Water quality, hematological parameters and biological performances of Snakehead fish (*Channa striata*) reared in different stocking densities in a recirculating aquaculture system

[1,2]Dewi Puspaningsih, [2]Eddy Supriyono, [2]Kukuh Nirmala, [3]Iman Rusmana, [4]Cecep Kusmana, [1]Ani Widiyati

[1] Research Institute for Freshwater Aquaculture and Fishery Extension, Bogor, Indonesia; [2] Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University (IPB), Bogor, Indonesia; [3] Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University (IPB), Bogor, Indonesia; [4] Department of Silviculture, Faculty of Forestry, Bogor Agricultural University (IPB), Bogor, Indonesia. Corresponding author: E. Supriyono, eddysupriyonoipb@gmail.com

Abstract. Snakehead fish (*Channa striata*) has been a very popular and important freshwater fish species in many Southeast Asian countries. The purpose of this study was to evaluate the water quality, hematological parameters and growth performances of snakehead reared at different stocking densities, in a recirculating aquaculture system. The experimental design used a completely randomized design with three different stocking densities as treatments: A - 2 fish L^{-1}; B - 4 fish L^{-1}; C - 6 fish L^{-1}. Each treatment consisted of three replications. Snakehead presented an average length of 6.07±0.10 cm and an average weight of 1.82±0.07 g. Water quality parameters were monitored: dissolved oxygen, pH, temperature, total ammonia nitrogen, nitrites, nitrates and orthophosphate. The hematological parameters analyzed were the red blood cells, white blood cells, hemoglobin, hematocrit and blood glucose level. The growth performances observed were the survival rate, specific growth rate, food conversion ratio, absolute length, absolute weight and biomass. Fish were cultured in a recirculating aquaculture system (RAS), a prototype built in the Research Institute for Freshwater Aquaculture and Fishery Extension, Bogor, Indonesia. The results showed that water quality parameters were within the optimal range for snakehead culture. Red blood cell count in treatment A was significantly different from B and C ($P<0.05$). The white blood cell count in treatment B was significantly different from C ($P<0.05$). The blood glucose levels were significantly different among the treatments ($P<0.05$). The survival rate and food conversion ratios were significantly different between A and B treatments ($P<0.05$). The specific growth rate, absolute length, absolute growth and biomass were significantly different among the treatments ($P<0.05$).

Key Words: fish biomass, growth performance, RAS, Snakehead.

Introduction. Snakeheads (Channidae family) are air breathing freshwater fish containing two genera, *Channa* with 26 species native to South and South East Asia, and *Parachanna* with 3 species native to tropical Africa (Courtenay & Williams 2004). In Indonesia, there are efforts to develop the snakehead fish culture, especially in public swamp waters, by utilizing larvae originating from natural waters (Gaffar et al 2012). Furthermore, efforts towards snakehead fish domestication and aquaculture have been carried out in Indonesia, including South Borneo (Bijaksana 2012), South Sumatra (Muthmainnah et al 2012; Hartini et al 2013) and West Java. The efforts consist in the transportation of fish (Wahyu et al 2015), culture using water hyacinths and probiotics (Saputra et al 2017), larvae rearing in green water systems (Saputra et al 2018) monitoring the dynamics of water quality (Puspaningsih et al 2018) and others.

Many authors state that the effect of stocking density on fish growth and survival are basically very dependent on water quality parameters in the culture medium, because poor water quality can subsequently have an impact on mortality and decrease production (Hosfeld et al 2009; Yuan et al 2010; Rahman et al 2012). The main source of potentially polluting waste is feed-derived, such as uneaten feed, undigested feed residues and excretion products, which either discharges in the farm effluent, or is made available for reuse within the farm (Cripps & Bergheim 2000). The accumulation of feed residue and fish excreta during cultivation often causes water quality deterioration in fishponds, resulting in toxic effects for the fish. Aquaculture farm discharge contains considerable quantities of organic matter, nitrogen and phosphorus and can further degrade the water quality in receiving waters (Lin et al 2002). The most important forms of nitrogenous waste are ammonia-N (53-68%) and urea-N (6-10%) (Kajimura et al 2004). Environmental problems arising from intensive culture include waste that can pollute the surrounding environment. The dynamics of water quality during snakehead culture without recirculation systems show a significant increase in nitrite levels since the third day, being proven that nitrite cannot turn to the nitrate maximally, resulting in fish mortality (Puspaningsih et al 2018). Furthermore, the use of recirculating aquaculture systems (RAS) is one approach used to limit the impact of aquaculture on aquatic environments (Pagand et al 2000). The advantages of RAS are water conservation, high production, avoidance of fish contamination from pollutants, easy management and it is eco-friendly (Setiadi et al 2019).

Stocking density is sometimes considered the main factor that affects the hematological parameters (Salah & Wael 2011). The hematological parameters are an important tool that can be used for effective and sensitive monitoring of the physiological and pathological state of fish (Kohanestani et al 2013), as these parameters can be changed because of the environment, nutrition and stress (Taufik & Setiadi 2015). Thus, hematological parameters can be used as an indicator in terms of fish physiological and pathological changes (Meraj et al 2016). Furthermore, the stocking density also plays an important role in fish farming because it affects growth and survival (Hosfeld et al 2009), microbial activity, water quality and production levels (Schveitzer et al 2013), nutrient recovery (Yuan et al 2010) and immune response (Salas-Leiton et al 2010). Research on the effect of stocking density of snakehead has been conducted on brood fish with a stocking density of 357.22 g m^{-2} and on larvae with stocking density of 2-6 larvae L^{-1} (Mollah et al 2009), on fingerlings of 8-10 cm length with a stocking density of 20-40 fish m^{-2} (Amin et al 2015) and others. The effect of stocking density on water quality and hematological parameters has not been studied in detail. The purpose of this experiment was to evaluate the effect of different stocking densities on water quality, hematological parameters and biological performances of snakehead reared in RAS.

Material and Method. The experiment was conducted at the Research Station for Freshwater Aquaculture Environmental Technology and Toxicology, Research Institute for Freshwater Aquaculture and Fishery Extension, Bogor, Indonesia from May to July 2018. Water quality parameters were analyzed at the laboratory of the previously mentioned research station. The hematological analysis was conducted at the Laboratory of Fish Health, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University (IPB), Bogor, Indonesia.

Experimental design. This experiment was conducted in a semi-outdoor area using a Completely Randomized Design. The treatments used in this study consisted of three different stocking densities, namely A - 2 fish L^{-1}; B - 4 fish L^{-1}; C - 6 fish L^{-1}. Each treatment had three replications. Fish were cultured for 8 weeks.

Experimental fish. Test fish were snakeheads with an average length of 6.07±0.10 cm and an average weight of 1.82±0.07 g. Fish were obtained from a fish farmer in Depok, West Java, Indonesia. Before treatment, fish were acclimatized in fiber tanks with the size of 200x100x50 cm^3 for 2 weeks.

Experimental tanks and mediums. The tanks used in this experiment were 9 units of fiber tanks with the size of 50x30x30 cm^3, filled with 25 L of groundwater. A physical filter with gravel stone and limestone was used along with a biological filter with bio balls.

The physical and biological filters had a 10 L capacity. The water flow rate in each tank was maintained at 1.0 L min^{-1}. The RAS had a trial run for 2 weeks and it was adjusted to ensure that ammonia oxidizing bacteria and nitrite oxidizing bacteria occurred in the system (FAO 2015). Aeration was introduced in the fiber tanks and nets were placed on top of the fish tanks in order to avoid the fish jumping out. In this experiment, a prototype RAS was made in the Research Station for Freshwater Aquaculture Environmental Technology and Toxicology, Research Institute for Freshwater Aquaculture and Fishery Extension, Bogor, Indonesia. Water from the fish tanks flowed into the physical filters and through the biological filters, then the water was pumped back in the fish tanks. The layout scheme of the RAS is presented in Figure 1.

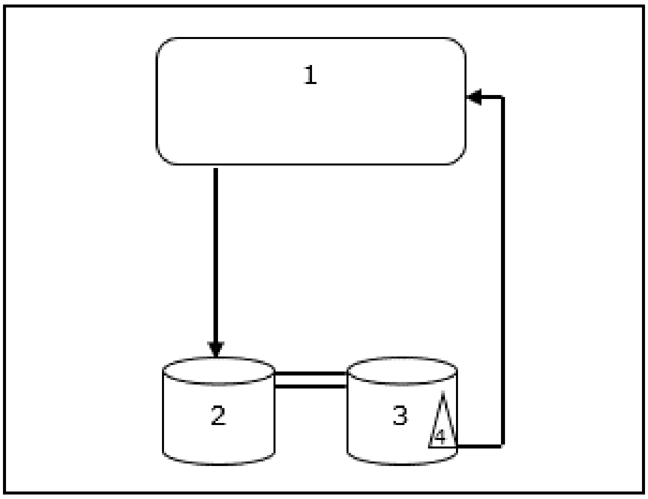

Figure 1. The layout scheme of the recirculating aquaculture system (1 - fish tank; 2 – physical filter; 3 – biological filter; 4 – pump).

Experimental procedures. Fish were fed with commercial pellets with a chemical composition studied previously Puspaningsih et al (2018). The feed was given as much as 3% biomass body weight per day (Ministry of Marine Affairs and Fisheries of the Republic of Indonesia 2014), with a feeding frequency of 3 times a day, at 08.00, 12.00 and at 16.00. Groundwater was used as a medium for fish culturing. It was filtered using a filter bag, thereafter the water was precipitated and aerated for 7 days. No water discharge or displacement was carried out, except for replacing water lost due to evaporation, transpiration and sludge removal (Endut et al 2009). Fish was cultured in the RAS for 8 weeks. In the RAS unit, a transparent shelter was made in order to anticipated the effects of adverse climate effects on water quality fluctuations in the culture media.

Experimental parameters. The measurement of water quality parameters such as total ammonia nitrogen (TAN), nitrite, nitrate and orthophosphate were performed by following the procedures described each by the Indonesian National Standards 06-6989.30-2005; Indonesian National Standards 06-6989.9-2004; Indonesian National Standards 6989.79-2011; Indonesian National Standards 06-6989.31-2005. The blood samples were collected at the end of the experiment using a 1 mL syringe by caudal vein puncture (Talpur et al 2014) for determining hematological parameters (red blood cell count, white blood cell count, hemoglobin, hematocrit and blood glucose levels). Sampling for determining growth performances was conducted every 2 weeks (Zhang et al 2011).

Data collection. The first initial data collection was carried out to determine the initial conditions before the study was conducted. The sampling of the fish and water quality parameters were conducted every 2 weeks (Zhang et al 2011), while the hematological parameters were determined at the end of the experiment. Water quality parameters measured were pH using Lovibond SensoDirect pH110, dissolved oxygen and temperature using Lovibond SensoDirect Oxi200, total ammonia nitrogen, nitrite, nitrate and orthophosphate analyses using UV/VIS Spectrometer PG Instruments T80^{+}. The digital analytical balance OHAUS Adventurer Model AX1502 d=0.01 g was used to measure the weight gain. Biological performances of snakehead, such as survival rate (SR), specific growth rate (SGR), Feed Conversion Ratio (FCR), absolute length, absolute growth and biomass were evaluated and calculated according to the following formula (Zehra & Khan 2012):

SR (%) = Final number of fish/Initial number of fish x 100

SGR (%) = (Ln final body weight - Ln initial body weight)/No. of days x 100

FCR = FCR = Dry feed fed (g)/(Final body weight + Dead fish weight) - Initial body weight

Absolute length/weight gain (cm fish^{-1}/g fish^{-1}) = Final body length/final body weight - Initial body length/initial body weight

Data analysis*.* Data on water quality were analyzed descriptively. Regarding the hematological parameters (hemoglobin, hematocrit, red blood cell count, white blood cell count and blood glucose level) and the growth performances data (SR, SGR, FCR, absolute length, absolute growth and biomass) one-way analysis of variance (ANOVA) was used. The analysis was continued with Duncan's test at a confidence level of 95%, if there were significant differences among the treatments.

Results and Discussion. The initial data collection describing the condition of water quality at the beginning of the experiment is presented in Table 1. During the experiment, the temperature of the fish tank for treatment A varied between 26.9 and 28.8°C, while in the filter it varied between 26.6 and 29.2°C. In the fish tank for B it varied between 26.7 and 31.6°C, while in the filter it varied between 24.3-31.0°C. In the fish tank C, it varied between 27.0 and 28.9°C, while in filter between 25.7 and 30.8°C. The temperature dynamics varied in the fish tanks and in the filter at all treatments. The trend was the same, temperature decreased to 25-27°C in the 8 weeks (Figure 2a). The dissolved oxygen concentration in the tank for A during the experimental period ranged from 1.73 to 5.66 mg L^{-1}, while in the filter it ranged from 2.48 to 6.38 mg L^{-1}. In fish tank B, it ranged from 1.98 to 5.56 mg L^{-1}, while in the filter it ranged from 3.03 to 3.59 mg L^{-1}. In fish tank C, it ranged from 2.22 to 4.97 mg L^{-1}, while in the filter it ranged from 2.71 to 3.36 mg L^{-1} (Figure 2b). All pH values measured showed the same trend at all treatments, which peaked on the 4th week of the culture period then decreased until the 6th week (Figure 2c).

Table 1

Water quality parameters at the beginning of the experiment

Parameters	*Value/concentration*
Temperature (°C)	27
Dissolved oxygen (mg L^{-1})	2.96
pH	7.2
Total Ammonia Nitrogen (mg L^{-1})	0.16
Nitrite (mg L^{-1})	0.0038
Nitrate (mg L^{-1})	5.6
Orthophosphate (mg L^{-1})	1.5

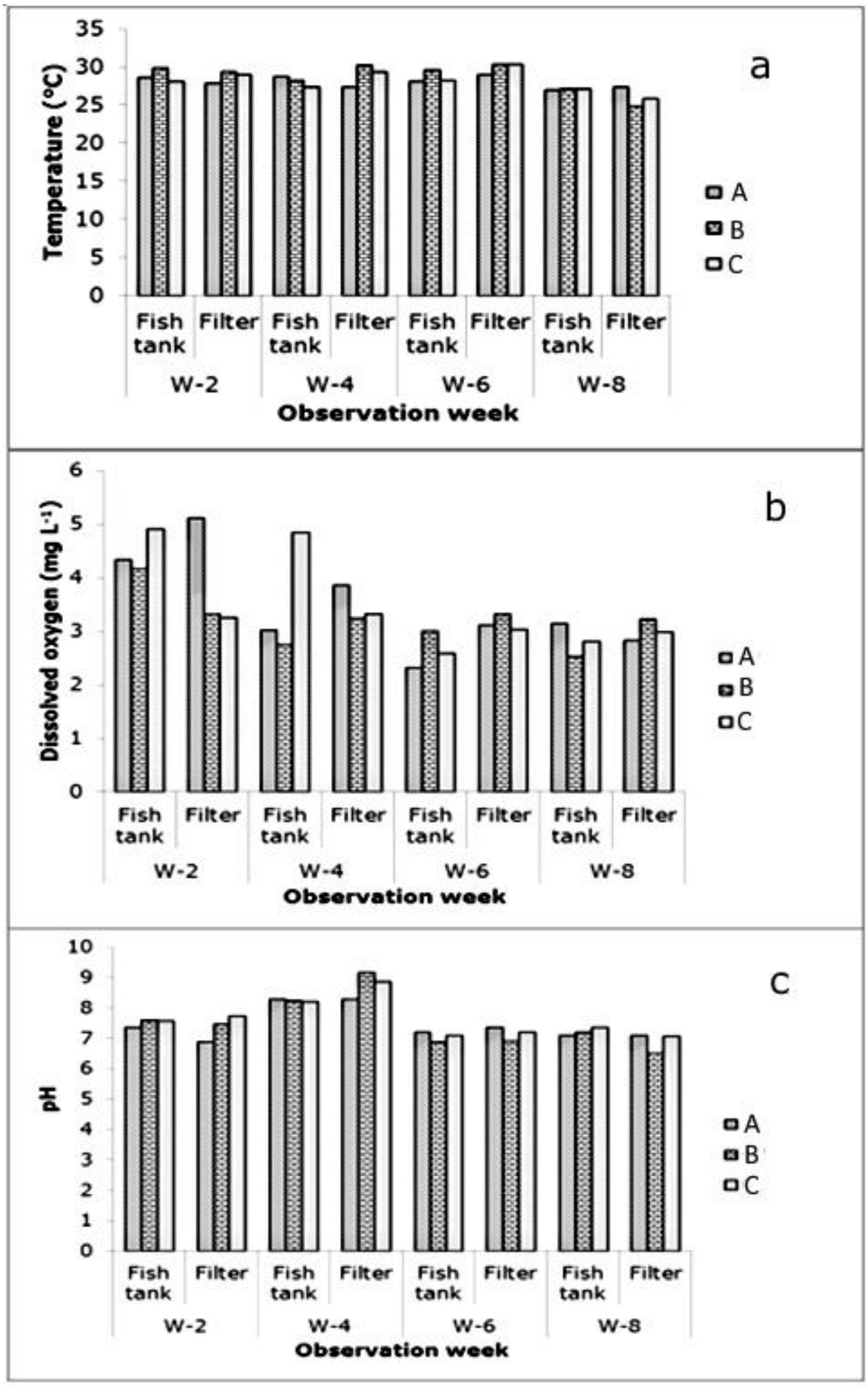

Figure 2. Water quality parameters measured during the experimental period: a – temperature; b - dissolved oxygen; c - pH.

Other water quality parameters measured (TAN, nitrite, nitrate and orthophosphate) during the experimental period can be seen in Figure 3. Generally, TAN concentration in the filter was lower than in the fish tanks. All the treatments showed the same patterns. The highest TAN concentration was observed in the 2nd week, decreasing afterwards in the 4th week, and following with slight fluctuations in the next week (Figure 3a). The nitrite concentration in all treatments showed the same patterns. The highest levels were observed in the 2nd week and then gradually decreased on the following week (Figure 3b). The nitrate concentrations showed the same trend among the treatments, where the peak was observed in the 6th week (Figure 3c). Overall orthophosphate concentrations in the filters were lower than in the fish tanks for all treatments. The B treatment showed fluctuations compared with treatments A and C (Figure 3d).

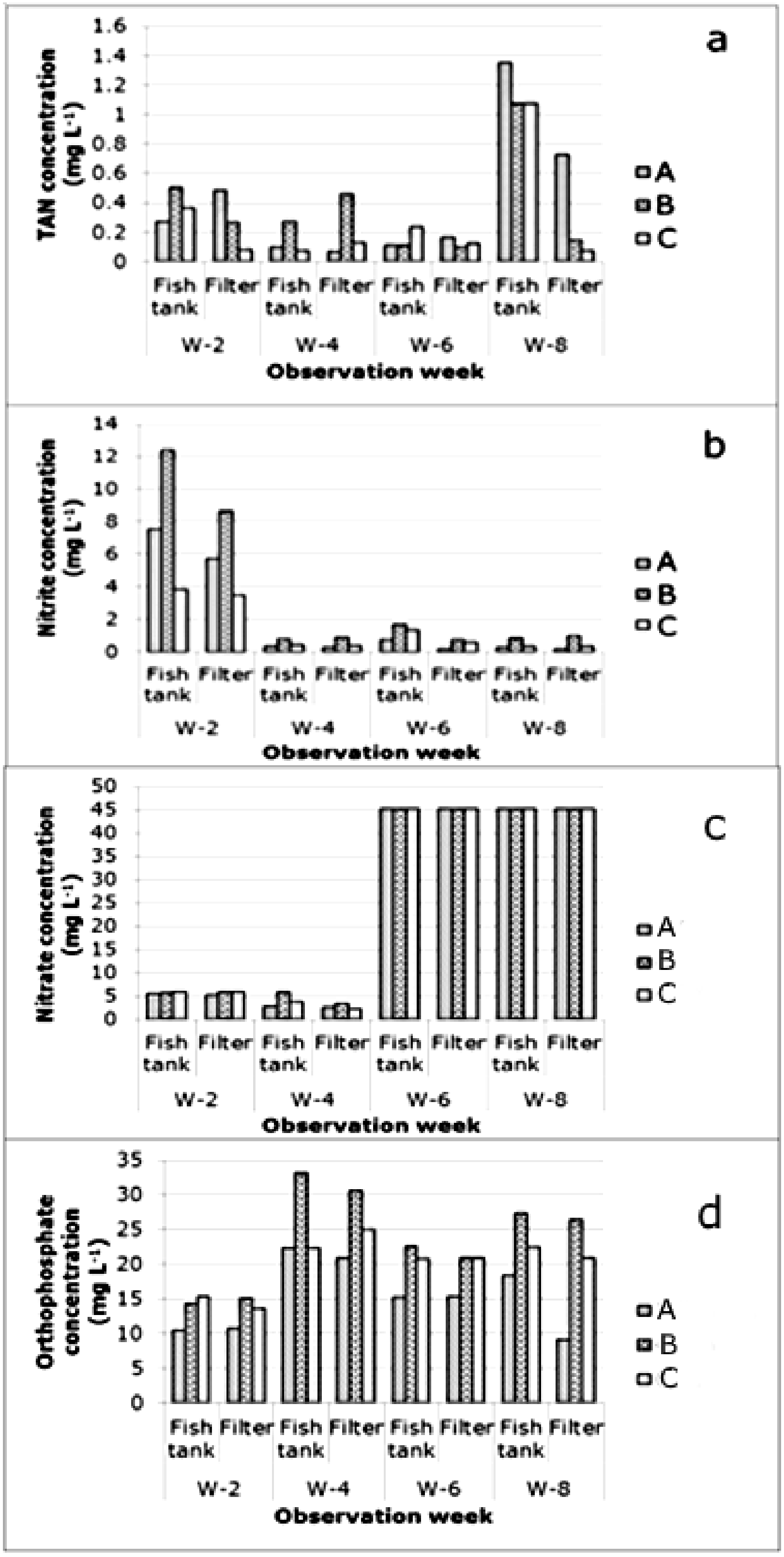

Figure 3. Water quality parameters measured during the experimental period; a – TAN (total ammonia nitrogen); b – nitrite; c – nitrate; d - orthophosphate.

Hematological parameters of snakehead varied among the treatments. Parameters such as red blood cell count, white blood cell count, hemoglobin and hematocrit values decreased with increasing stocking density. Blood glucose levels also increased (Table 2). Hematological parameters of snakehead cultured at different stocking densities showed that the red blood cell count, white blood cell count and blood glucose levels were affected by the stocking density (P<0.05), while hemoglobin and hematocrit were not.

Table 2

Hematological parameters of snakehead cultured at a different stocking density

Parameters	*Stocking density treatments*		
	2 fish L^{-1}	*4 fish L^{-1}*	*6 fish L^{-1}*
Red blood cell count ($x10^5$ cells mm^{-3})	27.38 ± 1.48^a	23.48 ± 0.99^b	20.85 ± 2.67^b
White blood cell count ($x10^5$ cells mm^{-3})	1.05 ± 0.16^{ab}	1.16 ± 0.08^b	0.91 ± 0.09^a
Hemoglobin (%)	7.30 ± 0.90^a	7.20 ± 0.69^a	6.75 ± 1.35^a
Hematocrit (%)	31.20 ± 2.69^a	31.58 ± 0.77^a	28.94 ± 4.13^a
Blood glucose level (mg/dL)	48.50 ± 2.12^a	75.00 ± 5.66^b	108.5 ± 0.71^c

Note: the mean value with different superscript on the same row showed a significant difference (P<0.05).

Data on the biological performances such as SR, SGR, FCR, absolute length, absolute weight growth and biomass of the fish are presented in Table 3. The highest SR, SGR, absolute length, absolute weight and biomass were observed in treatment B. SR and FCR of the snakehead at different stocking densities in the RAS after 8 weeks showed significant differences (P<0.05) between A and C, and also between B and C. SR in treatments A and B were 82% and 84%, respectively, while in C treatment it was 60.67%. The lowest FCR (0.80) was obtained in treatment B, but it was not significantly different (P>0.05) from A (0.89). It was significantly different (P<0.05) than C (1.12). The highest SGR was observed in treatment B (2.80%), followed by A (2.53%) and C (2.15%) and all the treatments were significantly different among each other (P<0.05). Absolute length and growth were significantly different (P<0.05) among the treatments, with the highest value observed in B, followed by A and C.

Table 3

Biological performances of snakehead fish at different stocking densities

Parameters	*Stocking density treatments*		
	A (2 fish L^{-1})	*B (4 fish L^{-1})*	*C (6 fish L^{-1})*
SR (%)	82 ± 5.29^a	84 ± 4.00^a	60.67 ± 2.00^b
SGR (%)	2.53 ± 0.01^a	2.80 ± 0.03^b	2.15 ± 0.01^c
FCR	0.89 ± 0.08^a	0.80 ± 0.09^a	1.12 ± 0.02^b
Absolute Length (cm)	3.43 ± 0.06^a	3.65 ± 0.16^b	2.97 ± 0.08^c
Absolute Weight growth (g)	4.87 ± 0.05^a	5.97 ± 0.14^b	3.57 ± 0.02^c
Biomass (g)	274.49 ± 19.55^a	654.91 ± 43.10^b	490.81 ± 18.45^c

Note: the mean value with different superscript on the same row showed a significant difference (P<0.05). SR – survival rate; SGR – specific growth rate; FCR – feed conversion ratio.

The TAN concentration in A ranged from 0.099 to 1.35 mg L^{-1}, higher than in B and C (Figure 3a). It is a lower concentration compared with other results for TAN concentrations in tanks without a recirculation system, with values up to 2 mg L^{-1} at a density of 2 fish L^{-1} (Puspaningsih et al 2018). TAN concentration in this experiment is similar with the one from other studies, for 5000-7500 fish ha^{-1} in ponds, where feed was administered twice per day. Ammonia nitrogen ranged between 0.01 and 1.45 mg L^{-1} (Rahman et al 2012). TAN is not toxic to fish, but the equilibrium with unionized ammonia is depended on the pH and temperature of the water (Effendi 2003). The application of the recirculation system in the present experiment has proven to decrease the nitrite concentration, whereas Puspaningsih et al (2018) reported that the nitrite concentration during the culture of snakehead without recirculation reach up to 15-20 mg L^{-1}. Nitrite concentration in this experiment presents higher values, between 3.5-12.4 mg L^{-1}, only in the 2nd week during the culture period, decreasing afterwards (Figure 3b). Nitrate concentration in this experiment ranged from 2.31 to 45.17 mg L^{-1}, which was higher than the one reported by Rahman et al (2012), of 0.94-1.60 mg L^{-}1, where no recirculation was used. The nitrification process might have appeared in the 4th week, when the nitrite is gradually turned into the nitrate form. The nitrate concentration itself tended to increase in the 6th week (Figure 3c). Such conditions were common in the RAS when the system was running, the highest concentration occurring in the 1st and 2nd week, especially ammonia and nitrite. The following week the nitrate concentration would exceed the ammonia and nitrite concentrations. This phenomenon is normal (Pungrasmi et al 2016). The concentration of orthophosphate in this experiment ranged from 9.17 to 33.15 mg L^{-1} (Figure 3d), higher compared to the values reported by Rahman et al (2012). The phosphate-phosphorous concentration in snakehead ponds without recirculation at different stocking densities ranged from 0.30 to 2.80 mg L^{-1} (Rahman et al 2012). The difference may occur because of the difference in the culture media.

The temperature measured in this experiment (Figure 2a) is not much different from the results reported by Qin & Fast (1998), which stated that the snakehead can be cultured in temperatures from 20.5°C to 28.8°C. Dissolved oxygen concentrations (Figure 2b) and pH values (Figure 2c) measured in this experiment are not much different from the ones reported by Rahman et al (2012), where dissolved oxygen concentrations ranged from 3.20 to 7.30 mg L^{-1} and pH values ranged between 7.20 and 8.40. The amount of feed given depends on the different stocking densities, thus it affects the amount of nutrient waste produced, which will ultimately influences the difference in TAN, nitrite, nitrate and orthophosphate concentrations. However, the RAS applied in this study enhanced the water quality, stabilizing it. The present experiment showed that the dynamics of all the water quality parameters during the experiment such as temperature, pH, dissolved oxygen, TAN, nitrite, nitrate and orthophosphate (Figure 2 and 3) are still within the tolerance interval for snakehead culture (Ministry of Marine Affairs and Fisheries of the Republic of Indonesia 2014). The biological filter used in the RAS is appropriate for snakehead culture, water quality parameters being stable during the fish culture. Therefore, the effect of stocking density differences on water quality is negligible.

Blood parameters, such as the number of red blood cells, white blood cells, hemoglobin and hematocrit were closely related to individual responses to change in environmental parameters. According to Fazio (2019), the complete blood cell count (CBC) is an important and powerful diagnostic tool, as well as a component of a minimum database, which can be used to monitor the health status of fish in response to changes related to nutrition, water quality and diseases. Docan et al (2011) mentioned that there is growing interest in the study of hematological parameters and structural features of fish blood cells regarded as important for aquaculture purposes. Barton (2002) also stated that one of the secondary stress responses in fish are changes in hematological features (hematocrit, leucosit, hemoglobin) and increases in glucose levels. The present experiment showed that red blood cells of fish in treatment A (27.38×10^5 cells mm^{-3}) were significantly different from the ones from treatment B (23.48×10^5 cells mm^{-3}) and C (20.85×10^5 cells mm^{-3}) ($P<0.05$) (Table 2). This value is higher if compared with the results of Docan et al (2011), which stated that the number of red blood cells in the case of rainbow trout (*Oncorhynchus mykiss*) reared in RAS ranged between 0.886 and 1.178×10^6/μL. This condition may occur because the snakehead can take oxygen directly from the air, so that it has a higher content of red blood cells than that the rainbow trout. Well et al (2005) reported a similar situation between the salmon catfish (*Arius leptaspis*) and tarpon (*Megalops cyprinoides*). It was observed that tarpon can take oxygen directly from the air and has a higher number of red blood cells compared with salmon catfish. The decreased number of red blood cells in the C treatment indicates that fish are affected by stocking densities. The hematocrit value also decreased. Low red blood cell count and hematocrit values are an indication of the anemia condition in fish, when the fish usually stop feeding (Talpur & Ikhwanuddin 2013). The normal red blood cells count in snakehead fish according to Wahyu et al (2015) was 1.98×10^6 cells mm^{-3}, while the normal hematocrit was 21.2%, which means that there were a decrease in red blood cells count and an increase in hematocrit level in this experiment. Docan et al (2011) also stated that the reduction in the red blood cell count may be due to the destruction of the red blood cells, caused by an increase in stocking densities.

The number of white blood cells in treatment A showed no significant differences compared with the other two treatments. The white blood cell count in treatment B was significantly different when compared with treatment C ($P<0.05$). An increasing number of white blood cells occurred when the density increased from 2 fish L^{-1} to 4 fish L^{-1}, then decreased at density 6 fish L^{-1} (Table 2). The hemoglobin content showed no significant differences among the treatments, the lowest hemoglobin content being observed in treatment C. Docan et al (2011) stated that certain hematological variables were influenced by density, although results were inconsistent. According to Wahyu et al (2015), the normal white blood cells count in snakehead fish was 1.86×10^5 cells mm^{-3}, while the normal hemoglobin was 9.05%, which means that there were a decrease in the white blood cells count and hemoglobin value in this experiment. The decreased hemoglobin content in treatment C linked with the decreasing of red blood cells, indicating that fish could have been under stress due to the high stocking density. The blood glucose levels of snakehead under different stocking densities were significantly different among the treatments ($P<0.05$). The level increases with increasing stocking densities. The highest blood glucose levels were reached in treatment C, while the lowest were reached in treatment A. Blood glucose is one of the secondary stress response parameters (Barton 2002). The stocking density of fish has caused some stress in this experiment. An increase in blood glucose levels associated with a decrease in red blood cell levels indicates that the fish have anemia, which reduced the appetite and, in some cases, totally halting the feeding process (Talpur & Ikhwanuddin 2013), causing differences in the amount of feed consumed. In stress conditions, the blood glucose would increase to keep homeostasys of the fish, resulting in the decline of insulin (Royan et al 2014). Furthermore, chromaffin cells would release catecholamine, adrenaline and noradrenaline in the blood stream (Reid et al 1998). Finally, stress hormones would join with cortisol, resulting in increased blood glucose via glucogenesis and glycogenolysis (Iwama et al 1999).

In this experiment, the increasing level of FCR in treatment C was followed by the decrease of SGR. This has the same tendency as in other cases (Zehra & Khan 2012; Amin et al 2015). Zehra & Khan (2012) mention that when SGR reaches 1.21%, then the FCR value is 3.27, while at a SGR of 1.82% the FCR value is 1.48. Amin et al (2015) also mention the same result, where the highest SGR (1.57%) matched the lowest FCR (0.98). The FCR value in the present experiment may be affected by the stocking density, the higher stocking density resulting in the higher FCR value. Generally, according to Rahman et al (2012), the FCR value increased with the increasing feed application rates above optimal . FCR value in the present experiment shows no significant differences between treatments A and B, but it is significantly different between A and C, and also B and C ($P<0.05$). Rahman et al (2012) also mention that growth, survival and production of snakehead are inversely related to the stocking density of fingerlings.

Conclusions. The design construction of RAS applied for snakehead culture is efficient in water use and water quality. Hematological parameters of snakehead reared in RAS, such as the red blood cell count, white blood cell count and blood glucose levels are influenced by stocking density, whereas the hemoglobin and hematocrit are not affected by stocking density, in this study. Furthermore, the optimal SR, SGR, absolute length growth, absolute weight growth, FCR and biomass of snakehead fish cultured in the RAS is optimal at a stocking density of 4 fish L^{-1}.

Acknowledgements. This research was conducted with funding support from the Ministry of Marine Affairs and Fisheries of the Republic of Indonesia, and research equipment support from Research Institute for Freshwater Aquaculture and Fishery Extension, Bogor, Indonesia.

References

Amin S. M. N., Muntaziana M. P. A., Kamarudin M. S., Rahim A. A., Rahman M. A., 2015 Effect of different stocking densities on growth and production performances of chevron snakehead *Channa striata* in fiberglass tanks. North American Journal of Aquaculture 77:289-294.

Barton B. A., 2002 Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integrative And Comparative Biology 42:517-525.

Bijaksana U., 2012 [Domestication of snakehead fish, *Channa striata*, efforts to optimize swamp waters in South Kalimantan Province]. Jurnal Lahan Suboptimal 1(1):92-101. [in Indonesian]

Courtenay W. R., Williams J. D., 2004 Snakeheads (Pisces, Channidae) - A biological synopsis and risk assessment. U.S. Geological Survey, Reston, 143 p.

Cripps S. J., Bergheim A., 2000 Solid management and removal for intensive land-based aquaculture production systems. Aquacultural Engineering 22:33-56.

Docan A., Cristea V., Dediu L., Mocanu M., Grecu I., 2011 The impact of level of the stocking density on the haematological parameters of rainbow trout (*Oncorhynchus mykiss*) reared in recirculating aquaculture systems. AACL Bioflux 4(4):536-541.

Effendi H., 2003 [Review of Water Quality for Management of Aquatic Resources and Environment]. Kanisius Publisher, Yogyakarta, Indonesia, 256 p. [in Indonesian]

Endut A., Jusoh A., Ali N., Wan Nik W. N. S., Hassan A., 2009 Effect of flow rate on water quality parameters and plant growth of water spinach (*Ipomoea aquatic*) in an aquaponic recirculating system. Desalination and water treatment 5:19-28.

Fazio F., 2019 Fish hematology analysis as an important tool of aquaculture: a review. Aquaculture 500:237-242.

Gaffar A. K., Muthmainnah D., Suryati, 2012 [Treatment of snakehead fish *Channa striata* with different stocking densities and differences in feed volume]. Prosiding InSINas 0727:303-306. [in Indonesian]

Hartini S., Sasanti A. D., Taqwa F. H., 2013 [Water quality, survival and growth of snakehead fish (*Channa striata*) which are maintained in the media with the addition of probiotics]. Jurnal Akuakultur Rawa Indonesia 1(2):192-202. [in Indonesian]

Hosfeld C. D., Hammer J., Handeland S. O., Fivelstad S., Stefansson S. O., 2009 Effects of fish density on growth and smoltification in intensive production of Atlantic salmon (*Salmo salar* L.). Aquaculture 294:236-241.

Indonesian National Standards 06-6989.30-2005 Water and waste water-part 30: ammonia test methode with spectrophotometer using phenat. Jakarta, Indonesia, 10 p.

Indonesian National Standards 06-6989.31-2005 Water and waste water-part 31: phosphate test methode with spectrophotometer using ascorbic acid. Jakarta, Indonesia, 10 p.

Indonesian National Standards 06-6989.9-2004 Water and waste water-part 9: nitrite test methode (NO_2-N) with spectrophotometer. Jakarta, Indonesia, 13 p.

Indonesian National Standards 6989.79-2011 Water and waste water-part 79: nitrate test methode with spectrophotometer. Jakarta, Indonesia, 18 p.

Iwama G. K., Vijayan M. M., Forsyth R. B., Ackerman, P. A., 1999 Heat shock proteins and physiological stress in fish. American Journal of Zoological Research 39:901-909.
Kajimura M., Croke S. J., Glover C. N., Wood C. M., 2004 Dogmas and controversies in the handling of nitrogenous wastes: The effect of feeding and fasting on the excretion of ammonia, urea and other nitrogenous waste products in rainbow trout. The Journal of Experimental Biology 207:1993-2002.
Kohanestani Z. M., Hajimoradloo A., Ghorbani R., Yulghi S., Hoseini A., Molaee M., 2013 Seasonal variations in hematological parameters of *Alburnoides eichwaldii* in Zaringol Stream-Golestan Province, Iran. World Journal of Fish and Marine Sciences 5:121-126.
Lin Y. F., Jing S. R., Lee D. Y., Wang T. W., 2002 Nutrient removal from aquaculture wastewater using a constructed wetlands system. Aquaculture 209:169-184.
Meraj M., Yousuf A. R., Bhat F. A., Ali M. N., Ganai B. A., Shahi N., Habiba B., Ganaie H. A., Shah N. U. D., Rahman M., Rather M. I., 2016 Hematological profiling of *Tryplophysa marmorata* (Heckel 1838) from water body of Khasmir Himalaya - a prespective. Journal of Fisheries and Aquatic Scince 11(4):296-303.
Ministry of Marine Affairs and Fisheries of the Republic of Indonesia, 2014 [Academic manuscript of haruan snakehead fish (*Channa striata* Bloch 1793) as a result of domestication]. Mandiangin, South Kalimantan, Indonesia, 74 p. [in Indonesian]
Mollah M. F. A., Mamun M. S. A., Sarowar M. N., Roy A., 2009 Effects of stocking density on the growth and breeding performance of brood fish and larval growth and survival of shol, *Channa striatus* (Bloch). Journal of Bangladesh Agricultural University 7(2):427-432.
Muthmainnah D., Nurdawati S., Aprianti S., 2012 [Cultivation of snakehead fish (*Channa striata*) in caramba containers in swamp]. Prosiding InSINas 0758:319-323. [in Indonesian]
Pagand P., Blancheton J. P., Lemoalle J., Casellas C., 2000 The use of high rate algal ponds for the treatment of marine effluent from a recirculating fish rearing system. Aquaculture Research 31(10):729-736.
Pungrasmi W., Phinitthanaphak P., Powtongsook S., 2016 Nitrogen removal from a recirculation aquaculture system using a pumice stone bottom substrate nitrification-denitrification tank. Ecological Engineering 95:357-363.
Puspaningsih D., Supriyono E., Nirmala K., Rusmana I., Kusmana C., Widiyati A., 2018 The dynamics of water quality during culture of snakehead fish (*Channa striata*) in the aquarium. Omni Akuatika 14 (2):123-131.
Qin J. G., Fast A. W., 1998 Effects of temperature, size and density on culture performance of snakehead, *Channa striatus* (Bloch), fed formulated feed. Aquaculture Research 29:299-303.
Rahman M. A., Arshad A., Amin S. M. N., 2012 Growth and production performance of threatened snakehead fish, *Channa striatus* (Bloch), at different stocking densities in earthen ponds. Aquaculture Research 43:297-302.
Reid S. G., Bernier N. J., Perry S. F., 1998 The adrenergic stress response in fish: Control of catecholamine storage and release. Comparative Biochemistry and Physiology Part C 120:1-27.
Royan F., Rejeki S., Haditomo A. H. C., 2014 Effect of salinity level on blood profile in nile tilapia *Oreochromis niloticus*. Journal of Aquaculture Management and Technology 3:109–117.
Salah M. K., Wael A. O., 2011 Effect of different stocking densities on hematological and biochemical parameters of silver carp, *Hypophthalmichthys molitrix* fingerlings. Life Science Journal 8:580-586.
Salas-Leiton E., Anguis V., Martin-Antonio B., Crespo D., Planas J. V., Infante C., Canavate J. P., Manchado M., 2010 Effects of stocking density and feed ration on growth and gene expression in the Senegalese sole (*Solea senegalensis*): Potential effects on the immune response. Fish & Shellfish Immunology 28:296-302.
Saputra A., Jusadi D., Suprayudi M. A., Supriyono E., Sunarno M. T. D., 2018 [Effects of different feeding frequencies using *Moina sp.* as initial food on growth and survival

of snakehead *Channa striata* larvae reared in green water system]. Jurnal Riset Akuakultur 13(3):239-249. [in Indonesian]
Saputra A., Setijaningsih L., Yosmaniar, Prihadi T. H., 2017 [Nitrogen and phosphorus dynamics in the culture media of snakehead *Channa striata* maintained with the application of probiotic and water hyacinth *Eichhornia crassipes*]. Jurnal Riset Akuakultur 12(4):379-388. [in Indonesian]
Schveitzer R., Arantes R., Baloi M. F., Costadio P. F., Arana L. V., Seiffert W. Q., Andreatta E. R., 2013 Use of artificial substrates in the culture of *Litopenaeus vannamei* (Biofloc System) at different stocking densities: Effects on microbial activity, water quality and production rates. Aquaculture Engineering 54:93-103.
Setiadi E., Taufik I., Widyastuti Y. R., Ardi I., Saputra A., 2019 Different substrate of trickling filter on growth, survival rate, and water quality of common carp (*Cyprinus carpio*) cultivation by using an intensive recirculation system. IOP Conference Series: Earth and Environmental Science 236 012027, DOI: 10.1088/1755-1315/236/1/012027.
Talpur A. D., Ikhwanuddin M., 2013 *Azadirachta indica* (neem) leaf dietary effects on the immunity response and disease resistance of Asian seabass, *Lates calcarifer* challenged with *Vibrio harveyi*. Fish and Shellfish Immunology 34:254-264.
Talpur A. D., Munir M. B., Mary A., Hashim R., 2014 Dietary probiotics and prebiotics improved food acceptability, growth performance, hematology and immunological parameters and disease resistance against *Aeromonas hydrophyla* in snakehead (*Channa striata*) fingerlings. Aquaculture 426-427:14-20.
Taufik I., Setiadi E., 2015 [Exposure of endosulfan insectisides at sublethal concentrations of hematological and histological conditions of carp (*Cyprinus carpio*)]. Jurnal Riset Akuakultur 10(1):109-115. [In Indonesian]
Wahyu, Supriyono E., Nirmala K., Harris E., 2015 [The effect of fish density during transportation on hematological parameters, blood pH value , and survival rate of juvenile snakeheads *Channa striata* (Bloch, 1793)]. Jurnal Iktiologi Indonesia 15(2):165-177. [In Indonesian]
Well R. M. G., Baldwin J., Seymour R. S., Christian K., Britain T., 2005 Blood cells function and hematology in two tropical freshwater fishes from Australia. Comparative Biochemistry and Physiology 141:87-93.
Yuan D., Yi Y., Yakupitiyage A., Fitzimmons K., Diana J. S., 2010 Effects of addition of red tilapia (*Oreochromis* spp.) at different densities and sizes on production, water quality and nutrient recovery of intensive culture of white shrimp (*Litopenaeus vannamei*) in cement tanks. Aquaculture 298:226-238.
Zehra S., Khan M. A., 2012 Dietary protein requirement for fingerling *Channa punctatus* (Bloch), based on growth, feed conversion, protein retention and biochemical composition. Aquaculture International 20:383-395.
Zhang S., Li G., Wu H., Liu X., Yao Y., Tao L., Liu H., 2011 An integrated recirculating aquaculture system (RAS) for land-based fish farming: The effects on water quality and fish production. Aquacultural Engineering 45:93-102.
***FAO Food and Agriculture Organization of the United Nations, 2015 Small-scale Aquaponic Food Production. Integrated Fish and Plant Farming. FAO Fisheries and Aquaculture Technical Paper No. 589, Rome, Italy, 288 p.

Received: 2 April 2019. Accepted: 7 July 2019. Published online: 27 September 2019.
Authors:
Dewi Puspaningsih, Ministry of Marine Affairs and Fisheries of the Republic of Indonesia, Research Institute for Freshwater Aquaculture and Fishery Extension, 16154, Bogor, West Java, Indonesia, e-mail: dewi.puspaningsih79@gmail.com
Eddy Supriyono, Bogor Agricultural University (IPB), Fisheries and Marine Sciences Faculty, Aquaculture Department, Agatis Street, Lingkar Kampus IPB Darmaga, 16680, Bogor, West Java, Indonesia, e-mail: eddysupriyonoipb@gmail.com
Kukuh Nirmala, Bogor Agricultural University (IPB), Fisheries and Marine Sciences Faculty, Aquaculture Department, Agatis Street, Lingkar Kampus IPB Darmaga, 16680, Bogor, West Java, Indonesia, e-mail: kukuhni25@gmail.com
Iman Rusmana, Bogor Agricultural University (IPB), Mathematics and Natural Sciences Faculty, Biology Department, Agatis Street, Lingkar Kampus IPB Darmaga, 16680, Bogor, West Java, Indonesia, e-mail: rusmana13@yahoo.com
Cecep Kusmana, Bogor Agricultural University (IPB), Forestry Faculty, Silviculture Department, Agatis Street, Lingkar Kampus IPB Darmaga, 16680, Bogor, West Java, Indonesia, e-mail: ckmangrove@gmail.com
Ani Widiyati, Ministry of Marine Affairs and Fisheries of the Republic of Indonesia, Research Institute for Freshwater Aquaculture and Fishery Extension, 16154, Bogor, West Java, Indonesia, e-mail: ani_widiyati@yahoo.co.id

How to cite this article:
Puspaningsih D., Supriyono E., Nirmala K., Rusmana I., Kusmana C., Widiyati A., 2019 Water quality, hematological parameters and biological performances of Snakehead fish (*Channa striata*) reared in different stocking densities in a recirculating aquaculture system. AACL Bioflux 12(5):1546-1558.