Growth performance, survival rate, cannibalism of Asian redtail catfish *Hemibagrus nemurus* juvenile, with different stocking density

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Abstract. Asian redtail catfish (*Hemibagrus nemurus*) is a freshwater fish that has a delicious taste, soft meat texture, no fine bones, and high economic value. To date, the fulfillment of *H. nemurus* fish needs still depends on catches in nature. Thus, aquaculture development is needed to increase sustainable national production. The problem in the development of *H. nemurus* fish cultivation is the limited number of juveniles produced from nursery. The purpose of this study was to determine the optimum stocking density that provides the best growth performance in rearing *H. nemurus*. The experimental fish used second-generation *H. nemurus*, with an initial length of 1.93±0.38 cm and an initial weight of 0.07±0.03 g. The research container was fiberglass with a capacity of 500 L. The research was conducted using a completely randomized design with 3 treatments and 4 replicates. The treatments tested were stocking density of 10 fish L^{-1}, 15 fish L^{-1} and 20 fish L^{-1}. Parameters measured were final length, final weight, total length, total weight, absolute length, absolute weight, length specific growth rate, weight specific growth rate, survival rate, cannibalism, condition factor and cortisol. The results showed that the stocking density of 15 fish L^{-1}, was the optimal stocking density that gave the best growth performance with final length of 4.70±0.46 cm, final weight of 0.62±0.16 g, absolute length of 2.78±0.06 cm, absolute weight of 0.55±0.03 g, length specific growth rate of 2.98±0.04 cm, weight specific growth rate of 7.27±0.15 g, survival rate of 77.8±3.63% and cannibalism of 11.9±2.18%.

Key Words: cannibalism, growth performance, *Hemibagrus nemurus*, stocking density, survival rate.

Introduction. Asian redtail catfish (*Hemibagrus nemurus*) is a freshwater fish with a delicious taste, soft meat texture, no fine spines, and high economic value (Gustiano et al 2015). National demand for *H. nemurus* is largely met from wild catch in waters. The total national production from aquaculture of *H. nemurus* in 2020 reached 6,535.19 tons (Ministry of Marine Affairs and Fisheries 2020). This data shows that the aquaculture sector needs to increase production to fulfill sustainable national needs.

The sustainability of aquaculture businesses in the nursery and enlargement segments is determined by the certainty of juveniles supply. However, until now, there are still obstacles in the provision of *H. nemurus* juvenile in mass. This condition is caused by low survival rate and high cannibalism rate (Kusdiarti et al 2020; Heltonika et al 2022). The low survival rate is due to the suboptimal rearing system, limited information on oxygen demand, water flow velocity, stocking density, and high cannibalism (Qin & Fast 1996; Heltonika et al 2022).

Increasing the production and productivity of *H. nemurus* juvenile in mass can be done through optimizing stocking density. Research related to the effect of stocking density on increasing production and productivity has been widely conducted, including on Seurukan fish (*Osteochilus vittatus*) (Azhari et al 2017), Siamese catfish (*Pangasianodon hypophthalmus*) (Heltonika et al 2021), patin Pasupati (*Pangasianodon hypophthalmus* × *Pangasius djambal*) (Darmawan et al 2016), and rainbow trout

(*Oncorhynchus mykiss*) (Timalsina et al 2017). Differences in stocking density were shown to have a significant effect on survival rate, cannibalism, total length, and specific growth rate in larvae of *H. nemurus* (Rahmah et al 2014).

Inappropriate stocking densities accelerate the decline in water quality leading to low survival rate. Extreme water quality degradation leads to increased toxicity and metabolic disorders, resulting in fish mortality. Many studies have been conducted on the effect of increasing stocking density on water quality degradation (Boyd & Trucker 1998; Fivelstad et al 1998; Garcia et al 2013; Feucht & Zander 2015; Araujo-Luna et al 2018; Costas et al 2008). Another impact of inappropriate stocking density is cannibalism. Factors that influence cannibalism include increased aggressiveness, feed shortage and high density (Kestemont et al 2003; Laiz-Carrion et al 2012; Heltonika et al 2021).

To date, there is no data and information related to the effect of stocking density on the growth performance of *H. nemurus* juvenile. The purpose of this research is to determine the optimum stocking density that produces the best growth performance at the juvenile stage. The output of this research is expected to be used to optimize the production system of *H. nemurus* farming, improve product quality and maximize profits.

Material and Method

Time and location of research. The research was conducted in August-December 2021 at the Multi Species Indoor Hatchery, Installation of Environment and Aquaculture Technology, Cibalagung, Research Institute for Freshwater Aquaculture and Fisheries Extension (RIFAFE), Ministry of Marine Affairs and Fisheries, Bogor, West Java, Indonesia.

Experimental design. The research was conducted using a completely randomized design (CRD) with 3 treatments and each treatment was repeated 4 times. The treatments tested were stocking density of 10 fish L^{-1}, 15 fish L^{-1} and 20 fish L^{-1}. The determination of stocking density refers to Sugihartono et al (2016). Each treatment was given a current of 10 cm sec^{-1}. The current source used SP 1200 series water pump with a capacity of 13 W, voltage 220V/240V/50 Hz, output 700 L H^{-1} and was installed in each experimental container. To ensure the stability of current velocity, measurements were taken every morning using Flowatch FL 03 s/n 17 111 859 made in Switzerland, produced by JDC electronic SA. The water pump was cleaned from the remaining feed and feces using clean water and sprayed using running water every 2 days.

Experimental fish. The experimental fish used were spawned *H. nemurus* from one pair of parents weighing 1.5 kg each. Spawning was done hormonally with stripping technique. Spawned eggs were hatched in a 40 × 60 x 40 cm^2 aquarium. The larvae were reared in blue round fiber tanks with a capacity of 1 (one) ton. Larval rearing until the size of the experimental fish was carried out for 30 days. During rearing, the larvae were fed with *Moina* sp. until 15 days of age. The adaptation process to artificial feed was carried out in stages, starting at the age of 10 days to the age of 15 days. Starting at 16 days of age, the fish were fed artificial feed with 40% protein content, until they were ready to be stocked in the experimental container. The size of the juvenile used as experimental fish was 1.93±0.38 cm long, with a weight of 0.07±0.03 g $fish^{-1}$. *H. nemurus* juvenile that had reached the size of the experimental fish were taken randomly from the rearing tanks, then stocked in each experimental container according to the treatment.

Rearing conditions. The rearing container used was a round fiber tub with a diameter of 150 cm, height of 30 cm with a water capacity of 500 L. Before the juvenile were introduced, the experimental container was filled with water sourced from a well and had been settled for 3 (three) days. Each tub was equipped with aeration from an aerator and a water pump as a source of current with a speed of 10 cm sec^{-1}. Furthermore, the water was given salt as much as 3 ppt in each experimental container for disinfection.

After the water had settled for 3 days, the experimental containers were ready to be stocked with the juvenile. Each container was filled with experimental fish which is taken randomly from the rearing tanks with a stocking density of 10 fish L^{-1}, 15 fish L^{-1} and 20 fish L^{-1}. During rearing, the juveniles were fed with commercial feed with a protein content of about 40% (Kusmini et al 2019). The frequency of feeding was 3 times a day (08.00, 12.00 and 16.00) as much as 10% of the total biomass. Maintenance was carried out for 30 days.

Every 2 (two) days, the rearing containers were cleaned of uneaten feed and feces, using the sipon technique. Water quality was monitored by measuring parameters in situ and ex situ. Water quality parameters measured in situ every 5 days were temperature, using a digital thermometer with two decimal accuracy; pH using a digital pH meter (pH 5 plus LaMotte pH meter) with two decimal accuracy and dissolved oxygen (DO) using a digital DO meter (DO meter YSI Pro 20) with two decimal accuracy. During the measurements, aeration and current speed were turned off. Water quality parameters analyzed ex situ were ammonia referring to SNI 06-6989.30-2005, nitrate referring to SNI 06-6989.79-2011, and nitrite referring to SNI 06-6989.29-2005. Water quality measured ex situ was carried out by taking water samples using an opaque 0.5 L sample bottle. Each sample bottle was labeled according to its origin. Furthermore, sample bottles that have been filled with water were put into a tightly closed container box, for preparation and analysis at the Environmental and Toxicology Laboratory, Cibalagung, Bogor, West Java, Indonesia.

Parameters. During rearing, fish mortality and the amount of feed given were recorded daily. At the end of rearing, the experimental fish were recorded for population size to calculate survival rate. Length and weight measurements were taken every 10 days using millimeter blocks. The sample size for length and weight measurement was 30 fish per container (Githukia et al 2015; Yogev et al 2020). During the measurement, the experimental fish were given stabilizer (brand Arowana Ocean) as much as 1 mL L^{-1} water. After measuring length and weight, the experimental fish were returned to the experimental container.

Parameters measured included absolute length (Satrawaha & Pilasamorn 2009), absolute weight (Thongprajukaew & Rodjaroen 2017), specific growth rate (Bake et al 2014), survival rate and cannibalism (Heltonika et al 2021), condition factor (Thongprajukaew & Rodjaroen 2017) and length-weight relationship (Satrawaha & Pilasamorn 2009). Laboratory analysis was conducted to measure cortisol levels of the experimental fish, which was done by taking 20 fish samples for each treatment (±20 g per treatment). The cortisol measurement test method refers to the Cortisol ELISA procedure EIA-1887.

Data analysis. Data on final length, final weight, absolute length, absolute weight, specific length growth rate, specific weight growth rate, survival rate and cannibalism, were statistically analyzed using analysis of variance (ANOVA) at 95% confidence level with SPSS software 20. Length-weight relationship data were analyzed using linear regression, condition factors and size uniformity were analyzed using line and bar graphs. Water quality parameters and cortisol were displayed in tabular form and analyzed descriptively.

Results. Water quality plays an important role in the growth and development of juveniles. Optimum water quality will improve growth performance, while bad water quality will increase mortality. During the experiment, water quality was measured in situ and ex situ with the measurement results shown in Table 1.

Juvenile reared at various stocking densities for 30 days, showed growth performance that was not significantly different in terms of final length and weight, absolute length and weight, and specific growth rate of length and weight ($p > 0.05$) (Table 2). The higher the stocking density, the higher the level of cannibalism which is inversely proportional to survival rate. This result indicates that the juvenile still have predatory traits as indicated by high cannibalism. This condition results in the mortality rate of *H. nemurus*. The results of statistical analysis on the survival rate of *H. nemurus* reared for 30 days showed a significant difference between densities of 20 fish L^{-1} and 10 fish L^{-1}, but not significantly different ($p > 0.05$) for stocking densities of 10 fish L^{-1} and 15 fish L^{-1} and 20 fish L^{-1} and 15 fish L^{-1} (Table 2).

Table 1

Water quality at various densities during the 30-day rearing period

Parameters	*Unit*	*Stocking density (fish L^{-1})*			*Standard*
		10	*15*	*20*	
Temperature	°C	25.20-26.40	25.20-27.10	25.20-26.00	25-30[1,2]
pH	-	6.77-6.89	6.79-7.23	6.73-6.89	6.50-8.50[1,2]
Dissolved oxygen	mg L^{-1}	3.80-6.16	3.16-6.16	2.30-6.16	Min 2 & optimal >5[3,4,5]
Ammonia	mg L^{-1}	0.198-0.263	0.263-0.495	0.263-0.720	< 0.02[6]
Nitrite	mg L^{-1}	0.003-0.170	0.003-0.173	0.003-0.359	< 0.06[6]
Nitrate	mg L^{-1}	4.688-8.430	4.688-8.74	4.688-9.420	< 20[6]

Note: [1)]Boyd (2017), [2)]SNI: 01-6484.4-2000, [3)]SNI: 01-7256-2006, [4)]Susanto (2006), [5)]Marx et al (2020), [6)]PP No. 82 of 2001.

Table 2

Growth performance of *H. nemurus* reared at various densities during the 30-day rearing period

Parameters	*Unit*	*Stocking density (fish L^{-1})*		
		10	*15*	*20*
Initial length	cm	1.93±0.35[a]	1.93±0.37[a]	1.93±0.34[a]
Final length	cm	4.57±0.40[a]	4.70±0.46[a]	4.61±0.42[a]
Initial body weight	g	0.07±0.03[a]	0.07±0.02[a]	0.07±0.02[a]
Final body weight	g	0.58±0.13[a]	0.62±0.16[a]	0.63±0.15[a]
Absolute length	cm	2.65±0.13[a]	2.78±0.06[a]	2.70±0.07[a]
Absolute body weight	g	0.51±0.04[a]	0.55±0.03[a]	0.56±0.04[a]
Specific growth rate (SGR)	% day^{-1}	7.06±0.23[a]	7.27±0.15[a]	7.31±0.20[a]
Survival rate	%	87.20±8.39[a]	77.80±3.63[ab]	69.90±5.19[b]
Cannibalism	%	10.90±0.41[a]	11.90±2.18[a]	16.20±0.60[b]

Note: Values were represented as mean±standard deviation. Different superscripts show significant differences between treatments ($p < 0.05$).

In addition to affecting cannibalism and survival rate, stocking density also affects the relationship between length and weight of *H. nemurus*. The results of the calculation of the relationship between the length and weight of the juvenile were < 3. The results of the calculation of the relationship between length and weight of fry reared at various densities are shown in Figures 1A, 1B, and 1C. The results of the analysis show that the higher the stocking density, the lower the coefficient of determination R^2. This result means that weight gain is strongly influenced by length gain and the growth pattern is negative allometric.

The condition factor is one of the indicators of the growth performance of the reared *H. nemurus* juvenile. The results of statistical analysis showed that the stocking density of *H. nemurus* did not significantly affect the condition factor (Figure 2). The results of the calculation of the condition factor of *H. nemurus* reared at various stocking densities are relatively the same and have a condition factor value > 1 (Figures 2A, 2B, and 2C). This condition indicates that the growth performance of *H. nemurus* reared at various stocking densities is still optimum.

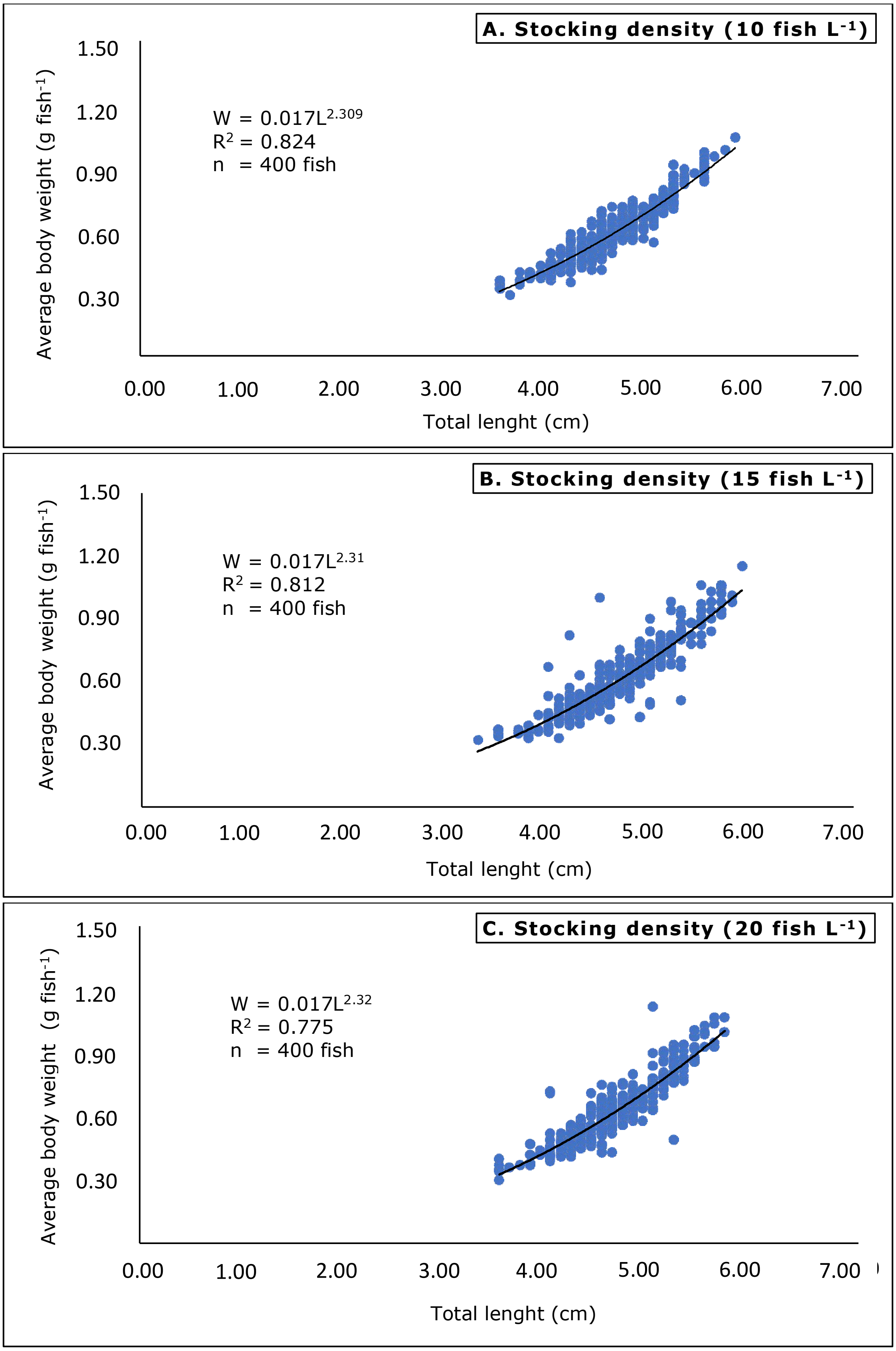

Figure 1. Relationship between length and weight of *H. nemurus* reared for 30 days at densities: A. 10 fish L^{-1}, B. 15 fish L^{-1}, and C. 20 fish L^{-1}.

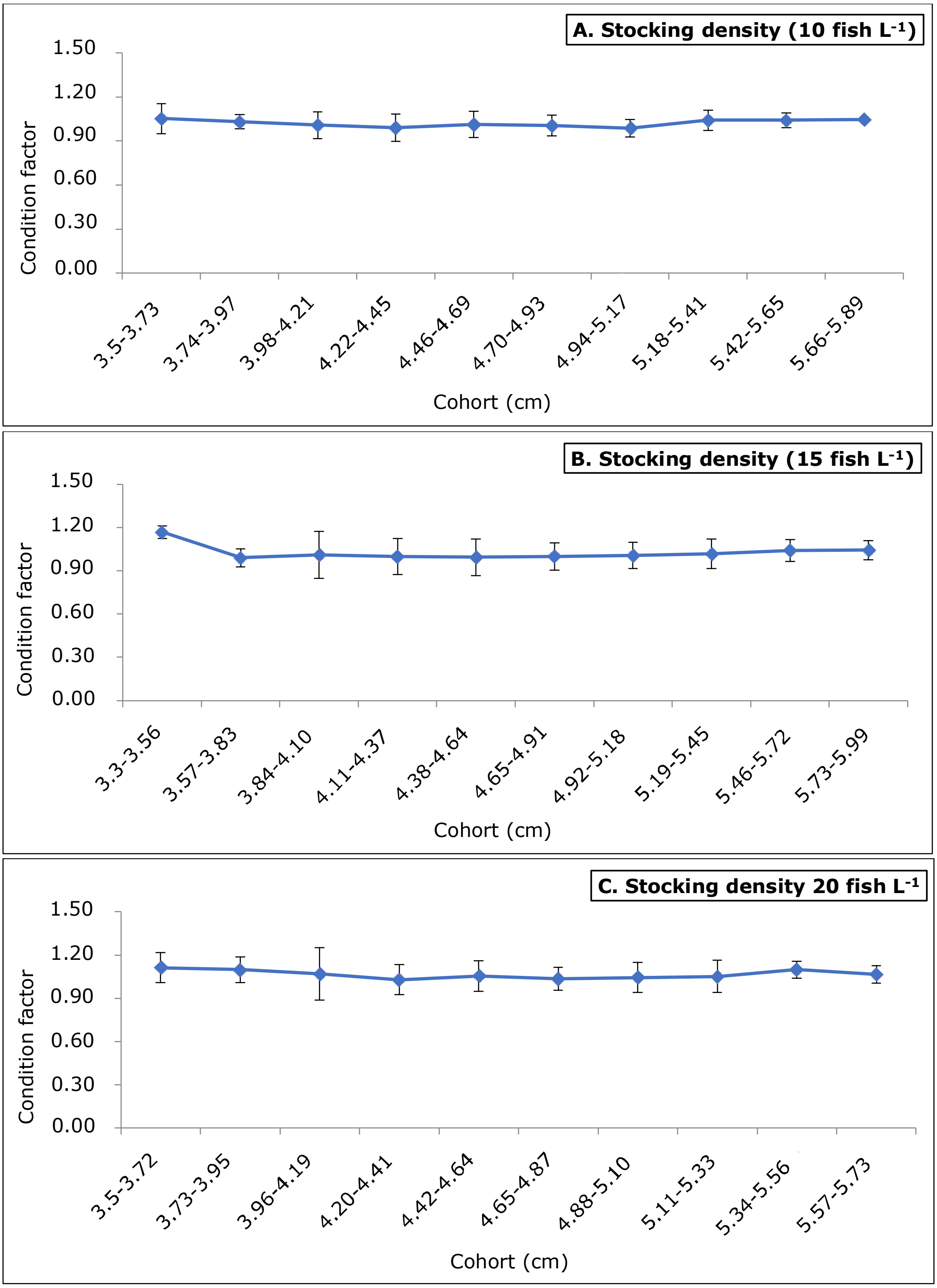

Figure 2. Condition factor of *H. nemurus* reared for 30 days at various stocking densities: A. 10 fish L^{-1}, B. 15 fish L^{-1}, and C. 20 fish L^{-1}.

The results of the measurement of stress response with cortisol indicator are presented in Table 3. These results indicate that the *H. nemurus* juvenile with the lowest indication of stress is found at a density of 15 fish L^{-1}.

Table 3

Cortisol concentration of *H. nemurus* at various densities during the 30-day rearing period

Stocking density (fish L^{-1})	*Cortisol level (ng mL^{-1})*
10	1.00-5.43
15	Nd-0.18
20	Nd-1.37

Note: nd = not detected.

Discussion. Water quality plays an important role in fish rearing including *H. nemurus*. The results of measurements of water temperature and pH for 30 days were in the optimum range for growth and development of *H. nemurus* (Boyd & Trucker 1998). Density has a significant effect on the concentration of DO and nitrite during the rearing period. The results of the analysis showed that an increase in density was directly proportional to a decrease in DO and an increase in nitrite concentration. These results are in accordance with previous studies (Fivelstad et al 1998; Costas et al 2008) which reported that increasing stocking density in fish rearing was shown to increase COD (chemical oxygen demand), $NH_4^+N^-$, and pH levels. The results of other studies state that stocking density affects oxygen consumption in waters, resulting in low DO concentrations (Garcia et al 2013; Araujo-Luna et al 2018). Low DO concentrations can increase the toxicity of nitrate and ammonia to the fish being reared. In addition to affecting the toxicity of waters, DO also affects the metabolic process so that it affects the mortality rate (Duan et al 2011; Prakoso et al 2016; Saputra et al 2021). The optimum DO value for fish growth is > 5 mg L^{-1} but should not be < 3 mg L^{-1} (Marx et al 2020). The results of other studies state that DO for fish growth is at least 2 mg L^{-1} and the optimum concentration is > 5 mg L^{-1} (Susanto 2006). DO levels in the rearing medium for *H. nemurus* with a density of 20 fish L^{-1} (2.30-6.16 mg L^{-1}) are in the range that can interfere with survival rate.

In addition to influencing water quality, stocking density also affects the survival rate of *H. nemurus*. The low survival rate of *H. nemurus* reared at a density of 20 fish L^{-1} was due to low DO (2.30-6.16 mg L^{-1}) and high nitrite concentration (0.003-0.359 mg L^{-1}). These results are in line with research on striped snakehead (*Channa striata*) fry which states that low stocking density (2 fish L^{-1}) produces a higher specific growth rate (0.9% day^{-1}) and survival rate reaches 95.42% compared to higher densities (Latifah et al 2022). The results of the analysis of the absolute weight and length of *H. nemurus* juvenile reared at various densities, were directly proportional to their specific growth rate. Meanwhile, the level of cannibalism of *H. nemurus* at various stocking densities shows results that are inversely proportional to survival rate. The results of this study indicate that the lower the stocking density, the lower the cannibalism and the higher the survival rate.

Cannibalism was highest at a density of 20 fish L^{-1} and lowest at a density of 10 fish L^{-1}. Factors that influence the increase in cannibalism include increased aggressiveness due to hormonal influences (Heltonika et al 2021), closer distance between individuals (Laiz-Carrion et al 2012), frequent contact (Garcia et al 2013) and size differences (Kestemont et al 2003). In addition, low survival rate is caused by competition for feed, limited space to move, and low DO content (Kadarini et al 2010; Laiz-Carrion et al 2012). These results are consistent with research on Barramundi fish (*Lates calcarifer*) that showed the highest cannibalism at high densities (Krol & Zielinski 2015). To suppress cannibalism, it is recommended to rear *H. nemurus* at a density of less than 20 fish L^{-1}.

In addition to affecting survival rate and cannibalism, stocking density also affects the growth performance of *H. nemurus*, because optimal metabolism will be achieved under optimum water quality conditions. Optimum water quality will be achieved at optimum density. Some research results show that stocking density has a significant effect on the growth performance of reared *H. nemurus* (Bjornsson & Olafsdottir 2006; Rahmah et al 2014; Krol & Zielinski 2015; Heltonika et al 2021; Paredes-Lopez et al 2021). However, it is different for *H. nemurus* reared during the 30-day rearing period.

The results of the analysis show that the specific growth rate performance of *H. nemurus* reared at various densities is not significantly different. This result indicates that the specific growth rate of *H. nemurus* is not affected by stocking density even up to 20 fish L^{-1}.

Other growth performance analyzed was the ratio of length and weight. These results show that weight gain is strongly influenced by length gain and the growth pattern is negative allometric. However, there is a tendency for the coefficient of determination to be higher in the rearing of *H. nemurus* with higher density and inversely proportional to the regression equation. This result shows that there is an effect of stocking density on increasing weight gain with length gain. The length-weight relationship data shows the value of b in the range of 2.31-2.32 (b < 3). Research by Borah et al (2020) states that the growth pattern of fish, with a value of b < 3, indicates a negative allometric growth pattern where length growth is faster than weight growth. Thus, the weight gain that will approach positive allometrics is a density of 20 fish L^{-1}.

The results of the calculation of the condition factor show that the value is > 1. A condition factor of > 1 indicates that fish grow and develop well, while a condition factor of < 1 is the opposite (Borah et al 2020). The condition factor value is caused by insufficient food availability or a decrease in immunity in fish (Jin et al 2015). Condition factor is a factor measured to see the growth performance and sensitivity of fish to the environment (Jin et al 2015). Condition factors can be influenced by intrinsic and extrinsic factors, such as the reproductive cycle, feed intake, life history and various stressors from the environment (Erasmus et al 2019). The results of the analysis show that *H. nemurus* juvenile during the 30-day rearing period can grow and develop optimally up to a density of 20 fish L^{-1}.

Optimum growth of *H. nemurus* reared at various densities can be known from their stress response. The indicator of stress response measured is cortisol (Odhiambo et al 2020). The results of the analysis show that *H. nemurus* reared at various densities are in optimum conditions. The low concentration of cortisol indicates that the fry are not stressed. This result is in accordance with research that has been conducted on *H. nemurus* (Pratama et al 2022), stating that *H. nemurus* reared at both high and low stocking densities, indicated that they are not stressed and their growth was optimal. However, there is a tendency for fish with densities that are too low (10 fish L^{-1}) or too high (20 fish L^{-1}) to experience symptoms of stress.

Conclusions. The rearing of *H. nemurus* juvenile at a stocking density of 15 fish L^{-1} produced the best growth performance in terms of final length 4.70±0.46 cm, final weight 0.62±0.16 g $fish^{-1}$, absolute length 2.78±0.06 cm, absolute weight 0.55±0.03 g $fish^{-1}$, specific length growth rate 2.98±0.04% day^{-1}, specific weight growth rate 7.27±0.15% day^{-1}; survival rate 77.80±3.63% and cannibalism 11.90±2.18%.

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Conflict of interest. The authors declare that there is no conflict of interest.

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