FULL PAPER

Growth, Development, and Survival of *Portunus pelagicus* Larvae and Juveniles in Different Feed Regimens, Rearing Media, and Stocking Densities

Nonita S. Cabacaba[1,2,*], Kimberly A. Badocdoc[1], Ed-Marie B. Boiser[1], Cristan Joy M. Campo[1], Shaira Lyle A. Josue[1]

[1]*National Fisheries Research and Development Center – Marine Fisheries Research andDevelopment Center (NFRDI-MFRDC)*
[2]*Bureau of Fisheries and Aquatic Resources – Guiuan Marine Fisheries Development Center (BFAR-GMFDC), Guiuan, Eastern Samar 6809*

─ A B S T R A C T ─

Various factors in *P. pelagicus* seed production and grow-out culture were investigated in this study. Experiments were conducted to 1) compare natural and artificial feeds for larval production, 2) evaluate indoor tank and outdoor net cages as rearing media, and 3) assess different stocking densities for grow-out culture. Growth, development, and survival were assessed for the performance of each variable. *(I)* In larval production, the development of megalopa larvae into crab instar was synchronous in both natural and artificial feed treatments in a 5-day rearing observation. Crab instars began to appear on Day 4 with 27% composition in both feeds. Although survival appeared to be relatively higher in natural feed (43.96±6.04%), this was not significant from survival in artificial feed treatment (33.33±13.34), (t=0.726, p>0.05). *(II)* In Phase I grow-out culture, a two-variable design experiment was conducted to assess growth performance and survival of crab juveniles reared in indoor tanks and outdoor net cages at different stocking densities. Specific growth rate (SGR) differed significantly (t=2.937, p<0.05) between indoor tanks (6.39±0.24%.d^{-1}) and outdoor net cages (8.31±1.11%.d^{-1}). However, mean survival rate was better in indoor tanks (20.83 ± 9.24%) than outdoor net cages (8.94±3.58% only), (t=2.938, p=0.015). In terms of stocking density, SGR was highest in 75 ind.m^{-2} (7.87±2.44%.d^{-1}). However, growth performance and survival of juveniles among different stocking densities were not significantly different (p>0.05). Furthermore, two-factor ANOVA results have shown that growth performance of the juveniles was influenced by both the differences in rearing medium and stocking density, but not in terms of survival (F=0.120, p=0.888). *(III)* In Phase II grow-out culture, juveniles attained highest SGR (3.54±0.56%.d^{-1}) at 5 ind.m^{-2} stocking density. This was followed by 15 ind.m^{-2} (3.45±2.39%.d^{-1}) and by 10 ind.m^{-2} (2.33±0.50%.d^{-1}) (p>0.05). However, survival rate was highest in 15 ind.m^{-2} (46.67±0.00%), but the differences among other stocking densities were not statistically significant (p>0.05). Overall, results suggest that artificial feed can be an alternative for *Artemia* in rearing megalopae to crab instar stage. Stocking density in Phase I and II grow-out culture did not substantially affect growth performance and survival of juvenile *P. pelagicus*. However, higher stocking density increases incidence of cannibalism among reared crabs.

1. INTRODUCTION

The blue swimming crab, Portunus pelagicus, is widely distributed throughout the coastal and estuarine areas of the Indo-Pacific region (Ikhwanuddin et al. 2011b). It supports significant commercial and recreational fisheries that sustain a large percentage of coastal communities within a wide geographic range (Moller et al. 2008). However, the increasing demand from both local and international markets has created a considerable strain on the wild population. Heavy fishing pressure coupled with

urbanization have resulted in natural habitat loss and reduced water quality. This in turn resulted in the significant decline of natural stock. In many countries particularly in Asia, this is an imminent problem that is set to create a massive impact on the blue swimming crab resources (Ikhwanuddin et al. 2012), which could threaten the livelihood of communities that depend on the blue swimming crab fishery.

Crab aquaculture has been established in response to the current decline in crab fishery (Andrés et al. 2010). Today, it is considered a very promising industry that is expected to generate high economic gains in the future (Moller et al. 2008). In many countries such as Malaysia and the Philippines, however, mass production remains almost entirely dependent on the collection of wild crab juveniles (Ikhwanuddin et al. 2011b). Rather than solve the problem, this practice only creates an even greater pressure on the wild population. In order to improve and sustain the now depleted wild stock and create a sustainable blue swimming crab industry, the generation of a reliable seed production technology is of utmost importance.

The basic technology for portunid crab seed production has been existing for some time. Since the early 1980s, *P. pelagicus* had already been extensively studied. However, results remained inconsistent (Laviña and Buling 1980; Sukumaran and Neelakantan 1996; Josileen and Menon 2004; Romano and Zeng 2006; Soundarapandian et al. 2007; Castine et al. 2008; Andrés et al. 2010; Ikwanuddin et al. 2011a; Ikwanuddin et al. 2011b). Moreover, seed production of *P. pelagicus* had been mostly experimental and a standardized hatchery protocol is yet to be published (Ikhwanuddin et al. 2012).

Several factors were identified as probable causes of larval and early juvenile mortality in *P. pelagicus*. These include poor water management, fluctuating water parameters (especially temperature and salinity level), food type, overstocking, and cannibalism. Among the aforementioned, cannibalism is considered as a major problem that greatly affects the survival of larvae and early juveniles. Stocking density is also vital as overstocking often leads to competition for space and food (Iribarne et al. 1994). By addressing major hatchery bottlenecks, the establishment of a reliable seed production technology for *P. pelagicus* is well on its way. Hence, this study aims to create a well-established scheme for seed production and grow-out culture that would increase production and reduce mortality rates. The study specifically aimed: 1) to evaluate different feed types (i.e., artificial feed and *Artemia*) in terms of survival of *P. pelagicus* from megalopa to crab instar; 2) to compare the growth and survival of early juvenile *P. pelagicus* reared at different stocking densities (50, 75, 100 individuals per square meter) in wooden tanks and in hapa net cages; and, 3) to determine the growth and survival of *P. pelagicus* juvenile crabs reared in grow-out cages at different stocking densities (5, 10, and 15 ind.m.$^{-2}$).

2. MATERIALS AND METHODS

2.1 Study Site and Organism

The study was conducted at the BFAR-Guiuan Marine Fisheries Development Center (BFAR-GMFDC), Eastern Samar. Materials for larval and juvenile rearing such as indoor tank systems and outdoor hapa net cages were prepared prior to the experiments. Wild-caught *P. pelagicus* berried crabs were allowed to hatch their eggs, and their offspring were used in the experiments. All crab zoea larvae produced were reared using current MFRDC rearing techniques following modified protocols of Soundarapandian et al. (2007) and Ikhwanuddin et al. (2011a).

2.2. Larval Rearing: Natural and Artificial Feed Experiment

The performance of natural (*Artemia*) and artificial feeds for larval production of *P. pelagicus* was assessed by determining the development and survival of megalopa larvae.

Culture units were held in 8 L transparent aquaria with ambient water temperature of 28-30°C, salinity of 30-35 ppt, pH of 8.0-9.0, and dissolved oxygen of 6 mg/L. These were covered with black cloth to achieve 12 hr-12 hr light and dark condition, and were provided with aeration. About 50% of the water was replenished daily to avoid possible fouling.

Larvae of the same brood were stocked at 20 ind.L^{-1} stocking density and were fed with 5 ind.mL^{-1} density of *Artemia* and with 2 mg commercial artificial feed in separate treatments. Both feeds were administered twice daily, in the morning and in the afternoon. The artificial feed used in this study was composed of 53% protein, 3% crude fiber, 2% calcium, 9% crude fat, 20% crude ash, and 1% phosphorous, and was equivalent to the dry weight portion of *Artemia*.

The development and survival were assessed daily for five days until the larvae reached the crab

instar stage. Each feed treatment was conducted in triplicates and two similar trials of the experiment were done in September 2018.

2.3 Grow-out Culture

2.3.1 Phase I Grow-out

The growth performance and survival rate of *P. pelagicus* juveniles reared in wooden tanks and in hapa net cages, and at different stocking densities were evaluated. Hence, a two-variable design was conducted for this experiment.

Wooden tanks (1 x 1 x 0.5 m) were cleaned and disinfected prior to usage. Sand-filtered seawater, aeration lines, sandy substrate, and B-net shelters were provided in the tanks. Water was replenished by installing a flow-through system. Moreover, outdoor hapa net cages with a dimension of 1 m x 1 m x 1.5 m (l x w x h) were installed in a floating platform at the nearby aquaculture site of BFAR-GMFDC. B-net shelters were also provided in the cages to prevent or lessen cannibalism.

Hatchery-bred 20-day-old juvenile crabs of the same brood with weight ranging from 0.2 to 1.0 g were stocked in both rearing media at 50, 75, and 100 ind.m^{-2} density. Each stocking density was duplicated in both rearing media, hence, a total of 900 juveniles were reared in all of the setups. The juveniles were fed daily with mixed *Acetes* and fish meat at 10% of the total body weight. Growth and survival were monitored weekly.

2.3.2 Phase II Grow-out

The crab juveniles produced in Phase I grow-out (~5 g) were transferred and cultured in 1 m x 1 m x 1.5 m B-net cages for five weeks. The B-net cages were installed at the nearby sea of BFAR-GMFDC at a shallow depth of 0.20-0.75 m. Additional net covers were attached around the cages to avoid other crab species from destroying them.

The crab juveniles were reared at stocking densities of 5, 10 and 15 ind.m^{-2}, each of which were duplicated. *Acetes* and fish meat mixed food were provided daily at 10% of the total body weight of crabs. The growth performance and survival rate of crabs were assessed at the end of rearing.

2.4 Growth, Development, and Survival Assessments

For the larval rearing, the development of megalopae into crab instar was daily monitored and assessed by determining each larval stage's percent composition. Moreover, the percentage of living individuals at the end of rearing was reported as survival rate.

For grow-out assessment, the growth and survival of crabs were monitored weekly. The carapace width and length were measured using a caliper, and the individual body weight was measured using an analytical balance. The growth performance of crabs was assessed by calculating the percent carapace length and width gain, total weight gain, and specific growth rate (SGR). Survival was assessed by determining the percentage of crabs harvested over the number of initially reared individuals.

2.5 Data and Statistical Analysis

Descriptive and inferential statistics were done using IBM SPSS Statistic version 21 software. Descriptive statistics were reported as means, standard deviation, and standard error of the mean, and were presented in tables and graphs. Inferential statistics utilized independent sample t-test for comparing two treatments, one-way ANOVA for three treatments, and two-way ANOVA for two independent variable experiments. Duncan and Tukey post hoc tests were also done. Significance was determined with an alpha level of 0.05.

3. RESULTS AND DISCUSSION

3.1 Natural and Artificial Food for Crab Larvae

The development of megalopae larvae fed with *Artemia* and with artificial feed was synchronous. The percent composition of megalopae and crab instar during the 5-day rearing is presented in Figure 1A and 1B. Larvae in both feeds began to develop into crab instars on Day 4 with crab instar composition of 27.50±9.17% in natural food treatment, and 27.19±9.06% in artificial food treatment, with no significant difference (t=0.024, p>0.05). On Day 5, the crab instar composition increased to 42.29±2.29% in natural food treatment, and to 35.31±5.52% in artificial feed treatment, also with no significant difference (t=1.168, p>0.05).
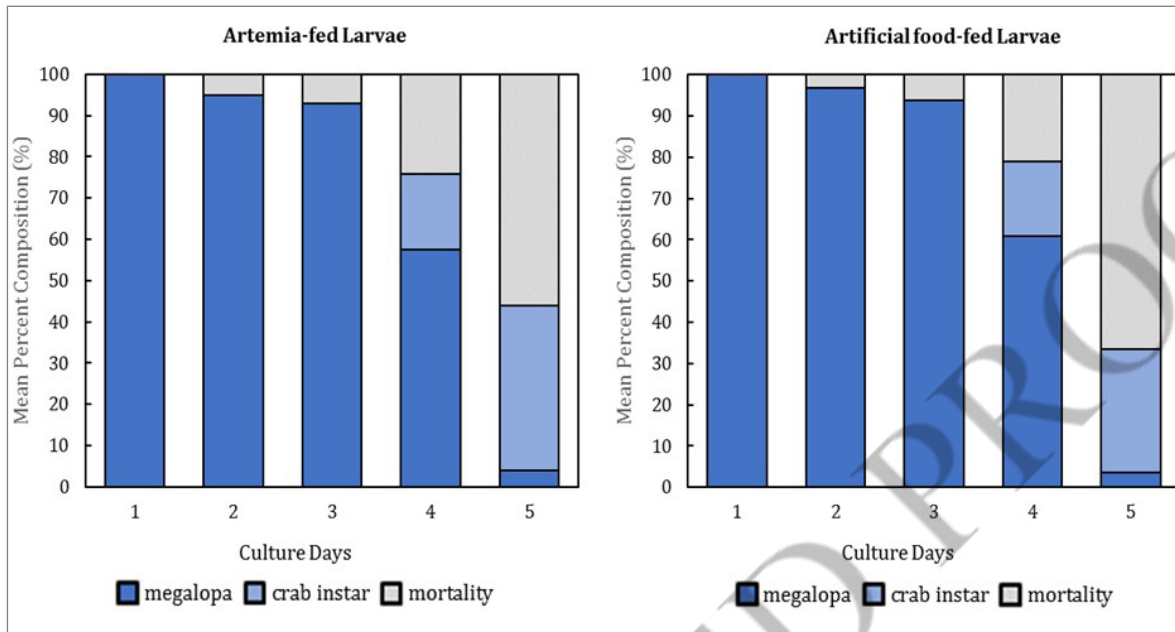
Figure 1. Development of crab larvae fed with *Artemia* (left), and artificial food (right). Graphs show percent composition of megalopa larvae, crab instars, and mortality during 5-day larval rearing.

After 5 days of rearing, the mean survival of megalopae fed with *Artemia* was 43.96±6.04%. On the other hand, megalopae fed with artificial feed had a mean survival rate of 33.33±13.34% (Figure 2). However, the survival rates of larvae in the two feed treatments did not differ significantly at $t=0.726$, $p>0.05$.

In the study of Castine et al. (2008), there was also no significant difference observed in the survival and development time between microbound diet and *Artemia*-fed megalopae. However, in the same study, the *Artemia*-fed megalopae metamorphosed into crabs with significantly wider carapaces and greater dry weights. Since the megalopa is constantly swimming when hunting for food, it was possible that the *Artemia*-fed megalopae had greater chances of encounter with actively swimming *Artemia* nauplii than the negatively buoyant artificial feed (Castine et al. 2008). The high foraging activities of *P. pelagicus* megalopae are attributed to their mechanosensory receptors that are capable of detecting vibrations emitted by the swimming *Artemia* nauplii (Anger 2001). Although the result of the experiment suggests that artificial feed may be given to supplement live feeds such as the *Artemia*, large-scale culture systems may also need to observe frequent water exchanges.

In Guiuan Marine Fisheries Development Center (GMFDC) where *P. pelagicus* megalopae are reared in large-scale, it was observed that megalopae no longer feed on the artificial feeds that sink to the bottom of the tank. The accumulation of unconsumed artificial food contributes to water fouling that is commonly observed in many hatcheries. Hatcheries may need to exert more effort in maintaining the water quality if the artificial feed is used. Since the study used smaller cultures, water fouling was not observed and the water parameters were within the normal range. The results suggest that artificial feed could be used as replacement for *Artemia* in rearing megalopae to crab instar stage.
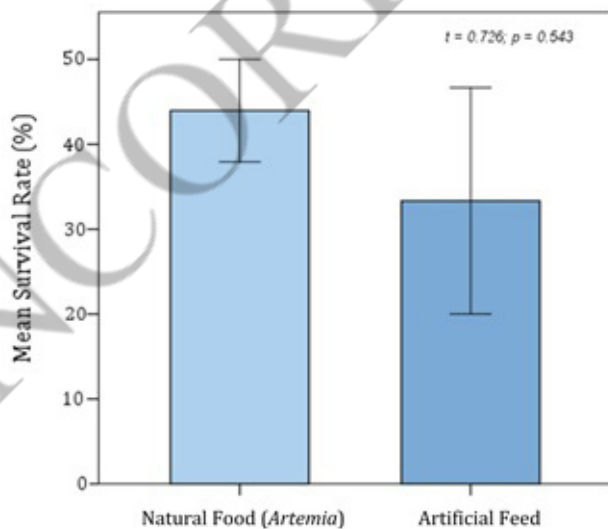

Figure 2. Survival rate (mean ± SE) of *P. pelagicus* megalopae fed with different diets.

3.2 Grow-out Culture: Phase I

Rearing medium and stocking density are also crucial factors for the success of portunid crab seed production. Thus, in creating a dependable hatchery protocol, it is necessary to determine the optimum stocking density and best medium for rearing. Hence, this study investigated the growth and survival of early juvenile crabs stocked at various densities in wooden tanks and hapa net cages.

3.2.1 Indoor Tanks Versus Outdoor Net Cages

The growth performance of *P. pelagicus* juveniles reared in indoor tanks and outdoor net cages did not differ significantly, more specifically, in terms of total carapace length and width gain and total weight gain (p > 0.05). The descriptive statistics and t-test results are presented in Table 1. However, the specific growth rate of juveniles in indoor tanks (6.39 ± 0.24%.d^{-1}) and outdoor cages (8.31 ± 1.11%.d^{-1}) differed significantly ($t=2.937$, $p< 0.05$).

The survival of juveniles was assessed weekly. The results were substantially better in indoor tanks than in outdoor net cages consistently from Week 1 until Week 5. Figure 3 shows the mean survival rates of *P. pelagicus* juveniles grown in both rearing media for 5 weeks. The final mean survival rate for juveniles reared in indoor tanks was 20.83 ± 9.24% and in outdoor net cages was 8.94 ± 3.58% only with significant difference at $t=2.938$, $p=0.015$.

Table 1. Descriptive statistics and t-test results on the growth performance of *P. pelagicus* juveniles reared in indoor tanks and outdoor net cages.

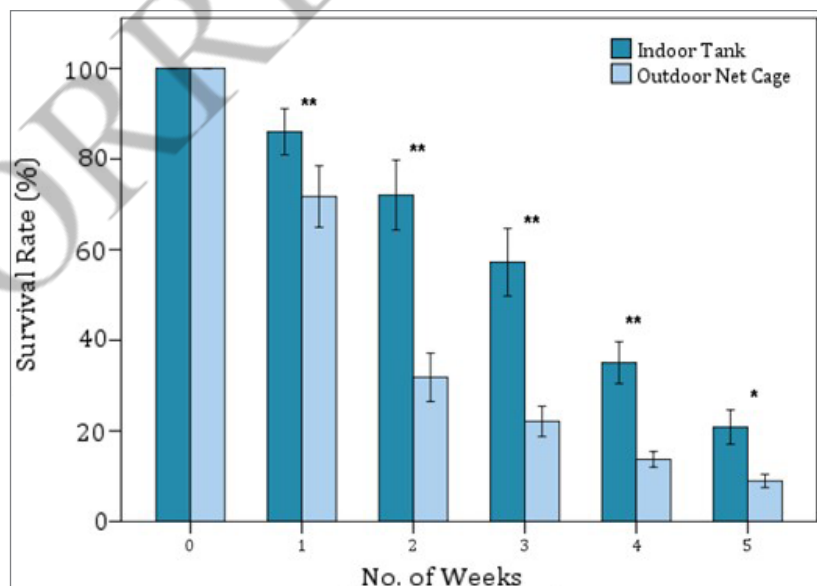|	Rearing Media		95% CI for Mean Difference	t	df	p
	Indoor Tank	Outdoor Net Cage				
Total carapace length gain (mm)	4.55 ± 2.67	8.51 ± 1.84	-3.967	-2.122	4	0.101
Total carapace width gain (mm)	11.63± 4.87	21.34 ± 4.00	-9.713	-2.670	4	0.056
Total weight gain (g)	2.60 ± 2.00	5.53 ± 1.46	-2.933	-2.049	4	0.110
Specific growth rate (%.d^{-1})	6.39 ± 0.24	8.31 ± 1.11	-1.920	-2.937	4	0.043

Figure 3. Mean survival rates of juvenile *P. pelagicus* reared in indoor tank and outdoor net cage for five weeks. Note: Values (mean ± SE) with asterisks show significant difference using t-test (*p<0.05, **p<0.01).

The superior results in growth performance of juveniles reared in outdoor hapa net cages could be attributed to the natural conditions of the rearing medium. However, these conditions might have caused greater molting frequency which left crabs more vulnerable to cannibalism than those that molted less often. This might have caused the increase in mortality leading to low survival obtained in the hapa net cages. On the other hand, it is also possible that better growth was observed due to lesser number of competitors for space and food. Ideally, a rearing medium should promote faster development and a larger growth increment without sacrificing survival (Daly et al. 2009).

The absence of substrate in the floating hapa net cages might also have caused an increase in cannibalism. Although B-nets were placed as shelters, the provision of sand substrate in rearing *P. pelagicus* is recommended to increase survival and reduce cannibalistic activity (Cabacaba and Salamida, unpublished data). Nevertheless, the addition of substrate might not still completely ensure a decrease in cannibalism as observed in the study of Ut et al. (2007) on the development of nursery culture techniques for mud crab *Scylla paramamosain*. Whereas, intermolt crabs would remain buried in the substrate for most of the day, newly molted crabs would be found above the sand surface, making them more vulnerable to cannibalism. Hence, future studies could include complex compartments as shelters to increase survival during molting period.

3.2.2 Different Stocking Densities

Juveniles reared at 50 ind.m^{-2} stocking density had the best growth performance assessed in terms of total carapace length and width gain and total weight gain (Table 2). SGR was highest in 75 ind.m^{-2} with 7.87±2.44%.d^{-1}. However, growth parameters were not significantly different among the various stocking densities (p>0.05). The results of the one-way ANOVA are found in Table 3.

Table 2. Mean (± SD, N = 6) values for total carapace length and width gain, total weight gain, and specific growth rate of *P. pelagicus* juveniles reared at different stocking densities.

	Stocking density (ind.m^{-2})		
	50	75	100
Total carapace length gain (mm)	7.13 ± 0.70 [a]	5.89 ± 3.85 [a]	6.58 ± 5.26 [a]
Total carapace width gain (mm)	17.01 ± 0.41 [a]	16.30 ± 7.27 [a]	16.15 ± 12.93 [a]
Total weight gain (g)	4.85 ± 0.02 [a]	3.28 ± 1.77 [a]	4.07 ± 4.43 [a]
Specific growth rate (%.d^{-1})	7.14 ± 0.75 [a]	7.87 ± 2.44 [a]	7.05 ± 0.88 [a]

Note: Values with similar letters did not have significant difference using Duncan post hoc test (subset for alpha=0.05)

Table 3. One-way ANOVA table results for total carapace length and width gain, total weight gain, and specific growth rate of *P. pelagicus* juveniles reared in different stocking densities.

		Sum of Squares	df	Mean Square	*F*	*p*
Total carapace length gain	Between Groups	1.545	2	0.773		
	Within Groups	43.018	3	14.339	0.054	0.948
	Total	44.563	5			
Total carapace width gain	Between Groups	0.844	2	0.422		
	Within Groups	220.087	3	73.362	0.006	0.994
	Total	220.931	5			
Total weight gain	Between Groups	2.465	2	1.233		
	Within Groups	22.744	3	7.581	0.163	0.857
	Total	25.209	5			
Specific growth rate	Between Groups	0.801	2	0.400		
	Within Groups	7.293	3	2.431	0.165	0.855
	Total	8.094	5			

The survival of juveniles decreased drastically through time as can be observed in Figure 4. The mean survival rates of *P. pelagicus* juveniles at different stocking densities did not differ significantly from Week 2 to Week 5. The differences were only significant after one week of rearing ($p<0.05$) with least survival in 100 ind.m^{-2} stocking density. The final mean survival rate was highest for 75 ind.d^{-1} density with 17.67±8.10%. This was followed by 50 ind.m^{-2} with 17.00±12.70%, and by 100 ind.m^{-2} with 10.00±5.48%. However, the differences among the mean survival rates were not significant at $p>0.05$.

The survival of crabs in different stocking densities did not differ significantly to each other, hence, it is recommended that future studies use wider range of stocking densities. In other studies, growth

can be inhibited at higher densities. The incongruity in the survival and growth may be attributed to the foraging behavior of conspecifics. Asynchronous molting may also cause size variability that could increase predation pressure on smaller crabs resulting to higher proportion of larger individuals (Broderson et al. 1989; Borisov et al. 2007). In the study of Triño et al. (1999), lower survival and growth at higher stocking densities may be a result of appetite suppression, feeding inhibition, sub-lethal injuries, or cannibalism as observed in other juvenile crab and shrimp species. Although sufficient food was provided to each of the treatments, antagonistic behavior may have caused the feeding inhibition or appetite suppression in high densities resulting to low survival and growth (Sainte-Marie and Lafrance 2002).
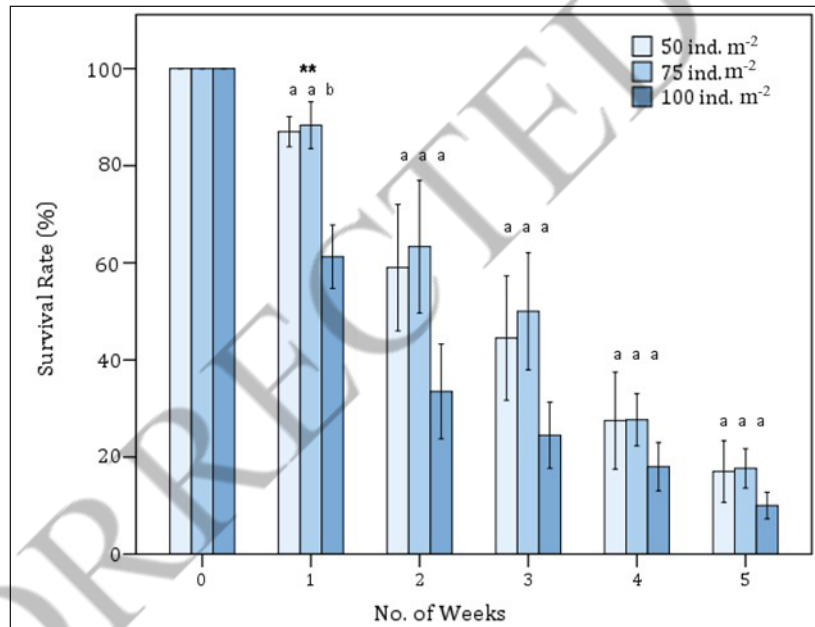

Figure 4. Mean (± SE) survival rates of P. pelagicus juveniles reared at different stocking densities for five weeks. Note: Values with different letters and asterisks (**) have significant difference (p < 0.01).

3.2.3 Effects of the Interaction Between Rearing Media and Stocking Densities

The effect of the interaction between the rearing media type and stocking density on the growth performance and survival rates of *P. pelagicus* was analyzed using a two-factor ANOVA. Results have shown that growth performance of the juveniles, particularly in terms of total carapace length and width gain and SGR were significantly influenced by both the differences in rearing medium and stocking

density. However, the interaction between rearing medium and stocking density did not significantly affect the survival of *P. pelagicus* juveniles (F=0.120, p=0.888). The summary of the results of the two-factor ANOVA is presented in Table 4.

Moreover, simple main effects analysis has shown that both growth performance and survival of juveniles significantly differ between indoor tanks and outdoor net cages. On the other hand, growth parameters except SGR and survival were not influenced by the differences in stocking densities.

Table 4. Two-way ANOVA results on the effects of rearing medium, stocking density, and the interaction of rearing medium and stocking density on the growth performance and survival rate of *P. pelagicus* juveniles reared in Phase I grow-out culture.

	Rearing media		Stocking density		Rearing media*stocking density	
	F-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value
Total carapace length gain	16.187	0.007	0.534	0.612	6.644	0.030
Total carapace width gain	21.041	0.004	0.063	0.940	5.837	0.039
Total weight gain	11.274	0.015	1.078	0.398	4.322	0.069
Specific growth rate	0.710	0.432	35.630	<0.001	78.734	<0.000
Survival rate	7.630	0.033	1.298	0.340	0.121	0.888

3.3 Grow-out Culture: Phase II

The hatchery-reared crab juveniles produced in Phase I were further cultured in B-net cages for 30 days. At the time of stocking, the average carapace width of the 60 crab juveniles ranged from 30.4–40.1 mm and the average body weight range from 4.2–5.3 g.

The growth performance of juveniles stocked at 5 ind.m^{-2} were highest compared to 10 and 15 ind.m^{-2} densities (Table 5). At this low stocking density, juveniles attained a mean specific growth rate of 3.54±0.56%.d^{-1}. This was followed by 15 ind.m^{-2} with 3.45±2.39%.d^{-1} and 10 ind.m^{-2} with 2.33 ± 0.50%.d^{-1}. Nevertheless, the values of growth parameters did not differ significantly among different stocking densities (p>0.05).

After one week of rearing, mortality was observed only in the setup with 15 ind.m.$^{-2}$ density. The graph for survival rates of juveniles stocked at different densities is presented in Figure 5. Survival rates among different stocking densities were inconsistent every week. During Week 2, 5 ind.m^{-2} yielded highest survival rate (90.00%) but this drastically became lowest during Week 3 (50.14%) and then further decreased during Week 4 (40.00%). At the end of the experiment, crab juveniles stocked at 15 ind.m^{-2} had the highest final mean survival rate (46.67±0.00%), but the differences among other stocking densities were not statistically significant (p>0.05).

Table 5. Mean (± SD, n=6) values of the total carapace length and width gain, total weight gain, specific growth rate, and survival rates of *P. pelagicus* reared in Phase II grow-out culture at different stocking densities.

	Stocking Density (ind.m^{-2})		
	5	10	15
Total carapace length gain (mm)	8.26 ± 2.36 [a]	6.32 ± 0.53 [a]	3.89 ± 1.86 [a]
Total carapace width gain (mm)	18.71 ± 7.71 [a]	12.91 ± 4.19 [a]	7.82 ± 7.50 [a]
Total weight gain (g)	8.05 ± 3.25 [a]	5.33 ± 1.37 [a]	4.41 ± 2.71 [a]
Specific growth rate (%.d^{-1})	3.54 ± 0.56 [a]	2.33 ± 0.50 [a]	3.45 ± 2.39 [a]
Survival rate (%)	40.00 ± 0.00 [a]	45.00 ± 7.07 [a]	46.67 ± 0.00 [a]

Table. One-way ANOVA table on total carapace length and width gain, total weight gain, specific growth rate, and survival rate of *P. pelagicus* juveniles reared in Phase II grow-out at different stocking densities.

		Sum of Squares	df	Mean Square	F-value	p-value
Total carapace length gain	Between Groups	19.170	2	9.585	1.543	0.346
	Within Groups	18.633	3	6.211		
	Total	37.803	5			
Total carapace width gain	Between Groups	118.750	2	59.375	1.338	0.384
	Within Groups	133.121	3	44.374		
	Total	251.871	5			

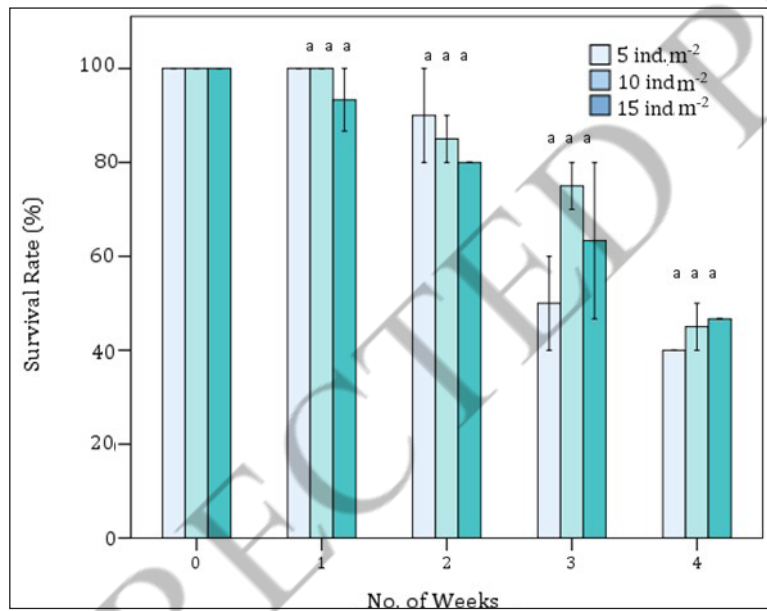|		Sum of Squares	df	Mean Square	F-value	p-value
Total weight gain	Between Groups	14.301	2	7.151	0.706	0.561
	Within Groups	30.373	3	10.124		
	Total	44.675	5			
Specific growth rate	Between Groups	1.838	2	0.919	0.440	0.680
	Within Groups	6.259	3	2.086		
	Total	8.097	5			
Survival rate	Between Groups	48.185	2	24.093	1.446	0.363
	Within Groups	50.000	3	16.667		
	Total	98.185	5			

Figure 5. Survival rates of *P. pelagicus* juveniles in Phase II grow-out culture reared at different stocking densities for four weeks.

The total carapace width of the crabs in this study is lesser compared to the findings of Josileen and Menon (2005). The growth and survival of all the treatments were not significantly different, suggesting that hatcheries can use higher stocking density such as 15 ind.m^{-2} to maximize production per unit effort, especially when considering rearing effort and utilization of space (Zamora et al. 2005; Ut et al. 2007). However, it is possible that no difference was found because only two replicates were prepared. Future studies can use three or more replicates and trials or increase the range of stocking densities to obtain significant results.

The study of Wilber and Wilber (1989) suggested that higher stocking densities can affect crab intermolt duration and increment. The numerous conspecific predators may act as environmental stressors delaying molting, hormonal activity, and suppressing appetite yielding lower growth and survival (Sante-Marie and Lafrance 2002).

The mortality in all treatments might have also been caused by cannibalism despite the availability of alternative food source. This was observed during the weekly sampling where only few dead crabs were seen (missing crabs). Although Kovatchera et al. (2006) solved this problem by isolating crabs to their individual cages. This technique is inefficient in hatchery setting because it would not maximize the production. Similar to Phase I, complex substrate and shelters may be added instead to culture cages to maximize the surface area, reduce encounter rates, and decrease cannibalism. Feeding regime may also be improved to increase the food availability in culture cages with complex structure (Broderson et al. 1989; Borisov et al. 2007).

4. CONCLUSION

Natural food and artificial commercial feed had similar results in terms of development and survival of crab larvae. This indicate that artificial feeds can be a good alternative for natural food in larval production. The use of artificial feeds has a great potential especially in small-scale systems, but in large-scale systems this must be used with an appropriate feeding management. Future studies on artificial feeds should focus more on formulating feeding regimens that would not lead to water fouling and would promote increase in feeding activity.

This present study also evaluated the survival and growth of Day 20 crab juveniles at three stocking densities in wooden tanks and in hapa net cages. After five weeks of rearing, growth performance (SGR) and survival were substantially better in outdoor net cages than in indoor tanks, and relatively similar in different stocking densities (50, 75 and 100 ind.m^{-2}). The better growth and survival in outdoor net cages was not extensively studied but this could be attributed to the natural conditions which stimulate frequent molting of crabs. On the other hand, stocking of crabs at 100 ind.m^{-2} may maximize rearing space. Also, the provision of B-net shelters favorably minimized incidence of cannibalism, which is a main issue that arises from increasing stocking density.

Furthermore, growth and survival of juveniles in Phase II grow-out culture did not differ significantly in terms of stocking density (5, 10, and 15 ind.m^{-2}). Experiments conducted in this study suggest that stocking density do not considerably affect the growth performance and survival of juvenile crabs. These findings, however, suffer from limited number of trials and a narrow range of stocking densities. Yet, this could imply maximizing rearing space by using 15 ind.m^{-2} as a starting density for grow-out of juvenile crabs.

5. ACKNOWLEDGMENT

6. REFERENCES

Andrés M, Rotllant G, Zeng C. 2010. Survival, development and growth of larvae of the blue swimmer crab, *Portunus pelagicus*, cultured under different photoperiod conditions. Aquaculture 300: 218-222.

Anger K. 2001. Nutrition. The Biology of Decapod Crustacean Larvae. A.A. Balkem Publishers, Lisse, The Netherlands. 109-144 pp.

Borisov RR, Epelbaum AB, Kryakhova NV, Tertiskaya AG, Kovatcheva NP. 2007. Cannibalism behavior in red king crabs reared under artificial conditions. Russ. J. Mar. Biol 33: 227-231.

Broderson CC, Rounds PJV, Bobcock MM. 1989. Diet influences cannibalism in laboratoryheld juvenile red king crabs (*Paralithodes camtschatica*). Proc. Int. Symp. on King and Tanner Crabs. Anchorage, Alaska. 1989. University of Alaska Sea Grant Program, Rept. No. 90-04: 109-122.

Castine S, Southgate PC, Zeng C. 2008. Evaluation of four dietary protein sources for use in microbound diets fed to megalopae of the blue swimmer crab, *Portunus pelagicus*. Aquaculture 281: 95–99.

Daly B, Swingle JS, Eckert GL. 2009. Effects of diet, stocking density, and substrate on survival and growth of hatchery-cultured red king crab (*Paralithodes camtschaticus*) juveniles in Alaska, USA. Aquaculture 293: 68-73.

Ikhwanuddin M, Adila TN, Azra MN, Hii YS, Talpur AD, Abol-Munafi AB. 2011a. Determination of live prey ingestion capability of *Portunus pelagicus* (Linnaeus 1758). World Journal of Fish and Marine Sciences 3(6): 570-575.

Ikhwanuddin M, Mansor JH, Bolong AMM Long ML. 2011b. Improved hatchery-rearing techniques for juvenile production of blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758). Aquaculture Research 43: 1251-1259.

Ikhwanuddin M, Azra MN, Talpur MAD, Abol-Munafi AB, Shabdin ML. 2012. Optimal water

temperature and salinity for production of blue swimming crab, *Portunus pelagicus* 1st day juvenile crab. AACL Bioflu 5: 4-8.

Iribarne O, Fernandez M, Armstrong D. 1994. Does space competition regulate density of juvenile Dungeness crab *Cancer magister* Dana in sheltered habitats. Journal of Experimental Marine Biology and Ecology 183: 259-271.

Josileen J, Menon NG. 2004. Larval stages of the blue swimmer crab, *Portunus pelagicus* (Linnaeus, 1758) (Decapoda, Brachyura) in captivity. Crustaceana 77: 785-803.

Josileen J, Menon NG. 2005. Growth of the blue swimmer crab, *Portunus pelagicus* Linnaeus, 1758) (Decapoda, Brachyura). Crustaceana 78(1): 1-18.

Kovatcheva N, Epelbaum A, Kalinin A, Borisov R, Lebedev R. 2006. Early history stages of the red king crab *Paralithodes camtschaticus* (Tilesius, 1815) Biology and Culture. VINRO Publishing.

Laviña AFD, Buling A. 1980. Seed production of *Portunus pelagicus* (Linnaeus) Crustacea: Portunidae in conical aquaria and raceways. APDEM III, SEAFDEC AQD.

Moller H, Lee SY, Paterson B, Mann H. 2008. Cannibalism contributes significantly to the diet of cultured sand crabs, *Portunus pelagicus* (L.): A dual stable isotope study. Journal of Experimental Marine Biology and Ecology 361: 75–82.

Romano N, Zeng C. 2006. The effects of salinity on the survival, growth and haemolymph osmolality of early juvenile blue swimmer crabs, *Portunus pelagicus*. Aquaculture 260: 151–162.

Sainte-Marie B, Lafrance M. 2002. Growth and survival of recently settled snow crab *hionoecetes opilio* in relatin to intra-and intercohort competition and cannibalism: a laboratory study. Mar. Ecol. Prog. Ser. 244: 191-203.

Soundarapandian P, Thamizhazhagan E, John Samuel N. 2007. Seed production of commercially important blue swimming crab *Portunus pelagicus* (Linnaeus). Journal of Fisheries and Aquatic Science 2: 302-309.

Sukumaran KK, Neelakantan B. 1996. Spawning biology of two marine portunid crabs, *Portunus (Portunus) sanguinolentus* (Herbst) and *Portunus (Portunus) pelagicus* (Linnaeus) from the Karnataka Coast. The Fourth Indian Fisheries Forum Proceedings 35-38.

Triño AT, Millamena OV, Keenan C. 1999. Commercial evaluation of monosex pond culture of mud crab Scylla species at three stocking densities in the Philippines. Aquaculture 174: 109-118.

Ut VN, LeVay L, Nghia TT, Hanh TTH. 2007. Development of nursery culture techniques for the mud crab *Scylla paramamosain* (Estampador). Aquac. Res. 38: 1563-1568.

Wilber DH, Wilber Jr. TP. 1989. Effects of holding space and diet on the growth of West Indian spider crab *Mithax spinosissimus* (Lamarck). J. Exp. Mar. Biol. Ecol. 131: 215-222.

Zamora O, Findiesen A, Stubblefield J, Frenkel V, Zohar Y. 2005. Large-scale juvenile production of the blue crab *Callinectes sapidus*. Aquaculture 244: 129-139.