

# Effects of stocking density on survival rate and larval development of blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758) under laboratory conditions

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**Abstract.** The objective of the present study was to evaluate the effects of stocking density on survival and larval development of *Portunus pelagicus* (Linnaeus, 1758) under laboratory conditions. First zoeal crabs were stocked in triplicate glass beaker at densities of 20, 30, 40, 50, and 60 larvae L<sup>-1</sup>. The result showed that high stocking density (40-60 larvae L<sup>-1</sup>) consistently produced low successful metamorphosis of first crab instar (19.11-20.00%). The low stocking density level (20-30 larvae L<sup>-1</sup>) tested in this study resulted in slightly higher C<sub>1</sub> survival ranging from 22.67 to 23.11%. However, no significant difference ( $p > 0.05$ ) was found in the treatments. The relationships between stocking density and larval development duration and successful metamorphosis of C<sub>1</sub> were linear and quadratic respectively. This aims to generate further information on the relationship between stocking density and survival of C<sub>1</sub> *P. pelagicus*. The result of this work will therefore provide a basis for future research on the effects of stocking density on the survival of C<sub>1</sub> *P. pelagicus*.

**Key Words:** crab instar, *Portunus pelagicus*, metamorphosis, zoeal.

**Introduction.** The blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758), supports substantial commercial fisheries and is an important component of many recreational fisheries in Australia and other parts of the world. In Australia, since 1991/1992 the commercial catch is increasing substantially, from 215 tons in that season to 740 tons in 1997/1998, principally due to the use of pots and improved technology (Kangas 2000). Increasing market prices and new market opportunities have also strongly been driving commercial interest to the species (Efrizal 2014; Hamid et al 2016). Because blue swimming crabs are high-valued and readily marketed worldwide, the economic potential for farming them is promising. However, little information on culture methods is available as most landing comes from the wild.

In general, maximization of crab farm yield has partially relied on the perfecting of exploitation and husbandry methods. In this regard, elevation of rearing density was initially viewed as a promising approach, which combined the maximization of water use with increased stock production. The use of high stocking density as a technique to maximize water usage and thus increase stock production in fish culture has been shown to exert severe adverse effects on growth (Fenderson & Carpenter 1971; Allen 1974; Refstie & Kittelsen 1976; Refstie 1977; Trzebiatowski et al 1981; Leatherland & Cho 1985) and performance of teleosts (Schreck et al 1985). In addition, density was also found to be a potential source of stress (Refstie 1977; Jobling 1985; Gatlin et al 1986; Vijayan & Leatherland 1988; Pickering 1993), capable of having consequential repercussions of fish growth rate (Papoutsoglou et al 1979, 1987; Holm et al 1990; Ross & Watten 1988). Acute and chronic stress trigger a series of defense mechanisms that are generally energy demanding and therefore induce an elevation of the animal metabolic rate (Barton & Iwama 1991). These are likely to be the source of growth depletion (Vijayan & Leatherland 1988; Jorgensen et al 1993). However, limited

information describing the effects of stocking density during the premetamorphic stages of crab larvae exists. The effect of stocking density on growth performance has been reported on several species of fish; for example, Azhari et al (2017) reported that the stocking density influenced the growth performance and survival rate of climbing perch *Anabas testudineus* and on common carp *Cyprinus carpio* (Widiastuti 2009). Hence, the objective of the present study is to examine the effects of stocking density on survival rate and larval development of blue swimming crab, *P. pelagicus* (Linnaeus, 1758), under cultured conditions.

## Material and Method

**Time and site.** Experiment was carried out at the Hatchery of Marine Research Station, Port Dickson, Negeri Sembilan, Malaysia from August to December 2014.

**Experimental design.** There is an ovigerous female, with mean body weight (BW) of 268.30 g and carapace width of 140.58 mm that spawned naturally by manipulating its environment in cylindrical black plastic tanks (Efrizal et al 2015), after which the female was transferred in 300 L circular fiberglass tank for incubation and hatching of eggs. The water quality in the incubation and hatching of eggs was maintained using a flow-through system ( $2 \text{ L min}^{-1}$ ) and gentle aeration. Samples of ova from ovigerous crab were examined at 2-3 day intervals. When hatching appeared imminent, the incoming and outgoing water were stopped. The hatched larvae were collected from dense photopositive aggregation at the surface using a bowl net. They were reared into triplicate beaker glass containers at different densities of 20, 30, 40, 50 and 60 larvae  $\text{L}^{-1}$ . Each container was filled with 3 L of sea water and provided an air supply.

The first zoea stage was fed a mixture of microalga *Nannochloropsis oculata* ( $5 \times 10^3 \text{ cells mL}^{-1}$ ), rotifers *Brachionus plicatilis* ( $5 \text{ ind mL}^{-1}$ ) and *Artemia* nauplii ( $5 \text{ ind mL}^{-1}$ ). A combination of rotifers *B. plicatilis* ( $5 \text{ ind mL}^{-1}$ ) + *Artemia* nauplii ( $5 \text{ ind mL}^{-1}$ ) and *Artemia* nauplii ( $10 \text{ ind mL}^{-1}$ ) alone were given in the  $Z_2$  and  $Z_3$  to first crab stage, respectively (Efrizal 2015).

**Water quality maintenance.** The salinity was maintained at 30 ppt salinity and a temperature at 27 to 28°C. Seawater collected from Port Dickson waters was successively filtered through a series of cartridge filters (10 and 5  $\mu\text{m}$ ) before being used. Organisms were maintained under continuous light conditions between 3000 and 3500 lx, and dissolved oxygen above 5.6 ppm. The range of pH was 7.94-8.07.

Daily water exchange was 100% in all the experimental tanks, with feces, 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.

**Data analysis.** The data of survival rate (%), premetamorphic survival, and successful metamorphosis) and duration of larval stages (days) was tested using one way ANOVA and followed by Duncan's Multiple Range test was used to compare the mean differences among treatments (Steel & Torrie 1980). Arcsine transformation was done in the analysis of the data in percentage.

**Results.** Premetamorphic survival and duration of larval stage of blue swimming crab reared under different stocking densities are summarized in Table 1. Changes in the number of respective larval stages with time after hatching are shown in Figure 1. Increasing densities from 20 to 60 larvae  $\text{L}^{-1}$  increased the premetamorphic  $Z_1$  survival ranging from 93.33 to 97.56%. However, no significant differences ( $p > 0.05$ ) were found within the treatments. The relationship between density and premetamorphic  $Z_1$  survival was found to be linear ( $PZ_1SR = 0.0916D + 92.0110$ ;  $R^2 = 0.3014$ ;  $p < 0.05$ ; Figure 2a). Mean development duration for 20 larvae  $\text{L}^{-1}$  (4.33 days) was significantly shorterst ( $p < 0.05$ ) than those for 40 to 60 larvae  $\text{L}^{-1}$  (5.33-6.00 days). The relationship was also linear ( $DuZ_1 = 0.0400D + 3.6667$ ,  $R^2 = 0.6923$ ,  $p < 0.05$ ; Figure 2b).

Mean surviving premetamorphic  $Z_2$  (Table 1) increased from 20 to 30 larvae  $L^{-1}$  and then started to decrease from 40 to 60 larvae  $L^{-1}$ . The premetamorphic survival at  $Z_2$  showed a polynomial cubic response to stocking density ( $PZ_2SR = 0.0018D^3 - 0.2188D^2 + 8.0543D - 10.3480$ ;  $R^2 = 0.674$ ;  $p < 0.05$ ; Figure 2c). Mean premetamorphic survival at densities 20 to 30 larvae  $L^{-1}$  was significantly higher ( $p < 0.05$ ) than those stocked at 50 to 60 larvae  $L^{-1}$ . The relationship between stocking density and development duration of  $Z_2$  was linear ( $DuZ_2 = 0.0333D + 4.0000$ ;  $R^2 = 0.6250$ ;  $p < 0.05$ ; Figure 2d).

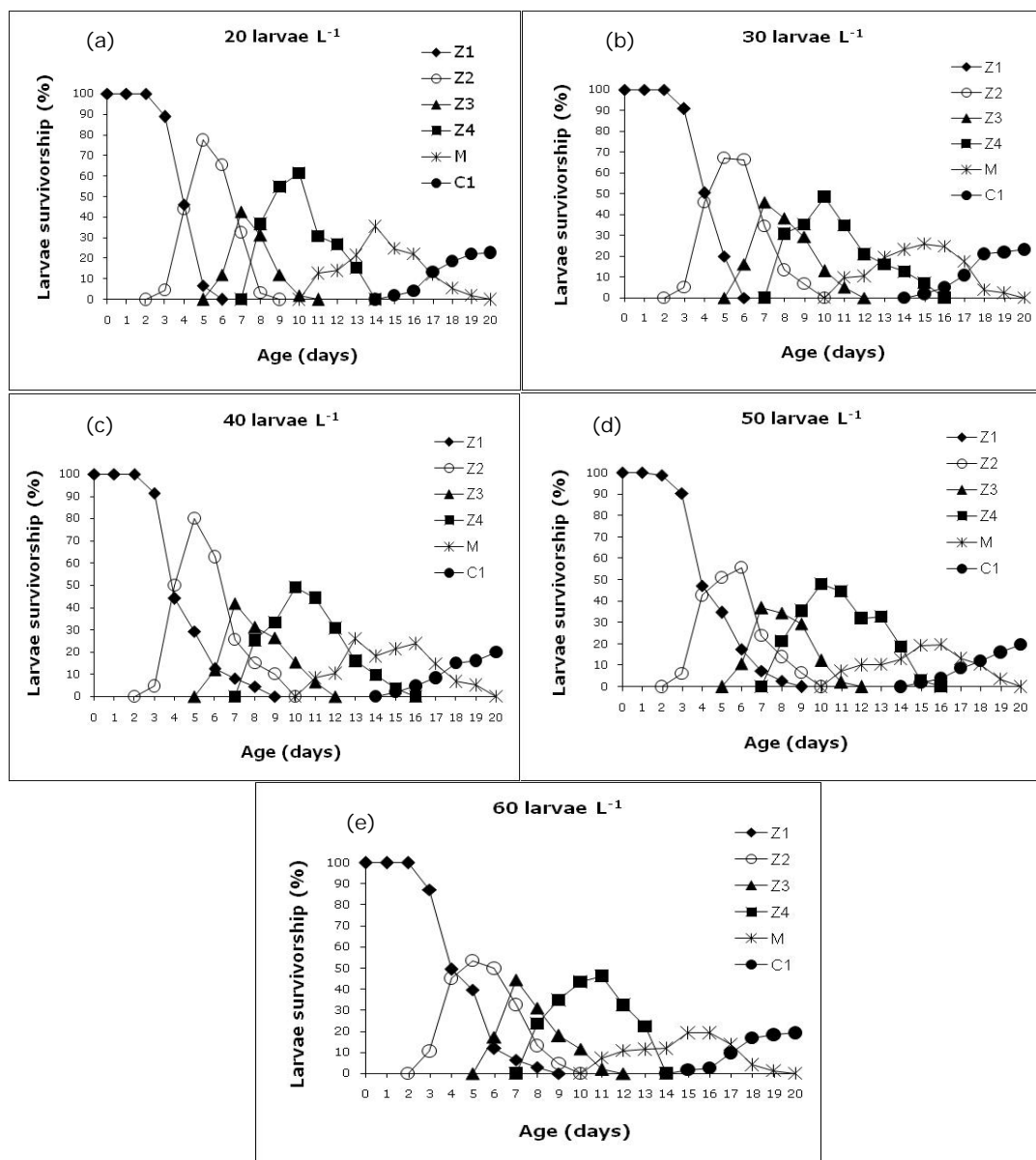


Figure 1. Survival rate and stage of larvae *P. pelagicus* reared at (a) stocking density 20 larvae  $L^{-1}$ , (b) stocking density 30 larvae  $L^{-1}$ , (c) stocking density 40 larvae  $L^{-1}$ , (d) stocking density 50 larvae  $L^{-1}$ , and (e) stocking density 60 larvae  $L^{-1}$ .

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 densities

Density	Variable	$Z_1$	$Z_2$	$Z_3$	$Z_4$	$M$	$C_1$
20 larvae L <sup>-1</sup>	%±SE	93.33±2.16 <sup>a</sup>	77.33±2.94 <sup>a</sup>	68.00±2.45 <sup>a</sup>	43.33±2.16 <sup>b</sup>	26.67±2.16 <sup>ab</sup>	22.67±3.56 <sup>a</sup>
	Du±SE	4.33±0.41 <sup>C</sup>	4.67±0.41 <sup>C</sup>	4.00±0.00 <sup>C</sup>	5.33±0.41 <sup>A</sup>	7.33±0.41 <sup>B</sup>	4.33±0.41 <sup>B</sup>
30 larvae L <sup>-1</sup>	%±SE	95.56±2.18 <sup>a</sup>	82.22±1.96 <sup>a</sup>	68.44±1.44 <sup>a</sup>	44.44±1.96 <sup>b</sup>	27.56±3.31 <sup>a</sup>	23.11±2.37 <sup>a</sup>
	Du±SE	5.00±0.00 <sup>BC</sup>	5.00±0.00 <sup>BC</sup>	4.67±0.41 <sup>BC</sup>	5.67±0.41 <sup>A</sup>	7.33±0.41 <sup>B</sup>	4.67±0.41 <sup>AB</sup>
40 larvae L <sup>-1</sup>	%±SE	95.67±0.41 <sup>a</sup>	74.67±3.56 <sup>ab</sup>	55.67±2.04 <sup>b</sup>	52.67±2.68 <sup>a</sup>	23.33±2.27 <sup>ab</sup>	20.00±1.41 <sup>a</sup>
	Du±SE	5.33±0.41 <sup>AB</sup>	5.33±0.41 <sup>ABC</sup>	5.00±0.00 <sup>AB</sup>	5.67±0.41 <sup>A</sup>	8.00±0.00 <sup>AB</sup>	5.33±0.41 <sup>AB</sup>
50 larvae L <sup>-1</sup>	%±SE	96.27±1.82 <sup>a</sup>	66.40±1.70 <sup>b</sup>	55.73±2.79 <sup>b</sup>	52.00±1.50 <sup>a</sup>	21.07±1.99 <sup>b</sup>	19.73±1.42 <sup>a</sup>
	Du±SE	5.67±0.41 <sup>AB</sup>	5.67±0.41 <sup>B</sup>	5.33±0.41 <sup>AB</sup>	6.00±0.00 <sup>A</sup>	8.67±0.41 <sup>A</sup>	5.67±0.41 <sup>A</sup>
60 larvae L <sup>-1</sup>	%±SE	97.56±0.98 <sup>a</sup>	66.89±6.28 <sup>b</sup>	54.67±2.16 <sup>b</sup>	53.33±1.63 <sup>a</sup>	20.67±1.4 <sup>b</sup>	19.11±1.44 <sup>a</sup>
	Du±SE	6.00±0.00 <sup>A</sup>	6.00±0.00 <sup>A</sup>	5.67±0.41 <sup>A</sup>	6.00±0.00 <sup>A</sup>	8.67±0.41 <sup>A</sup>	5.67±0.41 <sup>A</sup>

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

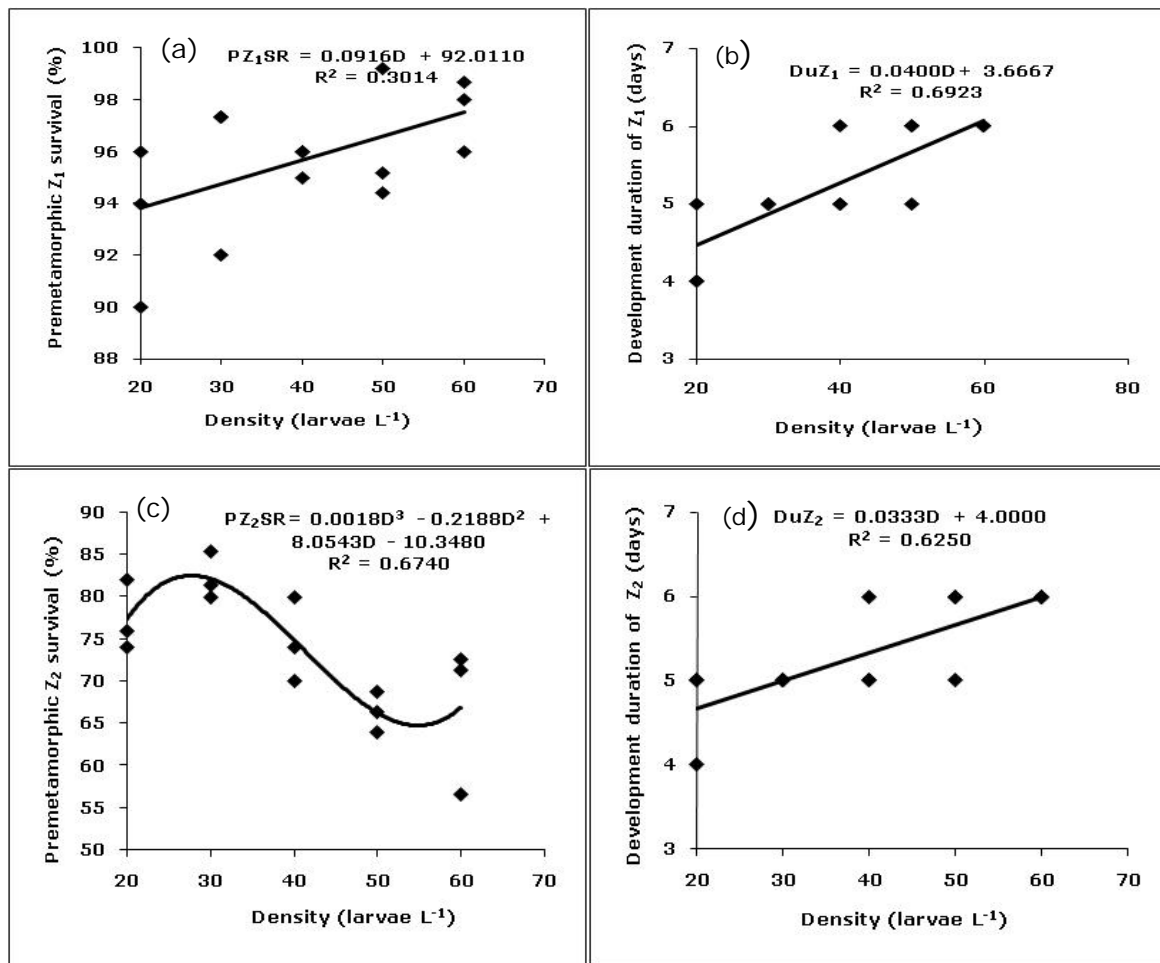


Figure 2. (a) Relationship between density and premetamorphic  $Z_1$  survival rate ( $PZ_1SR$ ) of *P. pelagicus* larvae, (b) Relationship between density and development duration of *P. pelagicus*  $Z_1$  larvae ( $DuZ_1$ ), (c) Relationship between density and premetamorphic  $Z_2$  survival rate ( $PZ_2SR$ ) of *P. pelagicus* larvae, and (d) Relationship between density and development duration of *P. pelagicus*  $Z_2$  larvae ( $DuZ_2$ ).

The relationship between stocking density and the premetamorphic  $Z_4$  survival was cubic ( $PZ_4SR = -0.0006D^3 + 0.7580D^2 + 29.6890D$ ;  $R^2 = 0.6672$ ;  $p < 0.05$ ; Figure 3c). The highest premetamorphic  $Z_4$  survival obtained at 20 to 30 larvae  $L^{-1}$  (43.33-44.44%) was significantly lower ( $p < 0.05$ ) than those of 40 to 60 larvae  $L^{-1}$  (52.00-53.33%). The duration of larvae at this phase was 5.33 to 6.00 days and no significant difference ( $p > 0.05$ ) was found among treatments. However, the relationship between stocking density and development duration of  $Z_4$  was linear ( $DuZ_4 = 0.0167D + 5.0667$ ;  $R^2 = 0.2841$ ;  $p < 0.05$ ; Figure 3d).

The influence of stocking density was more evident on survival and the larval development during the M stages. Higher in density (40 to 60 larvae  $L^{-1}$ ) caused a reduction of premetamorphic M survival (20.67-23.33%) with a longer larval development (8.00-8.67 days) compared to 26.67-27.56% (7.33 days) at 20 to 30 larvae  $L^{-1}$  (Table 1). Analysis of Duncan's multiple range test (DMRT) showed that mean premetamorphic M survival at stocking density 30 larvae  $L^{-1}$  was significantly higher ( $p < 0.05$ ) than those of 50 and 60 larvae  $L^{-1}$ . The relationship between stocking density and premetamorphic survival and development duration of M were quadratic ( $PMSR = -0.0004D^2 - 0.1496D + 30.6350$ ;  $R^2 = 0.4532$ ;  $p < 0.05$ ; Figure 4a) and linear ( $DuM = 0.0400D + 6.4000$ ;  $R^2 = 0.6000$ ;  $p < 0.05$ ; Figure 4b) respectively. In general, the high stocking density (40-60 larvae  $L^{-1}$ ) consistently produced low successful metamorphosis throughout the experiment resulting in only 19.11 to 20.00% final survivals at  $C_1$ . However, no significant ( $p > 0.05$ ) differences were observed among treatments. A cubic relationship of  $SuMpC_1 = 0.0003D^2 - 0.0315D^2 + 1.0453D + 12.375$ ;  $R^2 = 0.2629$ ;  $p <$

0.05 (Figure 4c) was found between stocking density and  $C_1$  survival. Mean larval development duration had a positive linear correlation ( $Du_{C_1} = 0.0367D + 3.667$ ;  $R^2 = 0.5216$ ;  $p < 0.05$ ; Figure 4d) with stocking density.

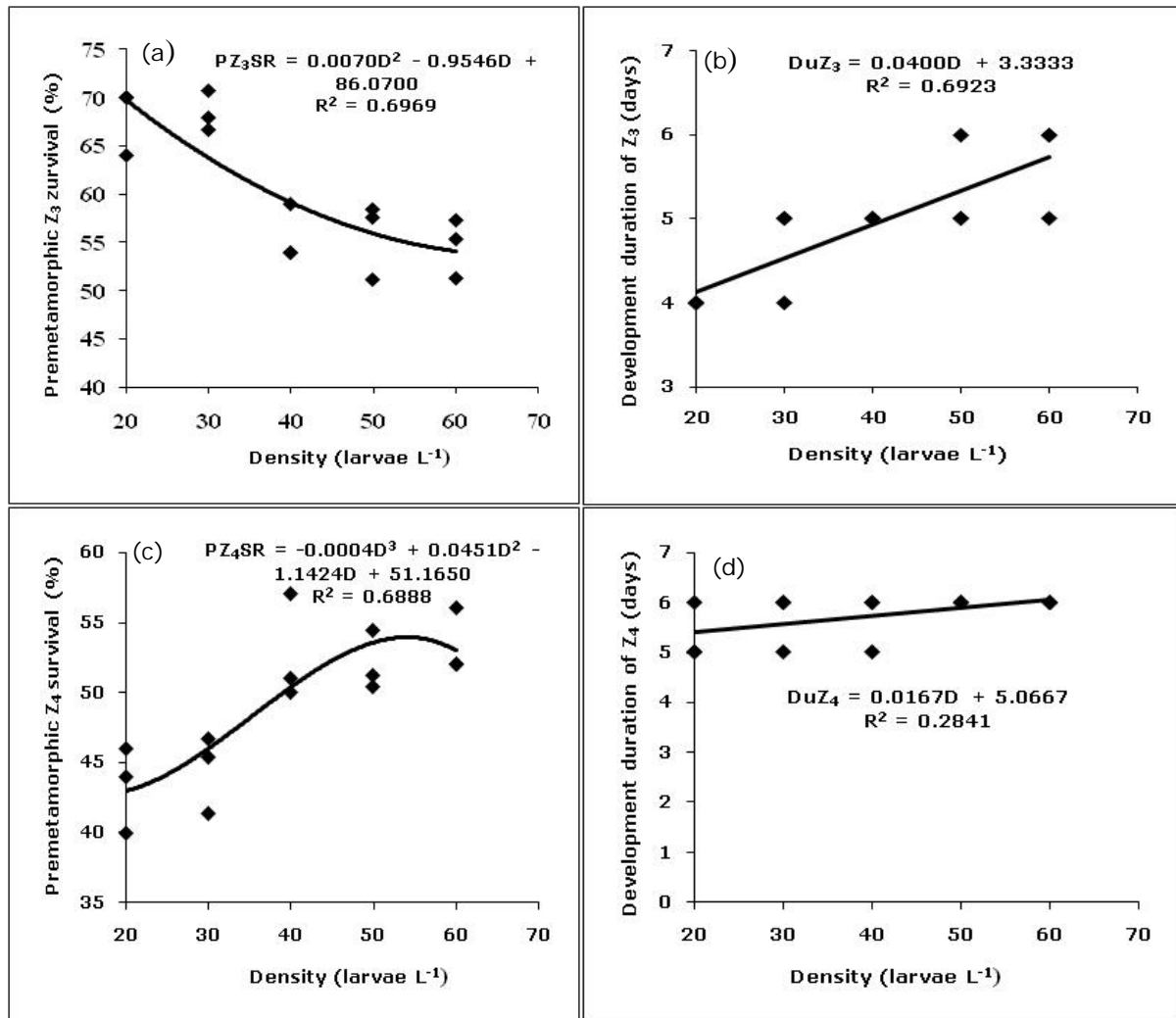


Figure 3. (a) Relationship between density (D) and premetamorphic  $Z_3$  survival rate (PZ<sub>3</sub>SR) of *P. pelagicus* larvae, (b) Relationship between density (D) and development duration of *P. pelagicus*  $Z_3$  larvae (DuZ<sub>3</sub>), (c) Relationship between density (D) and premetamorphic  $Z_4$  survival rate (PZ<sub>4</sub>SR) of *P. pelagicus* larvae, and (d) Relationship between density (D) and development duration of *P. pelagicus*  $Z_4$  larvae (DuZ<sub>4</sub>).

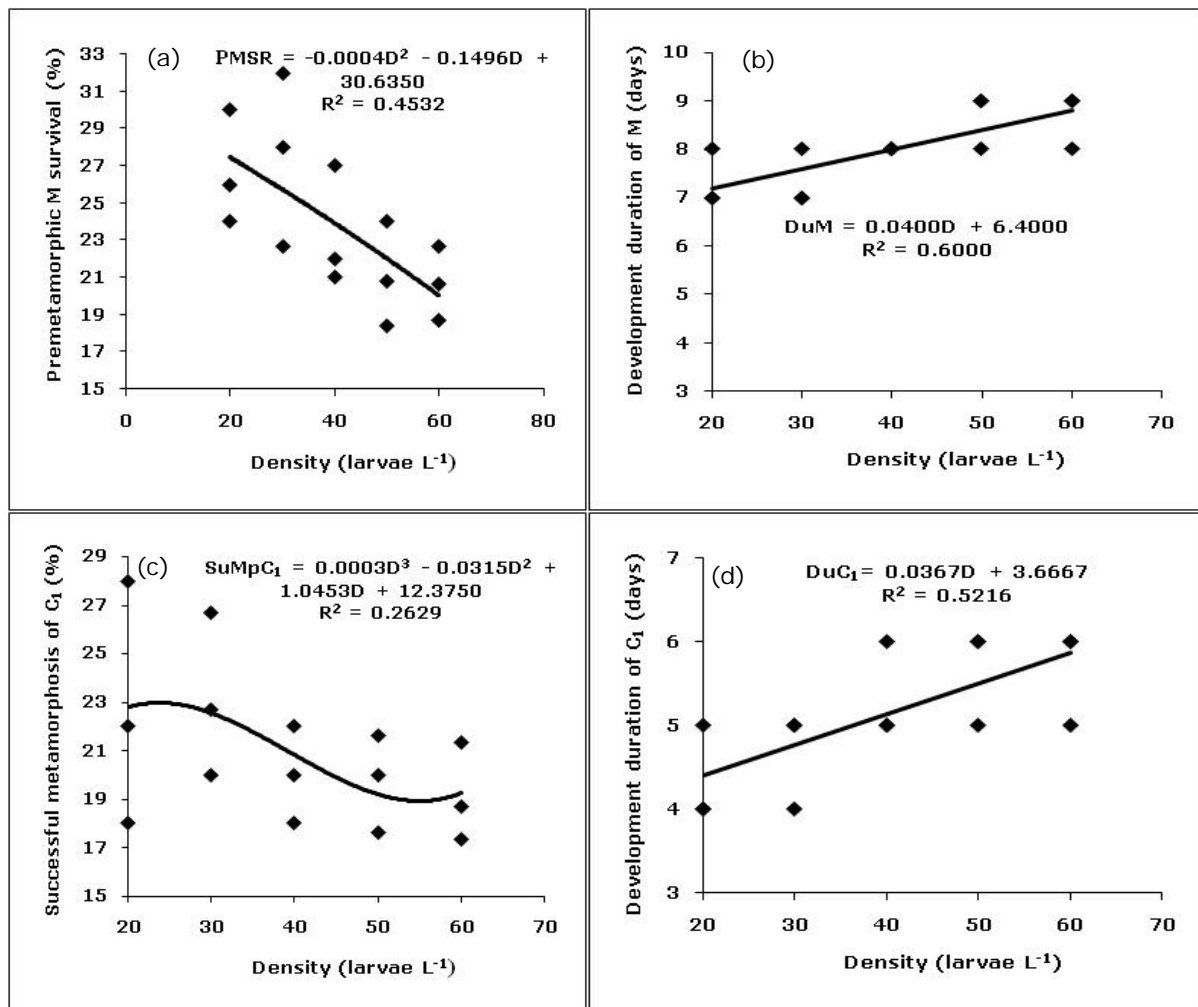


Figure 4. (a) Relationship between density (D) and premetamorphic M survival rate (PMSR) of *P. pelagicus* larvae, (b) Relationship between mean density and development duration of *P. pelagicus* M larvae, (c) Relationship between density (D) and successful metamorphosis of *P. pelagicus* C<sub>1</sub> larvae (SuMpC<sub>1</sub>), (d) Relationship between mean salinity and development duration of *P. pelagicus* C<sub>1</sub> larvae.

**Discussion.** It is very little known on the effects of stocking density on survival and larval development of crab belonging to genus *Portunus* despite its great economic value. However, stocking density effects on marine and freshwater fish have largely been investigated. Most of these studies showed that stocking density has shown similar effects on the growth rate in teleosts.

There are several possible factors which act alone, or in combination, to impair the growth rate of fish (Vijayan & Leatherland 1988), shrimp (Allan & Maguire 1992) and crab (Penha-Lopes et al 2006) stocked at high densities (Vijayan & Leatherland 1988). These include behavioral factors such as social interactions, the development of hierarchies and establishment of territorial borders (Symons 1970; Fenderson & Carpenter 1971; Refstie & Kittelsen 1976). The formation of hierarchies may lead to reduced feeding by low-ranking fish and in suppression of growth in these individuals (Ejike & Schreck 1980; Jobling 1985; Koebele 1985). High levels of spontaneous activity associated with agonistic behaviour have been shown to cause elevated metabolic rates in juvenile sockeye salmon (*Oncorhynchus nerka*) and rainbow trout (*Oncorhynchus mykiss*) (Brett 1964; Li & Brocksen 1977), possibly leading to decreased food utilization efficiency (Fagerlund et al 1981; Papoutsoglou et al 1987).

The results of this study suggested that stocking density has a marked effect on the premetamorphic survival and development duration of all of the blue swimming crab larvae. At Z<sub>1</sub>, stocking density did not influence the premetamorphic Z<sub>1</sub> survival. However, development duration of Z<sub>1</sub> was affected by stocking density ( $p < 0.05$ ), where

the stocking densities of 20 to 30 larvae L<sup>-1</sup> provided relatively shorter (1 to 2 days) development duration compared to the high density (40-60 larvae L<sup>-1</sup>). However, since there was no significant difference regarding survival, perhaps this may be due to adaptability of larvae to varying stocking densities.

The effects of stocking density on larval survival could be seen when the Z<sub>1</sub> larvae metamorphosed to the premetamorphic Z<sub>2</sub>. At this stage, the low stocking density of 20 to 30 larvae L<sup>-1</sup> gave a relatively high premetamorphic Z<sub>2</sub> survival of 77.33-82.22% compared to 66.40-74.67% at 40 to 60 larvae L<sup>-1</sup>. The significant effect might be due to interaction factors among the larvae for either space utility or competition of food and the lower feeding rate. The lower feeding rate at high density may be due to the decreased efficiency in search of food as proposed by Refstie & Kittelsen (1976). In aquacultured larvae crabs, food availability (quantity and quality) (Hartnoll 1982; Sheen 2000), overcrowding stress (Wilber & Wilber 1991; Dittel et al 1995) and cannibalism (Sainte-Marie & Lafrance 2002) seem to be the major factors influencing survivorship.

At the premetamorphic Z<sub>3</sub> survival, the effects of stocking densities were clearly demonstrated. At this stage, the high stocking density of 40-60 larvae L<sup>-1</sup> provided the lower premetamorphic survival and longer development duration than the low stocking density of 20-30 larvae L<sup>-1</sup>. Penha-Lopes et al (2006) also reported that the growth of juvenile *Mithraculus forceps* is strongly affected by stocking density. Poor survival rates at the higher densities may be associated with intensive agonistic behaviour among the crabs (Sainte-Marie & Lafrance 2002). Other factors such as sub-lethal injuries (e.g. limb autotomy), stress and interactions may also affect molt increment by reducing the energy available to growth (Sainte-Marie & Lafrance 2002). Although interactions were not observed during the day, the crabs are generally more active during the night (Reigada & Negreiro-Fransozo 2001). In the present study, most of the dead blue swimming crab larvae were found when checked in the morning. Sub-lethal injuries did not seem to be the major cause because of the low incidents of limb loss observed among the living and dead blue swimming crab larvae. Therefore, the high interaction between the crab larvae and the consequent physiological stress appear to be the main causes of the lower growth rate observed at high culture densities.

Contrary to premetamorphic Z<sub>3</sub> survival, at Z<sub>4</sub> stage high at stocking density of 40-60 larvae L<sup>-1</sup> gave the better premetamorphic survival than the low density (20-30 larvae L<sup>-1</sup>). In this study, different stocking densities had a more stable effect upon survival rate after the larvae developed into premetamorphic M survival and successful metamorphosis to C<sub>1</sub>. At this stage, the low densities of 20 to 30 larvae L<sup>-1</sup> consistently gave the highest premetamorphic M survival and successful metamorphosis to C<sub>1</sub> compared to high stocking densities (40 to 60 larvae L<sup>-1</sup>). Yet, at the end of the experiment, different stocking density did not show any significant effect on successful metamorphosis to C<sub>1</sub> and this was probably due to high mortality of the premetamorphic Z<sub>4</sub> survival at 20 to 30 larvae L<sup>-1</sup>, thus having a great influence on the survival rate of M and C<sub>1</sub>.

**Conclusions.** The high stocking density (40-60 larvae L<sup>-1</sup>) consistently produced low successful metamorphosis of first crab instar (C<sub>1</sub>), while the low stocking density (20-30 larvae L<sup>-1</sup>) resulted in higher of C<sub>1</sub> survival rate. There was a linear relationship between stocking density and larval development duration, and a quadratic relationship between stocking density and successful metamorphosis of C<sub>1</sub>.

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