

## Evaluation of Water Quality Parameters, Growth and Proximate Composition of Juvenile Crab, *Portunus pelagicus* Cultured in RAS and Non RAS System

(Penilaian Parameter Kualiti Air, Tumbesaran dan Komposisi Terhampir Ketam Juvenil *Portunus pelagicus* yang Dikultur dalam Sistem RAS dan Bukan RAS)

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### ABSTRACT

Juvenile blue swimming crab, *Portunus pelagicus* were reared over 31 days in two different systems namely recirculating aquaculture system (RAS) and conventional aquaculture system (CAS) to evaluate the water quality parameters, growth, and its body composition. The juvenile crab, weighing of  $0.95 \pm 0.18$  g and stocking was at 40 crabs  $m^{-2}$  and fed twice per day with a commercial shrimp pellet. During the experimental time, significantly ( $P < 0.05$ ) increment in dissolved oxygen (DO) ( $6.42 \pm 0.13$ ), low level of ammonia- nitrogen ( $0.04 \pm 0.10$ ) and nitrite-nitrogen ( $0.04 \pm 0.10$ ) were recorded in RAS than conventional aquaculture system (CAS) (DO:  $5.99 \pm 0.24$ ; ammonia- nitrogen:  $3.83 \pm 1.59$ ; nitrite-nitrogen:  $0.71 \pm 0.58$ ). The carapace width, weight gain and specific growth rate (SGR) were significantly ( $P < 0.05$ ) higher in RAS. Protein content ( $22.65 \pm 0.11\%$ ) in crab also were significantly ( $P < 0.05$ ) higher in RAS compared with crab protein ( $21.41 \pm 0.12\%$ ) cultured in CAS. Although the survival rate was slightly higher in the juveniles reared at CAS, however it was not significantly different comparing with the individuals reared at RAS. The results strongly suggested that the use of RAS may improve the growth performance and maintain the better water quality for the crabs in captivity.

**Keywords:** Conventional aquaculture system (CAS); growth; *Portunus pelagicus*; recirculating aquaculture system (RAS); survival

### ABSTRAK

Ketam juvenil renang biru, *Portunus pelagicus* telah dipelihara selama 31 hari dalam dua sistem berbeza iaitu sistem akuakultur udara berulang (RAS) dan sistem akuakultur konvensional (CAS) untuk menilai parameter kualiti air, tumbesaran dan komposisi badan. Ketam juvenil seberat  $0.95 \pm 0.18$  g dan penstockan adalah pada 40 ketam  $m^{-2}$  dan diberi makan dua kali sehari dengan pelet udang komersial. Semasa masa uji kaji percubaan, peningkatan ( $P < 0.05$ ) dengan ketara oksigen terlarut (DO) ( $6.42 \pm 0.13$ ), tahap rendah ammonia- nitrogen ( $0.04 \pm 0.10$ ) dan nitrit-nitrogen ( $0.02 \pm 0.07$ ) direkodkan dalam RAS daripada konvensional sistem akuakultur (CAS) (DO:  $5.99 \pm 0.24$ ; ammonia-nitrogen:  $3.83 \pm 1.59$ ; nitrit-nitrogen:  $0.71 \pm 0.58$ ). Lebar karapas, pertambahan berat dan kadar pertumbuhan khusus (SGR) adalah ketara ( $P < 0.05$ ) lebih tinggi dalam RAS. Kandungan protein ( $22.65 \pm 0.11\%$ ) pada ketam juga adalah ketara ( $P < 0.05$ ) lebih tinggi pada RAS berbanding dengan protein ketam ( $21.41 \pm 0.12\%$ ) yang dikultur dalam CAS. Walaupun kadar kemandirian adalah lebih tinggi dalam juvenil yang dipelihara dalam CAS, walau bagaimanapun, ia tidak berbeza dengan ketara berbanding dengan individu yang dipelihara dalam RAS. Keputusan menyarankan bahawa penggunaan RAS boleh meningkatkan prestasi tumbesaran dan mengekalkan kualiti air yang lebih baik untuk ketam dalam kurungan.

**Kata kunci:** Kemandirian; pertumbuhan; *Portunus pelagicus*; sistem akuakultur konvensional (CAS); sistem akuakultur udara berulang (RAS)

### INTRODUCTION

A processes of treating wastewater to maintain water quality and increases aquaculture production by recirculating aquaculture systems (RAS) have been receiving attention worldwide (Van Rijn 1996). RAS have been used to manage water usage to grow tilapia, crawfish, catfish, blue crabs, striped, oysters, mussels bass and multiplicities of aquarium pets (Van Rijn 1996). The processes seen to have the potential of reducing pollutant, stabilize water quality parameters such as pH, temperature, salinity, oxygen and among others are kept within culture range to maintain

fish or crustacean growth and health (Nicula et al. 2015). However, RAS have significant environmental impacts (Davis & Arnold 1998).

Crabs are a large group of invertebrates, due to the high palatability of their meat; they are in the prime focus of the wide commercial fisheries (Latyshev et al. 2009). Nowadays, the tendency of benefiting from seafood for an essential protein supply is increasing worldwide rapidly (Lee 1995). Crab tissue proteins contain 20 different amino acids of nutritional importance; this prompt crab production and are ranked second among the crustaceans

with shrimps leading (Barrento et al. 2010; CMFRI 2015; Konosu et al. 1978).

Increasing demand for marine crabs in domestic as well as in the global export industries such as China, France, Indonesia, Japan, Philippines, Spain, Thailand and United States (Lui & O'Connor 1977). In a larger extent crab contained essential amino acids (EAA) (Chen et al. 2007), which is an essential for growth, proper function of the physiology component and immunity for human (Fantle et al. 1999; Vilasoa et al. 2007). Marine crab lipids are also a vital nutrients for human health (Marques et al. 2010), because it contained eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that are believed to be protective for human health in many ways that can be acquired from seafood and thus human obtain EPA and DHA by consuming aquatic invertebrates like crustaceans (Carrillo-Dominguez et al. 2005). The crab breast meat and claw meat have higher amounts of EPA and DHA (Celik et al. 2004). Though several studies on different crab species had been conducted, to the best of our knowledge, no work has been done on the effect of different culture techniques on juvenile blue swimming crab, *Portunus pelagicus* in captivity. Therefore, the present work aims to determine the effects of different culture system on water management, growth and body composition of marine crab *P. pelagicus*.

## MATERIALS AND METHODS

### EXPERIMENTAL SITE

This experiment was carried out at the hatchery complex in the Marine Science Center of UPM, Port Dickson, Negeri Sembilan, Malaysia during February, 2017.

### BROODSTOCK COLLECTION AND HATCHING

Four ovigerous females (carrying fertilized eggs), weighing from 175 to 250 g were captured with a baited trap at Port Dickson (2°31'N and 101° 48'0"E), Negeri Seremban, Malaysia and brought to the Center of Marine Science of UPM, Port Dickson, Malaysia. After reaching the laboratory, the crabs were immersed in a static formalin bath (concentration 50 µl/L) for 6 h (Romano & Zeng 2006) and stocked them in indoor four individual 100 vL circular fiberglass tanks for hatching at a temperature of 27±1°C and salinity of 30 ± 2 ppt. The tank water was aerated to maintain sufficient dissolved oxygen (DO) level and the berried female was not fed. The discarded eggs and faeces of crab were siphoned daily from the tank, accompanied with about a 10% water exchange (treated).

After hatching, the newly hatched healthy zoea were stocked in 1000<sup>-1</sup> black round fiberglass tank at a stocking density of 50 larvae l<sup>-1</sup> and cultured at a salinity of 30-32 ppt at 28±1°C. The zoea- I were initially fed rotifers (*Branchionus* sp.) at 15-20 individuals mL<sup>-1</sup> and this rotifer density was maintained until the end of megalopa stages. From the Zoea- II stage onwards, *Artemia* nauplii and

shrimp nursery powder feed were added daily to the larval rearing tanks at the rate of 1-2/mL and 2 mg/L for Zoea- II; 3-5/mL and 2 mg/L for Zoea- III; 6-8/mL and 2 mg/L for Zoea- IV stages. In addition to rotifers and *Artemia* nauplii, shrimp crumbled feed (< 200 µm) were fed at the rate of 5 mg/L from the megalopae stage to first crab (C1). Larvae were fed manually at early morning (9.00 am) and late afternoon (6.00 pm) per day. In larval rearing, a volume of 30% water was exchanged from each larvae tank at every 5 days intervals. After the crab larvae had metamorphosed to the megalopae stage, the additional artificial seaweed substrates constructed from black orchid plastic nets (70%) were suspended within the larvae-culture tank to provide a surface for settlement of megalopae and C1 crabs. During the larvae culture, the strong aeration was provided into the tanks.

### SEAWATER TREATMENT FOR CULTURE SPECIES

Prior to disinfection with active chlorine for 24 h to remove bacteria the seawater (31 ppt) was filtered through a 10 µm nylon net. Ethylene-Diamine-Tetra-Acetic (EDTA) was used to settle down the heavy metals and then sodium thiosulphate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>) was added to neutralize the chlorine concentration at the beginning of the experiment according to Talpur et al. (2011).

### SOURCE OF EXPERIMENTAL CRABS

The juvenile crabs used for the present experiments were cultured in the laboratory. Briefly, towards the end of larval culture, when the postlarvae (megalopae) were undergone mass metamorphic induction to the C1 crabs, the healthy instar with intact appendages were collected using a 1 mm<sup>2</sup> sieve and briefly rinsed with fresh seawater before being placed communally into 3m<sup>2</sup> rectangular fiberglass tank (0.5 m water depth) contained a 10 cm layer of local sea sand at the bottom and 50 cm of free water surface above the sand layer. The animals were allowed to grow until reached the juvenile stage for 30 days. During this culture period, the first crab instars were fed at 15% body weight with shrimp pellet feeds No. 5002 (C.P. Aquaculture Private Limited) twice a day using feeding trays. At the end of day 30 of nursing or when they became grow more or less 25 mm carapace width, the healthy juvenile with intact appendages were harvested and briefly rinsed with fresh seawater before being started for growth performance trials.

### RECIRCULATING AQUACULTURE SYSTEM (RAS)

Generally, this system has involved the water recirculation from the rearing basins into the physical and biological filters that comprised of two individual 100 vL circular fiberglass tanks. Two foams of mechanical filters and biological filters made of bio-balls, sand, coral-chips and coal were used as filter media. Both filter tanks were provided with a mild aeration to maintain the sufficient

oxygen (DO) level; and the filters were covered with a black orchid plastic net (70%) to make suitable environmental conditions for growing beneficial microbes. No water discharge or displacement was occurred in the RAS during this study, except those compensations due to water losses by evaporation and syphoning. On the other hand, the Non-RAS system i.e. CAS consisted of three plastic basins (0.26 m<sup>2</sup>) with a water volume of approximately 50 L. During the culture period, about a 20% water was exchanged daily from each culture tank of CAS.

#### TANK SET UP

The growth and survival trails were conducted in 6 plastic basins (50 L, 0.26 m<sup>2</sup>). Each basin was stocked with 10 healthy intact juvenile crabs (40 m<sup>-2</sup>) with an average wet weight of 0.95 g, carapace length of 11.82 mm and carapace width of 23.16 mm. Each basin contained a 10 cm layer of local sea sand at the bottom and 15 cm of the free water surface above the sand layer. The sandy substratum was acclimatized to the culture condition for 15 days before starting the experiment. The commercial star shrimp pellets (code: 5004) contained around 40% crude protein, 5% fat, 3% fiber, and 12% moisture was given to the juveniles during the experimental period. The feed was fed to the juvenile crabs at 10% of the total biomass until the end of the culture period as advised by Oniam et al. (20011). The blue swimming crabs were fed by hand twice a day at early morning (9.00 am) and late afternoon (6.00 pm).

#### WATER PARAMETER

The main water parameters analyzed in the present study include; temperature, ammonia-nitrogen, pH and dissolved oxygen (DO), salinity and nitrite-nitrogen. All the water quality parameters were recorded once in a week. The salinity, temperature, pH and DO were measured using YSI 556 Multi-probe (USA) whereas API pharmaceutical test kit (commercial test kit) was used to measure the ammonia-nitrogen and nitrite-nitrogen.

#### EXPERIMENTAL DESIGN

Two treatments were conducted with triplicates in a completely randomized design (CRD). A total of 60 juvenile (C30) crabs were used over a 31-day period starting on April 17, 2017. Treated seawater (31-32 ppt) and sandy substratum were used as the rearing media. Water pH was maintained at a range between 8.0 and 8.3 using calcium carbonate in the water reservoir tank. Diffused aeration was provided to maintain dissolved oxygen (DO) level in each culture basin throughout the experiment. During each water exchange, the aeration was stopped temporarily to remove the leftover feed, dead juveniles, the settled particles from each bowl bottom by syphoning. The average carapace width and weight measurements were taken on day 1 of this study and then fortnightly 3 juvenile crabs were taken from each culture

tank to measure the growth performance of the crab until juvenile reach the double size of carapace width (CW) from the initial measurement. At the end of experiment, the final carapace width and length of all surviving crabs were measured using a digital calliper (0.01 mm; Mitutoyo, Japan) and final wet weight was measured using a digital balance (sensitivity = 0.01 g) to analyze the growth performance. Percentage survival was estimated using a digital calculator and then the samples were stored at -20°C for whole-body proximate composition analysis.

#### WHOLE-BODY PROXIMATE COMPOSITION

The proximate composition of the carbs samples and feed samples were determined according to the standard method of AOAC (1997). Crude protein was determined using the Kjeldahl method after an acid digestion method with the help of protein analyzer unit (2300 Kjeltex Analyzer Unit, Foss Tecator). Crude lipid was determined by petroleum ether extraction using a Soxtec system (Soxtec System 2050 Foss Tecator). Moisture was determined by oven drying at 105°C for 24 h and ash was determined using muffle furnace heating the mantle at 600°C for 4 h.

#### CALCULATIONS

Bio productive indices such Specific growth rate (SGR), body weight gain and feed conversion ratio (FCR) was calculated using the following formula:

Weight gain (%)

$$= \frac{\text{Final body weight (g)} - \text{Initial body weight (g)}}{\text{Initial body weight (g)}}$$

× 100 (Tina & Darumas 2014)

SGR (%)

$$= \frac{\ln \text{Final body weight (g)} - \ln \text{Initial body weight (g)}}{\text{Culture period (day)}}$$

× 100 (Ikhwanuddin et al. 2012)

$$\text{FCR (\%)} = \frac{\text{Total quantity of feed consumed (gm)}}{\text{Total weigh (gm)}}$$

(Maheswardue et al. 2008)

At the end of the experiment, percent survival of juvenile was also calculated using the formula of Talpur et al. (2011) as follows:

$$\text{Survival rate (\%)} = \frac{\text{Total number of Survived crablet}}{\text{Initial number of Stocked crablet}} \times 100$$

#### STATISTICAL ANALYSIS

Mean values of different parameters (viz., body weight gain, survival rate, SGR, and carapace length and

carapace width growth rate) from different replicates of each treatment was calculated using Microsoft Office Excel 2010. Data for carapace width and length, growth performance, proximate composition and survival of crabs was statistically analyzed using t-test (SPSS Ver. 16) to compare the two rearing units.

## RESULTS AND DISCUSSION

The result of water quality parameters was significantly affected by the rearing techniques (Table 1). However, a significant ( $P < 0.05$ ) amount of DO was observed in RAS, with low ammonia-N and nitrite-N level compared to CAS. Other parameters such as water temperature, pH and salinity was not affected by rearing techniques.

The growth performance, feed utilization and survival of blue swimming crab juveniles *P. pelagicus* were affected by their rearing techniques (Table 2). SGR, weight gain and carapace width and length were significantly ( $P < 0.05$ ) higher in RAS than in CAS. Although survival rate was slightly in favour of CAS, but no significant differences were observed in RAS.

Proximate composition of juvenile crab was not affected due to rearing techniques, except crude protein that was significantly ( $P < .05$ ) higher in RAS compared to CAS (Table 3).

In the present study, the physicochemical parameters of the water was reported almost within the optimum range for crab culture (Seemann et al. 2015) in both system, however, slight variation was noticed in CAS. The lack of natural productivity in both system and the absence of algal blooms have contributed to maintaining the optimal oxygen concentration in (Badiola et al. 2012). After the administration of feed, dissolved oxygen showed a significant difference in treatments, but none of the treatment is below 5.5 mg/L (Nicula et al. 2015). The supplied experimental diet had no effect on water pH in the present study. Promya and Chitmanat (2011) reported that the higher pH in a pond maybe due to the presence of algae in the pond. With the recirculation treatment, the water quality parameters were found at acceptable levels for crab optimal growth over the 30 days of feeding trial. The low amount of ammonia and another harmful nitrogenous compound nitrate in the water had positive effect on the health, feeding efficiencies and growth performance of the crab (Li et al. 2013). This was mainly attributed to the combined treatment by the biological filter, as compared to the conventional system. The growth of swimming crabs is usually expressed in carapace width or length increment (Catacutan 2002; Takeuchi et al. 1999). In the present experiment, SGR, weight gain and carapace width and length were significantly higher in RAS than

TABLE 1. Analysis of water quality parameters on weekly basis from RAS and CAS systems over a period of 30 days

Parameters	RAS	CAS
Water temperature (°C)	28.17±0.83 <sup>a</sup>	28.25±0.87 <sup>a</sup>
Dissolved oxygen (mg/L)	6.42±0.13 <sup>a</sup>	5.99±0.24 <sup>b</sup>
Salinity (ppt)	31.83±0.42 <sup>a</sup>	31.89±0.50 <sup>a</sup>
pH	8.11±0.12 <sup>a</sup>	8.06±0.15 <sup>a</sup>
Ammonia-nitrogen (mg/L)	0.04±0.10 <sup>b</sup>	3.83±1.59 <sup>a</sup>
Nitrite-nitrogen (mg/L)	0.02±0.07 <sup>b</sup>	0.71±0.58 <sup>a</sup>

All values were given in mean of three replicates ± standard deviation (SD). Different superscript letters in the same row are significantly different ( $P < 0.05$ ).

\*RAS= Recirculating aquaculture system, \*CAS = Conventional aquaculture system

TABLE 2. Growth performance, feed utilization and survival of blue swimming crab juveniles *P. pelagicus*

Parameters	RAS	CAS
Initial weight (g)	0.95±0.18 <sup>a</sup>	0.95±0.18 <sup>a</sup>
Final weight (g)	7.00±3.52 <sup>a</sup>	3.83±1.23 <sup>b</sup>
Weight gain (g)	6.05±3.52 <sup>a</sup>	2.84±1.16 <sup>b</sup>
Weight gain (%)	636.96±370.02 <sup>a</sup>	299.30±122.17 <sup>b</sup>
SGR (%)	3.00±1.38 <sup>a</sup>	2.26±1.23 <sup>b</sup>
FCR	1.16±0.10 <sup>a</sup>	2.13±0.13 <sup>a</sup>
Survival (%)	38.93±5.0 <sup>a</sup>	40.10±8.66 <sup>a</sup>
Initial carapace length (mm)	11.82±0.60 <sup>a</sup>	11.82±0.60 <sup>a</sup>
Final carapace length (mm)	23.68±3.67 <sup>a</sup>	18.15±2.65 <sup>b</sup>
Initial carapace width (mm)	23.16±1.34 <sup>a</sup>	23.16±1.34 <sup>a</sup>
Final carapace width (mm)	46.56±7.05 <sup>a</sup>	35.95±5.17 <sup>b</sup>

All values were given in mean of three replicates ± standard deviation (SD). The mean values bearing the different superscripts in the same row are significantly different ( $P < 0.05$ ).

\*RAS= Recirculating aquaculture system, \*CAS = Conventional aquaculture system

TABLE 3. Proximal composition (mean  $\pm$  SD) in crab harvest at the end of cultivation

Treatment	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
Initial	72.54 $\pm$ 0.33	21.02 $\pm$ 0.21	1.69 $\pm$ 0.03	3.57 $\pm$ 1.04
RAS	70.83 $\pm$ 1.43 <sup>a</sup>	22.65 $\pm$ 0.11 <sup>a</sup>	1.72 $\pm$ 0.28 <sup>a</sup>	2.19 $\pm$ 0.23 <sup>a</sup>
CAS	71.49 $\pm$ 1.17 <sup>a</sup>	21.41 $\pm$ 0.12 <sup>b</sup>	1.70 $\pm$ 0.26 <sup>a</sup>	2.30 $\pm$ 0.63 <sup>a</sup>

All values were given in mean of three replicates  $\pm$  standard deviation (SD). Different superscript letters in the same column represent significant difference ( $P < 0.05$ ).

\*RAS= Recirculating aquaculture system, \*CAS = Conventional aquaculture system

the CAS which is an evidence that the rearing condition has significant influence on the growth performance and feeding utilization which is in agreement with Pinheiro and Fransozo (1993) and Pinheiro and Hattori (2006).

Although survival rate was slightly in favour of the conventional system over RAS, no significant differences were observed in the present study. However, during experimental rearing condition cannibalism is a major cause of low survival of crustaceans during experimental rearing condition. Even though various techniques have been developed to minimize cannibalism including rearing in isolation than in groups of three, rearing under zero light condition as in the red frog crab, *Ranina ranina* and the addition of substrates as in *Penaeus japonicus* (Celada et al. 1989; Kevrekidis & Kevrekidis 1996; Minagawa 1994). Sheen and Wu (1999) reported 90-100% survival was achieved when *S. serrata* were reared individually in darkness. Generally, survival was enhanced by maintaining the individual crabs in darkness per cultured unit.

RAS offer advantages in terms of reduced water consumption (Verdegem et al. 2006), improved opportunities for waste management and nutrient recycling (Piedrahita 2003), better hygienic, disease management and biological pollution control (Tal et al. 2009; Zohar et al. 2005). Although the feeding behaviour of crab and other aquatic animals is strongly influenced by the environmental conditions, however, water quality improvement in a RAS may increase feed consumption, feeding efficiency and metabolism, which may improve the whole-body chemical composition and meat quality of crab (Buentello et al. 2000). In the current study, the proximate composition of juvenile crab was not affected by rearing techniques, except for the crude protein which were significantly higher in RAS compared to CAS that is in agreement with studies on *Portunus pelagicus* (Gokoolu & Yerlikaya 2003), *P. sanguinotantus* (Siddique et al. 1987), *Scylla serrata* (Sheen 2000) and *Callinectes sapidus* (Farragut 1965). Moreover, the significant improvement of body protein of juvenile crabs in RAS over CAS system in the present study may be link to better dissolved oxygen, reduced ammonia and nitrite-nitrogen compound in RAS as several studies have shown increases in oxygen consumption after feeding due to the metabolic cost of digesting and assimilating the nutrients from the feed with low amount of ammonia and nitrite- nitrogen improved the body composition of culture species (Buentello et al. 2000).

## CONCLUSION

Overall, the study showed that the RAS system produced better results in maintaining the water quality parameters. Also, RAS has more advantages, especially in reducing water waste management, human recourses and space requirements. In addition, it can be concluded that the culture method using RAS is significantly better in terms of low ammonia and nitrite –nitrogen, higher growth and body protein content than the conventional method for blue swimming crab juvenile.

## ACKNOWLEDGEMENTS

The authors are grateful to those who have directly and indirectly contributed to this study especially to Comas staff, Port Dickson, Malaysia. This study was funded by a grant from Universiti Putra Malaysia (UPM via project no. GP- IPB/2014/9440400).

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Received: 14 October 2018

Accepted: 13 September 2019