated as following:

Survival (newly-hatched larvae) = -41.545 – 1.919 Temperature + 4.682 Salinity

Survival (newly-settled juveniles) = 209.630 – 6.800 Temperature + 2.222 Salinity

Survival (fully juveniles) = 156.658 – 7.704 Temperature + 4.568 Salinity

The response surface plots summarizing percentage survival of *H. asinina* under different

Table 2. Results of two-way ANOVA for survival of larvae, newly-settled juveniles and fully juveniles *H. asinina* through 96 h under different temperature and salinity combinations (95% confidence interval)

Parameters	Sum of square	*df*	Mean square	F-value	P-value
Larvae					
Intercept	30875.297	1	30875.297	1.384E	0.000
Temperature	1572.865	2	786.432	35.256	0.000
Salinity	2401.797	2	1200.899	53.837	0.000
Temperature x salinity	255.891	4	63.973	2.868	0.087
Error	200.757	9	22.306		
Newly – settled juveniles					
Intercept	141122.370	1	141122.370	6067.363	0.000
Temperature	27037.630	2	13518.815	581.223	0.000
Salinity	835.852	2	417.926	17.968	0.000
Temperature x salinity	1481.481	4	370.370	15.924	0.000
Error	418.667	18	23.259		
Fully juveniles					
Intercept	106024.480	1	106024.480	5312.744	0.000
Temperature	30103.963	2	15051.982	754.235	0.000
Salinity	3388.020	2	1694.010	84.885	0.000
Temperature x salinity	573.429	4	143.357	7.183	0.001
Error	359.219	18	19.957		

temperature and salinity combinations over 96 h showed that high survival of newly-hatched larvae (60%), newly-settled juveniles (100%) and fully juveniles (100%) were obtained at 27-30°C and 31-33 ppt, 26-29°C and 27-33 ppt, and 26-27°C and 31-33 ppt, respectively (Fig 1).

DISCUSSION

It is clear that for the tested ranges of temperature and salinity; it is latter which mostly affect survival of the newly-hatched larvae, newly - settled juveniles and fully juveniles of *H. asinina*, especially at the lower ranges. The lowest survival was found at the lowest salinity tested of 27 ppt and the highest temperature tested of 35°C for larvae, newly - settled juveniles and fully juveniles. The results of this study suggested that salinity has a strong effect on larvae and juveniles of abalone *H. asinine*, which agreed with the study in *Haliotis diversicolor supertexta*. Chen and Chen (2000) found that juvenile abalone *Haliotis diversicolor supertexta* maintained 35‰ or higher at 20°C or lower survival salinity higher than 45‰ when salinity is increased. They have also suggested that juveniles maintained in 25‰ or a lower salinity at 30°C or a higher temperature survived salinities lower than 14‰ when salinity is decreased. Cheng *et al*., (2002) also found that hemolymph osmolality of Taiwan abalone *Haliotis diversicolor supertexta* stabilized within two days after they were transferred to different salinities from 33 psu and hemolymph osmolality (Cl^-, Na^+ and K^+ concentrations) increased directly with medium salinity. In addition, Romo *et al*., (2010) found that oxygen consumption rate of pink abalone *Haliotis corrugate* was not affected by temperature and salinity. Ammonium excretion was inversely related to salinity. The O:N ratio indicated that abalone maintained in lower salinities had an interval of 4.9-7.7, which is indicative of protein-dominated metabolism, whereas the O:N ratio in 35‰ was 28.8-35.5 for temperatures of 20 and 24°C, suggesting that carbohydrates were used as energy substrate. He also concluded that optimized culture of pink abalone should be cultivated at 24°C in a salinity of 35‰. The results of this study were in agreement with various studies that salinity has a strong effect on larvae and juveniles of various mollusks such as spotted babylon *Babylonia areolata* (Xue, 2010), scallop *Argopecten purpuratus* (Soria *et al*., 2007), green mussel

Table 3. Turkey test applied to different temperature and salinity treatments for survival of larvae, newly-settled juveniles and fully juveniles *H. asinina* at 95% confidence interval

Temperature (°C)	Post-test	Salinity (‰)	Post-test
Larvae			
25	≠ 35	27	≠ 30; 33
30	≠ 35	30	≠ 27; 33
35	≠ 25; 30	33	≠ 27; 30
Newly-settled juveniles			
25	≠ 35	27	≠ 33
30	≠ 35	30	≠ 33
35	≠ 25; 30	33	≠27; 33
Fully juveniles			
25	≠ 30; 35	27	≠ 30; 33
30	≠ 25; 35	30	≠ 27; 33
35	≠ 25; 30	33	≠ 27; 30

Perna viridis (Nair and Appukuttan, 2003), sydney rock oysters *Saccostrea glomerata* (Dove and O'Oconner 2007), European flat oyster *Ostrea edulis* (Robert *et al*., 1988), conch *Strombus gigas* (Davis, 2000), black-lip pearl oyster *Pinctada margaritifera* (Doroudi *et al*., 1999), silver-lip pearl oyster *Pintada maxima* (Taylor *et al*., (2004), northern Bay scallop *Argopecten irradians* (Tettelbach and Rhode, 1981). Xue, (2010) found that survival of juvenile spotted babylon *Babylonia areolata* was significantly different among temperature and salinity combination treatments due to temperatures but not due to salinities. The optimal condition for culturing juvenile *B. areolata* was obtained at temperatures from 26 to 30°C and salinity from 26 to 30 g/l. Soria *et al*., (2007) explained that higher survival rates of juvenile scallop *Argopecten purpuratus* at higher salinity was due to an increase in salinity that produced a reduction in NH_3 - N proportion and under hypersaline conditions juvenile scallop tend to decrease excretion as a way of osmoconformation. Nair and Appukuttan, (2003) found that total mortality of the green mussel larvae *Perna viridis* occurred after 24 hr at the temperature of 33°C and 35°C and no significant difference in settlement of larvae at 29°C and 31°C. Dove and O'Oconner (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

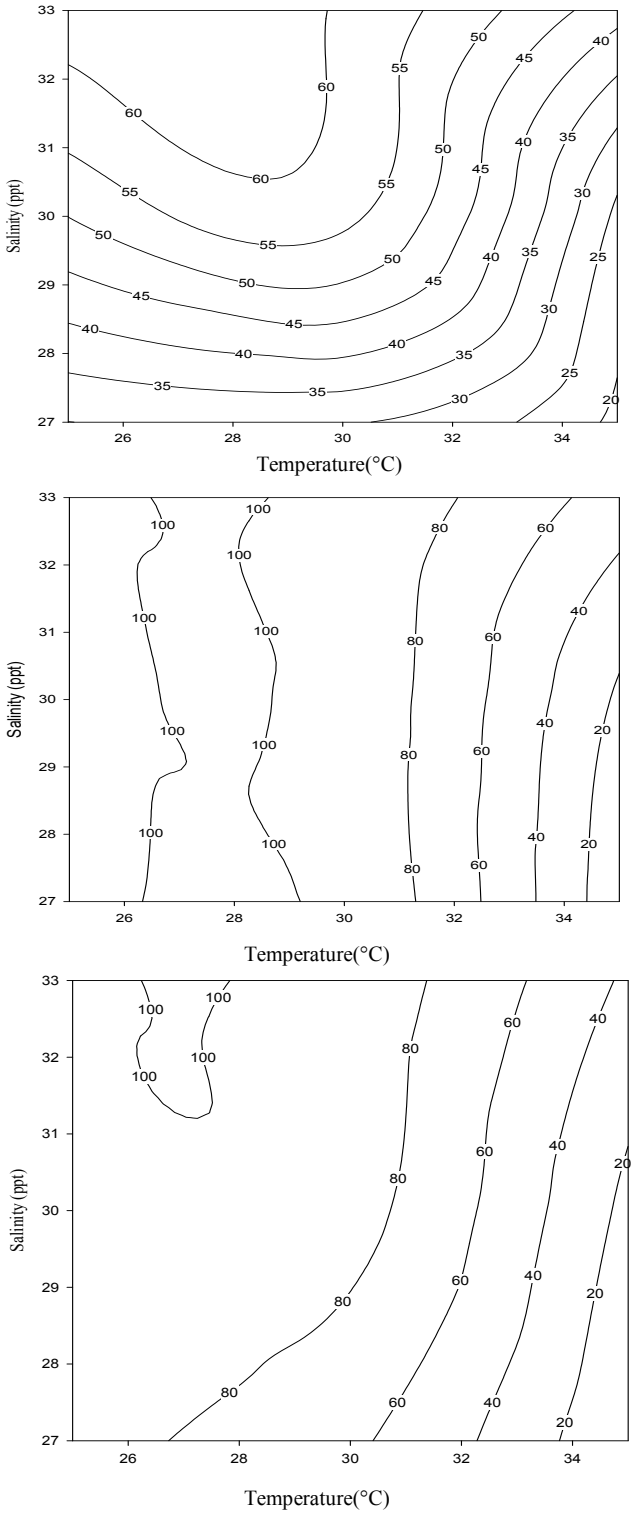

Fig 1. Response surface contour diagram showing maximized survival rate of larvae (upper), newly-settled juvenile (middle) and fully juvenile (lower) *H. asinina* through 96 h under different combinations of temperature and salinity.

Table 4. Multiple regression analysis on survival of larvae, newly-settled juveniles and fully juveniles *H. asinina* through 96 h under different temperature and salinity combinations on 95% confidence interval

Parameters	B	Standard error	Beta	t-value	p-value
Larvae					
Intercept	-41454	26.984		-1.536	0.145
Temperature	-1.919	0.462	-0.499	-4.158	0.001
Salinity	4.682	0.769	0.731	6.085	0.000
R^2 = 0.784; F = 27.155; p < 0.000					
Newly-settled juveniles					
Intercept	209.630	50.826		4.124	0.000
Temperature	-6.800	0.870	-0.836	-7.820	0.000
Salinity	2.222	1.449	0.164	1.533	0.138
R^2 = 0.726; F = 31.755; p < 0.000					
Fully juveniles					
Intercept	156.658	38.096		4.112	0.000
Temperature	-7.704	0.652	-0.878	-11.820	0.000
Salinity	4.568	1.086	0.312	4.205	0.000
R^2 = 0.868; F = 78.695; p < 0.000					

salinity and temperature for D-veliger larval survival. While spot survival was significantly affected by salinity only and no interaction was detected between salinity and temperature for spat survival. 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 grown at 24°C and at salinity of 30, 35 and 40‰. 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 and 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.

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REFERENCES

Albuquerque EF, Meurer B and Netto GCG. 2009. Effects of temperature and salinity on the survival rates of *coxicerberus ramosae* (Albuquerque, 1978), an interstitial isopod of a sandy beach on the coast of Brazil. Brazilian Archives of Biology and Technology 52:1179-1187.

Cheng W, Yeh SP, Wang CS and Chen JC. 2002. Osmotic and ionic changes in Taiwan abalone *Haliotis diversicolor supertexta* at different salinity levels. Aquaculture 203:349-357.

Chen JC and Chen WC. 2000. Salinity tolerance of *Haliotis diversicolor supertexta* at different salinity and

temperature levels. Aquaculture 181:191-203.

Doroudi MS, Southgate PC and Mayer RJ. 1999. The combined effects of temperature and salinity on embryos and larvae of the black-lip pearl oyster *Pinctada margaritifera*. Aquaculture Research 30:271-277.

Davis M. 2000. The combined effects of temperature and salinity on growth, development, and survival for tropical gastropod veligers of *Strombus gigas*. Journal of Shellfish Research 19:883-889.

Dove MC and O'Oconner WA. 2007. Salinity and temperatures tolerance of Sydney rock oysters *Saccostrea glomerata* during early ontogeny. Journal of Shellfish Research 26:939-947.

Jarayabhand P and Paphavasit N. 1996. A review of the culture of tropical abalone with special reference to Thailand. Aquaculture 140:159-168.

Lu J, Lin Q, Sun Y, Sheng J and Chen Q. 2004. Effect of temperature on the early development of *Haliotis diversicolor* Reev. Journal of Shellfish research 23:963-966.

Nair M and Appukuttan KK. 2003. Effect of temperature on the development, growth, survival and settlement of green mussel *Perna viridis* (Linnaeus, 1758). Aquaculture Research 34:1037-1045.

Robert R, His E and Dinet A. 1988. Combined effects of temperature and salinity on fed and starved larvae of the European flat oyster *Ostrea edulis*. Marine Biology 97:95-100.

Romo ZM, Re AD, Diaz F and Mena A. 2010. Physiological responses of pink abalone *Haliotis corrugate* (Gray, 1828) exposed to different combinations of temperature and salinity. Aquaculture Research 41:953-960.

Shi YH, Zhang GY, Zhu YZ, Liu JZ and Zang WI. 2010. Effects of temperature on fertilized eggs and larvae of tawny puffer *Takifugu flavidus*. Aquaculture Research 41:1741-1747.

Soria G, Merino G and Brand EV. 2007. Effect of increasing salinity on physiological response in juvenile scallops *Argopecten purpuratus* at two rearing temperatures. Aquaculture 270:451-463

Taylor JJ, Southgate PC and Rose RA. 2004. Effects of salinity on growth and survival of silver-lip pearl oyster, *Pinctada maxima* spat. Journal of Shellfish Research 23:375-377.

Tettelbach ST and Rhodes EW. 1981. Combined effects of temperature and salinity on embryos and larvae of the northern bay scallop *Argopecten irradiant irradians*. Marine Biology 63:249-256.

Xue M. 2010. The combined effects of temperature and salinity on growth and survival of hatchery-reared juvenile spotted Babylon *Babylonia areolata* (Link 1807). Journal of the World Aquaculture Society 41:116-122.

Zheng H, Zhu J, Ke C, Zhou S and Li F. 2000. Effects of temperature and salinity on embryonic development of *Babylonia formosae habei* (Gastropoda: Buccinidae). Journal of Oceanography in Taiwan Strait 19:1-5.