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Optimal Water Temperature and Salinity for Production of Blue Swimming Crab, *Portunus pelagicus* 1st Day Juvenile Crab

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Abstract. A study was carried out to determine the optimal rearing conditions of the water temperature and salinity on the survival rate and molting of larvae stage until the 1st day juvenile crab (C1) of the blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758). Trials were carried out at water temperature 30ºC with water salinity 30 ppt and low salinity 20 ppt, ambient water temperature between 24-28ºC with salinity 30 ppt and low salinity 20 ppt. Results of the present study shows that both water temperature and salinity significantly affected survival of the crab larvae. Replicates treated with water temperature at 30ºC with water salinity 30 ppt produced C1 juvenile with mean survival rate 0.25% ±0.21. The larvae rearing for all the other three treatments did not survive up to C1. The study shows that the zoea reached the megalopa stage in 13-14 days and reached the C1 stage in 16-17 days for the larvae rearing batch treated with water temperature at 30ºC and water salinity at 30 ppt. The study recommended that the optimal water temperature and salinity for the larvae rearing of *P. pelagicus* is 30ºC and 30 ppt.

Key Words: Blue swimming crab, juvenile, *Portunus pelagicus*, salinity, water temperature.

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Introduction

Blue swimming crab (*Portunus pelagicus*, Portunidae) is a commercially important species, especially as a possible alternative culture species to shrimps/prawns. The crab fishery and culture operations are expected to continue to grow in the future. At present no appropriate techniques established for the commercial production of *P. pelagicus* juvenile crabs. Literature review shows that quite a number research on the water temperature and salinity rearing conditions of the Portunidae crab (*Portunus* spp., *Charybdis* spp. and *Scylla* spp.) larvae has been carried out. Study by Baylon & Suzuki (2007) shows that both salinity and temperature significantly affected survival of Crucifix crab (*Charybdis feriatus*) larvae and there was a significant interaction between salinity and temperature. Study shows that temperature and salinity had a direct effect on the development of mud crab (*Scylla serrata*) larvae through metamorphosis, survival and production (Marichamy & Rajapackiam 1992). Study by Sun *et al* (1984) on *Portunus trituberculatus* shows that the development from zoea 1 to 1st day juvenile crab required 15-18 days at a temperature of 22-25ºC. Study by Bryars & Havenhard (2006) shows that at constant temperature of 22.5ºC and 25ºC, *P. pelagicus* larvae survival was far greater than at low temperatures down to 17ºC and development period of the larval period was inversely related to (constant) temperature. Study by Baylon and Suzuki (2007) shows that the optimum temperature recommended for the larval rearing of Crucifix crab (*Charybdis feriatus*) is 26-32°C. Study by Marichamy & Rajapackiam (1992) on *S. serrata* shows that the maximum production and fast growth, with an increased survival at each stage, was observed in the higher temperature of 28.5-31ºC. The study by Marichamy & Rajapackiam (1992) also shows that at temperature of 27-28ºC, the inter-moult period was prolonged (30 days) and the survival rate was lower and even prolonged until 35 days at 25-26ºC with still less production for zoea 1 to reached 1st day juvenile crab. Temperature shock causing mud crab (*S. serrata*) larval stress and mortality has been surmised when unintentional temperature fluctuations due to equipment failure has led to abnormally high mortality rates (Mann *et al* 1999). Study on *S. serrata* by Mann *et al* (1999) shows that the temperature fluctuations of 5ºC over the range 23-28ºC within a daily cycle have been typically followed by dramatic mortality events. Study on mud crab, *Scylla paramamosain* shows that 25-30ºC was optimal temperature range for zoea l development (Li *et al* 1999). However, only early mud crab larvae appeared generally more tolerant to lower temperature, while megalopa could survive well at temperatures as high as 35ºC (Zeng & Li 1992). Study by Hoang (1999) shows that the most suitable

Table 1. Larvae rearing batches conducted at different water temperatures and salinity level with replicates.

Batch	Sub batches	Treatment parameters
1	1a, 1b and 1c	Water temperature at 30ºC with water salinity 30 ppt.
2	2a, 2b and 2c	Water temperature at 30ºC with low water salinity 20 ppt.
3	3a, 3b and 3c	Ambient water temperature 24-28ºC with water salinity 30 ppt.
4	4a, 4b and 4c	Ambient water temperature 24-28ºC with low water salinity at 20 ppt.

range of temperature is 28-30ºC for the larvae rearing of mud crab, *S. paramamosain*. Identifying the relationship between salinity and survival rate of crab larvae are necessary to determine an optimal salinity for artificial production. Study by Baylon & Suzuki (2007) on the Crucifix crab, *C. feriatus* larval rearing shows that the optimum salinity recommended is 25-35 ppt. Study by Li *et al* (1999) on the mud crab, *S. paramamosain* shows that the most optimal salinity for the duration of larval development appeared to be 27 ppt. In another study by Hoang (1999) on *S. paramamosain* shows that the most suitable range of salinity was 29-31 ppt for larval zoea stage and 22-25 ppt for megalopa and salinity of 30 ppt was good for developing zoea larval stages (Nguyen & Truong 2004). Not only water salinity affects survival rate, but also the moult and metamorphosis time of crab larvae. There is a need to develop hatchery-rearing techniques to provide steady and reliable supplies of *P. pelagicus* seed. To achieve this, optimal rearing conditions must be determined. The present study aimed to define optimal rearing conditions of water temperature and salinity on the survival rate and molting of larval stage until the 1st day juvenile crab of *P. pelagicus*.

Materials and Methods

Seawater

The sea water was passed through cotton beg filter (10 µm) after settling overnight in the sedimentation tanks. The water was disinfected with 25 ppm of active chlorine and neutralised with sodium thiosulphate at the beginning of the experiment.

Broodstock Management

Portumus pelagicus berried females were caught from the wild using gill net. The berried females were acclimated and kept in a circular fibreglass holding tank with stocking density one crab per tone of water. Chopped fish meat was given once daily as food. Moderate aeration was provided throughout the incubation period. When the berried females become matured with black eggs, the crabs were then transferred to the circular Polyethylene (PE) hatching tanks (500L capacity) with stocking density one crab per tank. The hatching tank filled up to 400L disinfected seawater provided with moderate aeration throughout the experimental period. Seawater within the holding tanks and hatching tanks exchanged at rate 100 % daily. To reduce the exposure of larvae to high bacteria levels, infections, the larvae were removed from the hatching tank within the second hour of hatching. Before moving of larvae from hatching tank, the aeration was turn-off for 10 to 15 minutes to allow the vigorously swimming, photo-positive larvae to aggregate at the surface, where they were collected for experimental trials.

Larval stocking

Fibreglass circular tanks located indoor under transparent roofs were used as culture tanks. Each culture tank has a volume of 5,000 L capacity with dimension of 1.4 m radium, 80 cm high and 1.2 mm thickness with grey background colour. Each culture tank filled with sterilised seawater at a volume 4,000 L with the stocking density at 50 larvae L^{-1}. About 200,000 larvae stocked per culture tank. A volume of 20-30% water was exchanged daily.

Feeding regimes

Three types of feeding regimes were given to the crab larvae throughout the study trials including *Artemia* nauplii at 0.5-1.0 individual mL^{-1}, rotifer (*Branchionus* spp.) at 30-35 individual mL^{-1} and encapsulated *Spirulina* at 10 g per tonne. The encapsulated *Spirulina* (Seastar International Inc., Salt Lake City, USA) was given to larvae after four hours hatching until the end of zoea 2 stages (about 6 days after hatching). Rotifers were also fed after four hour hatching until the end of zoea 4 stages (about 12 days after hatching). *Artemia* nauplii (Great Salt Lake USA) was given from zoea 2 until the juvenile stage. Larvae were fed once daily in the morning at about 10.00h.

Management of larvae

When the crab larvae metamorphosed to the megalopa stage, substrate was provided within the larvae culture tank to facilitate a shelter to reduce the cannibalism. The substrate was made up of strips of nylon strings, and suspended vertically from the tank bottom with weighted at one end. Dead larvae and debris was removed daily to prevent contamination. Larval numbers were estimated and monitored daily through volumetric method using 50-100 mL beaker from rearing tanks. The larvae rearing trial terminated when all the crab larvae metamorphosed to 1st day juvenile crabs (C1). Any 1st day juvenile crab (C1) found was removed from the larvae rearing tanks at the time of the daily count. Moderate aeration was provided in the larval rearing tanks.

Experimental setup

Four treatments were conducted with three replicates for water temperature and salinity as shown in Table 1. Throughout the study trials the water temperature was maintained using water heater.

Survival rate

Larvae were directly counted and the percent survival to C1 was determined by using following formula:

$$\text{Survival rate} = \frac{\text{Total number of survived larvae}}{\text{Initial number of stocked larvae}} \times 100$$

Data analysis

Data presented as mean ± standard deviation (SD). Statistical significance of differences between treatments for survival rates from zoea 1 (Z1) to 1st day juvenile crab (C1) was determined using Microsoft Excel 2007 and Completely Random Design (CRD) for unequal number of replications using SPSS version 16.0 for windows. The difference was displayed as statistically significant when $P < 0.05$.

Results

A reduction in the survival of zoea 2 day six (D6) in the 1st larvae rearing batches at water temperature at 30ºC and water salinity 30 ppt (1a, 1c) was observed as shown in Table 2. However, in larvae rearing batch (1b), a significant reduction was achieved on the ninth day (D9) when zoea 2 moulted to zoea 3. All three run (1a, 1b, and 1c) produced 1st day juvenile crabs with survival rate 0.29%, 0.02% and 0.45% respectively in Table 2. The study shows that the zoea reached the megalopa stage in 13–14 days and reached to C1 in 16–17 days (Table 2). The study showed that larvae treated at water temperature 30ºC with low water salinity 20 ppt for 2a, 2b, 2c did not survive till 1st day juvenile crab (Table 2).

No larval survival for 3a; 3b; 3c up to C1 treated with ambient water temperature between 24–28ºC and salinity 30 ppt. Larvae survived until day 7 for 3a, day 5 for 3b and 3c respectively (Table 3). However, larvae at water temperature between 24–28ºC and with low water salinity 20 ppt for 4a, 4b, 4c, the zoea survived until day 1 for 6a, day 8 for 6b and day 3 for 6c respectively (Table 3). There was significant difference ($P < 0.05$) in larvae rearing batches with and without water heater.

Discussion

Studies shows that water temperature and salinity are two environmental factors which may have a profound effect on the survival and rate of development of Portunidae crab larvae (Marichamy & Rajapackiam 1992; Baylon & Suzuki 2007). Study by Anger (2001) shows that survival and development rate in decapods larvae, temperature has a weak effect on survival. The results of the present study clearly shows that temperature and salinity affected survival rate and development of *P. pelagicus* larvae.

Consistently high survival of zoea stages and complete metamorphosis to 1st juvenile crab observed when larvae reared at 30ºC water temperature with water salinity 30 ppt. From the literature it has been found that both water temperature and salinity are affecting factors to Portunidae crab larvae. For the larval and nursery rearing of the crucifix crab, *C. feriatus* the optimum temperature and salinity combination recommended is 26-30ºC and 25-35 ppt (Baylon & Suzuki 2007). Study by Baylon *et al* (2001) shows that the best survival of the mud crab, *S. serrata* zoea with highest metamorphosis to megalopa occurred at 32 ppt with temperature 26-29ºC. The optimal water salinity and temperatures favourable for survival and development of decapods crustacean larvae and juveniles in laboratory conditions

Table 2. Survival rate of larvae stage till the 1st day juvenile crab of *P. pelagicus* larvae treated with water temperature at 30°C and water salinity at 30 ppt for larvae rearing batch No. 1 (1a, 1b, and 1c) and water temperature at 30°C and low water salinity at 20 ppt for larvae rearing batch No.2 (2a, 2b, and 2c)

Batch No:	1a		1b		1c		2a		2b		2c	
Day	Stages	%	Stages	%	Stages	%	Stages	%	Stages	%	Stages	%
1	Z1	100	Z1	100	Z1	100	Z1	100	Z1	100	Z1	100
2	Z1	100	Z1	93.20	Z1	91.31	Z1	96.27	Z1	85.31	Z1	96.27
3	Z1	81.33	Z1	93.20	Z1	89.09	Z1	93.44	Z1	76.28	Z1	93.44
4	Z1	69.94	Z1	77.20	Z1	85.66	Z1	74.48	Z1	0.00	Z1	74.48
5	Z1	61.27	Z2	77.20	Z1	79.40	Z1	52.38			Z1	52.38
6	Z2	46.04	Z2	53.09	Z2	27.03	Z2	52.38			Z2	52.38
7	Z2	22.40	Z2	53.09	Z2	9.49	Z2	21.91			Z2	21.91
8	Z2	4.48	Z2	53.09	Z2	7.72	Z2	0.00			Z2	0.00
9	Z3	2.05	Z3	12.56	Z3	7.28						
10	Z3	0.82	Z3	0.11	Z3	6.17						
11	Z3	0.33	Z4	0.11	Z4	4.21						
12	Z4	0.33	Z4	0.08	Z4	3.84						
13	Z4	0.33	M	0.08	M	3.72						
14	M	0.29	M	0.06	M	3.02						
15	M	0.29	M	0.06	M	0.75						
16	M	0.29	M	0.02	C1	0.45						
17	C1	0.29	C1	0.02								

Note: a = Z1 = Zoea 1; Z2 = Zoea 2; Z3 = Zoea 3; Z4 = Zoea 4; M = Megalopa; C1 = 1st day juvenile crab. b * = Significant different at 5% level ($P<0.05$) between for larvae rearing batch no. 1 (1a, 1b, and 1c) and water temperature of 30°C and low water salinity of 20 ppt for larvae rearing batch No.2 (2a, 2b, and 2c).

Table 3. Survival rate of larvae stage till the 1st day juvenile crab of *P. pelagicus* larvae treated with ambient water temperature between 24-28ºC and water salinity of 30 ppt for larvae rearing batch No. 3 (3a, 3b, and 3c) and ambient water temperature between 24-28ºC and water salinity of 20 ppt for larvae rearing batch No. 4 (4a, 4b, and 4c).

Batch No:	3a		3b		3c		4a		4b		4c	
Day	Stages	%	Stages	%	Stages	%	Stages	%	Stages	%	Stages	%
1	Z1	100	Z1	100	Z1	100	Z1	100	Z1	100	Z1	100
2	Z1	87.26	Z1	96.22	Z1	84.28	Z1	0.00	Z1	63.31	Z1	90.61
3	Z1	73.78	Z1	89.09	Z1	84.28			Z1	59.61	Z1	66.30
4	Z1	46.88	Z1	60.60	Z1	84.28			Z1	56.36	Z1	0.00
5	Z2	27.79	Z2	22.26	Z2	68.57			Z2	52.42		
6	Z2	14.34	Z2	0.00	Z2	0.00			Z2	50.56		
7	Z2	3.36							Z2	48.12		
8	Z3	0.00							Z3	43.01		
9									Z3	0.00		

Note: a = Z1 = Zoea 1; Z2 = Zoea 2; Z3 = Zoea 3; Z4 = Zoea 4; M = Megalopa; C1 = 1st day juvenile crab. b * = Significant different at level between for larvae rearing batch No. 3 (3a, 3b and 3c) and ambient water temperature between 24-28°C and water salinity 20 ppt for larvae rearing batch No. 4 (4a, 4b and 4c).

were found to be closely similar to the salinity and temperature of their natural environment (Anger 2001). *Portunus pelagicus* larvae reared in 20 ppt during the present study failed to metamorphose to megalopa. In the present study most of the mortality of zoea stages occurred during the process of molting. When examined, the dead Z1 larvae reared at 20 ppt were found to have developed some morphological features of a Z2, but failed to shed completely their exuvia which could have caused death. This observation shows that mortality caused by incomplete molting may be similar to the "moult death syndrome" reported by Blackshaw *et al* (1999) on the mud crab, *S. serrata* larvae. Further to explain that highest survival rate of *P. pelagicus* larvae was achieved at 30 °C with salinity 30 ppt. The result of present study matches with the previously findings. Study by Anger (2001) shows that the effects of salinity stress in crustaceans have been observed in delayed development, reduced survival, feeding and growth rates, as well as extended molting. Study on stone crab, *Menippe mercenaria* larvae shows that salinity stress produced extended development at a reduced salinity of 20 ppt compared to high salinity of 30-40 ppt (Ong & Costlow 1970). Other study by Nagaraj (1992) on *Liocarcinus puber* larvae when reared at lower water salinity of 20 and 25 ppt compared to 30 and 35 ppt also produced extended development. Results of the present study are also in agreement of previously mentioned. It can be said that salinity is one of the affecting factor in survival and molting process *P. pelagicus* larvae.

Mann *et al* (1999) reported that the temperature fluctuation of 5ºC over the range 23-28ºC within a daily cycle have been typically followed by dramatic mortality events in *S. serrata* larvae. No any larvae of *P. pelagicus* reached to megalopa stage reared at 24-28ºC in the present, this fluctuated temperature optimistically affected the survival of the larvae. Study by Brown *et al* (1992) in stone crab, *Menippe mercenaria* shows that the warm water temperature promoted rapid growth whiles molting was delayed when temperature was low. In the present study, the *P. pelagicus* zoea reached the megalopa stage between 13-15 days and reached the C1 between 16-18 days when tested at 30ºC. It can be said that temperature is a parameter to affect the survival and molting as observed under this study. Other studies also show that prolonged molting especially in later zoea 1 stages resulted in higher cannibalism of megalopa on zoea, in the mass seed production of *S. serrata* (Quinitio *et al* 2001) and *P. pelagicus* (Hamasaki 2003).

Conclusion

The results revealed that zoea stages of *P. pelagicus* were highly sensitive to fluctuation of temperature and low water salinity 20 ppt which suggests that both factors limit the successful survival rate. The study concludes that the *P. pelagicus* larvae required an optimal rearing condition under a constant water temperature 30ºC and salinity 30 ppt for better survival and molting.

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