OPTIMAL CONDITIONS FOR LARVAL REARING OF SPOTTED BABYLON SNAIL (*Babylonia areolata* LINK 1807)

[1]ABOL-MUNAFI, A. B., [2]PITAGSALEE, C., *[1]HAYIMAD, T., [1]NORBAIZURA, S., [1]NORMADIHA, M. N. AND [1]FONG, P. W.

[1] Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, 21300 Kuala Terengganu, Terengganu Darul Iman, Malaysia. [2]Fisheries Technology Division, Faculty of Science and Technology, Prince of Songkla University, Pattani Campus, Thailand.

**Corresponding author: Taufiq_hayimad@hotmail.com*

Abstract: The spotted babylon snail (*Babylonia areolata* Link 1807) is a new commercial gastropod that has been cultured and consumed widespread throughout Asia. In recent years, market demand for this snail has increased, causing a reduction in wild populations and shortage of seed stock to support its aquaculture industry. A large quantity of quality spotted babylon snail larvae should be produced artificially in captivity to support the increasing demand. The optimal conditions for spotted babylon snail larvae have been studied. A salinity level at 30-34 ppt and stocking density for spotted babylon snail larvae of 300 larvae $L^{-1}$is recommended for high growth and survival rates. During the larval rearing period, rotifer should be added with phytoplankton for feeding on the 6th day after hatching to increase growth and survival rates of the larvae.

KEYWORDS: Salinity, Stocking density, Rotifer, Spotted babylon snails

---

Introduction

Many species from the genus *Babylonia* have been cultured for human consumption, including *B. areolata*, *B. formosae formosae*, *B.formosae habei B. japonica*, *B. zeylonica* and *B. spirata* (Chaitanawisuthi and Kritsananpuntu, 1999a; Zheng *et al*., 2005). The spotted babylon snail (*B. areolata*) is a marine gastropod widely consumed throughout Asia including China, India, Taiwan, Thailand and Vietnam. *B. areolata* is the most popular species for captive breeding due to its fast growth (4-6 months to marketable size), high tolerance to the environment, a high market value (USD $4.91 per kg), relatively simple culture techniques and delicious meat (Chaitanawisuthi and Kritsananpuntu, 1999b; Zhou *et al*., 2007). Moreover, this species can be reared in a polyculture system in earthen ponds with sea bass (*Lates calcarifer*) and milkfish (*Chanos chanos*) in order to increase yield profits (Chaitanawisuthi *et al*., 2001a; Kritsanapuntu *et al*., 2006a). Unfortunately, the rapidly growing demand for *B. areolata* has caused a catastrophic reduction in wildstock due to overfishing. A shortage of wild seed stock limits captive production of spotted babylon snail. In order to alleviate fishing pressure on wild spotted babylon snail populations, quality juvenile snails should be produced in captivity to support its aquaculture industry (Kritsanapuntu *et al*., 2007). The optimum larval culture conditions must be determined in order to maximize the survival and growth rate of juvenile spotted babylon snails. Previous research on this gastropod has focused on the juveniles including its economic value (Chaitanawisuti *et al*., 2002), culture system (Chaitanawisuti and Kritsanapuntu, 1999b; Chaitanawisuti and Kritsanapuntu, 2000; Kritsanapuntu *et al*., 2006a; Kritsanapuntu *et al*., 2006b) and biological aspects (Chaitanawisuti and Kritsanapuntu, 1999a; Chaitanawisuti *et al*., 2001b; Chaitanawisuti *et al*., 2001c; Hayimad *et al*., 2008; Zhou *et al*., 2007). The aim of this study was to optimize rearing conditions in order to increase the survival and growth rates of *B. areolata* larvae.

Materials and Methods

The study was conducted at Marine Hatchery, Universiti Malaysia Terengganu (UMT), Kuala Terengganu, Malaysia. Three experiments were performed on the larva of spotted babylon snail, namely studies on the effects of salinity, stocking density, and delayed feeding on growth and survival.

Acquisition of larvae

Spotted babylon snail larvae used in the study were obtained from natural breeding of broodstock maintained in fiber tanks (size 1.0 x 0.5 x 0.4 m, 50 liters of water volume) at temperature 30±1^{O}C and salinity 33±1 ppt with aeration supplied 20 hours (except during feeding period). Sea water system used for broodstock maintenance was recirculating water system (unfiltered). Produced eggs capsuled were dipped in freshwater (tap water) for ten seconds to eliminate parasites and copepods (Hayimad *et al*., 2008) before being transferred to an aquarium for incubation. In each experiment conducted, the larvae were obtained from the same batch of larval breeding to avoid growth retardation and genetic differences (Chaitanawisuthi *et al*., 2001a). During the incubation period, photoperiod was 12:12 (day: night) and aeration supplied was moderately 24 hours.

Experimental design

All experiments were conducted in 1000 ml rectangular plastic aquariums, except the effect of delayed feeding experiment, that was conducted in 6000 ml plastic aquariums. Temperature was controlled at 29±1^{O}C (room temperature without air condition), salinity was monitored daily at 30±1 ppt (except in effect of salinity experiment), dissolved oxygen was monitored daily to > 5 mg L^{-1} and pH was ranged between 7.5 to 8.0. All water quality parameters were monitored daily by using YSI Multimeter model 556. Microalgae fed to the larvae in the effect of salinity and stocking density experiments was *Tetraselmis* sp. Before the beginning of the delayed feeding experiment, *Tetraselmis* sp. was dropped. The larvae were fed with *Nannochloropsis* sp. instead (because *Nannochloropsis* sp. was not bigger than *Tetraselmis* sp.). The larvae were fed once a day (9.00 am) with density of $2x10^{7}$cell mL^{-1} (the microalgae were counted by using counting chamber with an improved Neubauer ruling). Static water system was used in all experiments with 70% water changing in every 2 days. Seawater was treated via UV exposure and filtered through a 10 µm filter net. Growth rate was measured in larval shell length according to the Brito-Manzano and Aranda (2004) method as average growth rate in µm day^{-1}= (average shell length at the end of the experiment – average shell length at the beginning)/ total growth period in days. The survival rate was evaluated by counting the numbers of living settle juvenile at the end of the experiment. Thirty percent (for effects of salinity experiment) and ten percent of the larvae (for effects of stocking density and delayed feeding experiments) in each treatment were measured using the shell length (maximum anterior to posterior distance of shell) at every 3 days using compound microscope attached with moticam 480 program. The experiment on effects of salinity and stock density on growth and survival were conducted for 3 weeks, and the effect of delayed feeding experiment was conducted for 4 weeks.

Effects of salinity on growth and survival of spotted babylon snail larvae

To determine the effect of salinity on growth and survival, four treatments of salinity level; 28, 30, 32 and 34 ppt were assessed in 3 replicates with larval stocking density 500 larvae L^{-1} , as according to Brito-Manzano and Aranda (2004), the larvae should be stocked in moderate stocking

density during culture period. Ambient sea water during this experiment was 30±1 ppt. To reduce and increase salinity level in this experiment, freshwater and sodium chloride (NaCl) was added into seawater, respectively. The sea water was monitered by using YSI Multimeter model 556. This experiment was conducted for 3 weeks.

Effects of stocking density on growth and survival

Four treatments of stocking density; 300, 500, 800 and 1,000 larvae L^{-1} were assessed in 3 replicates. To determine accuracy of stocking density, the larvae were counted and transferred individually from incubation tank to experimental aquariums by using disposable pipette. This experiment was conducted for 3 weeks.

Effects of delayed feeding on growth and survival

Four treatments with 3 replicates of delayed feeding started on 3rd, 6th, 9th day after hatching (DAH) and control (without feeding rotifer). Marine Rotifer (*Brachionus* sp.) sized 100-300 µm at stocking density 7 individuals mL^{-1} were added daily with *Nannochloropsis* sp. into the experimental aquariums with the same amount in other experiment. The rotifer preparation was conducted two weeks prior to the beginning of the experiment with stocking density 5-10 individuals mL^{-1} and fed with *Isochrysis* sp. This experiment was conducted for 4 weeks.

Statistical analysis

All statistical analyses were performed using SPSS System (version 11.5). Data were tested for equivalent and normality. Statistical differences in survival rate and shell length among treatments were determined through one way analysis of variance (ANOVA) at = 0.01.

Results

Effects of salinity on growth and survival of spotted babylon snail larvae

The results of the experiment on the effect of salinity on growth and survival rates of spotted babylon snail larvae are shown in Table 1 and Figure 1. The larvae cultured in salinity level of 32 ppt showed the highest growth and survival rates (26.79 µm day^{-1} and 6.4%, respectively), which was significantly different ($P<0.05$) from larvae cultured in salinity level of 28 ppt (20.39 µm day^{-1} and 3%) but not significantly different ($P>0.05$) from larvae cultured in a salinity level of 30 (25.48 µm day^{-1} and 5.4%) ppt and 34 ppt (25.27 µm day^{-1} and 4.2%).

Table 1: Mean shell length, growth and survival rates for the effects of salinity, stocking density and different period of adding rotifer on growth and survival of spotted babylon snail larvae

Experiment	Treatment	Mean shell length at hatching (µm)	Mean shell length at metamorphosis (µm)	Experimental period (day)	Growth rate (µm day^{-1})	Survival (%)
Salinity	28 ppt	459.30±17.27	826.41±12.26	18	20.39±4.23	3.00±2.64
	30 ppt	465.70±12.26	924.43±12.44	18	25.48±2.16	5.40±1.73
	32 ppt	495.34±12.29	977.66±15.74	18	26.79±2.43	6.40±2.00
	34 ppt	456.15±15.38	911.10±9.76	18	25.27±1.45	4.20±2.64
Stocking density	300 larvae L^{-1}	320.31±2.17	838.24±2.17	15	34.53±2.45	31.00±2.11
	500 larvae L^{-1}	312.71±1.09	832.09±2.73	15	34.62±1.87	19.10±2.34
	800 larvae L^{-1}	309.45±1.88	813.99±22.26	15	33.64±2.67	13.40±1.65
	1000 larvae L^{-1}	305.47±0.63	756.81±7.12	15	30.09±4.11	11.00±2.05
Delayed feeding	3 days after hatching	543.03±4.23	1000.60±43.40	19	24.08±3.01	13.34±6.81
	6 days after hatching	531.45±6.72	1020.10±11.11	19	25.72±2.97	18.00±5.76
	9 days after hatching	539.06±1.65	1013.30±22.50	19	24.96±3.42	15.50±6.32
	No adding rotifer	533.77±4.91	964.90±30.90	19	22.69±2.55	12.60±1.63

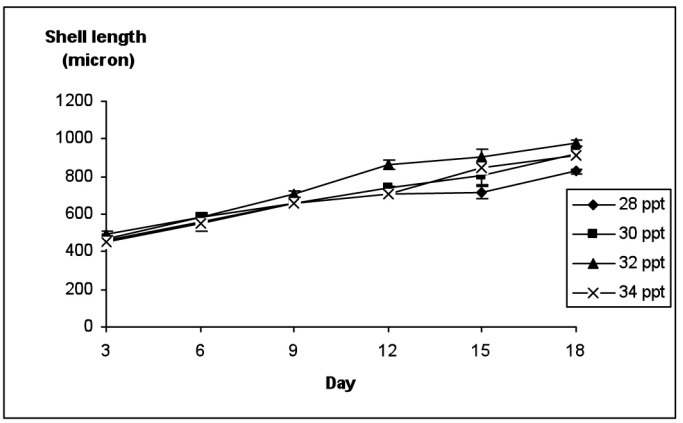

Figure 1**:** Growth pattern of spotted babylon snail larvae cultured in different level of salinity

Effects of stocking density on growth and survival

The results on experiment of stocking density on growth and survival rates of spotted babylon snail larva were shown in Table 1 and Figure 2. The highest growth rate was found in the 500 larvae L^{-1} treatment (34.64 µm day^{-1}), but not significantly different ($P>0.05$) from all treatments. The highest survival rate was found in the larvae cultured in 300 larvae L^{-1} treatment (31%) which was significantly different ($P<0.05$) from other treatments (19.1%, 13.4% and 11.0%).

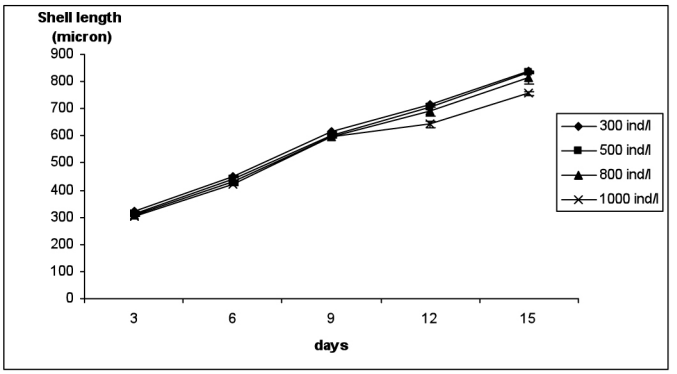

Figure 2**:** Growth pattern of spotted babylon snail larvae cultured in different stocking density

Effects of delayed feeding on growth and survival

The results of delayed feeding on growth and survival rates of spotted babylon snail larva experiment were shown in Table 1 and Figure 3. The highest growth (25.72µm day^{-1}) and survival rates (18.0%) were found in the third treatment (delayed feeding on the 6th day after hatching). But there was no significant difference in either growth and survival rate in all treatments.

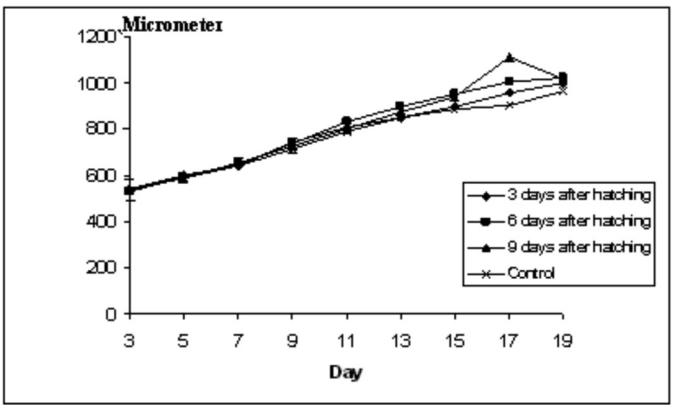

Figure 3: Growth pattern of spotted babylon snail larvae fed with phytoplankton by adding rotifer at different period of rearing

Discussion

Various studies have reported the effect of salinity on different gastropods, but no research has been conducted on *B. areolata* larvae. The results from this study show that different salinity levels would affect the growth and survival rates of spotted babylon snail larvae. The experiment shows that the larvae should not be reared below 28 ppt; however, this is not consistent with the results obtained by Pachenik *et al.* (2003) and Genio *et al.* (2008). According to Pachenik *et al.* (2003), the larvae of estuarine palmonate gastropod (*Amphibola cretana*), an endemic species distributed in New Zealand, could metamorphose to the juvenile stage at 12 ppt. But the growth rate was reduced if compared to the larvae reared at 18 ppt. Genio *et al*. (2008) reported that the optimal salinity level for *Nassarius reticulus* larvae (distributed from the Canaries and Azores to Norway and throughout

the Mediterranean and Black Seas (Fretter and Graham, 1994)) ranged from 21 to 33 ppt. Whereas high larval mortality and low development rate could be found if the larvae were exposed to a salinity level below 17 ppt for more than 48 hours. The authors indicated that the different species of gastropod larvae had different optimal salinity level. Xue *et al*. (2010) studied on survival and growth rates of spotted babylon snail juvenile, and reported that good growth and survival rates of juveniles reared in 26-30 ppt were obtained in the study. The previous authors explained that the optimal salinities for *B. areolata* embryo were 30 to 38 due to the fact that embryo and larval phases adapt to oceanic salinities by suspended style, while the juvenile adapted to estuarine salinities by benthic style. For the spotted babylon snail larvae, optimal salinity levels ranged from 30-34 ppt.

The results from the effect of stocking density experiment show that low or moderate stocking density should be used during larval rearing. The larvae reared at 300 larvae L^{-1} showed the highest survival rate (31.0%). This study supports the view of Orensanz *et al.* (1991) who described that the stocking density could influence on larval survival more than the growth. The authors reported further that high stocking density could cause food, oxygen depletion and environmental stresses. According to Liu *et al.* (2006), the moderate stocking density (10-20 larvae mL^{-1}) was recommended for clams (*Meretrix meretrix*) where this stocking density could shorten the planktonic stage of the larvae and reduce mortality. From the present study, three hundred larvae per liter is the suitable stocking density for spotted babylon snail larvae.

The spotted babylon snail larvae could be fed with both phytoplankton and rotifer. The growth and survival rates of the larvae fed with phytoplankton added with rotifer were not significantly different from the control treatment (no supplement with rotifer) but a high survival was obtained in this experiment (Table 1). The highest growth (25.72 μm day^{-1}) and survival (18.00%) rates were found in the larvae which were given rotifer on the 6^{th} day after hatching due to the size of larva (595.89 μm) which made it able to accept rotifer. The present study introduces rotifer to the *B. areolata* larvae as it has been used to feed many commercial aquatic larvae such as shrimp, fish and crab. Nevertheless, the rotifer was introduced to the larvae at 7 individuals mL^{-1} because rotifer is a type of zooplankton, the same as the *B. areolata* larva, and increasing of number zooplankton could deplete the number of phytoplankton fed to the larvae. Hayimad *et al.* (2008) explained that spotted babylon snail larvae solely fed with phytoplankton (*Isochrysis* sp. and *Chaetoceros* sp.) had relatively low survival (2.75% and 2.01%, respectively). According to Darunchu and Thongsriphong (2004), the spotted babylon snail larvae were able to feed with phytoplankton supplemented with brine shrimp (*Artemia* sp.) after 10 days of hatching. The authors also recommended that the larvae fed with phytoplankton supplemented with brine shrimp had a high survival rate (28.69%) if compared to the larvae solely fed with phytoplankton. Samocha *et al*. (1988) described that *Penaeus semisulcatus* larvae fed with brine shrimp (*Artemia* sp.) and rotifer (*Branchionus* sp., at stocking density 20 individuals mL^{-1}) had high survival and growth rates, but the larvae fed with rotifer solely reduced energy intake as dry weight was lower. The authors reported further that the prey organisms used in the experiment (brine shrimp and rotifer) could cease and limit food supply to predator, because it grew quickly by consuming huge amount of phytoplankton and become invulnerable to predation. The previous researchers concluded that the *P. semisulcatus* larvae could be fed with brine shrimp solely to support sufficient growth and survival rates. From this present study, rotifer does not influence supplemented feed for *B. areolata* larvae, and the larvae can be fed solely with phytoplankton.

Many aspects of spotted babylon snail larvae should be studied further such as water quality, exchange water regime and biological larvae. The results of the present study showed that spotted babylon snail larva was successfully cultured at a salinity level of 30-34 ppt using a stocking density of 300 larvae per liter to maintain economic value. The larvae could be fed with phytoplankton

solely, although to increase growth and survival rates marine rotifer could be added during culture period as supplemented feed on 6th day after larval hatching.

Acknowledgements

Many thanks to Dr. Hamid Khoda Bakhsh, Mr. Masduki Mohammad Morni, Mr. Sawaet Chaimong kol and Supat Khongpuang for their suggestion and guidance on this study. We are also grateful to Miss Mariah Arnold for helping to edit of the article.

References

Brito-Manzano, N. and Aranda, D. A. (2004). Development, growth and survival of the larvae of queen conch *Strombus gigas* under laboratory conditions. *Aquaculture*. (242), 479-487.

Chaitanawisuti, N., and Kritsanapuntu A. (1999a). Experimental culture of juvenile spotted babylon, *Babylonia areolata* Link 1807 (Neogastropoda: Buccinidae) in Thailand. *Asian Fisheries Science*. (12), 77-82.

Chaitanawisuti, N., and Kritsanapuntu A. (1999b). Effects of different feeding regimes on growth, survival and feed conversion of hatchery-reared juveniles of the gastropod mollusk spotted babylon *Babylonia areolata* (Link 1807) in flow through culture system. *Aquaculture Research*. (30), 589-593.

Chaitanawisuti, N., and Kritsanapuntu, A. (2000). Growth and production of hatchery-reared juvenile spotted babylon *Babylonia areolata* Link 1807 cultured to marketable size in intensive flow through and semi-closed recirculating water systems. *Aquaculture Research*. (31), 415-419.

Chaitanawisuti, N., Kritsanapuntu, A., Natsukari, Y. (2002). Economic analysis of a pilot commercial production for spotted babylon, *Babylonia areolata* (Link 1807), of marketable sizes using a flow-through culture system in Thailand. *Aquaculture Research*. (33), 1265-1272.

Chaitanawisuti, N., Kritsanapuntu, A., Natsukari, Y., and Kathinmai, S. (2001a). Growth trials for polyculture of hatchery-reared juvenile spotted babylon (*Babylonia areolata*) and sea bass (*Lates calcarifer*) in a flow-through sea water system. *Aquaculture Research*. (32), 247-250.

Chaitanawisuti, N., Kritsanapuntu, A., Natsukari, Y., and Kathinmai, S. (2001b). Comparative study on growth, feed effiency, survival of hatchery-reared juvenile spotted babylon *Babylonia areolata* Link 1807 (Neogastropoda: Buccinidae) fed with formulated diets. *Asian Fisheries Science*. (14), 53-59.

Chaitanawisuti, N., Kritsanapuntu, A., Natsukari, Y., and Kathinmai, S. (2001c). Effects of different types of substrate on growth and survival of juvenile spotted babylon, *Babylonia areolata* Link 1807 reared to marketable size in a flow-through seawater system. *Asian Fisheries Science*. (14), 279-284.

Darunchu, L., and Thongsriphong, T. (2004). Nursing on veliger larvae of spotted babylon *Babylonia areolata* Link 1807, by adding brine shrimp. *Technical paper. No. 57*, 12 pp.

Fretter, V., Graham, A. (1994). British Prosobranch Molluscs, their functional anatomy and Ecology. *The Ray Society*. Dorset press, Dorchester.

Genio, L., Sousa, A., Nuna, V., Dias, J. M., and Barroso, C. (2008). Effect of low salinity on the survival of recently hatched veliger of *Nassarius reticulates* (L.) in estuarine habitats: A case study of Ria de Aveiro. *Journal of Sea Research*. (59), 133-143.

Hayimad, T., Abol-Munafi, A. B., and Pitagsalee, C. (2008). Effect of different diets on the growth and survival of the larvae and juveniles of spotted babylon snails (*Babylonia areolata* Link 1807). *Journal of Sustainability Science and Management*. (3(2)), 58-65.

Kritsanapuntu, S., Chaitanawisuti, N., Santhaweesuk, W., and Natsukari, S.Y. (2006a). Pilot study on polyculture of juvenile spotted babylon, *Babylonia areolata*, with milkfish, *Chanos chanos*, to marketable sizes using large-scale operation of earthen porns in Thailand. *Aquaculture Research*. (37), 618-624.

Kritsanapuntu, S., Chaitanawisuti, N., Santhaweesuk, W., and Natsukari, S.Y. (2006b). Combined effects of water exchange regimes and calcium carbonate addintions on growth and survival of hatchery-reared juvenile spotted babylon (*Babylonia areolata* Link 1807) in recirculating grow-out system. *Aquaculture Research*. (37), 664-670.

Kritsanapuntu, S., Chaitanawisuti, N., and Natsukari, Y. (2007). Effects of different diets and seawater systems on egg production and quality of the broodstock *Babylonia areolata* L. under hatchery condition. *Aquaculture Research*. (38), 1311-1316.

Liu, B., Dong, B., Tang, B., Zhang, T., and Xiang, J. (2006). Effect of stocking density on growth, settlement and survival of clam larvae, *Meretrix meretrix*. *Aquaculture*. (258), 344-349.

Orensanz, J.M., Parma, A.M., Iribarne, O.O. (1991). Population dynamics and management of natural stocks. In: Shumway, S.E.(Ed.), Scallops: Biology, Ecology and Aquaculture. Elsevier, NewYork, pp. 625–713.

Pechenik, J.A., Marsden, I.D., and Pechenik, O. (2003). Effects of temperature, salinity, and air exposure on development of the estuarine pulmonate gastropod *Amphibola cretana*. *Aquaculture*. 292, 159-176.

Samocha, T. M., Uziel, N., and Browdy, C., L. (1989). The effect of feeding two prey organisms, nauplii of *Artemia* and rotifer, *Brachionus plicatilis* (Muller), upon survival and growth of larval marine shrimp, *Penaeus semisulcatus* (De haan). *Aquaculture*. 77, 11-19.

Xue, M., Ke, C., Wang, D., Wei, Y., and Xu, Y. (2010). The combination 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*. 40 (S1), 116-122.

Zheng, H., Ke, C., Zhou, S., and Li, F. (2005). Effects of starvation on larval growth, survival and metamorphosis of Ivory shell *Babylonia formosae habei* Altena *et al*., 1981 (Neogastropoda: Buccinidae). *Aquaculture*. (243), 357-366.

Zhou, J.B., Zhou, Q.C., Chi, S.Y., Yang, Q.H., and Liu, C.W. (2007). Optimal dietary protein requirement for juvenile ivory shell, *Babylonia areolate*. *Aquaculture*. (270), 186-192.