http://www.pjbs.org

PJBS

ISSN 1028-8880

# Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2018.275.283

# Research Article
# Protein Profile and Ammonia Excretion of Mud Crab *Scylla serrata* with Recirculation System

Yuni Puji Hastuti, Kukuh Nirmala, Rifa Syarifah and Siska Tridesianti

Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University, 16680, West Java, Indonesia

## Abstract

**Background and Objective:** Mud crabs have a high level of cannibalism so as to increase the growth of maximum mud crab and reduce the level of stress it is necessary environmental manipulation that supports it as a container maintenance with dark and bright conditions. This study aimed to determine the optimum container condition for survival and growth of mud crab by protein profile and ammonia excretion. **Materials and Methods:** Mud crab derived from the fisherman Pasuruan East Java with initial weight 45-65 g kept in containers ($60\times40\times30$ cm$^3$) in recirculation systems for 42 days with feeding trash fish restricted by 5% twice daily. The study was conducted with two treatments and three replications of bright containers and dark containers. Several stress indicators including protein retention, total serum protein, ammonia secretion were determined and growth performance of the mud crab was also evaluated. **Results:** The dark container resulted in optimum condition for mud crab indicated by the highest survival $30\pm6$%, specific growth rate $0.44\pm0.02$%/day, absolute longevity $1.29\pm0.03$ cm, absolute weight growth $10.5\pm0.31$ g, protein retention $15.59\pm0.46$% and feed conversion ratio $6.38\pm0.27$. **Conclusion:** This study concluded that better growth performance was observed in dark container for nursery of mud crabs.

Key words: Mud crab, *Scylla serrata*, protein profile, dark containers, environmental manipulation, response physiological, biomassa

Citation: Yuni Puji Hastuti, Kukuh Nirmala, Rifa Syarifah and Siska Tridesianti, 2018. Protein profile and ammonia excretion of mud crab *Scylla serrata* with recirculation system. Pak. J. Biol. Sci., 21: 275-283.

Corresponding Author: Yuni Puji Hastuti, Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University, 16680, West Java, Indonesia Tel: +62-813-1049-9728



Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Mud crab *Scylla serrata* has been known as commonly cultured commodity in Indonesia, particularly in West Java, Central Java, East Java, Bengkulu, north Sumatra, south Sulawesi, Southeast Sulawesi and Papua. Shelle and Lovatelli[1] reported that there is a remarkable increase in the world production of crustacea up to 9.7% (1990-2010), while crab production reached 200.000 t in 2010. Mud crab demand also increased 183.5% in 2000-2007 and it was expected to increase 188% in 2014. In 2013, crab export reached 34.173 t, but then decreased 28.091 t in 2014. This possibly related to the limitation of mud crab supply. Mud crab supply is still dependent on natural stock due to limited cultivation techniques. This led to exploitation, which contributed to a decreased population of the crabs in their natural habitat.

Mud crab is regarded susceptible to mortality because of cannibalism, which accounted for the major factor of decreased crab production. This species experienced molting during the growth phase in which cannibalism occurred, leading to a reduced crab population[2].

In a previous study, a manipulated container was prepared to rear sand lobster juveniles which were nocturnal feeders and recognized to have a high cannibalism. The light intensity was manipulated in dark and bright containers. To deal with high cannibalism among crabs, there is a need to design crab container with proper bright and dark condition, which contributed to improvement of growth performance through stress reduction. Presence of high light intensity could promote stress and mortality in some aquatic organisms[3]. The colors of rearing media and container play a significant role in reducing light penetrating to water[4] and also could affect stress response of fish.

There is limited published data pertaining optimum dark and bright condition of the container for mud crab. Hence, this current study aimed to investigate survival rate and growth performance of mud crab (*Scylla serrate*) cultivated in dark and bright container with recirculation system. Several stress indicators including protein retention, total serum protein, ammonia secretion were determined and growth performance of the mud crab was also evaluated. This research is expected to offer meaningful data for aquaculturists in enhancing their mud crab production.

## MATERIALS AND METHODS

This study was conducted from January to March, 2017, in the Laboratory of Environmental Aquaculture 2, Environmental Laboratory 1, Fish Nutrition Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences and Laboratory of Animal Physiology Faculty of Veterinary Medicine, Bogor Agricultural University.

**Mud crab seeds:** A total of 60 seeds (initial length 5.5-7.5 cm and weight 45-65 g) were obtained from Pasuruan, East Java.

**Feed:** Trash fish obtained from fish sellers in Muara Angke, North Jakarta was used to feed mud crabs.

**Container:** A total of 6 plastic boxes ($60\times40\times30$ $cm^3$) were used as containers, 3 plastic boxes were covered by black plastic (front, back, bottom, right and left side). The containers were also covered with net in top side. Each treatment consisted of 3 containers with 2 filter tanks (diameter 50 cm, height 100 cm), 1 water pump, 1 gutter roof, 1 recirulated pipe for each container, 6 shelters for each container, 1 water tap for each container and 2 aerators for each container to maintain level of dissolved oxygen.

**Rearing media:** Sea water (salinity 35 g $L^{-1}$) was obtained from Ancol, north Jakarta, while freshwater was supplied from laboratory facilities in Faculty of Fishery and Marine Science.

**Experimental design:** Completely randomized design consisting of 2 factors with triplicates was arranged. The treatments included ; Treatment A (mud crabs reared in bright container) and treatment B (mud crabs reared in dark container).

**Experimental animals:** A total of 60 seeds (initial length 5.5-7.5 cm and weight 45-65 g) were obtained from Pasuruan, East Java. Prior to main experiment, mud crabs were stocked in container ($60\times40\times30$ $cm^3$) for 7 days. For experiment, 10 mud crabs were stocked in each container. The water quality was controlled using recirculation system and the mud crabs were fed by trash fish twice a day (08.00 am and 08.00 pm) at satiation.

**Sampling:** The mud crabs (3 crab per replication) were sampled once per week by weighing their weight and measuring carapace length.

**Feeding:** The experimental animals were fed (twice a day at 9 am and 5 pm) by fresh fish (using restricted method) at feeding rate of 5% in the week 1-5 and 4.5% in the week[5] 6-9.

**Management of water quality:** To obtain optimum water quality, shiponication (before and after feeding) was performed to remove any unwanted materials in the bottom of chamber. Parameters of water quality (temperature, pH, dissolved oxygen) were controlled once a day, while other parameters (alkalinity, turbidity, nitrates, nitrites) were weekly measured[6]. Water sample was collected in maintenance container column and filter.

**Specific growth rate:** The specific growth rate of experimental mud crabs was calculated using the equation[7]:

$$SGR\ (\%) = \left( \sqrt[t]{\frac{Wt}{Wo}} - 1 \right) \times 100$$

Where:
SGR = Specific growth rate (%)
t = Rearing period (days)
Wt = Average body weight in certain time (g)
Wo = Initial average body weight (g)

**Feed conversion ratio:** The FCR evaluate only end experimental and this using the equation[8]:

$$FCR = \left( \frac{F}{(Wt + Wd) - Wo} \right)$$

Where:
FCR = Feed conversion ratio
Wo = Biomass of dead mud crabs during the experiment (g)
Wd = Initial mud crabs biomass (g)
Wt = Final fish biomass (g)

**Survival rate:** The survival rate of experimental mud crabs was calculated using the equation[8]:

$$Survival\ rate\ (\%) = \frac{Nt}{No} \times 100$$

Where:
Nt = Final mud crabs population
No = Initial mud crabs population

**Length growth:** The length growth was calculated using this following equation[4]:

$$Length\ growth\ (cm) = Lt\text{-}Lo$$

Where:
Lt = Average body length in certain time (cm)
Lo = Initial average body length (cm)

**Weight growth:** The weight growth was calculated using this following equation[4]:

$$Weight\ growth\ (g) = Wt\text{-}Wo$$

Where:
Wt = Final average body weight (g)
Wo = Initial average body weight (g)

**Protein retention:** Analysis of protein was carried out by proximate method. The observed protein retention was a change in protein content (Pr) to determine the mud crab protein. Analysis is done at the beginning and end of the experiment. Furthermore, by multiplying the food protein content with the amount of dry food consumed, the amount of protein can be determined. The retention (%) is calculated by referring to Takeuchi[9]:

$$Etention\ protein\ (\%) = \frac{Increase\ protein\ content\ (Pr)}{Total\ Pr\ consumed} \times 100$$

**Total serum protein:** Total protein was analyzed using biuret method. The test principle is that the protein in the sample will react with Cuprum ($Cu^{++}$). Then the analysis continued with measurements using a spectrophotometer at a wavelength of 546 nm. The tests were conducted at the beginning and end of the experiment based on Nugroho[10].

$$Total\ serum\ protein\ (g\ dL^{-1}) = 6 \times \left( \frac{As}{Ast} \right)$$

Where:
As = Sample absorbant
Ast = Blanko absorbant

**Ammonia excretion:** Ammonia excretion analysis was carried out at the end of the experiment. First, the crab sample is fasted for 24 h and then given restricted food. Then it is transferred to another container that has been filled with aerated water for 24 h. The water samples were taken every 1, 2, 4, 8 and 16 h, then the ammonia content was followed. During measurements the aeration is turned off and the crabs are not fed. Measurements were made using a spectrophotometer with a wavelength of 630 nm[11]. Ammonia levels are calculated by the formula:

$$\text{Mmonia excretion} = \frac{\left[\text{Nt} - \text{No}\right] \times \text{V}}{\text{W}} / \text{T}$$

Where:

Nt = Concentration of TAN at rearing period (mg TAN $L^{-1}$)
No = Concentration of TAN at certain time (mg TAN $L^{-1}$)
V = Volume water (mL)
W = Body weight (g)
T = Rearing period (days)

**Statistical analysis:** It is conducted that, analysis of variance (ANOVA) on physiological responses and growth response. ANOVA used is a one-way test followed by variable analysis using Duncan test.

## RESULTS

**Water quality:** As Table 1 represented water quality during 42 days experiment. The results indicated that parameters of water quality for both bright (A) and dark (B) container were observed in the optimal range which enabled to positively support the growth of mud crabs.

**Total serum protein (TSP):** The TSP of mud crabs cultured in A and B container at day 0 reached 7.95%, while TSP of mud crabs at day 42 was depicted in Fig. 1. The results showed that TSP seemed to decrease for both containers in day 42. The lowest TSP was attributed to mud crabs cultured in dark container (3.3%), compared to TSP of mud crabs cultured in bright container of 5.06% ($p>0.05$). Low TSP in B treatment may associate with the use of protein for growth of mud crabs.

**Protein retention (PR):** Protein retention of 42 day mud crabs was presented in Fig. 2. The results showed that dark container resulted in higher PR (15.59%) than bright container (13.24%). Statistical analysis revealed that PR in container B showed significant difference compared to other treatments ($p<0.05$).

**Ammonia excretion:** Results in Fig. 3 exhibited level of ammonia excreted by mud crabs under bright and dark container for 42 day experiment. The results demonstrated that ammonia excretion in bright container was 0.0008 mg TAN $g^{-1}$ body weight/h, which was not significantly different form ammonia excretion in dark container, i.e., 0.0014 mg TAN $g^{-1}$ body weight/ h ($p>0.05$).

**Survival rate (SR):** The data in Fig. 4 exhibited survival rate of mud crabs cultured in bright and dark container for 42 days. The SR of mud crabs cultured in bright container reached 10%, which was significantly lower ($p<0.05$) than that of mud crabs cultured in dark container, namely 30%.

Table 1: Parameters of water quality observed in bright and dark containers during experimental period

| | Treatments | | |
|---|---|---|---|
| Parameters | A (bright) | B (dark) | Optimum value |
| Salinity (g $L^{-1}$) | 25 | 25 | 25 (Hastuti *et al.*[5]) |
| Temperature (°C) | 25.5-26.7 | 25.3-26.7 | 23-35 (Shelle and Lovatelli[1]) |
| pH | 4.14-6.06 | 4.11-6.19 | 7 (Hastuti *et al.*[5]) |
| Dissolved oxygen (mg $L^{-1}$) | 3.9-8.1 | 4.2-8.6 | $\geq$5 (Shelle and Lovatelli[1]) |
| Alkalinity (mg $L^{-1}$) | 22.9-183.2 | 22.9-217.55 | $\geq$80 (Shelle and Lovatelli[1]) |
| TAN (mg $L^{-1}$) | 0.15-1.08 | 0.1-1.43 | <3 (Shelle and Lovatelli[1]) |

Different letters in the bar shows a significant difference at $p<0.05$

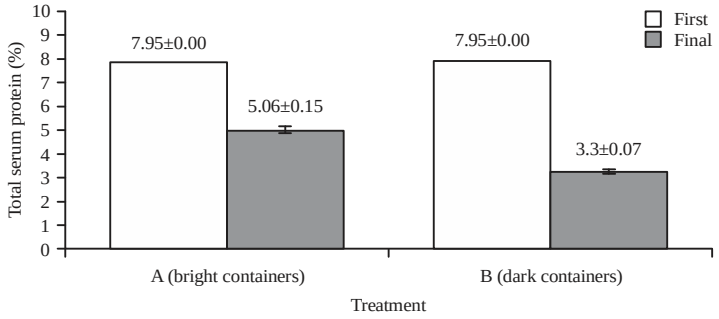


Fig. 1: Total serum protein of mud crabs cultured in bright and dark container for 42 days
Different letters in the bar shows a significant difference at $p<0.05$

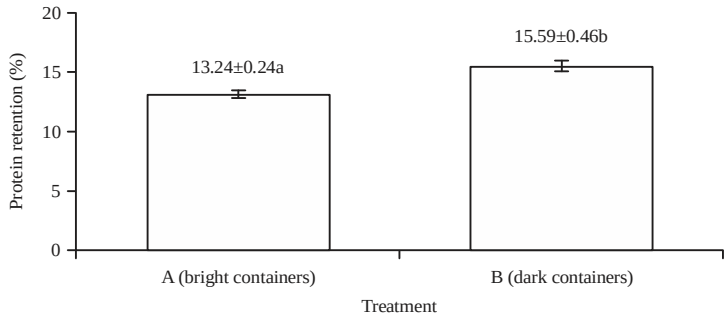


Fig. 2: Protein retention of mud crabs cultured in bright and dark container for 42 days
Different letters in the bar shows a significant difference at p<0.05

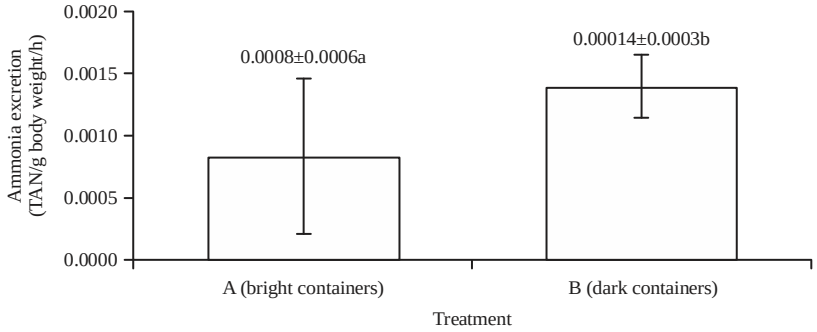


Fig. 3: Ammonia excretion of mud crabs cultured in bright and dark container for 42 days
Different letters in the bar shows a significant difference at p<0.05

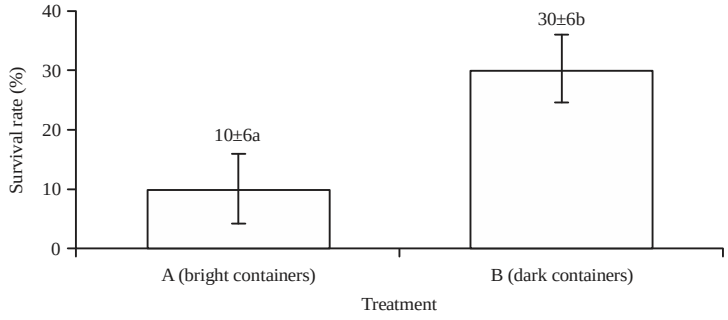


Fig. 4: Survival rate of mud crabs cultured in bright and dark container for 42 days
Different letters in the bar shows a significant difference at p<0.05

**Specific growth rate (SGR):** Specific growth rate of mud crabs reared in different containers for 42 days was exhibited in Fig. 5. The SGR of mud crabs cultured in bright container (0.28%) was significantly lower ($p<0.05$) than that of mud crabs cultured in dark container (0.44%).

**Length growth (LG):** Length growth of mud crabs cultured in bright and dark containers for 42 days was depicted in Fig. 6. The results showed that LG of mud crabs cultured in bright container (0.83 cm) was significantly lower ($p<0.05$) in comparison with that of mud crabs cultured in dark container (1.29 cm).

**Weight growth (WG):** Result in Fig. 7 presented weight growth of mud crabs cultured in bright and dark container for 42 days. The results demonstrated that WG of mud crabs cultured in bright container (5.85 g) was significantly lower ($p<0.05$) than in dark container (10.50 g).

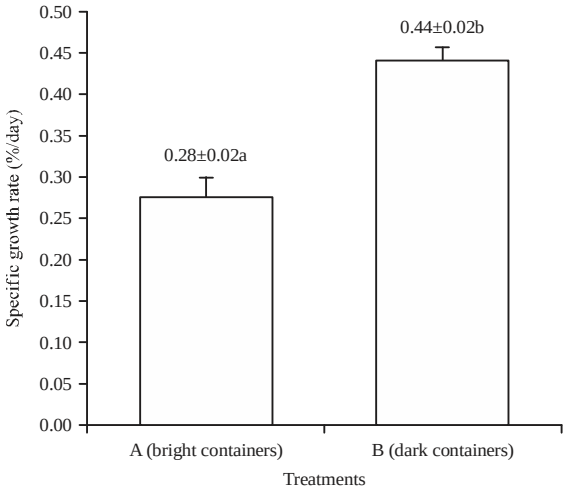


Fig. 5: Specific growth rate of mud crabs cultured in bright and dark container for 42 days
Different letters in the bar shows a significant difference at p<0.05

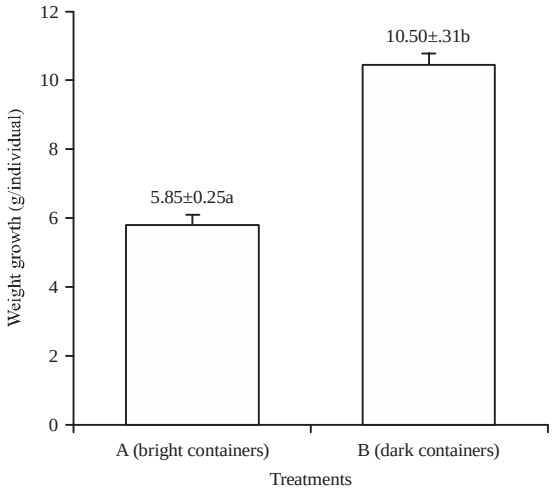


Fig. 7: Weight growth rate of mud crabs cultured in bright and dark container for 42 days
Different letters in the bar shows a significant difference at p<0.05

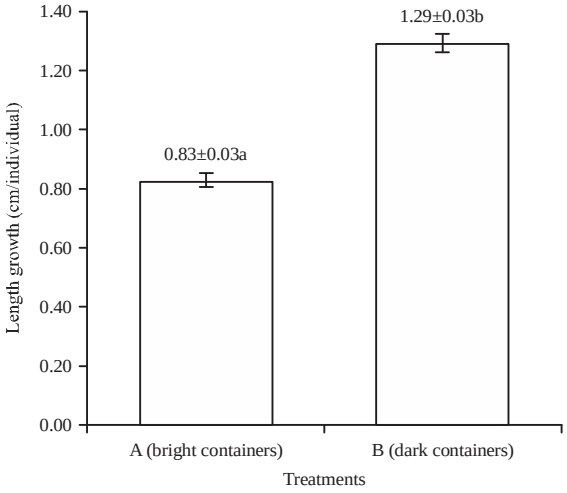


Fig. 6: Length growth rate of mud crabs cultured in bright and dark container for 42 days
Different letters in the bar shows a significant difference at p<0.05

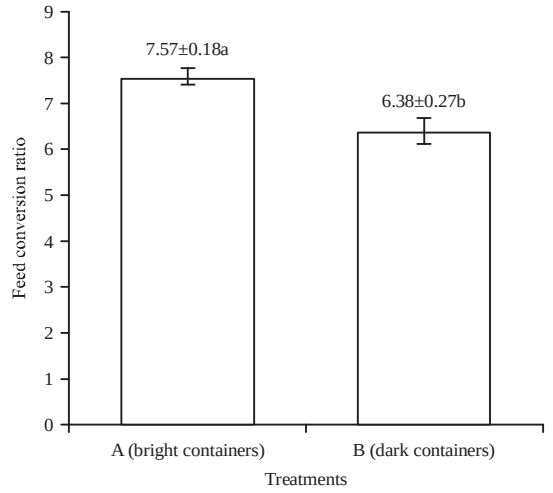


Fig. 8: Feed conversion ratio of mud crabs cultured in bright and dark container for 42 days

**Feed conversion ratio (FCR):** As presented in Fig. 8, the results showed that FCR of mud crabs cultured in bright container reached 7.57, which was significantly higher ($p<0.05$) than that of mud crabs culture in dark container, i.e., 6.38.

## DISCUSSION

Intensive crab farming could increase production efficiency, but it also produced more waste generated from debris and metabolic residue of the crabs. In this research, recirculation system was applied to intensify crab culture, which could reduce waste. Recirculation system is regarded as an effective alternative effort in maintaining water quality during growth phase of crab, thereby reducing water replacement for culture media. In this system, filtration system was performed by physical filter, chemical filter and biological filter. In the case of physical filtration, cotton filter and sand filter were used, which served to capture solid suspension in water. Smooth caves present in sand filter enables to trap suspended particles such as feces and debris[12]. In the case of chemical filter, zeolite stone was applicated to absorb ammonia through aluminosilicate minerals[13]. Furthermore, bioball was used as biological filter which enabled to serve as media for nitrifying bacteria that turned nitrogen ammonia into nitrate which was less deadly to aquatic life[14]. Based on

measurement of water quality (Table 1), application of both treatments unaltered parameters of water quality. It found that value of salinity, temperature, dissolved oxygen (DO) and total absolute nitrogen (TAN) in both bright and dark container was at acceptable range which could improve growth of mud crabs. The DO of bright and dark container was 3.9-8.1 and 4.2-8.6 mg $L^{-1}$, respectively. These values suggested that the treatments seemed to have DO values greater than optimum range[15], i.e., $\geq$5 mg $L^{-1}$. This may relate to under performed recirculated system, which inhibits oxygen diffusion into water. In the case of pH, the value reached 4.14-6.06 for bright container and 4.11-6.19 for dark container. This pH condition was also under optimum value[5], i.e., pH 7. Decreased pH was caused by the increased waste in the culture system. The pH tends to acid, which links to low alkalinity level in the culture media, i.e., 22.9-183.2 mg $L^{-1}$ for bright container and 22.9-217.55 mg $L^{-1}$ for dark container. These results indicate that there is value having value less than optimum condition, i.e., $\geq$80 mg $L^{-1}$ [11]. Alkalinity serves as buffer which compensate considerable pH changes, as well as total base concentration of water containing carbonate, bicarbonate, hydroxide, phosphate and borate[14]. Moreover, dark container showed higher range of TAN (0.1-1.43 mg $L^{-1}$) than bright container (0.15-1.08 mg $L^{-1}$). This result closely relates to the higher feed supply and ammonia secretion in dark container than in bright container.

The use of different colors covering the container enables to manipulate the light intensity coming to the culture media. The visible color shows varying responses to wavelength of the light and has different characteristics such as brightness and lightness. The use of white cover in bright container could reflect the most incoming light, but dark container enables to absorb the light[7]. Light intensity absorbed in bright and dark container was 400 lux and 150 lux, respectively, in which the light had intensity of 800 lux. Presence of excessive light may promote stress and mortality to aquatic organism. Some species could have better growth in low light intensity[3]. High stress condition would affect physiological responses of mud crabs and in turn affect their growth performance. They included total protein serum, protein retention and ammonia secretion.

Hemolymph mostly consists of hemocyanin (>60%) and the remaining was protein including coagulogen, apohemocyanin, hormone and lipoprotein. Serum protein serves to transport oxygen and plays important in reproduction, growth and stress response system. Protein content in blood could indicate the nutritional status of a crustaceae[16]. Total serum protein (TSP) of mud crabs cultured in dark container was 3.30%, which was significantly lower ($p<0.05$) than that of mud crabs cultured in bright container (5.06%). This suggests that protein is allocated for growth of mud crabs. Level of blood protein was lower before moulting, since protein was used to formation of exoskeleton[17]. This finding is augmented by the high protein retention of mud crabs cultured in dark container. Rameshkumar *et al.*[15] reported that total protein serum of mud crabs reached 10.97%.

Protein is a fundamental nutrition which is responsible for formation of tissues and growth performance. Trash fish contains protein content of 14.95%. Feed containing appropriate protein content would support growth performance and body proteins efficiently. The results demonstrated that dark container showed better protein retention (15.59%) in comparison with protein retention of mud crabs cultured in bright container (13.24%). In dark condition, feed consumption was higher, which could generate sufficient energy required for metabolic activities, thus digested protein could be stored[10].

Furthermore, ammonia excretion of mud crabs cultured in dark container was 0.0014 mg TAN $g^{-1}$ individual/h, which was significantly higher than that of mud crabs cultured in bright container, i.e., 0.0008 mg TAN $g^{-1}$ individual/h. Ammonia excretion was affected by protein content of feed and protein retention. The excessive of amino acids would be changed to be deaminated form. Nitrogen from deamination process was excreted[18]. This suggests that mud crabs cultured in dark container more consumed the feed, thus the digested protein was excessive as indicated by protein retention. Consequently, their growth was faster in comparison with mud crabs cultured in bright container. Light intensity penetrating the container also remarkably affected survival rate of mud crabs. High mortality is regarded as one of the constraints for intensive mud crab farming and influenced by several factors such as environmental condition, cannibalism and water quality. Hence, minimalizing the mortality of mud crabs is necessary.

In bright container, bright intensity was higher than in dark container. Thus, mud crabs cultured in bright container gathered in shelter, which caused higher physical contact among mud crabs. They are nocturnal feeders. Similar to sand lobster, mud crabs also had territorial behavior which enhanced the competition for space and feed, thus increasing cannibalism. This may reduce survival rate of mud crabs in bright container (10%) in comparison with dark container (30%) as depicted in Fig. 4.

Biomass is another important indicator for production performance of mud crabs. Dark container significantly demonstrated higher weight growth of mud crabs (10.50 g) in comparison with bright container (5.85 g) as exhibited in Fig. 7. Specific growth rate of mud crabs cultured in dark container exhibited better value, i.e., 0.44%, which was significantly higher than that of mud crabs cultured in bright container, i.e., 0.28% (Fig. 5). However, length growth of mud crabs cultured in dark container (1.29 cm) was significantly better than that of mud crabs cultured in bright container (0.83 cm) as shown in Fig. 6. Growth of mud crabs constituted a fundamental parameter for their growth performance, affecting feed need[19]. Furthermore, mud crabs were restrictly fed using transfish at 5%. In the case of feed conversion ratio (FCR), the lower value was observed in FCR of mud crabs cultured in dark container (6.38) in comparison with FCR of mud crabs cultured in bright container (7.57) as depicted in Fig. 8. Feed is required for generating energy that is necessary for metabolic activity and adaptation to environmental changes. At optimum environmental condition, the remaining energy was then used for improving the growth of mud crabs. Considering that mud crabs are nocturnal feeders, their growth in dark container is better than that in bright container. Dark container may offer an environment conducive for continuous feeding activity, which contribute to higher feed consumption and growth[20]. This could explain that dark container had more desirable feed efficiency as indicated by lower FCR.

Based on the explanation above, it can be seen that the treatment of dark containers resulted in a lower value (significant $p<0.05$) on the stress response than the treatment in bright containers. This shows that mud crabs with dark container treatment experience lower stress levels than mud crabs with bright container treatment. Low stress or suspected no stress on mud crabs by treatment of dark containers certainly has a positive effect on growth response such as specific growth rate, length growth rate, survival rate, weight growth rate, feed conversion ratio. Where in this study it has been known that the growth response in the treatment of dark containers is better than the treatment of bright containers. Thus it can be stated that the treatment of dark containers has a significant effect on the growth of mud crabs so that this study can provide important information in the development of large-scale mud crab cultivation technology.

## CONCLUSION

This study concluded that better growth performance was observed in dark container for nursery of mud crabs, as indicated by survival rate of 30%, growth rate of 0.44%/day, protein retention of 15.59%, total protein serum of 3.3%. Therefore, based on the results, nursery for mud crabs was better performed by using dark container to enhance survival rate, growth rate and intensive crab farming could be conducted in controlled condition.

## SIGNIFICANCE STATEMENT

It can be stated that the treatment of dark containers has a significant effect on growth of mud crabs so that this study can provide important information in the development of large-scale mud crab cultivation technology.

## REFERENCES


1. Shelley, C. and A. Lovatelli, 2011. Mud crab aquaculture: A practical manual FAO. Fisheries and Aquaculture Technical Paper No. 567, FAO, Rome, Italy, pp: 78.
2. Sulaiman and A. Hanafi, 1992. Pengamatan terhadap berbagai tingkah laku kepiting bakau *Scylla serrata* forskal. Warta Balitida, 4: 8-12.
3. Boeuf, G. and P.Y. Le Bail, 1999. Does light have an influence on fish growth? Aquaculture, 177: 129-152.
4. Dosdat, A., F. Servais, R. Metailler, C. Huelvan and E. Desbruyeres, 1996. Comparison of nitrogenous losses in five teleost fish species. Aquaculture, 141: 107-127.
5. Hastuti, Y.P., R. Affandi, M.D. Safrina, K. Faturrohman and W. Nurussalam, 2015. Optimum salinity for growth of mangrove crab Scylla serrata seed in recirculation systems. J. Akuakultur Indonesia, 14: 50-57.
6. APHA., 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edn., APPHA., AWWA and WPCF., Washington DC., USA., pp: 1193.
7. Fitch, T. and D. Lankford, 2013. Why do black material absorb light and white material reflect it? Columbia Daily Tribune, September 8, 2013.
8. Goddard, S., 1996. Feed Management in Intensive Aquaculture. Chapman and Hall, New York, pp: 50-57.
9. Takeuchi, T., 1988. Laboratory Work-Chemical Evaluation of Dietary Nutrients. In: Fish Nutrition and Mariculture, Watanabe, T. (Ed.). Kanagawa International Fisheries Training Center, Japan International Cooperation Agency (JICA), Japan, pp: 179-233.
10. Karim, M.Y., 2005. Kinerja pertumbuhan kepiting bakau betina (*Scylla serrata* Forsskal) pada berbagai salinitas media dan evaluasinya pada salinitas optimum dengan kadar protein pakan berbeda. Ph.D. Thesis, Bogor Agricultural University, Bogor, Indonesia.

11. Serang, A.M., M.A. Suprayudi, D. Jusadi and I. Mokoginta, 2007. Effect of dietary protein level and protein to energi ratio on the growth of swimming crab *Portunus pelagicus*. J. Akuakultur Indones., 6: 55-63.
12. Maishela, B., Suparmono, R. Diantari and M. Muhaemin, 2013. Pengaruh fotoperiode terhadap pertumbuhan lele dumbo (*Clarias gariepinus*). E-J. Rekayasa Teknologi Budidaya Perairan, 1: 145-150.
13. Schulz, C., U. Knaus, M. Wirth and B. Rennert, 2005. Effects of varying dietary fatty acid profile on growth performance, fatty acid, body and tissue composition of juvenile pike perch (*Sander lucioperca*). Aquacult. Nutr., 11: 403-413.
14. Dewi, Y.S. and M. Masithoh, 2013. Efektivitas teknik biofiltrasi dengan media bio-ball terhadap penurunan kadar nitrogen total. J. Limit's, 9: 45-53.
15. Rameshkumar, G., S. Ravichandran, G. Kaliyavarathan and T.T. Ajithkumar, 2009. Comparison of protein content in the haemolymph of brachyuran crabs. Middle-East J. Scient. Res., 4: 32-35.
16. Lorenzon, S., M. Martinis and E.A. Ferrero, 2011. Ecological relevance of hemolymph total protein concentration in seven unrelated crustacean species from different habitats measured predictively by a density-salinity refractometer. J. Mar. Biol., Vol. 2011. 10.1155/2011/153654.
17. Terwilliger, N.B., 1999. Hemolymph proteins and molting in crustaceans and insects. Am. Zool., 39: 589-599.
18. Silaban, T.F., L. Santoso and Suparmono, 2012. Addition of zeolite decrease ammonia concentration in common carp (*Cyprinus carpio*) cultured. E-J. Rekayasa Teknologi Budidaya Perairan, 1: 47-56.
19. Tzafrir Prag, T., M.P. Schreibman, I. Lupatsch and C.B. Zarnoch, 2010. Preliminary studies of energy and protein requirements of Atlantic horseshoe crabs, *Limulus polyphemus*, grown in captivity. J. World Aquacult. Soc., 41: 874-883.
20. Nurhidayat, K. Nirmala and D. Djokosetyanto, 2012. Efektivitas kinerja media biofilter dalam sistem resirkulasi terhadap kualitas air untuk pertumbuhan dan sintasan ikan red rainbow (*Glossolepis incises* Weber). J. Riset Akuakultur, 7: 279-292.