Actual activity of nitrifying bacteria in culture of mud crab *Scylla serrata* under recirculating system with various light treatments

Yuni P. Hastuti, Kukuh Nirmala, Daniela Merani, Siska Tridesianti

Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University, West Java, Indonesia. Corresponding author: Y. P. Hastuti, yuni_ph2@yahoo.com; yuniha@ipb.ac.id

Abstract. Water quality in aquaculture activities can be improved by using nitrifying bacteria. Nitrification constitutes an oxidation process of ammonium to form nitrite and nitrate. This current work aimed to investigate the effects of color treatments on the improved water quality of mud crab (*Scylla serrata*) aquaculture system through activity of nitrifying bacteria. Mud crab (average weight 52.6±7.2 g) was reared in container (60×40×40 cm) for 45 days and fed 5% of their weight twice a day. Dark and light containers with light intensity of 40-100 lux and 310-380 lux respectively were used. The best performance of nitrifying bacteria was found in light containers with abundance of 4.40±0.14 (10^7 CFU mL^{-1}) on day 0, 4.92±0.18 (10^7 CFU mL^{-1}) on day 21, and 5.44±0.18 (10^7 CFU mL^{-1}) on day 42, ammonium oxidation activity of 89.51-98.81%, nitrite formation activity of 50.85-87.20%, and nitrate reduction activity of 6.40-47.31%. Types of nitrifying bacteria were dominated by *Pseudomonas* sp. (12 isolates), *Bacillus* sp. (6 isolates), and *Acinetobacter* sp. (6 isolates). Our experiment concluded that light containers (310-380 lux) was suggested to improve water quality associated with the performance of nitrifying bacteria.
Key Words: container, aquaculture, water quality, nitrification, recirculation.

Introduction. Mud crab (*Scylla serrata*) is one of the high prospecting fishery commodities in Indonesia (Iromo & Farizah 2014). According to FAO (2017), the price of *S. serrata* in the world reaches 7.5-10.0 USD/kg. Sianturi et al (2013) reported that *S. serrata* was in great demand due to its taste and high nutrition content, with protein 13.6 g, fat 3.8 g, ash 14.1 g, and water 68.1 g for 100 g of fresh *S. serrata*. In addition, Karim (2005) stated that *S. serrata* also contains high levels of minerals and omega-3 fatty acids. The demand for *S. serrata* continues to increase, both in domestic and international markets. Importation of *S. serrata* showed a remarkable increase, from 279,400 tons in 2013 to 286,900 tons in 2014 (FAO 2015). This increased demand leads to elevated catching activities in nature as current production still relies on natural catches (Sentosa & Syam 2011). In Indonesia, production of *S. serrata* was recorded 34,173 tons in 2013, and then decreased to 28,091 tons in 2014 (KKP 2015). This exploitation would lead to overfishing. In addition, poor handling seems to be a problem which is associated with lower economic value of the mud crab (WWF 2015).

In response to the overfishing activities, Ministry of Marine Affairs and Fisheries Republic of Indonesia, issued a regulation Number 1 of 2015 (KKP 2015) of lobster *Panulirus* sp., crab *Scylla* sp., and Portunus *pelagicus* sp. Currently, *S. serrata* farming included enlargement activities, fattening, and crab egg production (Hastuti et al 2015). To reach optimum condition for *S. serrata* culture, environmental engineering is considerable. The natural habitat of *S. serrata* is in the shallow waters of mangrove area with pH 7.5-8.5, salinity of 10-25 ppt, and temperatures of 25-35°C (FAO 2011).

Recirculation aquaculture system (RAS) has received importance as a trend in the aquaculture. RAS is designed to maintain water quality in the crab farming (Fauzzia et al 2013). It is a system in which water in re-used after undergoing treatment including physical, chemical, and biological filtration. A living organism such as nitrifying bacteria

could serve as a biological filter by which toxic compounds in culture media are reduced through nitrification process under aerobic condition. RAS is applied since it has several advantages such as enhancing production with limited water and land availability, controlling the quality of aquaculture, and applicable in a location close to the *S. serrata* market (Helfrich & Libey 2013).

The optimal conditions for *S. serrata* farming could be obtained using environmental engineering. In this approach, the background color of container was regarded as the important part since it affected the intensity of either absorbed or reflected light (Fitch & Lankford 2013). While black container enables to absorp light, white container is capable of reflecting it. Culture condition with too high light intensity promotes stress and mortality. Furthermore, such condition also promote less feed consumption, contributing to higher unconsumed feed. The remaining feed and residual metabolism of the cultivated organisms lead to the accumulation of organic matter resulting in decreased quality of water (Widanarni et al 2010). Rough fish, which contains protein of 17% wet basis, is commonly used as a feed for *S. serrata* (Hein et al 2015). The use of nitrifying bacteria such as *Nitrosomonas* sp., *Nitrobacter* sp., and *Nitrosococcus* sp. has been proposed to serve as a meaningful solution regarding to improvement of water quality (Kiding et al 2015). Using nitrifying bacteria, this current work is designed to maintain concentration of inorganic nitrogen compounds (ammonia, nitrite, and nitrate) in optimum level. Therefore, the purpose of this study was to observe actual activity of nitrifying bacteria on the *S. serrata* recirculation system with different colors of the container.

Material and Method

Experimental fish and preparation. A total of 60 *S. serrata* (carapace length of 6.2±0.5 cm and weight of 52.6±7.2 g) were obtained from Pasuruan, East Java. The experiment was carried out in the Laboratory of Aquaculture Production Technology and Management, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Indonesia, from February to June 2017.

Experimental design. Two background colors (dark and light) of the culture container were used, as presented in Table 1. The experiment was performed in triplicate.

Table 1

Background colors of culture container applied in this experiment

Treatment	Treatment code
Light container wall (light intensity 310-380 lux)	T
Dark container wall (light intensity 40-100 lux)	G

Recirculating aquaculture system. The culture containers consisted of 6 plastic units (60×40×40 cm each). The containers were set in a maintenance rack and filled with water up to a height of 25 cm. Dark containers were prepared by covering with black plastic, while light container was not covered. The installation of recirculation system involved two container units (220 L) for three culture containers (for 1 treatment), with totally using 4 units of drum. A water pump of 20 Watt and 60 Watt were set on each drum. One drum of each treatment was prepared by a filter system consisting of dakron cotton, bioball, sand, and zeolite. The PVC pipe was mounted on the water pump up to the culture container with the faucet on each maintenance container. *S. serrata* was reared for 45 days in the culture containers (at density of 10 individuals/container) installed with 2 aeration systems and 6 shelters made of 2 pieces of water gutter (15×15 cm). Culture media was sea water with salinity of 25 g L^{-1}. *S. serrata* were acclimatized with 25 g of L^{-1} salinity medium for 7 days (Hastuti et al 2015). The initial sampling was conducted by weighing and measuring the carapace length of the *S. serrata*. *S. serrata* (3 individuals) was sampled for observing the growth (every 14 days). The crab was fed with rough fish twice a day at feeding rate (FR) of 5% (Sadinar et al 2013).

Screening of nitrifying bacteria. Water sample used for isolation of nitrifying bacteria was obtained from oxidation drum of *S*. *serrata* recirculation system. The medium used for bacteria isolation was a specific nitrification medium. Water sample was diluted serially to 10^{-6} with NaCl solution. Diluted sample (10^{-4}, 10^{-5}, and 10^{-6}) was cultured from different nitrifying medium to obtain pure colonies or single colonies. The colony was identified using morphological observations (colour, shape, edges, and elevations), gram staining, and biochemical tests (oxidative/fermentative, motility, catalase, and oxidase).

The actual activities of nitrifying bacteria. The nitrifying bacteria at diluted solution of 10^{-4}, 10^{-5}, and 10^{-6} from culture medium were extracted and fed into 5 mL of liquid nitrification medium in a threaded tube, then incubated for 5 days at a shaker at a speed of 80 rpm (Hastuti et al 2017). Then, this bacterial activity was measured on day 0 and day 5 after identification. This bacterial activity was measured using a spectrophotometer, including ammonium oxidation activity, nitrite acumulation, and nitrate reduction (APHA 2005). Tests of bacterial activity were performed in day 0, 21, and 42 (Suantika et al 2016). A control medium with no inoculated bacteria was used. The parameters observed included water quality including concentration of ammonium, nitrite, and nitrate (Cappucino & Sherman 1987).

Purification of bacteria. The morphology of nitrifying bacteria isolate was determined according to gram staining, shape, colony color, elevation, edges, and could be carried out using biochemical tests including oxidativeor fermentative tests, catalase, oxidase, and motility according Cowan & Steel (2003). Gram-positive bacteria were characterized by purple in color, which means that the bacteria are capable of binding violet crystals. Gram-negative bacteria were characterized by formation of pink in color, indicating that they are not able to bind violet crystals and only colored by safranin.

Water quality. During experiment, water quality concerning biological oxygen demand (BOD), total ammonia nitrogen (TAN), ammonia, ammonium, nitrite, nitrate, and C/N ratio were determined every 10 days. Water quality measurement was presented in Table 2.

Table 2
Water quality of *Scylla serrata* culture maintained on different coloured containers

Parameters	T (Light container)	G (Dark container)	Standard
BOD (mg L^{-1})	0.6-5.8	0.5-6.4	<5 (Mocuba 2010)
C/N Ratio	1.54-5.17	1.32-3.30	±5 (Pramanik et al 2012)
Ammonia (mg L^{-1})	0.001-0.007	0.001-0.005	<0.25 (FAO 2011)
Ammonium (mg $^{L-1}$)	0.21-0.79	0.17-0.93	-
Nitrite (mg L^{-1})	0.06-0.14	0.09-0.22	<10 (FAO 2011)
Nitrate (mg L^{-1})	0.38-0.98	0.39-1.27	<80 (Kwong & Chowdhury 2016)
Light intensity (lux)	310-380	40-100	400-700 for fish (Dellabio 2015)

Statistical analysis. Data were expressed as mean ± standard deviation, and evaluated using analysis of variance (ANOVA) at significance of 95% in SPSS 20.0 software. Significant difference between means was evaluated using Duncan test.

Result and Discussion

Actual activity of nitrifying bacteria. The results showed that 24 isolates of the bacteria were obtained (12 isolates from light container filter - T treatment, 12 isolates from dark container - G treatment). All isolates were able to live aerobically in medium with ammonium as a source of nitrogen and succinate as a carbon source. Nitrifying

bacteria were purified using quadrant method to produce pure colony or single colony (Figure 1). Bacteria were then identified morphologically and biochemically. We found that most isolated nitrifying bacteria had circular colonies, whole edges, and raised elevations with white color, and they were confirmed as Gram-negative bacteria. Nitrifying bacteria are generally Gram-negative cells, and capable of forming colonies in both freshwater and seawater in which the oxidation of ammonium and nitrite occured (Cheatham 2009).

Figure 1. Bacterial purification using quadrant method (A) and Gram staining (B) (original).

We found that the isolates from both dark and light container showed a similar colony morphology, i.e circular, whole edges, and raised elevation. The isolates mostly had a white colony, while other isolates were yellow. Based on Gram staining, formation of pink color indicated that the bacterial cells were regarded as Gram-negative, while presence of purple color indicated Gram-positive bacterial cells. A total of 6 isolates (coccus) were attributed to Gram-positive cells, while 18 isolates (bacillus) were recorded as Gram-negative cells. Based on Cowan & Steel (2003) showed that nitrifying bacteria were suspected to be 12 isolates of *Pseudomonas* sp., 6 isolates of *Bacillus* sp., and 6 isolates of *Acinetobacter* sp. Nitrification could be performed by bacteria such as *Pseudomonas stutzeri* (Novita 2006), *Bacillus* sp. (Lin et al 2007), and *Acinetobacter calcoaceticus* (Su et al 2015).

The ammonium oxidation activity on day 0, 21 and 42 is depicted in Figure 2. The results showed that the activity of nitrifying bacteria in light and dark container was significantly different. Bacteria from container T (light container) demonstrated a higher ammonium oxidation activity ($P<0.05$) than bacteria from container G (dark container). We also found that bacteria from container T were more stable in oxidizing ammonium over bacteria from container G. Nitrification is an oxidation process of ammonium to form hydroxylamine, then oxidized to nitrite and nitrate. The reactions occured in the nitrification process are caused by different enzymes, i.e. ammonia monooxygenase (AMO), hydroxylamine oxidoreductase (HAO), and nitrite oxidoreductase (NO). The nitrification process can occur in autotroph or heterotrophic conditions (Widanarni et al 2010). Agustiyani et al (2004) found that autotrophic ammonium oxidizing bacteria played an important role in the oxidation of ammonium to nitrite in the nitrogen cycle. Autotrophic nitrifying bacteria can utilize carbondioxide as an inorganic carbon source (Cheatham 2009). In addition, Agustiyani et al (2004) reported that the autotrophic nitrifying bacteria derive its energy from the oxidation of ammonium or nitrite and have a relatively slow rate of growth compared to heterotrophic nitrifying bacteria. Cheatham (2009) stated that autotrophic nitrifying bacteria have slow growth because the bacterial doubling time is 7-8 hours, whereas in situ condition, this doubling process occurs within 26 hours. Nitrite oxidizing bacteria are more sensitive to light than ammonium oxidizing bacteria. The nitrification process can run quickly in dark conditions. *Nitrosomonas* sp. would be inhibited at light intensity of <420 lux (Nguyen 2013).
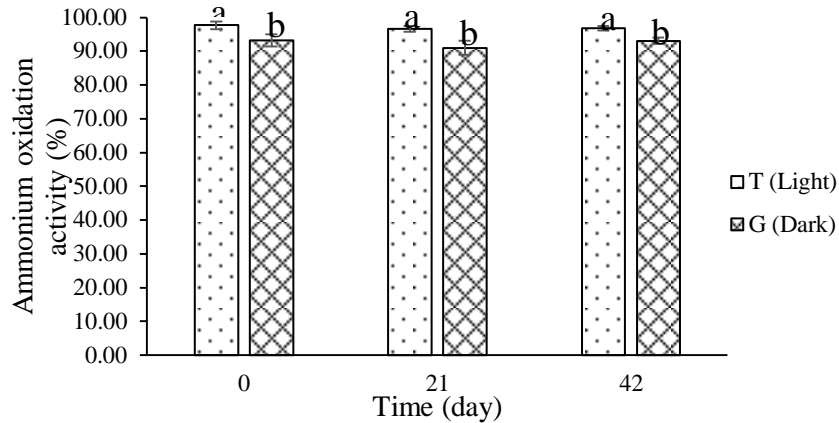
Figure 2. Ammonium oxidation activity of nitrifying bacteria in recirculation system of *Scylla serrata* culture. Different letters above the bars in the same period of measurement indicates significant difference.

Additionally, ammonium level present in the rearing water for 45 days of experiment also showed a significant difference (Figure 3). Ammonium oxidation activity by bacteria from container T ranged from 89.51% to 98.81%, whereas bacteria from container G ranged from 85.43% to 98.21%. The activity of nitrifying bacteria was influenced by oxygen availability in their environment. FAO (2014) reported that optimum nitrification process could be reached at DO 4-8 mg L^{-1}. We found that nitrification in both dark and light container could reach optimum condition due to indirect light exposure and presence of ammonium, thus allowing the desired growth of nitrifying bacteria. *Bacillus* sp. was capable of reducing ammonium nitrogen up to 74.7% (Lin et al 2007).
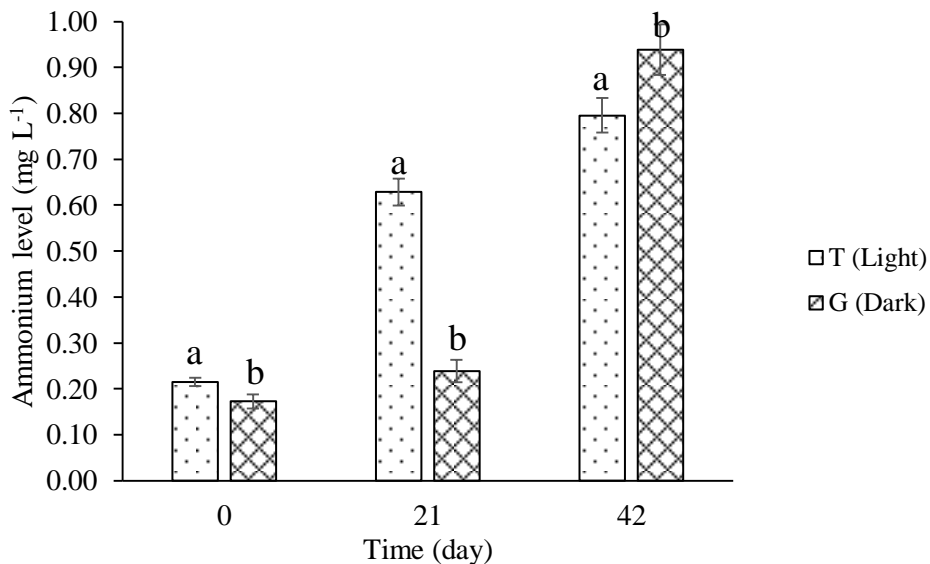

Figure 3. Ammonium level in recirculation system of *Scylla serrata* culture. Different letters above the bars in the same period of measurement indicates significant difference.

Nitrite formation. Figure 4 shows the formation of nitrite by nitrifying bacteria observed on day 0, 21, and 42. The results demonstrated that there was no significant effect on nitrite formation between bacteria from container T and bacteria from container G on day 0, but acitivty of nitrite formation by bacteria from container T was higher (P<0.05) than bacteria from container G on day 21 and day 42. EPA (2002) reported that factors affecting the growth of nitrifying bacteria included temperature, pH, light intensity, DO,

and composition of microbial groups. Agustiyani et al (2004) suggested that pH may affect bacterial growth and activity. Nitrifying bacteria can grow optimally at 17-34ºC and pH of 6-8.5 (FAO 2014). In addition, light conditions can affect the nitrification process. Cheatham (2009) found that nitrification by *Nitrosomonas* sp. was inhibited about 10 minutes due to direct exposure to light, and this could be solved by application of dark condition for 3-4 hours. Light exposure may affect the condition of nitrifying bacteria in the expansion phase and does not affect in the stationary phase (Cheatham 2009). According to FAO (2014), nitrification can run optimally at DO 4-8 mg L^{-1} in water condition.
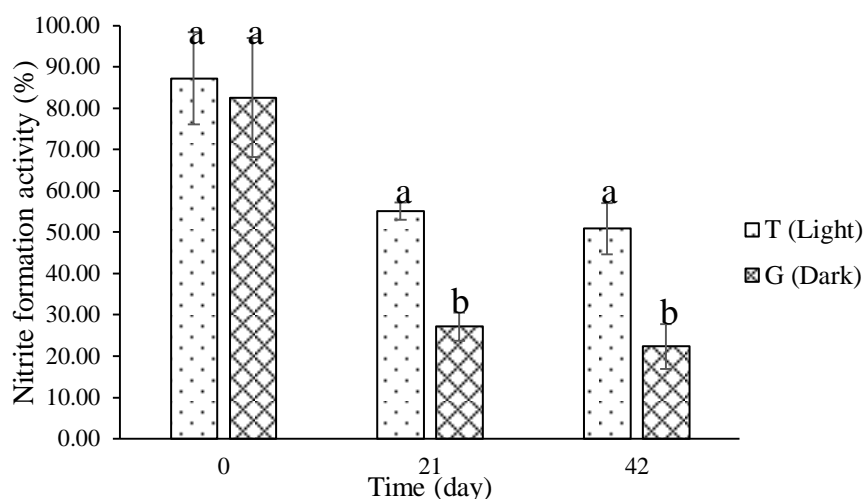

Figure 4. Nitrite formation activity by nitrifying bacteria in recirculation system of *Scylla serrata* culture. Different letters above the bars in the same period of measurement indicates significant difference.

In the present study the optimum temperature for growth of *S. serrata* was 25.3°C-26.7°C, but the crab could also grow optimally at 25-35°C (FAO 2011). In our experiment, the recorded salinity was 25 g L^{-1}, which is the optimum condition for *S. serrata* culture as reported by Hastuti et al (2015). In addition, pH 7.5-8.5 was regarded as the optimum range (FAO 2011), although the condition could not consistently reached in our experiment. This inconsistency resulted from accumulation of organic material derived from feces, residual metabolism, and debris.

Water quality in the culture media is presented in Table 2. We found a decrease in DO at week 3, then increased until week 5. DO ranged from 4.0 to 8.6 mg L^{-1}, while the optimum value for *S. serrata* culture was >5 mg L^{-1} (FAO 2011). FAO (2011) stated that *S. serrata* was tolerant of low DO. Fluctuation in DO value could be linked with the accumulation of organic materials derived from the remaining feed, feces, and other metabolic wastes.

BOD is the amount of oxygen used by bacteria and other microorganisms to stabilize organic matter under aerobic conditions (Mocuba 2010). The results showed that BOD ranged from 0.5 to 6.4 mg L^{-1}. This is consistent with Mocuba (2010) that the optimal BOD value for crab farming is <5 mg L^{-1}, and low BOD could affect the degradation of organic and inorganic wastes by decomposting bacteria.

Additionally, the results showed that C/N ratio ranged from 1.32 to 5.17. The level of ammonia and ammonium in culture media was 0.001-0.007 mg L^{-1} and 0.17-0.93 mg L^{-1}, respectively. In *S. serrata* farming, the ammonia level should be <0.25 mg L^{-1} (FAO 2011). The experimental crab could not accept excessive levels of free ammonia as it interfered oxygen binding by the blood, which in turn caused mortality.

Abundance of nitrifying bacteria. In our experiment, total plate count (TPC) was applied to determine abundance of nitrifying bacteria on day 0, 21, and 42 (Figure 5).

The results exhibited that the abundance of bacteria from container T and G on day 0 and 42 showed a significant difference (P<0.05). We found that bacteria from container T was more abundant in comparison with bacteria from container G. This may indicate that nitrifying bacteria in light containers are photosynthetic bacria capable of utilizing light as reported by FAO (2014). Suantika et al (2016) found that abundance of nitrifying bacteria in recirculation system using bioball reached 1.85 (10^3 CFU mL^{-1}), then increased up to 2.07 (10^7 CFU mL^{-1}), and increased again up to 1.35 (10^{10} CFU mL^{-1}). The optimal nitrite level for *S. serrata* farming was <10 mg L^{-1} (FAO 2011), while optimal nitrate level was <80 mg L^{-1} (Kwong & Chowdhury 2016). We also reported that light intensity during *S. serrata* rearing in the light and dark containers ranged from 310 lux to 380 lux and 40 lux to 100 lux, respectively. Dellabio (2015) found that the optimal light intensity for fish ranged from 400 lux to 700 lux.
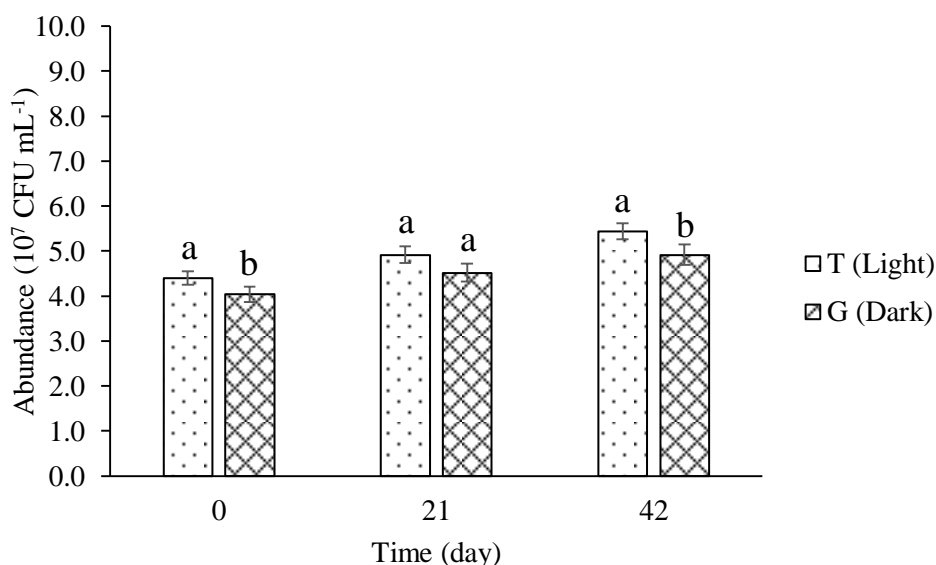

Figure 5. Abundance of nitrifying bacteria in recirculation system of *Scylla serrata* culture. Different letters above the bars in the same period of measurement indicates significant difference.

Conclusions. Based on the results, *S. serrata* culture using light container at light intensity of 310-380 lux demonstrated the better result in comparison with using dark container(40-100 lux). This technique was applicable to improve water quality by involving the role of nitrifying bacteria. In both light and dark container, the bacteria were dominantly from *Pseudomonas* sp., *Bacillus* sp., and *Acinetobacter* sp. In addition, we also concluded that bacteria from container T showed a higher ammonium oxidation activity (P<0.05) than bacteria from container G. Bacteria from container T was more stable in oxidizing ammonium than bacteria from container G.

References

Agustiyani D., Imamuddin H., Faridah E. N., Oedjijono, 2004 The effect of pH and organic compound on the growth and ammonia oxidizing bacteria activity. Jurnal Biodiversitas 5:43-47.
Cappucino J. G., Sherman N., 1987 Microbiology. A laboratory manual. California. Menko Park, pp. 31-179.
Cheatham A. K., 2009 Responses of nitrifying bacteria to aquaculture chemotherapeutic agents. Dissertation, Virginia (US) Virginia Polytechnic Institute and State University.

Cowan I. S. T., Steel K. J., 2003 Cowan and Steel's manual for the identification of medical bacteria. Barrow G. I., Feltham R. K. A. (eds), Cambridge (GB): Cambridge University Press.

Dellabio J., 2015 Advantages of LED lightning systems in larval fish culture. World Aquaculture, September 2015, pp. 25-29.

Fauzzia M. F., Rahmawati I., Widiasa I. N., 2013 Penyisihan amonia dan kekeruhan pada sistem resirkulasi budidaya kepiting dengan teknologi membran biofilter. Jurnal Teknologi Kimia dan Industri 2:155-161.

Fitch T., Lankford D., 2013 Why do black material absorb light and white material reflect it. MU's Office of Science Outreach.

Hastuti Y. P., Affandi R., Safrina M. D., Faturrohman K., Nurussalam W., 2015 Salinitas optimum untuk pertumbuhan benih kepiting bakau Scylla serrata dalam sistem resirkulasi. Jurnal Akuakultur Indonesia 14:50-57.

Hastuti Y. P., Rusmana I., Nirmala K., Affandi R., 2017 Activities of NH_4^+ and NO_2^- oxidizing bacteria in a recirculating system of mud crab Scylla serrata culture with different number of shelter. Research Journal of Microbiology pp. 1-9.

Hein T. T., Dinh T. D., Phu T. M., Bengston D. A., 2015 Assessment of the trash fish diet for snakehead aquaculture in Vietnam: Species composition and chemical characterisation. Asian Fisheries Science Journal 28:165-173.

Helfrich L. A., Libey G., 2013 Fish farming in recirculating aquaculture systems (RAS). Virginia Technology, pp. 1-19.

Iromo H., Farizah N., 2014 Analisis kandungan hormon tiroksin dengan metode ELISA pada induk betina kepiting bakau (Scylla serrata). Jurnal Harpodon Borneo 7:1-8.

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. Thesis, Bogor (ID): Institut Pertanian Bogor.

Kiding A., Khotimah S., Linda R., 2015 Karakterisasi dan kepadatan bakteri nitrifikasi pada tingkat kematangan tanah gambut yang berbeda di kawasan hutan lindung Gunung Ambawang Kabupaten Kubu Raya. Jurnal Protobiont 4:17-21.

Kwong K. O., Chowdhury A., 2016 The beneficial effects of multispecies Bacillus as probiotics in enhancing culture performance for mud crab Scylla. Aquaculture International 1:1-19.

Lin Y., Kong H. N., He Y. L., Liu B. B., Inamori Y., Yan L., 2007 Isolation and characterization of a new heterotrophic nitrifying Bacillus sp. strain. Journal of Biomedical and Environmental Sciences 20:450-455.

Mocuba J. J., 2010 Dissolved oxygen and biochemical oxygen demand in the waters close to the Quelimane sewage discharge. MSc Thesis, University of Bergen, Norway.

Nguyen T. T., 2013 Ammonium removal by means of algae and nitrifying bacteria treating swine waste manure. Thesis submitted in partial fulfilment of the requirements for the joint academic degree of: International Master of Science in Environmental Technology and Engineering an Erasmus Mundus Master Course from Ghent University (Belgium), ICTP (Czech Republic), UNESCO-IHE (the Netherlands), 93 p.

Novita L., 2006 Aktivitas Oksidasi Amonium dan Reduksi Nitrat Pseudomonas Stutzeri ASLT2 pada Sumber Karbon yang Berbeda [Skripsi]. Bogor (ID) Institut Pertanian Bogor.

Pramanik B. K., Fatihah S., Shahrom Z., Ahmed E., 2012 Biological aerated filters (BAFs) for carbon and nitrogen removal: A review. Journal of Engineering Science and Technology 7:428-446.

Sadinar B., Samidjan I., Rachmawati D., 2013 Pengaruh perbedaan dosis pakan keong mas dan ikan rucah pada kepiting bakau (Scylla paramamosain) terhadap pertumbuhan dan kelulushidupan dengan sistem battery di Tambak Tugu, Semarang. Journal of Aquaculture Management and Technology 2:84-93.

Santoso B. D., 2013 Pertumbuhan dan kelangsungan hidup juvenil lobster pasir Panulirus homarus di dalam wadah yang berbeda warna. Assay, Bogor (ID): Institut Pertanian Bogor.

Sentosa A. A., Syam A. R., 2011 Sebaran temporal faktor kondisi kepiting bakau (*Scylla serrata*) di Perairan Pantai Mayangan, Kabupaten Subang, Jawa Barat. Jurnal Perikanan (Journal of Fisheries and Sciences) 13:35-43.

Sianturi A., Basyuni M., Apandy Z., 2013 Tingkat kematangan gonad kepiting bakau (*Scylla serrata*) di Kawasan Hutan Mangrove Sicanang, Kecamatan Medan, Belawan, Sumatera Utara. Jurnal Universitas Sumatera Utara, pp. 1-10.

Su J. F., Zhang K., Huang T. L., Wen G., Guo L., Yang S. F., 2015 Heterotrophic nitrifiation and aerobic denitrification at low nutrient conditions by a newly isolated bacterium *Acinetobacter* sp. SYF26. Journal of Microbiology 161:829-837.

Suantika G., Pratiwi M. I., Situmorang M. L., Djohan Y. A., Muhammad A., Astuti D. I., 2016 Ammonium removal by nitrifying bacteria biofilm on limestone and bioball substrate established in freshwater trickling biofilter. Journal of Poultry, Fisheries, and Wildlife Sciences 4:157-162.

Widanarni, Pranoto S. H., Sukenda, 2010 Seleksi bakteri nitrifikasi dan denitrifikasi serta aplikasinya pada media budidaya udang vaname (*Litopenaeus vannamei*). Jurnal Akuakultur Indonesia 9:184-195.

*** APHA (American Public Health Association), 2005 Standards methods for the examination of water and waste water. 17th edition, Washington, 1193 p.

*** EPA (Environmental Protection Agency), 2002 Nitrification. US Environmental Protection Agency. Office of Ground Water and Drinking Water, Office of Water. Washington DC [Internet]. [Accessed on: 02 April 2017]. Available at: http:/www.epa.govogwdwdisinfectiontcrpdfswhite paper_tcr_ nitrification.pdf.

*** FAO (Food and Agriculture Organization of United Nations), 2011 Mud crab aquaculture: A practical manual. Rome (IT): FAO.

*** FAO (Food and Agriculture Organization of the United Nations), 2014 Small-scale aquaponic food production: Integrated fish and plant farming. FAO Fisheries and Aquaculture Technical Paper, 589:75-81.

*** FAO (Food and Agriculture Organization of the United Nations), 2015 Globefish: Highlights a quarterly update on world seafood markets. Rome (IT): FAO.

*** FAO (Food and Agriculture Organization of the United Nations), 2017 *Scylla serrata* (Null, 2001): Species Fact Sheets. Rome (IT): FAO.

*** KKP (Ministry of Marine Affairs and Fisheries), 2015 Volume ekspor kepiting bakau [Accesses on: 30 March 2017]. Available at: http://statistik.kkp.go.id/sidatik-dev/ Publikasi/src/kpda2015.pdf

*** WWF (World Wide Fund for Nature), 2015 Better management practices. Kepiting Bakau (*Scylla* sp.) Panduan Penangkapan dan Penanganan. Indonesia (ID): WWF.

Authors:
Yuni Puji Hastuti, Bogor Agricultural University, Faculty of Fisheries and Marine Science, Department of Aquaculture, Indonesia, West Java, 16680, e-mail: yuni_ph2@yahoo.com; yuniha@ipb.ac.id
Kukuh Nirmala, Bogor Agricultural University, Faculty of Fisheries and Marine Science, Department of Aquaculture, Indonesia, West Java, 16680, e-mail: kukuhnirmala@yahoo.com
Daniela Merani, Bogor Agricultural University, Faculty of Fisheries and Marine Science, Department of Aquaculture, Indonesia, West Java, 16680, e-mail: danielamerani37@gmail.com
Siska Tridesianti, Bogor Agricultural University, Faculty of Fisheries and Marine Science, Department of Aquaculture, Indonesia, West Java, 16680, e-mail: tridesiantisiska@gmail.com