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Original Article

Effect of feeding frequency and various shelter of blue swimming crab larvae, *Portunus pelagicus* (Linnaeus, 1758)

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Abstract

The objectives of this experiment were to improve blue swimming crab, *Portunus pelagicus*, rearing techniques by investigating (1) the applied frequency of feeding and (2) the effect of shelter on crab larvae survival. The results showed that the feeding frequency at 2 times (0900 hrs and 1500 hrs), 4 times (0700 hrs, 1300 hrs, 1900 hrs and 2300 hrs) and 6 times (0700 hrs, 1100 hrs, 1500 hrs, 1900 hrs, 2300 hrs and 0300 hrs) per day did not affect the survival rate of crab larvae from the zoea I (Z1) to first crab (C1) stages. The survival rate of crab larvae from the megalopa (M) to C1 stages with shelter was higher than without shelter, and the type of shelter affected the survival rate of the crab larvae. The survival rate of the C1 stage with artificial seaweed made of plastic shield membrane (36.61±3.64%) and artificial seaweed made of polyvinyl rope (35.79±6.04%) as shelters were not significantly different and higher than for artificial plastic grass as shelter (22.18±4.00%). In addition, the survival rates of the C1 stage with vertical shelter, horizontal shelter and a combination of vertical and horizontal shelter on the bottom of the tank were not significantly different. This study recommends that crab larvae rearing with shelters is important for increasing the survival rate and that the type of shelter affected the survival rate of crab larvae.

Keywords: *Portunus pelagicus*, survival rate of crab larvae, feeding frequency, shelter

1. Introduction

The blue swimming crab, *Portunus pelagicus*, a commercially important species, is distributed throughout the coastal waters of tropical regions of the western Indian Ocean and the Eastern Pacific (FAO, 2009). In Thailand, *P. pelagicus* is caught in the Andaman Sea and the Gulf of Thailand for direct consumption and for use as a raw material in the processing industry. Export of fresh, chilled, or frozen crabs to the United States, Japan, Taiwan and other countries, is annual, and is a multi-milliondollar business for Thailand. However, due to overfishing and marine pollution, the natural resource and fisheries production of *P. pelagicus* shows a downward trend in the seas of Thailand since 1999. For example, in 2004, the production of *P. pelagicus* was 29,500 t and in 2010 it was 22,800 t (Department of Fisheries, 2012). Therefore, the culturing of *P. pelagicus* is believed to be a way to increase productivity without placing undue pressure on the wild stock and also the job stability of those involved in commercial crab culture.

Currently, many countries are actively involved in crab culture and associated research, e.g., the Philippines, Indonesia, India, Australia, and Malaysia (Romano and Zeng, 2008). In Thailand, *P. pelagicus* culturing methods of breeding, nursing, and rearing to gain higher productivity and survival rates have been developed. The method of rearing crab broodstock in an earthen pond is also well developed (Oniam and Taparhudee, 2010; Oniam *et al*., 2009; 2010).

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However, in most of the countries to date, hatchery seed production of *P. pelagicus* has been experimental. Rearing technologies for the production of juvenile crabs have not been established on a commercial scale and rearing alone cannot maintain a farmer´s income because of low productivity. Factors that contribute to the low survival of crab larvae were identified as feedstock (Castine *et al*., 2008; Ikhwanuddin *et al*., 2012), cannibalism (Marshall *et al*., 2005), water quality (Bryars and Havenhand, 2006; Romano and Zeng, 2006; Liao *et al*., 2011) and bacterial diseases, parasites and fungi (Morado, 2011; Wang, 2011), among others. To improve the income from *P. pelagicus*, optimal rearing conditions must be determined, e.g., feeding management and methods to reduce cannibalism at nursery stage. Thus, the objectives of this study were to improve rearing techniques by investigating (1) the applied frequency of feeding, and (2) the impact of shelters on crab larvae survival. The knowledge gained from the research will be useful for seed production and the development of crab farming, which are important factors regarding farmers' job stability in the future.

2. Materials and Methods

2.1 Study site and source of experimental larvae

This study was conducted at the hatchery of the Klongwan Fisheries Research Station (KFRS), Prachuap Khiri Khan Province, Thailand. *P. pelagicus* broodstock were caught using a small scale crab trap by small scale fishermen in the coastal area of Prachuap Bay, Prachuap Khiri Khan Province, Thailand. Individual berried females with dark grey eggs were transferred into 200-L plastic tanks for hatching. After hatching, the crab larvae were transferred outdoors to 3,000-L concrete tanks for rearing at densities of 100 crabs L^{-1}. Crab larvae were fed with phytoplankton (diatom) and zooplankton (rotifers, *Branchionus* sp. and *Artemia* nauplii) until the experiment commenced.

2.2 Experimental design and set-up

Experiment 1 – Feeding frequency: Newly hatched crab larvae were transfer to 200-L plastic tanks for rearing at densities of 100 larvae L^{-1}. The crab larvae from the zoea I (Z1) to first crab (C1) stages were fed with diatom (*Thalassiosira spp*.), rotifer (*Branchionus* sp.) and *Artemia* nauplii according to Castine *et al*. (2008) and Arkronrat and Oniam (2012) (Table 1). After the crab larvae metamorphosed to the megalopa (M) stage they were provided with artificial seaweed made of plastic shield membranes as a shelter. Three treatments were applied to feeding frequency, 2 times at 0900 hrs and 1500 hrs (control), 4 times at 0700 hrs, 1300 hrs, 1900 hrs and 2300 hrs and 6 times at 0700 hrs, 1100 hrs, 1500 hrs, 1900 hrs, 2300 hrs and 0300 hrs, with three replicates per treatment.

Experiment 2 - Provision of shelter: A study was carried out on the effect of different types of shelter on the survival rate of crab larvae from the M to C1 stages. Different types of shelter were provided to crabs when they metamorphosed into the M stage. M stage larvae were transferred to 200-L plastic tanks for rearing at densities of 50 crabs L^{-1}. The crab larvae from the M to C1 stages were fed with *Artemia* nauplii using a feeding frequency based on the results of Experiment 1 explained above.

Four treatments involved crab rearing, 1) without shelter (control), 2) with artificial seaweed made of plastic shield membrane (T1), 3) with artificial seaweed made of polyvinyl rope (T2), and 4) with artificial grass made of plastic (T3) all as vertical shelter (Figure 1). Each treatment had three replicates. Then crab larvae were reared from the M to C1 stages using the optimal shelter based on the results of the previous shelter survival trial. Three treatments were crab rearing 1) with vertical shelter (control), 2) with horizontal shelter (T1), and 3) with a combination of vertical and horizontal shelter (T2) on the bottom of the tank.

2.3 Data collection

Main data collected during the study trial were the survival rates of the Z2, Z3, Z4, M, and C1 stages. The larval stage was determined using a dissecting microscope at 10×magnification to ensure that the larvae had reached the stage in the time period. Larval stages were differentiated by identification of the telson, eye-stalk, abdomen segmentation, spine, and setae according to Arshad *et al.* (2006). The survival rate was calculated using following equation:

Table 1. Feeding schedule for blue swimming crab larvae (per day).

Crab larval stage				
zoea I	zoea II	zoea III - IV	zoea IV - magalopa	magalopa-1st crab
Thalassiosira spp. ($1\text{-}2 \times 10^4$ cells/ml)	*Thalassiosira* spp. ($1\text{-}2 \times 10^4$ cells/ml)+ Rotifer (density of 10/ml)	*Thalassiosira* spp. ($1\text{-}2 \times 10^4$ cells/ml)+ Rotifer (density of 10/ml)+ *Artemia* nauplii (density of 5/ml)	Rotifer (density of 10/ml)+ *Artemia* nauplii (density of 5/ml)	*Artemia* nauplii (density of 5/ml)

(a) (b) (c)

Figure 1. Types of shelter : (a) artificial seaweed made of plastic shield membrane, (b) artificial seaweed made of polyvinyl rope, and (c) artificial grass made of plastic.

$$Survival\ rate = \frac{number\ of\ crab\ larvae\ left \times 100}{number\ of\ initial\ crab\ larvae}$$

In larval rearing, water exchange was undertaken every three days with about 20% of the total volume during the Z1 to Z4 stages, and daily water exchange (about 20%) was carried out during the M to C1 stages. To remove left over feed, detritus, and dead larvae each water exchange, the aeration was stopped temporarily and settled particles were removed from tank bottom by siphoning. Water quality was analyzed every three days. Salinity was measured by a refractometer from Prima tech, pH by a pH meter Cyber Scan pH 11, temperature, dissolved oxygen concentration (DO) by an oxygen meter YSI 550A, total ammonia by Koroleff's Indophenol blue method, nitrite by the colorimetric method and alkalinity by the titration method according to APHA, AWWA, and WPCF (2009).

2.4 Statistical analysis

At the end of the experiments, data on the survival rate were analyzed using analysis of variance and the difference between means was tested using Duncan's multiple range test at the 95% level of confidence using the SPSS program.

3. Results

3.1 Feeding frequency

Results showed that the mean survival rate of *P. pelagicus* larvae from the Z1 to C1 stages at different feeding frequencies were not significantly different (P>0.05) (Table 2). This study showed that the feeding frequency did not affect the survival rate of the crab larvae. In addition, the average water qualities in crab rearing tanks from Z1 to C1 stages were not significantly different (P>0.05) (Table 3).

3.2 Provision of shelter

The study on the effect of shelter on the survival rate of *P. pelagicus* larvae from the M to C1 stages showed that C1 production with shelter had a significantly higher survival rate compared to those produced without shelter (7.06± 4.46%) (P<0.05). The survival rate of the C1 stage with artificial seaweed made of plastic shield membrane (36.61±3.64%) and artificial seaweed made of polyvinyl rope (35.79±6.04%) as shelter were not significantly different (P>0.05) and were higher than artificial grass made of plastic as shelter (22.18± 4.00%) (P<0.05) (Figure 2). In addition, the survival rate of crab larvae reared with artificial seaweed made of plastic shield membrane as vertical shelter (41.86±3.35%) horizontal shelter (38.30±6.38%) and combination of vertical and horizontal shelter (45.30±4.18%) on the bottom of the tank were not significantly different (P>0.05) (Figure 3). This study recommends that crab larvae rearing with shelter is important to increase the survival rate, and the type of shelter affected the survival rate of the crab larvae.

3.3 Larval development

In this study, larval development from the Z1 to M stages took 10-12 days and from the M to C1 stages took 5-6 days in each treatment. Similar results for this species were

Table 2. Survival rate (%) of *Portunu pelagicus* larval stages at different feeding frequencies (mean ± SD).

Larval stage	Feeding frequency (per day)			*P*-value
	2 times	4 times	6 times	
zoea I	100±0.0%	100±0.0%	100±0.0%	-
zoea II	81.00±12.76%	90.66±3.05%	93.00±4.00%	0.224
zoea III	63.33±4.50%	73.00±11.13%	59.33±4.16%	0.142
zoea IV	52.33±11.93%	61.00±9.64%	51.66±6.50%	0.462
magalopa	33.66±5.68%	30.33±4.93%	35.66±3.78%	0.448
first crab	2.33±1.28%	1.15±0.76%	1.45±0.85%	0.381

Table 3. Water qualities in *Portunu pelagicus* rearing tanks from zoea I to first crab stages at different feeding frequencies (mean ± SD).

Parameters	Feeding frequency (per day)			*P*-value
	2 times	4 times	6 times	
Salinity (ppt)	31.83 ± 0.40	31.83 ± 0.40	31.66 ± 0.51	0.761
Temperature (°C)	27.46 ± 0.42	27.48 ± 0.40	27.46 ± 0.42	0.996
Dissolved oxygen (mg/l)	5.11 ± 0.21	5.16 ± 0.15	5.21 ± 0.24	0.700
pH	8.04 ± 0.13	8.06 ± 0.11	8.03 ± 0.08	0.896
Total ammonia (mg-N/l)	0.162 ± 0.109	0.206 ± 0.145	0.218 ± 0.165	0.771
Nitrite (mg-N/l)	0.059 ± 0.041	0.070 ± 0.048	0.080 ± 0.047	0.753
Alkalinity (mg/l as $CaCO_3$)	122.83 ± 9.96	122.50 ± 6.94	121.66 ± 6.21	0.965

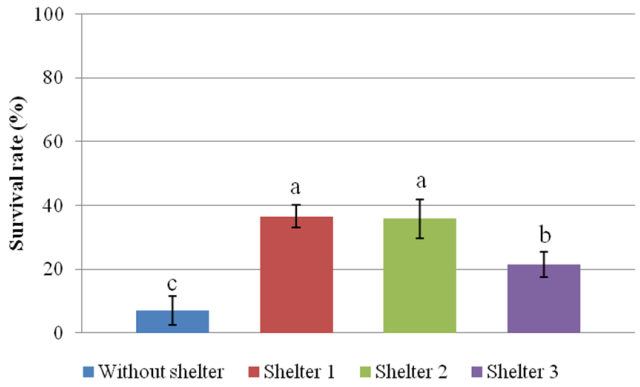

Figure 2. Percentage of successful metamorphosis of *Portunu pelagicus* megalopa to first crab stage with different shelter types: control (without shelter), artificial seaweed made of plastic shield membrane (Shelter 1), artificial seaweed made of polyvinyl rope (Shelter 2), and artificial grass made of plastic (Shelter 3) as vertical shelter. Different letters indicate significant differences (P<0.05) between all treatments.

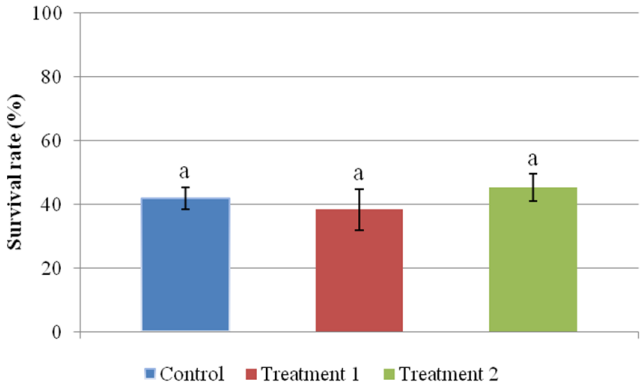

Figure 3. Percentage of successful metamorphosis of *Portunu pelagicus* megalopa to first crab stage with artificial seaweed made of plastic shield membrane as vertical shelter (control), horizontal shelter (treatment 1) and a combination of vertical and horizontal shelter (treatment 2) on the bottom of the tank. Same letters indicates no significant difference (P>0.05) between all treatments.

reported by Arshad *et al*. (2006), Oniam *et al*. (2010), and Andrés *et al*. (2010). Results of water quality sampling during the rearing periods (salinity 31-32 ppt, temperature 26.8-29.7°C, DO 4.83-5.61 mg/l, pH 7.85-8.47, total ammonia 0.014-0.414 mg-N/l, nitrite 0.000-0.134 mg-N/l, and alkalinity 112-146 mg/l as $CaCO_3$) did not affect the development stages and survival rates of *P. pelagicus* larvae (Arshad *et al*., 2006; Oniam *et al*., 2009; 2010; 2012).

4. Discussion

Feeding frequency is an important factor to be con-sidered as it can affect growth and survival; also water quality and feeding at the optimum frequency can result in tremendous savings in feed cost (Robertson *et al*., 1993; Nair and Sridhar, 2003). In the current experiment, the percentage of survival of *P. pelagicus* larvae until the C1 stage with a feeding frequency of 2, 4, and 6 times per day was not affected. Similar results for other crustacean species were reported earlier, e.g., the giant tiger prawn, *Penaeus monodon* (Robertson *et al*., 1993), the Indian prawn, *Penaeus indicus* (Nair and Sridhar, 2003), the blue crab, *Callinectes sapidus* (Zmora *et al*., 2005), the narrow-clawed crayfish, *Astacus leptodactylus* (Ulikowski and Krzywosz, 2006), and the mud crab, *Scylla paramamosain* (Nghia *et al*., 2007). However, a proper feeding frequency and the amount of food given to the reared species were paramount to improve growth and production. For example, in *P. monodon*, Robertson *et al*. (1993) evaluated the effects of feeding time and frequency, finding that instantaneous growth rates were improved significantly with daytime feeding and switching frequency from 1 to 4 times per day. Ulikowski and Krzywosz (2006) reported that the growth rate of *A. leptodactylus* juvenile was decreased as the feeding frequency decreased.

Many researchers reported that the factors that contribute to low survival of crab larvae were identified as feedstock (Castine *et al*., 2008; Ikhwanuddin *et al*., 2012), water quality (Bryars and Havenhand, 2006; Romana and Zeng, 2006; Liao *et al*., 2011), light intensity and photoperiod (Andrés *et al*., 2010), among others. Wu *et al*. (2010) reported that crab larvae at the zoea I stage produced by wild-caught

P. trituberculatus broodstock had a significantly higher survival rate than that of pond-reared *P. trituberculatus* broodstock. In contrast, Oniam *et al*. (2009) reported that seed production of *P. pelagicus* from wild-caught broodstock and pond-reared broodstock was not significantly different. Zoea produced by pond-reared *P. pelagicus* broodstock younger than 120 days had a significantly lower survival rate compared to those produced by older female broodstock (Oniam *et al*., 2010). Oniam *et al*. (2012) reported that zoea produced by *P. pelagicus* broodstock fed with mixed feeds had a significantly higher survival rate compared to those produced by crab broodstock fed with trash fish and shrimp feed. In addition, the causes of crab larvae mortality were moult death syndrome (MDS or death associated with moulting), bacterial diseases, parasites, and fungi (Morado, 2011; Wang, 2011). Marshall *et al*. (2005) reported that the factors that contribute to low survival of megalopa to first crab stages were MDS and cannibalism. While MDS may have occurred in the ponds, cannibalism was the main factor affecting mortality.

Cannibalism commonly occurs at the megalopa instar stage of crab development when the larvae develop chelipeds. Cannibalism, especially at the megalopa and juvenile stages, was one of the main reasons for failures in the development of rearing methods for a variety of crab species (Arshad *et al*., 2006; Ventura *et al*., 2008). The availability of refuge has been considered as one of the important factors affecting cannibalism in crabs (Luppi *et al*., 2001; Marshall *et al*., 2005; Oniam *et al*., 2011). In the current study, the survival rate of *P. pelagicus* larvae from the M to C1 stages with shelter was higher than without shelter and the type of shelter affected the survival rate of the crab larvae. Similarly, cannibalism in other crabs was reduced with the presence of artificial refuge areas (Luppi *et al*., 2001; Ut *et al*., 2007). Marshall *et al.* (2005) reported that cannibalism in juvenile crab of *P. pelagicus* might partially be controlled by refuge availability and increased refuge density. Mirera and Moksnes (2013) reported that the varying degree of shelter (seaweed, plastic strings, bamboo tubes, and open sand substratum) affected the survival rate of *S. serrata*. Cannibalism in *S. serrata* juveniles decreased by >50% in all the shelters compared with the sand substratum. These methods increased the survival of crab larvae proportionally. However, a study of shelter quality is necessary with further studies considering the number, size, and type of the shelter.

5. Conclusions

This study has demonstrated that a feeding frequency of 2, 4, and 6 times per day did not affect the survival rate of *P. pelagicus* larvae from Z1 to M stages but it is not clear for M to C1 stages because cannibalism is the main factor affecting mortality of M to C1 stages. On the other hand, the crab larvae from M to C1 stages with shelter had a higher survival rate than without shelter, and the type of shelter affected the survival rate of C1 stage. In addition, the survival rates of M to C1 stages reared with vertical shelter, horizontal shelter and a combination of vertical and horizontal shelter on the bottom of the tank were not significantly different.

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