Optimization of stocking density in intensification of mud crab *Scylla serrata* cultivation in the resirculation system

Optimalisasi padat tebar pada pemeliharaan kepiting bakau *Scylla serrata* dalam sistem resirkulasi

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ABSTRACT

This study aimed to determine optimum stocking density of mud crab *Scylla serrata* through the applied of different stocking density in every treatment in recirculation system. Experimental design used was complete randomized design (CRD) with three density treatments which were 5 (P1), 10 (P2), and 15 ind/container (P3). All treatments replicated three times. The crab with the average of body weight 150 g/ind cultured in a plastic box (40×30×30 cm^3). Crab was cultured within 60 days and were fed two times a day by at satiation method. The result showed that P2 treatment gave the best result of mangrove crabs production performance among all treatments with 73.33±5.77% survival rate, 0.68±0.01 g/ind/day absolute growth rate and food conversion ratio 10.11±0.01. Treatment P1 gave the good response of stress, it indicated by the lowest glucose of all tretamnets at the level of 31.91 mg/dL in the end of treatment periods. The water quality during study period was fluctuative as affected by different stocking density in the treatments.

Keywords: mud crab, stocking density, production performance

ABSTRAK

Penelitian ini bertujuan untuk menentukan padat tebar optimal kepiting bakau *Scylla serrata* melalui penerapan kepadatan tebar yang berbeda pada setiap perlakuan dalam sistem resirkulasi. Rancangan penelitian yang digunakan adalah rancangan acak lengkap dengan tiga perlakuan yaitu 5 (P1), 10 (P2), dan 15 ekor/wadah pemeliharaan (P3) dengan tiga ulangan. Kepiting bakau yang digunakan memiliki berat rata-rata 150 g/ekor. Wadah pemeliharaan yang digunakan selama pemeliharaan adalah kontainer plastik yang berukuran 40×30×30 cm^3. Pemeliharaan kepiting bakau dilaksanakan selama 60 hari dan diberikan pakan berupa ikan rucah dua kali sehari secara *at satiation*. Penelitian menunjukkan bahwa perlakuan P2 memberikan hasil kinerja produksi terbaik dibandingkan perlakuan lainnya dengan nilai kelangsungan hidup 73,33±5,77%, laju pertumbuhan mutlak 0,68±0,01 g/ekor/hari, dan rasio konversi pakan 10,11±0,01. Perlakuan P1 menunjukkan respons stres yang baik dengan memiliki nilai kadar glukosa paling rendah dari perlakuan lain yaitu 31,91 mg/dL pada akhir masa pemeliharaan. Kualitas air selama penelitian memiliki nilai yang fluktuatif di setiap perlakuan sebagai efek adanya perbedaan jumlah padat tebar setiap wadahnya

Kata kunci: kepiting bakau, padat tebar, kinerja produksi

INTRODUCTION

Mud crab *Scylla serrata* is an excellent fishery product having both high economic value and great developmental potential. Its increasing demand on both local and international markets leads to an increment of the production target, reaching up to 188% (KKP, 2014). Mud crab production mainly relies on wild catches that fluctuates considerably. Thus, there is a need to

develop a controlled mud crab farming system that can support the continuity of the production.

Mud crab *S. serrata* production in Indonesia consists of enlargement (or fattening), matured (in terms of eggs) crab production, and soft shell crab. The production is usually done by maintaining crabs in crab box at a density of 1 crab/box in ponds (land) or open water around the mangroves. Extensive traditional farming without crab box is carried out at a stocking density of 1 crab per 5–10 m^2 of the rearing container/tank. Meanwhile, a dense farming system would only have a stocking density of 1 crab for each 2–5 m^2 of the rearing container/tank (KKP, 2014). Mud crab has been widely produced in some coastal (ponds) areas in Indonesia, such as Karawang, Pemalang, Demak, Jepara and other coastal areas, especially those with mangrove forests.

Both land and rearing container efficiencies are needed in order to increase the quality and quantity of the mud crab production. In addition, optimal density and environmental manipulations are also necessary for creating optimal conditions which support growth and production. Suitabke environmental conditions (FAO, 2011) included shallow waters (with muddy ground), temperature (25–35 °C), pH (7.5–9.0), dissolved oxygen (DO>5 mg/L), and salinity (10–25 g/L). Previous research demonstrated that the optimum salinity for mud crab *S. serrata* optimal growth and survival in a water recirculating system is 25 g/L with an optimum pH of 7 (Hastuti *et al.*, 2015; Hastuti *et al.*, 2016).

Mud crab intensive production in a recirculating system is not only a more efficient way of farming but also important for the development of fisheries products (Siahainenia, 2009). The recirlating system is aimed at reusing the rearing water after it goes through filtration, sedimentation and bacterial purification processes in order to maintian bacteria and nitrogen at optimum levels. It is referred to as a closed culturing system, since both environment and media are tailored to the need of the farmed organism (Mulyadi *et al.*, 2014), and it has the advantge of being adaptable to the needs of the farmed organism through various manipulations. The system could be also applied on narrow field in order to intensively increase mud crab production (Schreier *et al.*, 2010). Mud crab stocking density optimization in a recirculating system is one of a breakthrough research in increasing its production.

This study was aimed to determine an optimum stocking density for mud crab production and eveluate its impacts on growth performance. The results of the present study will serve as a reference for further research on the recirculating system.

MATERIALS AND METHODS

Research design

A completely randomized design with three treatments (three different stocking densities) was used in this study, consisting of 5 (P1), 10 (P2), and 15 (P3) crabs/container. Each treatment was replicated three times.

Environmental preparation

As much as nine plastic containers (60×40×40 cm^3) were used in this research. The containers were cleaned with detergent, rinsed (with clean water) and dried, before placing them on rearing shelves and compiled based on the recirculating scheme for each treatment (Figure 1). The recirculating system was aimed at maintaining optimal conditions for mud crab production by reducing the accumulation of feces and feed residues in the rearing containers. Three different filters were used i.e. biological, chemical, and physical (Figure 2). The physical filter consisted of cotton and sand layers (Malang sand), while the chemical filter was made of zeolith stone. Bioball were used as biological filter.

A recirculating system was built for each treatment, thus a total of three recirculating systems were created, and made of three plastic containers and two drums (220 L) that served one as filter and the second as storage for filtered water. Each plastic container represented a replicate in each treatment and container was equipped with two aeration stones (0.125 L/s) and six shelters bound together using gutters in a cuboid shape (10×10×10 cm^3). The drums were also equipped with water pumps (60 W) serving to drain water from the filtering containers towards the rearing containers.

Both fresh and sea waters were used in the present study. The latter was brought from Ancol, West Java, at a salinity of 35 g/L, while tap water (from the Faculty of Fisheries and Marine Science, Bogor Agricultural University) was used as freshwater. Prior to use, water was first sterilized and aerated for 24 hour in order to avoid pathogenic microorganisms and chemicals as well that may harm the tested crab. In order to reach a salinity of 25 g/L, which is the optimum for mud crab growth performance (Hastuti *et al.*, 2015)
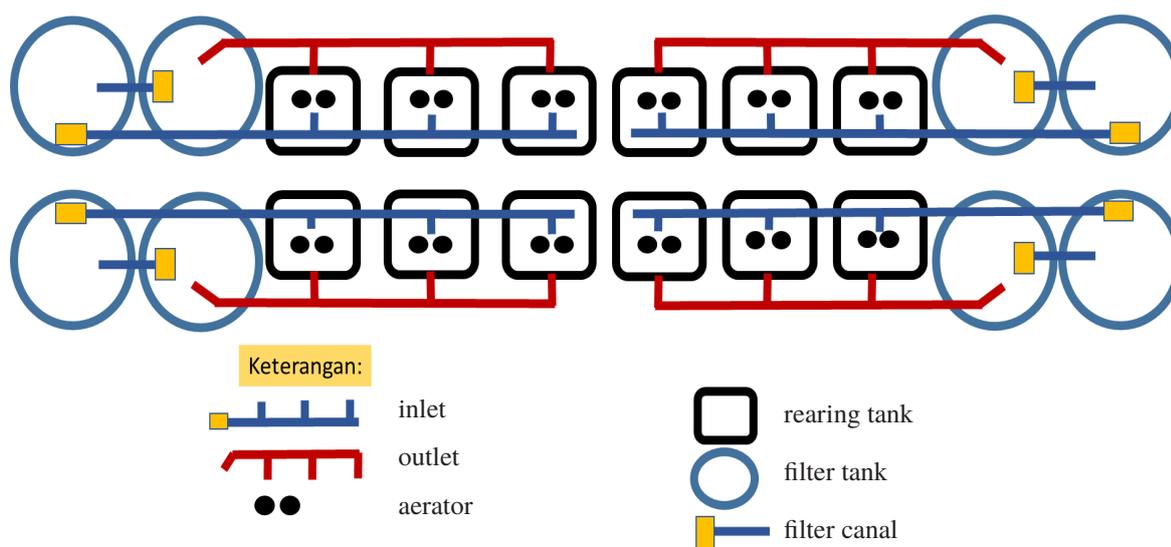
Figure 1. Recirculation scheme.

Figure 2. Filtering drum composition.

Preparation and rearing of the tested crabs

Ninety mud crabs at an average initial body weight of 150 g were used in the present research. Crabs were brought from Banjarmasin, South Kalimantan. Prior to stocking, crab were acclimatized to a medium saline water (25 g/L) for three days, and intial samples such as body weight and carapace width were taken prior to placing the crabs into containers.

Trash fish, derived from fish markets (TPI) in Muara Angke, was used to feed the crabs, which were fed twice daily (8 am and 4 pm) at satiation. The rearing period lasted for 60 days and water quality was maintained at an optimum level by smoothly running the recirculation system and making sure that no parts of both inlet and outlet were clogged. A stable water flow was maintained and 5% of the water was sponged out once there was waste (feed and feces). The tested parameters are presented in Table 1.

Water quality parameters such as temperature, pH, and water DO were recorded daily (8 am, 12 am, 5 pm, and 10 pm), while the others were measured weekly. Production performance parameters were also observed weekly, while stress response parameters were measured at the beginning, middle, and the end of rearing period.

Data analysis

The data were analyzed and tabulated using Microsoft Excel 2007 and SPSS and presented in forms of tables and graphs. Analysis of variance (ANOVA) with F-test at 95% of confidence interval was used to analyse data on production, while descriptive analysis was used for stress response and water quality profile.

RESULTS AND DISCUSSION

Results
Production performance

A 60 days period research on mud crab genrated data on production parameters such as survival rate (SR), absolute growth rate (AGR), and feed conversion ratio (FCR), presented in Table 2. Analysis of variance test (ANOVA) results showed that stocking density had significant effects on production parameters such as survival rate (SR), absolute growth rate (AGR) and feed conversion ratio (FCR) ($P<0.05$). No significant differences were observed between P1 and P2 treatments, but they significantly differed when compared to P3 treatment in survival rate and feed convertion

ratio parameters. The highest survival rates were observed in P1 treatment (80.00±0.00%) and no significantly different with P2 treatment (73.33±5.77). There is no significant differences were observed between P1, P2 and P3 treatments in absolute growth rate parameter (0.68±0.02 g/crab/day, 0.68±0.01 g/crab/day, and 0.66±0.01 g/crab/day). P1 and P2 treatments had no significant differences in the FCR value (10.31±0.15 and 10.11±0.11) but they differed significantly when compared to P3 treatment (9.33 ±0.22).

Stress response

Hemolymph glucose levels, which were determined as physiological response parameter of mud crab in each treatment (during the rearing

Table 1. Tested parameters

	Parameter	Unit	Measuring tool and method
Production performance	Survival rate (SR)	%	Goddard (1996)
	Absolute growth rate (AGR)	g/crab/day	Goddard (1996)
	Feed conversion ratio (FCR)	-	Goddard (1996)
Stress response	Hemolymph glucose levels	nmol/L	Blaxhall and Daysley (1973)
Water quality	Temperature	°C	Thermometer
	Turbidity	NTU	Turbidimeter
	Dissolved oxygen (DO)	mg/L	DO-meter
	Biochemical oxygen demand (BOD)	mg/L	BOD kit
	pH	-	pH-meter
	Salinity	g/L	Salinometer
	Ammonia	mg/L	Spectrophotometer
	Nitrite	mg/L	Spectrophotometer
	Nitrate	mg/L	Spectrophotometer
	Total organic matter (TOM)	mg/L KMnSO$_4$	Titration
	Alkalinity	mg/L CaCO$_3$	Titration

Table 2. Production performance of mud crab *Scylla serrata* at the end of the rearing period

Parameter	Stoking densities (ind/container)		
	P1 (5)	P2 (10)	P3 (15)
Survival rate (%)	80.00±0.00a	73.33±5.77a	48.89±10.18b
Biomass (g)	754.20±3.53a	1380.10±110.04b	1370.77±287.08b
Total consumption feed (g)	1849.67±106.73a	3181.00±332.65b	3826.67±304.16c
Absolute growth rate (g/ind/day)	0.68±0.02a	0.68±0.01a	0.66±0.01b
Feed conversion ratio	10.31±0.15a	10.11±0.11a	9.33±0.22b

Numbers on the same line followed by the same letter are not significantly different (at confidence level of 95%).

period) are presented in Figure 3. Hemolymph glucose levels that determined as physiological response parameter of mud crab in each treatment (during the rearing period) are presented in Figure 3. Decreases in hemolymph glucose levels were observed in all treatments at the end of the rearing period. The highest decrease levels were shown in the P1 (36.71 mg/dL) at the beginning of the rearing, it decreased to 31.91 mg/dL at the end of rearing. P3 treatment has the lowest decrease levels, it decreased from 37.95 mg/dL at the beginning of rearing to 36.36 mg/dL at the end of rearing.

Water quality

Water quality parameters such temperature, turbidity, dissolved oxygen, biochemical oxygen demand (BOD), pH, salinity, ammonia, nitrate, nitrite, alkalinity, and total organic matter (TOM) are presented in Table 3, and were still within the normal range for mud crab rearing in a recirculating system.

Discussion

Optimizing mud crabs *S. serrata* stocking density in a recirculation system was observed to have positive effects on environmental conditions. Indeed, physicochemical parameters are known as ecological factors that could directly affect oxygen consumption, metabolism, reproduction, growth, moulting, hormone production, phagocytosis, osmoregulation, and survival of
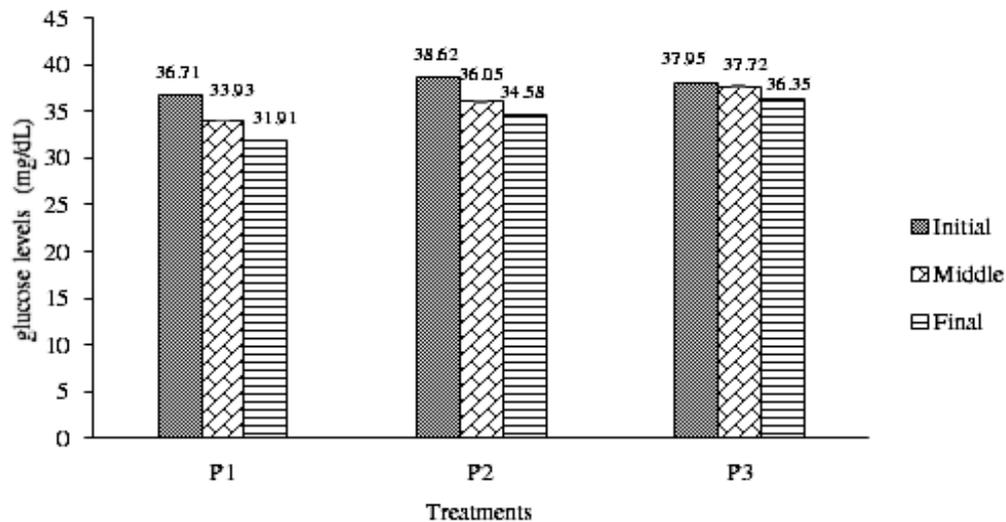

Figure 3. Hemolymph glucose levels in mud crab during the 60 days rearing period. Treatment: 5 (P1), 10 (P2), and 15 (P3) crabs/rearing container.

Table 3. Water quality parameters of rearing crab reared for 60 days

Parameter	Stocking densities (ind/container)		
	P1 (5)	P2 (10)	P3 (15)
Temperature (°C)	26–29	25–29	26–29
Dissolved oxygen (mg/L)	3.5–8.0	3.5–8.0	3.5–8.0
Turbidity (NTU)	0.45–2.70	1.10–3.80	0.80–4.80
Salinity (g/L)	25	25	25
Biochemical oxygen demand (mg/L)	0.0032–0.0144	0.0032–0.0164	0.0048–0.0144
pH	4.69–6.92	4.65–7.45	4.79–7.0
Alkalinity (mg/L CaCO$_3$)	11.46–57.31	11.46–80.23	11.46–68.77
Total organic matter (mg/L)	24–102	40–90	26–100
Ammonia (mg/L)	0.00002–0.0043	0.00003–0.0026	0.00003–0.00172
Nitrite (mg/L)	0.04–0.39	0.10–0.47	0.01–0.61
Nitrate (mg/L)	0.50–0.70	0.50–0.82	0.50–1.00

the crab (Handeland *et al*., 2008; Abbink *et al*., 2011; Gao *et al*., 2011). Based on the results of the water quality test (Table 2), water quality parameters were still within the recommended ranges for mud crab production. The TOM parameter was observed to be above the optimum range for mud crab culture in all treaments and treatment A had the highest TOM value (ranged between 24–102 mg/L) while the optimal TOM range in water is 20–30 mg/L (FAO, 2011). In afct, the total organic matter (TOM) content of marine sediment is generally very low (Zamora & Jeffs, 2011). However, a high range did not affect the rearing media opacities, which was evidenced by the turbidity range that were still within the recommended limits (<5 NTU) in all of the treaments (FAO, 2011). Water turbidity could be due to the dissolved organic matters and pigments caused by the decomposition of organic material (Bhatnagar & Devi, 2013).

The amount of organic matter contained in the water will also affect the BOD, which is the amount of oxygen used by microorganisms to stabilize organic materials (Varadharajan *et al*., 2013). BOD is a biological test procedures that measures the oxygen consumed by bacteria during organic matter decomposition. It ranged from 0.0032 to 0.0164 mg/L in the present stuudy, which was way below the optimal range of 20 mg/L (FAO, 2011). The low BOD could be a consequence of the degradation of both organic and inorganic wastes carried out by bacteria, which also affect ammonia, nitrite, and nitrate concentration in rearing media. The ammonia concentration in the water is known to infulence the osmoregulation process of crustaceans through the gills and an exposure to high ammonia levels can result in an increment in Na^+/K^+ -ATPase activity in the gill (Romano & Zeng, 2011). Ammonia accumulation in the hemolymph can affect metabolic processes such as oxygen transport and osmotic pressure balance (Unnikrishnan & Paulraj, 2010). The oxygen availability at an optimum level, in both rearing containers and filter, increased the success rate of nitrifying bacteria in degrading waste (Schreier *et al*., 2010). DO level in mud crab rearing containers during the 60 days period in a recirculating system was in accordance the FAO (2011) standard for crabs i.e >5 mg/L. In addition, ammonia reform process in an environment is strongly influenced by temperature (Zhang *et al*., 2015), which was observed to be within the normal range for mud crab production in a recirculation system (25–35 °C). Ammonia, nitrite and nitrate

contents of the rearing containers were measured and observed to be within the optimal ranges set by FAO (2001), e.i. <0.01 mg/L for ammonia, <0.5 mg/L for nitrite, and 0.1–1 mg/L for nitrate.

Total aquaculture waste (input) in a rearing container may lead to a decrease in pH, which corresponds to the equilibrium formula between pH and ammonia (NH_3). The equilibrum states that the amonia level of a given environment will increase along with an increasing pH, and vice versa (Yuechai, 2010). In the present study, the pH was observed to be lower (4.65 to 7.45) tending to an acid environment that was associated to a low alkalinity level. Water alkalinity refers to as the ability of the water to buffer pH changes and is part of the measurement of the basal concentration parameters of the water including carbonate, bicarbonate, hydroxide, phosphate, and borate (Ekubo & Abowei, 2011). The optimal alkalinity for mud crab growth performance is above 80 ppm and the ideal was determined to be 120 ppm (FAO, 2011). Meanwhile, a low alkalinity level was observed in the present study (from 11.46 to 80.23 mg/L $CaCO_3$), which could be due to the activity of forming new carapace. One of the effects of stocking mud crab at high densities on production is the competition for feed and space that could lead to stress, which is a physiological response against adverse environmental conditions. Blood glucose level is a parameter that best describe physiological responses in animals when maintaining homeostasis during changes that occur around them (Harianto *et al*., 2014). The highest glucose level was observed at the beginning of the rearing period, indicating hyperglisemia (indicator for early stress), and progressively decreased, which indicated a decrease in stress throuought the rearing period. The high glucose levels in the rearing containers, ranged between 31–39 mg/dL among treatments, could be a result of stress and the activity of the crab to maintian a stable body, which needs energy. The energy use could also be derived from blood glucose. When under stress condition, the glucose level in the blood increases due to the stress hormone that inhibits the secretion of insulin. Meanwhile, enzymes in the body proceed to gluconeogenesis, resulting in an increase in blood glucose (Tzafrir & Schreibman, 2010).

Stoking density determination is of capital importance in mud crab production, since it is related to the competition for both feed and space, which may lead to cannibalism that strongly affects the survival rate. In the present study, no

significant differences were observed between P1 and P2 in terms of survival rate (P>0.05). However, the number of crabs in each container differed at the end the rearing period (4 crabs in P1 and 7 crabs in P2). The lowest survival rate was observed in P3 treatment, which could be a consequence of cannibalism in the rearing container. Cannibalism could be defined as a type of predation of a dominant group of crabs on the other crabs. A common strategy used by farmers to overcome cannibalism is the reduction of the stocking density, feeding rate, or rearing the crabs individually (Laranja *et al*., 2010; Zhao *et al*., 2015).

Absolute growth rate is another parameter that should be closely monitored in the production process, and is defined as the actual increase in size of an individual per unit time under specific conditions (NRC, 1977). Growth is influenced by both internal and external factors (Sartje, 2010). Internal factors includes the resistance to disease and genetic, while the external factors include the availability of food in the living environment. Based on Table 2, there is no significant differences were observed between P1, P2, and P3 treatments in absolute growth rate parameter (0.68±0.02 g/crab/day, 0.68±0.01 g/crab/day and 0.66±0.01 g/crab/day).

Feed conversion ratio is a ratio measuring the efficiency with which the fish bodies convert animal feed into the desired output (NRC, 1977). During the rearing period, crabs were fed on fresh fish at satiation (Table 2). Analysis of variance showed that stocking density had a significant effect (P<0.05) on the feed conversion ratio and the best FCR was observed in P2 treatment (10.11±0.11) support with the best survival rate and total biomass value (73.33±5.77 % and 1380.10±110.04 g). The stocking density of 10 crabs/container showed the best results support with survival rate value indicating that the stocking density is efficient in recirculation system for spesific container.

CONCLUSION

The best stocking density for mud crab *Scylla serrata* production is 10 ind/container (P2) with a survival rate of 73.33±5.77%.

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