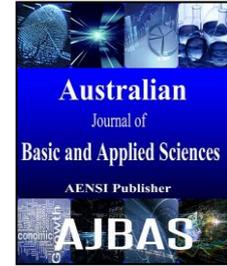




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Effects of Salinity on Survival Rate and Larval Development of Blue Swimming Crab *Portunus pelagicus* (Linnaeus, 1758) Under Laboratory Conditions

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ABSTRACT

The survival rate and larval development of the blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758) were studied in the laboratory at different constant salinity (26, 28, 30, 32 and 34 ppt). In this experiment, the first zoeal larval at lowest salinities of 26 ppt showed the low survival rate to C1 (21.11±1.36%) compared to 33.33 ± 4.08 % at 28 ppt, 36.67 ± 2.36% at 30 ppt, 27.78 ± 4.91% at 32 ppt and 24.44 ± 1.36% at 34 ppt. The development duration of C1 range from 3.33-4.00 days in all salinity levels and statistically not significant (P>0.05). The relationships between salinity and successful metamorphosis and development duration of C1 were quadratic. Based on the survival and larval development, the best salinity for the larvae culture of *Portunus pelagicus* was 28 -30 ppt.

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INTRODUCTION

Salinity is one of the important environmental variables, particularly for any organisms inhabiting coastal and estuarine regions. The important physiological and ecological effects of salinity on marine and brackishwater animals have previously been reviewed (Kinne, 1964; Levinton, 1982; Ponce-Palafox *et al.* 1997). The large Indo-West Pacific crab *Portunus pelagicus* (Linnaeus, 1758) spends much of its life cycle in nearshore marine and estuaries waters (Stephenson, 1962; Kailola *et al.* 1993). After mating, the mature females migrate into the open sea where they extrude their eggs and attached them to the hairs on the female's abdomen. The eggs are incubated under abdominal flap by the female. They are bright orange when first spawned and change progressively to dark grey as the embryos develop and use up the yolk. Small grey remnants of the egg mass may remain for a short period after developing eggs have been released into the water (Potter *et al.* 1983).

The eggs and larvae of blue swimming crab are planktonic. Consequently, during this period they are strongly be influenced by the ecological and productivity of the pelagic systems. Benthic recruitment also depends to great extent on their survival and successful settlement. It is, therefore, important to know the relationship between the occurrence of planktonic phase and the main environmental factors. Besides temperature, light

and availability of food, salinity is considered a key factor controlling reproduction and release of free-swimming development stages from benthos into pelagic environment.

Tolerance to salinity may vary with the life stages of the blue swimming crab. In general, salinity is not as influential as temperature to egg development and hatching, but the larvae are highly sensitive to changes in salinity (Buchanan and Milleman, 1969). A Knowledge on the salinity tolerance of the *P. pelagicus* larval stages could give an indication not only of their requirements but also of the conditions under which these stages normally live. The objective of this study is to determine the influence of salinity on the survival and development of larval blue swimming crab under laboratory conditions.

MATERIALS AND METHODS

Adult females, with mean body weight (BW) of 73.0-268.3 g and carapace width of 102.25-140.58 mm were collected from the coastal region of Port Dickson, Malaysia and brought to the hatchery in 10 liter plastic aquaria provide with good aeration. The crabs were held in 300L circular fiberglass tanks for disinfection 100 ppm formaldehyde for 30 minutes prior to stocking in maturation tanks.

Blue swimming crab females were randomly stocked in seven units of 1 m³ circular black plastic tanks at three crabs per tank. Tanks were provided

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with substrate of around 15 cm thick sand and adequate aeration. The crab are maintained in constant water depth of 30 cm at salinity of 30-32 ppt, pH 7.77-7.96, and temperature 26.5-27.0°C, with a shelter made of PVC pipe 13 cm in diameter and 40 cm in length, to serve as refuge during molting and attack by fellow crabs. Fresh bivalve mollusks meat was given as food daily at 1700-1800 h, and uneaten food was removed every morning. Mother crab were monitored for spawning twice weekly and berried females was transferred to hatching tank (300L) having similar environmental conditions for incubation of eggs. The water quality in the incubation tanks is maintained using a flow-through system (2 L/min) and gentle aeration. When hatching appeared imminent, as indicated dark ocular pigmentation and movement of the embryo inside the egg membrane, water inlet and outlet are stopped. Hatching occurred during both day and nighttime hours, larvae are transferred to a separate aquarium for observation and rinsing before being placed into the experimental rearing containers.

First stage zoea hatched from a single female were transferred without previous acclimatization from rearing salinity (30 ppt) to experimental tanks of 26, 28, 30, 32 and 34 ppt. Thirty Z_1 in each salinity treatment (three replicates per treatment) were placed in 3.7L cylindrical plastic larval rearing containers. Seawater was used and filtered through a series of 10 and 5 μm filters and diluted with distilled water to obtain the tested salinities. The high-salinity media were prepared by adjusting hypersaline solution separated from partly dried sea water under the sunshine. The larvae were maintained at 28 to 30°C, a continuous light condition (3000-3500 lx) and a constant aeration. The range of pH was 7.79 to 8.01 and dissolved oxygen was maintained above 6 ppm. The first zoea stage were fed a mixture of micro-algae *Nannochloropsis oculata* (5×10^3 cells/mL), rotifers *Brachionus plicatilis* (5 ind/mL) and *Artemia* nauplii (5 ind/mL). A combination of rotifers *B. plicatilis* (5 ind/mL) + *Artemia* nauplii (5 ind/mL) and *Artemia* nauplii (10 ind/mL) alone were given in the Z_2 and Z_3 to first crab stage, respectively. Daily water exchange was 100% in all the experimental tanks, with faeces, sheds, and remaining food being siphoned out from all containers. The larvae were examined daily and all dead larvae or molts were recorded. Dead larvae were removed at time of each observation. Daily water exchange was 100% in all the experimental tanks, with faeces, sheds, and remaining food being siphoned out from all containers. The larvae were examined daily and all dead larvae or molts were recorded. Dead larvae were removed at time of each observation. The absences of response to touch were the criteria for death. Dead larvae were removed at time of each observation. The data of survival rate (premetamorphic survival and successful metamorphosis) and duration of larval stages (days) were tested using one way ANOVA and Duncan's Multiple Range test to compare the mean differences among treatments (Steel and

Torrie, 1980). Arcsin transformation was done in the analysis of the data in percentage.

RESULTS AND DISCUSSION

The relative effects of salinity levels on survival rates of premetamorphic and successful metamorphosis and development duration of *P. pelagicus* larvae are summarized in Table 1. Changes in the development rate of respective larval stages with time after hatching are shown in Figure 1.

Treatment of different levels of salinity (26, 28, 30, 32 and 34 ppt) showed different effects on survival and development duration of Z_1 . The highest ($P < 0.05$) premetamorphic Z_4 survival was achieved by larvae reared at 34 ppt (98.89%) compared to larvae reared at 26 ppt (92.22%). While the development duration from Z_1 to the premetamorphic Z_1 survival was found shorter ($P < 0.05$) when the larvae reared at 28 to 34 ppt (4.00 days) compared to larvae reared at 26 ppt (5.33 days). The relationships between salinity and premetamorphic survival and development duration of Z_4 were linear ($PZ_1SR = 0.7782S + 72.2100$; $R^2 = 0.4263$; $P < 0.05$; Figure 2, and $DuZ_1 = -0.1333S + 8.2667$, $R^2 = 0.4324$; $P < 0.05$; Figure 3), respectively.

There were no differences ($P > 0.05$) in the premetamorphic survival rate at stages Z_2 and Z_3 among all the salinities. However, the development duration of Z_2 and Z_3 stages were found to be longer ($P < 0.05$) at lower salinity (26 ppt) than those higher salinities (28-34 ppt). The relationships between salinity and premetamorphic survival and development duration of Z_2 and Z_3 were quadratic ($PZ_2SR = -0.2380S^2 + 13.8370S - 118.54$; $R^2 = 0.2162$; $P < 0.05$; Figure 4, and $PZ_3SR = 0.0992S^2 - 6.6720S + 181.450$; $R^2 = 0.1011$; $P < 0.05$; Figure 6) and linear ($DuZ_2 = -0.2167S + 11.1670$; $R^2 = 0.7682$; $P < 0.05$; Figure 5, and $DuZ_3 = -0.0667S + 6.1333$; $R^2 = 0.3077$; $P < 0.05$; Figure 7), respectively.

The highest ($P < 0.05$) premetamorphic Z_4 survival was achieved by larvae reared at 28 (67.78%) compared to larvae reared at 26 ppt (54.44%). While the development duration from Z_3 to the premetamorphic Z_4 survival was found slightly shorter ($P > 0.05$) when the larvae reared at 28 to 34 ppt (6.33-7.00 days) compared to larvae reared at 26 ppt (7.33 days). The relationships between salinity and premetamorphic survival and development duration of Z_4 were quadratic ($PZ_4SR = -0.4961S^2 + 29.7120S - 380.4400$; $R^2 = 0.2270$; $P < 0.05$; Figure 8) and linear ($DuZ_4 = -0.1167S + 10.3000$, $R^2 = 0.3712$; $P < 0.05$; Figure 9), respectively.

Drastic increase in mortality was observed when larvae reached the megalopa stage. However high water salinity (28-34 ppt) gave better megalopa survival (34.44 – 44.44%) compared with the low salinity of 26 ppt with survival rate (33.33%). Duncan's Multiple Range test showed that significant differences ($P < 0.05$) were found in the rate of premetamorphic M survival between treatment 30 ppt and treatments 26 and 34 ppt, with

polynomial regression analysis showed a quadratic response ($PMSR = -0.5755S^2 + 34.4760S - 473.4800$; $R^2 = 0.4386$; $P < 0.05$; Figure 10). The longest larval development (7.00 -7.67 days) occurred at 26 to 30 ppt. At 32 and 34 ppt, larval developments were around 6.67 days. Significant

differences in larval development duration were found between treatment 26 ppt and treatments 32 and 34 ppt. The relationship between salinity and development duration was quadratic ($DuM = 0.0179S^2 - 1.1881S + 26.4260$; $R^2 = 0.4619$; $P < 0.05$; Figure 11).

Table 1: Survival rate (%), premetamorphic survival* and successful metamorphosis** and development duration (days) of different stages of zoea (Z_1 - Z_4 *), megalopa (M*), and first crab (C_1 ***) of blue swimming crab, *P. pelagicus*, reared under different salinity¹

Salinity	Variable	Z_1	Z_2	Z_3	Z_4	M	C_1
26 ppt	% ± SE	92.22 ± 1.36 ^b	78.89 ± 1.36 ^a	73.33 ± 6.24 ^a	54.44 ± 5.93 ^b	33.33 ± 2.36 ^b	21.11 ± 1.36 ^c
	Du ± SE	5.33 ± 0.41 ^A	5.67 ± 0.41 ^A	4.67 ± 0.41 ^A	7.33 ± 0.41 ^A	7.67 ± 0.41 ^A	4.00 ± 0.00 ^A
28 ppt	% ± SE	94.44 ± 1.36 ^{ab}	85.56 ± 3.60 ^a	75.56 ± 3.60 ^a	67.78 ± 3.60 ^a	41.11 ± 3.60 ^{ab}	33.33 ± 4.08 ^{ab}
	Du ± SE	4.00 ± 0.00 ^B	5.00 ± 0.00 ^B	4.00 ± 0.00 ^B	7.00 ± 0.00 ^A	7.00 ± 0.00 ^{AB}	3.33 ± 0.41 ^A
30 ppt	% ± SE	95.56 ± 3.60 ^{ab}	81.11 ± 1.36 ^a	71.11 ± 5.44 ^a	62.22 ± 4.91 ^{ab}	44.44 ± 4.91 ^a	36.67 ± 2.36 ^a
	Du ± SE	4.00 ± 0.00 ^B	4.67 ± 0.41 ^{BC}	4.00 ± 0.00 ^B	6.67 ± 0.41 ^A	7.00 ± 0.00 ^{AB}	3.33 ± 0.41 ^A
32 ppt	% ± SE	96.67 ± 2.36 ^{ab}	78.89 ± 3.60 ^a	65.56 ± 5.93 ^a	60.00 ± 4.71 ^{ab}	37.78 ± 2.72 ^{ab}	27.78 ± 4.91 ^{bc}
	Du ± SE	4.00 ± 0.00 ^B	4.00 ± 0.00 ^C	4.00 ± 0.00 ^B	6.67 ± 0.41 ^A	6.67 ± 0.41 ^B	3.33 ± 0.41 ^A
34 ppt	% ± SE	98.89 ± 1.36 ^a	77.78 ± 3.60 ^a	71.11 ± 3.60 ^a	57.78 ± 3.60 ^{ab}	34.44 ± 3.60 ^b	24.44 ± 1.36 ^c
	Du ± SE	4.00 ± 0.00 ^B	4.00 ± 0.00 ^C	4.00 ± 0.00 ^B	6.33 ± 0.41 ^A	6.67 ± 0.41 ^B	3.67 ± 0.41 ^A

Means within a given column with different superscripts are significantly different ($P < 0.05$). ¹Values are means ± standard errors from three replicate groups of larvae of the *P. pelagicus* (means ± SE; n = 3) Values; Du, development duration of larval stages (days); initial number: 90 zoea.

In all treatments, 21.11 to 36.67% of larvae successfully metamorphosed to C_1 . In this study, increasing salinity levels up to 30 ppt resulted in the increased of successful metamorphosis to C_1 (36.67%). However, at 32 and 34 ppt., metamorphosis rate to C_1 was reduced to 27.78% and 24.44% respectively. The higher and lowest successful metamorphosis survival of C_1 were obtained at 30 ppt and 26 ppt. The second best metamorphosis survival rate was found at 28 ppt and it was not significantly different ($P > 0.05$) compared to at 30 ppt. The development duration of C_1 were relatively shorter (3.33-4.00 days) at all salinity levels with no significant differences ($P > 0.05$). The relationships between salinity and successful metamorphosis and development duration of C_1 were quadratic ($SuMpC_1 = -0.7739S^2 + 46.4910S - 663.3400$; $R^2 = 0.5885$; $P < 0.05$; Figure 12 and $DuC_1 = 0.0357S^2 - 2.1762S + 36.3900$; $R^2 = 0.2653$; $P < 0.05$; Figure 13) respectively.

Salinity tolerance among decapod larvae varies with the different life stages. In general, salinity is not as important as temperature to egg development and hatching, but the larvae are highly sensitive to the changes in salinity (Buchanan and Milamen, 1969). The present study showed that salinity is an extremely important parameter as it affects development and survival rate of blue swimming crab larvae. Cadman and Weinstein (1988) and Ruscoe *et al.* (2004) have also found that salinity affects the growth of the blue crab *Callinectes sapidus* and mud crab *Scylla serrata* larvae, respectively.

A premetamorphic Z_1 survival, increasing salinity levels showed an increasing trend of survival rate which their relationship was found to be linear (Figure 2). At premetamorphic Z_2 and Z_3 , the relationships between salinity and premetamorphic survival showed a different pattern with that of premetamorphic Z_1 survival.

Increasing salinity beyond the optimum point decreased the total premetamorphic survival rate. However, salinity did not give a significant affect ($P > 0.05$) on the premetamorphic survival. The later

stages, the relationships between of the premetamorphic Z_4 and M survival and successful metamorphosis of C_1 were a similar pattern (a quadratic) to premetamorphic survival of Z_2 and Z_3 . This study showed that 30 ppt gave a higher survival both during the premetamorphic M and C_1 . This would further support the hypothesis that survival rate was correlated with salinity.

Contrary to premetamorphic survival, different salinity levels significantly affected on larval development duration from larval hatching to the premetamorphic Z_1 and Z_3 survival. At the premetamorphic Z_4 survival and C_1 , Salinity did not affect on larval development period. Generally, the number of days from hatching to achievement of each larval stage decreased with increasing salinity, but number of days at lowest salinity of 26 ppt tended to prolonged compare to those at 28-34 ppt.

In general, results of this study suggested that a relatively low salinity (26 ppt) caused a slow larval development and lower survival rate as compared to higher salinities (28, 30 and 34 ppt). Buchanan and Milleman (1969) reported that development duration of prezoa Dugness crab was reduced from about 60 minutes to less those 11 minutes when salinity was increased from 15 ppt to 32 ppt. In that study, no prezoa molt to zoeae at salinity of 10 ppt, but 100% molt at 30 ppt. Reed (1969) also reported that the highest survival of Dugness crab larvae is obtained at 25 to 30 ppt and percentage of survival rate decrease when the larvae held at 15 ppt. Similar results have been reported for *S. serrata* juveniles (Hai *et al.* 1998). He found that a reduction in salinity affects the molting frequency of *S. serrata* juveniles, whereby crabs held between 18 ppt and 30 ppt molt more frequently than crabs held at 6 ppt and 12 ppt, while all crabs held at 0 ppt died. Van Engel (1958) also stated that larvae of blue crab (*Callinectes sapidus*) may hatch prematurely and die in the prezoal stage, when salinity is very low.

The optimal salinity for survival of *S. serrata* larvae is obtained 32 ppt (Baylon *et al.* 2001). In contrast, Hamasaki (2003) found that larval molting is most synchronized at 29°C, so that optimum

temperature for larval rearing of *S. serrata* seem to be 29°C from the viewpoint of prevention of cannibalism in a mass seed production tank. Based on the findings of this experiment, blue swimming

crab *Portunus pelagicus* larvae should be reared at salinities ranging from 28 to 30 ppt in order to achieve optimal growth and survival.

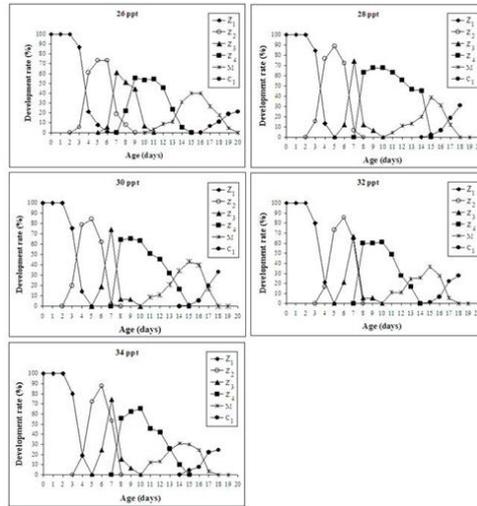


Fig. 1: Rate of larval development of *P. pelagicus* reared at 26 ppt, 28 ppt, 30 ppt, 32 ppt and 34 ppt.

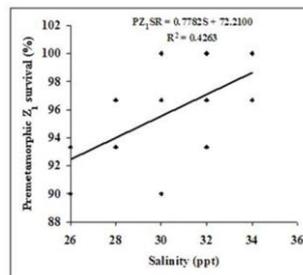


Fig. 2: Relationship between salinity (S) and premetamorphic Z₁ survival rate (PZ₁SR) of *P. pelagicus* larvae.

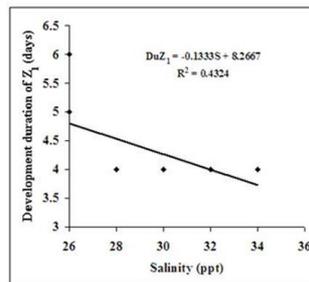


Fig. 3: Relationship between salinity (S) and development duration of *P. pelagicus* Z₁ larvae (DuZ₁).

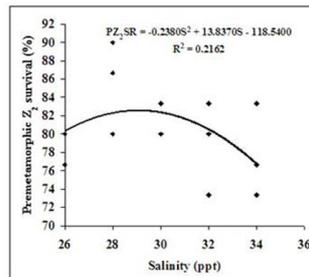


Fig. 4: Relationship between salinity (S) and premetamorphic Z₂ survival rate (PZ₂SR) of *P. pelagicus* larvae.

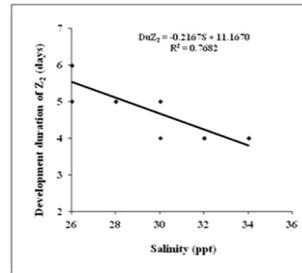


Fig. 5: Relationship between salinity (S) and development duration of *P. pelagicus* Z_2 larvae (DuZ_2).

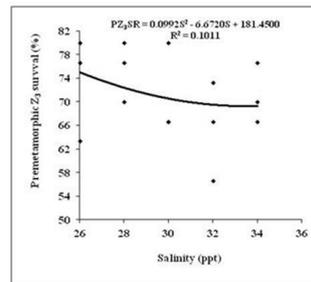


Fig. 6: Relationship between salinity (S) and premetamorphic Z_3 survival rate (PZ_3SR) of *P. pelagicus* larvae.

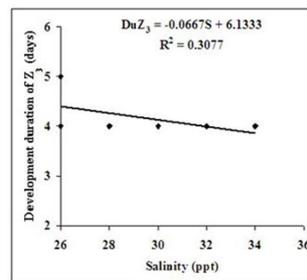


Fig. 7: Relationship between salinity (S) and development duration of *P. pelagicus* Z_3 larvae (DuZ_3).

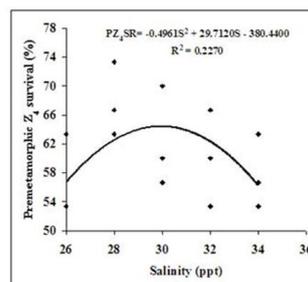


Fig. 8: Relationship between salinity (S) and premetamorphic Z_4 survival rate (PZ_4SR) of *P. pelagicus* larvae.

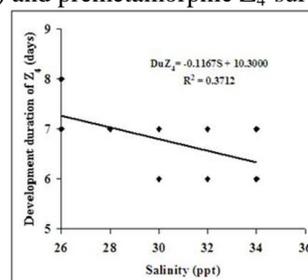


Fig. 9: Relationship between salinity (S) and development duration of *P. pelagicus* Z_3 larvae (DuZ_3).

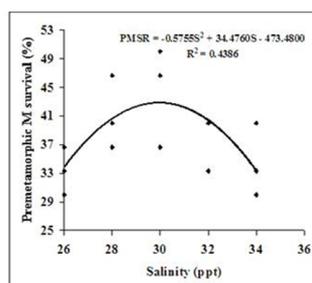


Fig. 10: Relationship between salinity (S) and premetamorphic M survival rate (PMSR) of *P. pelagicus* larvae.

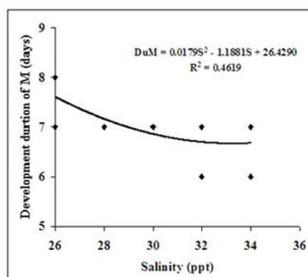


Fig. 11: Relationship between salinity (S) and development duration of *P. pelagicus* M larvae (DuM).

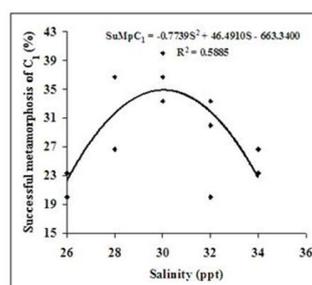


Fig. 12: Relationship between salinity (S) and successful metamorphosis of *P. pelagicus* C₁ larvae (SuMpC₁).

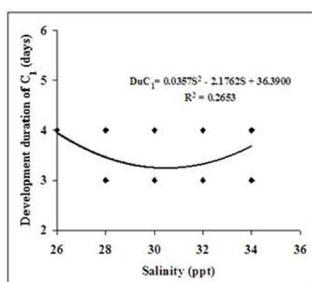


Fig. 13: Relationship between salinity (S) and development duration of *P. pelagicus* C₁ larvae (DuC₁).

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