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Optimizing Water Flow Rate to Improve Growth, Feed Efficiency, and Stress Response in Sustainable *Hemibagrus nemurus* Farming

Adang Saputra[1], Lusi Herawati Suryaningrum[2]*, Rini Widya Sari[3], Sinung Rahardjo[3], Siti Murniasih[1], Edy Farid Wadjdy[1]

[1]Research Center for Fisheries, National Research and Innovation Agency of Indonesia (BRIN)
[2]Research Center for Applied Zoology, National Research and Innovation Agency of Indonesia (BRIN)
[3]Technical University of Fisheries, Ministry of Marine Affairs and Fisheries of Indonesia

***Corresponding Author: lusi006@brin.go.id**

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ABSTRACT

The Asian red tail catfish (*Hemibagrus nemurus*) is a native fish from the Indonesian freshwater. The fish is targeted as a potential fisheries commodity; thus, its farming technology has been actively developed. This work aimed to investigate the water flow rate's effects on the growth performance, feed efficiency, and stress response of *H. nemurus*. The fish seed (an average length of 1.64± 0.18cm and an average weight of 0.06± 0.004g/ fish) was obtained from the spawning of selected broodstock weighing about 1.5kg/ fish. A 500L round fiberglass was used as the experimental container, filled with 150L of water. Each pond contained 1.500 fish seeds, randomly collected from an acclimatization container. They were daily fed at 10% of their total biomass thrice. The fish rearing was conducted at a water flow rate of 10, 20, and 30cm/ s. The following parameters were recorded: absolute length, absolute weight, specific growth rate, protein retention, survival rate, cannibalism, and length–weight relationship. The water flow rate has been shown to significantly improve water quality in aquaculture systems. Specifically, a flow rate of 20cm/ s produced the most favorable conditions for the rearing of *H. nemurus*, resulting in the highest specific growth rate (5.02%), the highest survival rate (47.81%), and the best feed efficiency (85.69%). These findings indicate that maintaining a water flow rate of 20cm/ s is optimal for promoting the health and growth of *H. nemurus* in aquaculture settings.

INTRODUCTION

Fish farming is crucial in meeting the increasing demand for fish protein worldwide. Efficient farming practices are essential for sustainable aquaculture production. In this context, the water flow rate is a critical environmental factor that significantly influences the performance and well-being of farmed fish species. Understanding the impact of water flow rate optimization on growth, feed efficiency, and stress response is crucial for improving farming practices and enhancing the productivity of aquaculture systems.

The Asian red tail catfish (*H. nemurus*) is commonly found in Southeast Asia countries, such as Indonesia. This species has excellent aquaculture potential and has received economic importance in freshwater farming **(Pamukas *et al*., 2021)**. *H. nemurus* grows well in low-water flow habitats such as rivers and lakes. Historically, most consumed *H. nemurus* were wild-caught, and the species has faced concerns about population decline due to overfishing and habitat degradation **(Heltonika *et al.*, 2021)**. Thus, efforts have been made to develop *H. nemurus* farming practices to ensure sustainable production and conservation of the species. Some efforts have been taken to raise the production of *H. nemurus*, including hormonal induction for broodstock and feed management system **(Thongprajukaew & Rodjaroen, 2017)**, in addition to domestication and reproductive performance enhancement **(Ina-Salwany *et al*., 2019)**.

However, farming technology for *H. nemurus* is hindered by some difficulties, including gonad maturity in a controlled condition **(Zulperi *et al*., 2017)**, undesirable growth performance **(Hasan *et al*., 2019)**, as well as the low survival rate of seed **(Heltonika *et al*., 2021)**. In addition, the most problematic issue of *H. nemurus* farming is associated with water quality maintenance in the cultural media. Achieving optimal growth and feed efficiency while minimizing stress in *H. nemurus* farming remains challenging. The water flow rate is a fundamental component of water quality and is vital in shaping fish physiology, behavior, and overall performance. Recognized as a critical factor, optimizing the water flow rate becomes imperative in enhancing farming practices and unlocking the maximum production potential. Moreover, understanding the effects of optimizing water flow rate on the growth, feed efficiency, and stress response of *H. nemurus* holds immense importance, as it enables us to make informed decisions toward improving aquaculture techniques and achieving optimal productivity.

Some previous works have been concerned with this problem. Developing comprehensive *H. nemurus* farming technologies, including optimizing water flow rate, is vital in this endeavor. **Voorhees *et al.* (2020)** modified the water flow rate, while some found that the water flow rate control in aquaculture successfully improved growth performance, efficiency, and feed utilization **(Obirikorang *et al*., 2017; Nilsen *et al*., 2019)**. Moreover, the water flow rate contributed to the rise of dissolved oxygen concentration and removed metabolic residues, debris, and feces **(Is-haak *et al*., 2019)**. The positive impacts of water flow rate on fish production have been reported, i.e., the lumpfish (*Cyclopterus lumpus*) **(Jorgensen *et al*., 2017)**, the tilapia (*Oreochromis niloticus*) **(Obirikorang *et al*., 2017)**, the Atlantic salmon **(Nilsen *et al*., 2019)**, the Murray cod (*Maccullochella peelii*) **(Shiau *et al*., 2020)**, and the rainbow trout (*Oncorhynchus mykiss*) **(Voorhees *et al*., 2020)**. However, limited research has been conducted on optimizing the water flow rate for *H. nemurus*, which possesses specific characteristics and cultivation requirements. Therefore, it is necessary to investigate the potential benefits of water flow rate optimization in *H. nemurus* farming to enhance their growth performance and well-being.

This study aimed to address the research gap by evaluating the effects of optimizing the water flow rate on the growth, feed efficiency, and stress response of *H. nemurus* at a hatchery scale. By comprehensively analyzing these aspects, valuable insights can be gained to improve farming practices, conserve wild populations, and promote the sustainability of *H. nemurus*.

MATERIALS AND METHODS

1. Experimental fish

The seeds of *H. nemurus* were produced from the artificial spawning of broodstock (1.5kg/ fish) with a hormonal program conducted on the Installation of Germplasm in Cijeruk (RIFAFE), Bogor, Indonesia. After the spawned eggs were hatched, larvae were transferred into a rearing container at a stock density of 3.000 larvae/container. A round fiber tank (capacity 1000L) was filled with 300L of water. The larvae were fed by artemia till they reached 11 days, then adapted to commercial feed for five days. Subsequently, they were completely fed by commercial feed consisting of protein (40.04%), lipid (4.21%), ash (10.11%), crude fiber (2.28%), and NFE–nitrogen free extract (43.36%). They were fed thrice daily, at ad-satiation level, for 30 rearing days. Once the larvae reached a length of 1.64± 0.18cm and weighed 0.06± 0.004g/ fish, they were randomly sampled and transferred into the experimental tank. Before exposure to the treatments, the initial weight and length of 30 larvae from each tank were recorded. During measurement, they were stabilized by a stabilizer (Arwana Ocean) at a 1mL/ L water dose. The fish sample was immediately returned to the experimental tank after measurement.

2. Rearing condition

The experimental tank (capacity 500L) was filled with 150L of water (settled for 3 days) supplied from a local well. Each tank was aerated by an aerator, with a stock density of 1500 fish taken from the larval rearing tank. The seeds were acclimatized for 3 days (till there was no mortality). After acclimatization, the fish seeds were exposed to different water flow levels for 30 rearing days. During the rearing, they were fed commercial feed containing 40% protein at a frequency of thrice daily (8:00 a.m., 12:00 a.m., and 4:00 p.m.), with a dosage of 10% total biomass. All experimental fish have been conducted in accordance with the SNI 6484.4:2014 as a pertinent national animal welfare and/or ethical regulations regarding the care and use of the catfish in Indonesia as the country of residence of all authors. The rearing tank was siphoned every two days to maintain cleanliness, and water quality was monitored every five days. *In situ* observations included measurements of temperature, pH, and dissolved oxygen. *Ex-situ* analyses were conducted to assess nitrate, ammonia, and nitrite levels. The nitrate content was measured using the standard method SNI 06-6989.79-2011; ammonia levels were determined using SNI 06-6989.30-2005, and nitrite content was assessed using SNI 06-6989.29-2005. For *ex-situ* analysis, water was sampled using a 0.5L opaque plastic bottle, labelled, and kept in a cool box. The analysis was performed in the Laboratory of Environment and Toxicology, Cibalagung, Bogor, Indonesia.

3. Experimental design

A completely randomized design with three treatments and four replications was conducted for this work, namely, the water flow rate of 10, 20, and 30cm/ s, referring to **Beecham *et al*. (2009)**. The water flow rate of 10, 20, and 30cm/ s was formed by the water pump with the technical specifications, namely Yamano SP 1200 (capacity 13 W; output 700 L/h), Yamano SP 1600 (capacity 23 W; output 900 L/h), and Yamano SP 1800 (capacity 28 W; output 1500 L/h), respectively. All of them have the voltage of 220V/240V/50 Hz. Flowatch JDC electronic SA-Switzerland, FL 03 serial number s/n 17 111 859 verified the water flow rate and measured each at 09:00 a.m.

4. Collecting data

Data were collected at different periods. Daily records were kept on mortality and feed consumption. At the end of the rearing period, the final length, final weight, and survival were recorded. The growth parameters were also determined, including total length **(Alam *et al*., 2013)**, protein retention and specific growth rate **(Bake *et al*., 2014)**, total weight, condition factor, and length–weight relationship **(Thongprajukaew & Rodjaroen, 2017)**, and cannibalism and survival rate **(Heltonika *et al*., 2021)**. The test parameters were calculated as follows **(Campos *et al*., 2019)**:

Absolute length (cm) = Final length (cm) - Initial length (cm)
Absolute weight (g) = Final weight (g) - Initial weight (g)
Protein retention (%) = 100 × (protein gain/protein feed)
Lipid retention (%) = 100 x (lipid gain)/lipid feed
Feed consumption (g) = total amount of feed
Protein efficiency ratio = wet weight gain/protein intake
Feed efficiency (%) = 100 × (wet weight gain/dry feed intake)
Cannibalism (%) = 100 × (number of missing and dead fish/number of initial fish)
Survival rate (%) = 100 × (number of final a live fish/number of initial fish)
Condition factor (g/cm^3) = 100 × W (g)/L (cm)^3
Specific growth rate (SGR) (%/day) = 100 × [(ln final weight - ln initial weight)/day]

In addition, the content of protein and stress response were determined. For protein analysis, 40 fish samples from each treatment were used, amounting to approximately ±40g per treatment. The protein quantification followed a standard of SNI 01–2891-1992 method. The stress response was approached by determining cortisol collected from 100 fish for each treatment (20 fish from each replication). The cortisol analysis was performed according to the protocol of DRG®Cortisol ELISA (EIA-1887).

5. Data analysis

The data (absolute length, absolute weight, SGR, protein retention, cannibalism, survival rate) were statistically analyzed using ANOVA (variance analysis) at a confidence interval of 95% using SPSS version 26. The remaining data (water quality and cortisol) were elaborated by descriptive analysis.

RESULTS

Water quality is crucial for fish culture, including *H. nemurus* seeds. **Hoar *et al.* (1979)** explained that the water quality parameters, i.e., oxygen, pH, temperature, dissolved oxygen (DO), nitrate, ammonia, and nitrite, were crucial to fish farming. In this work, we found that water temperature and pH met the criteria for the growth of *H. nemurus*. The level of DO in the water flow rate of 20cm/ s was relatively higher than that in 10 and 30cm/ s. Meanwhile, the flow rate of 20cm/ s resulted in lower ammonia, nitrite, and nitrate levels in the culture water relative to other flow rate treatments. The parameters of water quality are presented in Table (1).

Water quality is indicated by the chemical composition in the culture water and the growth performance of *H. nemurus*. The results showed that the total length and individual weight of *H. nemurus* did not significantly differ ($P > 0.05$) in all treatments. The SGR of fish in a flow rate of 20cm/ s varied significantly ($P < 0.05$) compared with a flow rate of 10 and 30cm/ s ($P < 0.05$). However, SGR between samples in 10 and 30cm/ s was not significantly different ($P > 0.05$). Moreover, cannibalism and survival rate of *H. nemurus* did not differ significantly ($P > 0.05$) in all treatments. The growth performance of *H. nemurus* in a 30-day trial is presented in Table (2).

Table 1. Water quality parameters of *H. nemurus* culture for a 30-day experiment at different water flow rates

Parameter	Unit	Water flow rates (cm/s)			Standards
		10	20	30	
Water temperature	°C	24.10–26.20	24.00–26.40	24.30–26.10	25-30[1,2]
pH	-	6.62–7.13	6.50–7.13	6.39–7.13	6.50-8.50[1,2]
Dissolved oxygen	mg/L	2.76–4.05	2.76–5.40	2.76–4.79	Min 2 and optimal >5[3,4,5]
Ammonia	mg/L	0.005–0.006	0.005–0.006	0.005–0.006	<0.02[6]
Nitrite	mg/L	0.003–0.170	0.003–0.163	0.003–0.359	<0.06[6]
Nitrate	mg/L	4.125–8,290	4.088–9.062	4.688–10.977	<20[6]

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* culture for a 30-day experiment at different water flow rates

Parameter	Unit	Water flow rates (cm/s)		
		10	20	30
Initial length	cm	1.64±0.18[a]	1.64±0.18[a]	1.64±0.18[a]
Final length	cm	4.34±0.55[a]	4.82±0.90[a]	4.48±0.90[a]
Initial weight	g	0.06±0.004[a]	0.06±0.004[a]	0.06±0.004[a]
Final weight	g	0.51±0.16[a]	0.65±0.20[a]	0.57±0.21[a]
SGR	%/day	4.91±0.03[b]	5.02±0.08[a]	4.90±0.03[b]
Cannibalism	%	52.76±4.95[a]	51.49±3.81[a]	52.60±2.53[a]
Survival rate	%	46.50±4.70[a]	47.81±4.10[a]	46.80±2.70[a]

The mean ± standard deviation was used to express the values. Different superscripts reveal significant treatment differences (P< 0.05).

In aquaculture, water quality plays a crucial role in feed consumption and overall growth performance. Feed represents a significant portion of operational costs, making feed efficiency a critical factor in fish farming. Several indicators, such as feed consumption, protein retention, lipid retention, protein efficiency ratio, and feed efficiency, can measure feed efficiency. In this experiment, fish reared at a water flow rate of 20cm/ s demonstrated a significantly lower feed consumption of 603.41±51.16 compared to those at 30 (760.58± 90.10) and 10cm/ s (789.86± 81.93) (P< 0.05). However, there was no significant difference in feed consumption between the 30 and 10cm/ s treatments (P> 0.05).

Protein retention was at its highest at the 20cm/ s flow rate (22.02± 0.76), followed by 19.82± 0.54 at 30cm/ s and 18.77± 1.15 at 10cm/ s. Lipid retention varied significantly among treatments (P< 0.05), with the highest value observed at 30cm/ s (38.75± 1.32). The protein efficiency ratio was most efficient at 20 (2.40±0.07) and 30 cm/s (2.39±0.87), both significantly higher than at 10cm/ s (2.12±0.11) (P< 0.05). Feed efficiency was similar considering the 20 (85.69± 2.92) and 30cm/ s (84.38± 2.40) flow rates, with no significant difference (P> 0.05). However, both were significantly more efficient than the 10cm/ s flow rate (75.57± 4.62) (P< 0.05). These findings are summarized in Table (3).

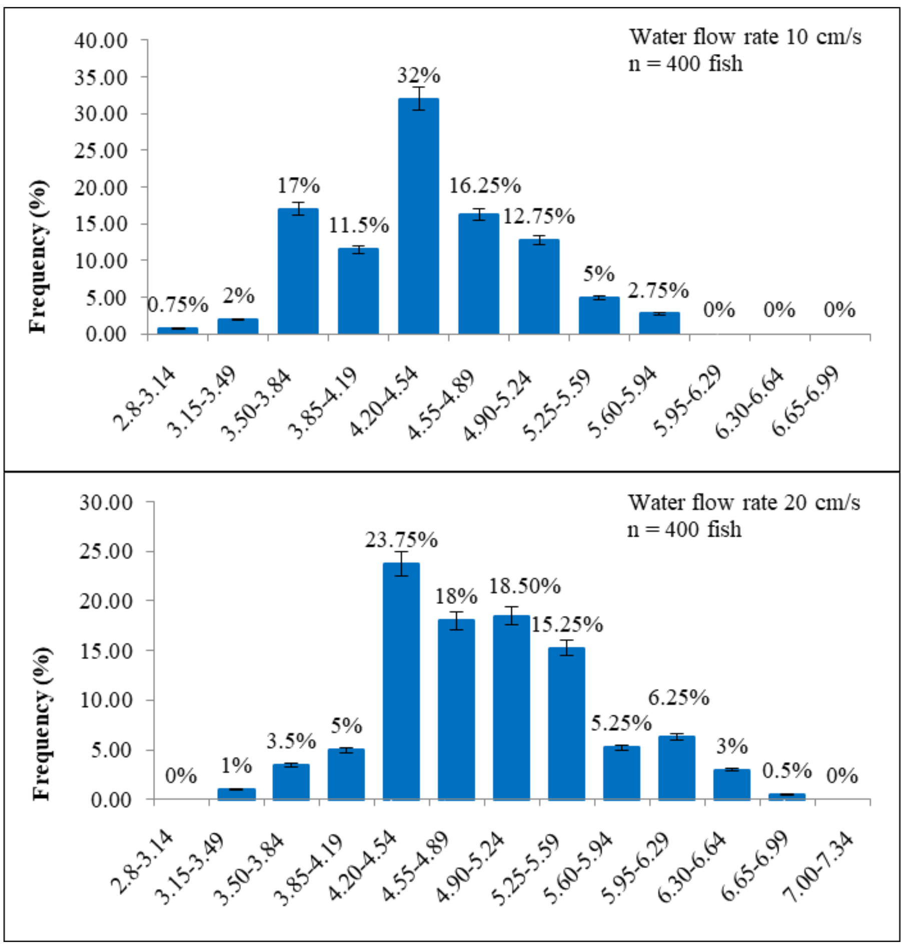
The experiment also highlighted that the water flow rate significantly influenced the size distribution of *H. nemurus* over 30 days (Fig. 1). At a flow rate of 10cm/ s, the fish sizes ranged from 4.20–4.54cm, making up 32% of the population (n = 400 fish), with the rest distributed between 2.80–4.19cm (31.25%) and 4.55–5.94cm (36.75%). Fish at the 20cm/ s flow rate showed more homogeneity in size, with 75.50% of the fish measuring 4.20–5.59cm, while the remaining sizes were 3.15–4.19cm (9.50%) and 5.60–6.99cm (15%). Fish reared at 30cm/ s also exhibited size variation, with 82.25% falling between 3.50–5.24cm, 4% between 2.80–3.49cm, and 13.75% between 5.25–6.29cm.

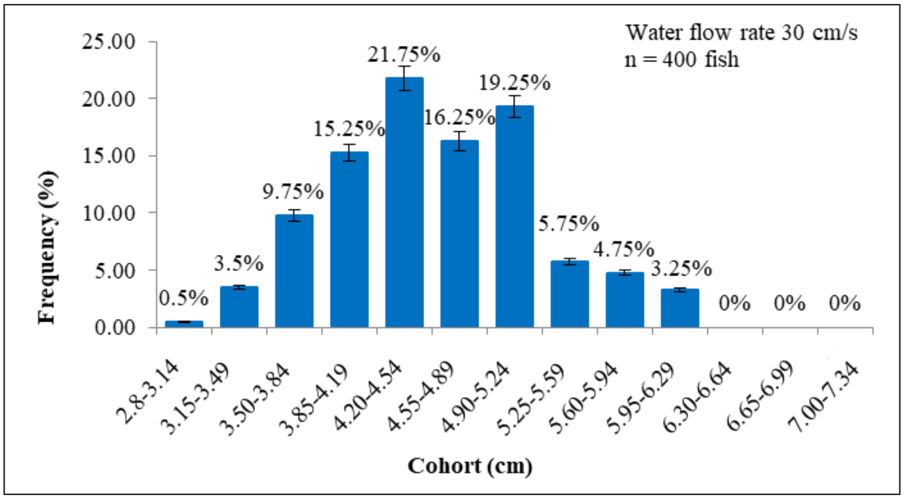
The results suggest that a water flow rate of 20cm/ s not only yielded the most uniform fish size but also resulted in the lowest cannibalism rates, likely due to the high similarity in fish length.

Table 3. Feed consumption, protein retention, lipid retention, protein efficiency ratio, feed efficiency of *H. nemurus* culture for a 30-day experiment

Parameter	Unit	Water flow rates (cm/s)		
		10	20	30
Feed consumption	g	789.86±81.93^{b}	603.41±51.16^{a}	760.58±90.10^{b}
Protein retention	%	18.77±1.15^{b}	22.02±0.76^{a}	19.82±0.54^{b}
Lipid retention	%	26.13±1.58^{c}	38.75±1.32^{a}	31.39±0.87^{b}
Protein efficiency ratio	-	2.12±0.11^{b}	2.40±0.07^{a}	2.39±0.87^{a}
Feed efficiency	%	75.57±4.62^{b}	85.69±2.92^{a}	84.38±2.40^{a}

The mean ± standard deviation was used to express the values. Different superscripts reveal significant treatment differences (P< 0.05).

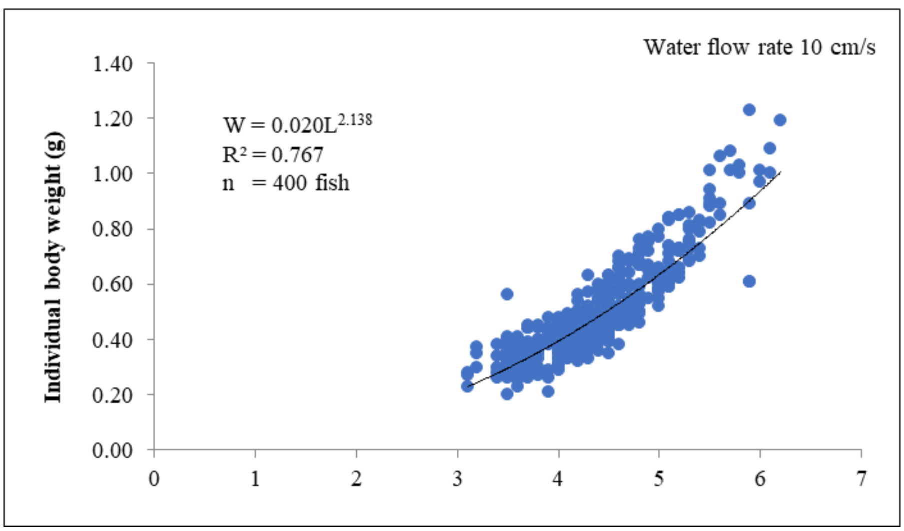




n is the number of fish tested

Fig. 1. Distribution of *H. nemurus* after 30 days of the experiment

Moreover, the water flow rate also affected the length–weight relationship of *H. nemurus*, resulting in a value of < 3 (Fig. 2). The data showed that the highest R^2 was achieved in the water flow rate of 20cm/ s, indicating that the weight gain was markedly influenced by the rise of fish length, with negative allometry.



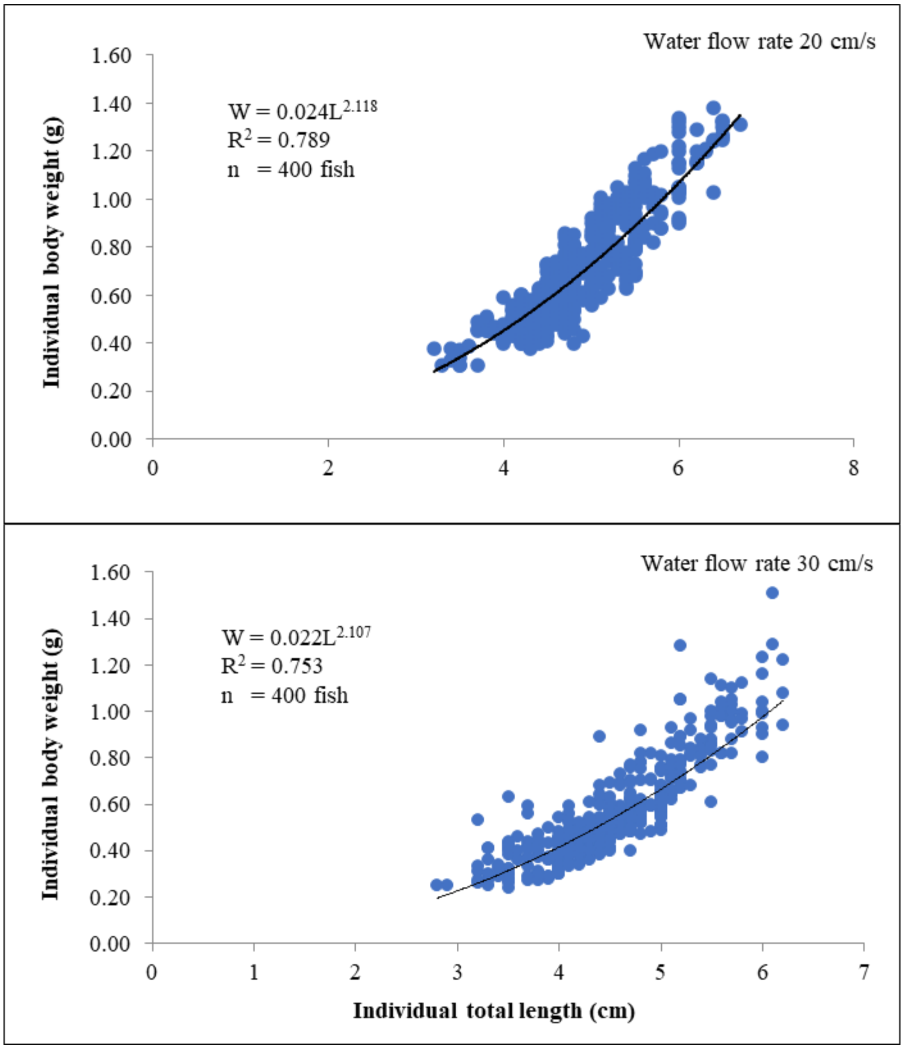


Fig. 2. The relationship of length-weight of *H. nemurus* as affected by water flow rate during a 30-day experiment

This work revealed the absence of stress response for *H. nemurus* fish during a 30-day experiment. The concentration of cortisol in all treatment groups was comparable ($P <$ 0.05) (Fig. 3), but the highest and lowest cortisol level was found in fish treated with a flow rate of 20 and 30cm/ s, respectively.

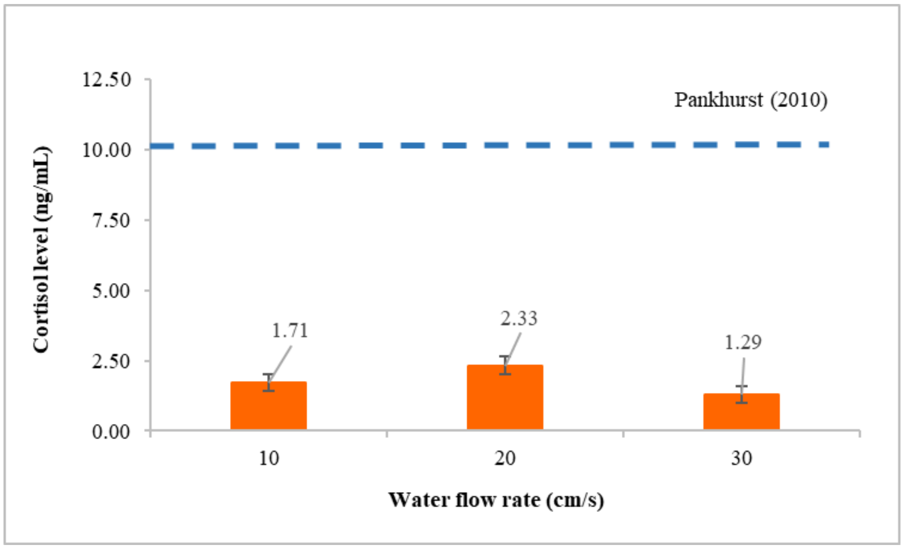


Fig. 3. The content of cortisol (ng/mL) in *H. nemurus* plasma

DISCUSSION

Water quality plays a pivotal role in fish culture. As explained by **Sun and Chen (2014)**, good water quality optimizes physiological and bioenergetic processes, as well as improves fish growth **(Nugraha *et al.*, 2021)**. One of the critical water quality parameters for fish culture is dissolved oxygen (DO), as stated previously by **Hoar *et al.* (1979)**. The concentration of DO is a critical factor in fish culture and essentially affects fish growth **(Xu *et al.*, 2019; Saputra *et al.*, 2021; Ali *et al.*, 2022)**. The lowest DO leads to a decline in metabolic rate, reducing oxygen consumption and fish growth **(Hui *et al.*, 2020)**. In this work, controlling the water flow rate aims to enhance the content of DO in the culture water **(Nilsen *et al.*, 2018)**. The water's relative temperature is relatively stable in a sufficient DO concentration. **Assar *et al.* (2023)** followed this, finding that the water flow rate could rise to DO. **Whangchai *et al.* (2018)** argued that controlling the flow rate prevented the temperature of the culture water of the Nile tilapia from fluctuating.

Furthermore, DO also affects water hardness, which influences the decomposition of debris and feces. The pollutant generated from the debris and feces breakdown is represented by ammonia, nitrate, and nitrite content. In this work, these chemicals were tolerable for *H. nemurus* fish in all treatments. This aligns with **Belal**'s **(2015)** finding that controlling the water flow rate in *O. niloticus* culture did not affect feed consumption. In our study, treatment of 20cm/ s produced the most effective result compared with the flow rate of 10 and 30cm/ s.

In addition to enhancing water quality, an appropriate flow rate can improve the growth of *H. nemurus*. Fish growth requires energy allocation occuring after completing basic needs **(NRC, 2011)**. In this work, the water flow rate for *H. nemurus* culture affected the SGR ($P< 0.05$). Treatment of 20cm/ s produced the most optimum SGR (5.02±0.08 %/day) and differed significantly between 10 and 30cm/ s. We argued that the relationship between water flow rate, DO content, fish growth, and feed was complicated. However, the SGR values followed the result of **Obirikorang *et al*. (2019)**, finding that daily specific growth of the tilapia (*O. niloticus*) increased with a higher flow rate.

The results demonstrated that the water flow rate of *H. nemurus* culture significantly affected protein retention, lipid retention, protein efficiency ratio, and feed efficiency. Protein retention reflects the percentage of protein stored in body tissue for a particular period **(Lee *et al*., 2020; Suryaningrum & Samsudin, 2020)**. In fish, protein retention is highly affected by protein intake, protein digestibility, and the balance of amino acids in feed **(Suryaningrum *et al*., 2017; Nafiqoh *et al*., 2021)**. However, in some cases, protein retention relates to energy and fish activity, in which protein is converted into energy, thus reducing protein retention **(Furuya *et al*., 2023)**. In this study, the water flow rate of 20cm/ s resulted in the most excellent retention of protein and lipid, indicating that the fish in this system requires less energy for movement. Thus, more protein and lipid were manifested in the tissue **(Ridwanudin *et al*., 2023)**. **Soerensen *et al*. (2020)** argued that flow rate and pattern intensified energy consumption in fish. Thus, more energy storage was used. The protein and lipid in tissue were hydrolyzed and oxidized, turning into energy; therefore, protein and lipid retention declined.

The most favorable level of protein efficiency ratio was achieved in the flow rate of 20 and 30cm/ s, achieving up to 2.40 and 2.39, respectively. This value also corresponds to the retention of protein and lipid. Protein is efficiently used and stored in tissue when the energy for metabolism is minimally used. The highest feed efficiency was found in the 20 and 30cm/ s water flow rates, corresponding to 85.69 and 84.38%. Therefore, the water flow rate always positively impacts feed efficiency and feed conversion ratio. This aligns with a previous study of the rainbow trout **(Welker *et al*., 2019)**.

Considering the fish length distribution, the experimental fish tends to be in a 3.50–5.59cm range. The population generally showed normal distribution in all treatments, despite a small population of fish with a size < 3.49cm found in the group of 10 and 30cm/ s. Notably, the high heterogeneity of large fish size (6.65–6.99cm; 0.5%) often induced cannibalism **(Pereira *et al*., 2017)**.

The results revealed that the water flow rate did not affect cannibalism ($P > 0.05$). The treatment of 20cm/ s demonstrated lower cannibalism (51.49± 3.81%) over other treatments. The rise of cannibalism is in line with the higher flow rate, presumably caused by the higher chance of interaction. **Ribeiro *et al*. (2020)** explained that, in addition to cannibalistic behavior, fish size, and feed availability, the cannibalism was triggered by the high intensity of fish interaction. The high flow rate induces more energy intake, inducing cannibalism, as found in *Heterobranchus longifilis* **(Baras *et al*., 2014)**. The cannibalism incidence positively relates to the survival rate of fish tested. In this work, the water flow rate of 20cm/ s gave a high survival percentage over other treatments, reaching 47.81± 4.10% (Table 1).

Furthermore, based on the length-weight relationship (n=400), all treatments demonstrated similar characteristics, with a correlation value of 2,107–2,138. This clearly explained that length growth was more dominant than weight in all populations. Similarly, **Pepin *et al*. (2015)** reported that, in many cases, fish length grew more intensively than fish weight. In our experiment, the growth trend in all treatments is consistent with the length-weight relationship. Overall, the experimental fish condition factor is found at < 3. The factor represents fish health, where a more significant value indicates a better fish condition **(Kim & Cho, 2019)**. Fish condition factor highly depends on habitat, gonad development, and other factors such as environmental condition and feed **(Faradonbeh *et al*., 2015)**.

Intriguingly, the flow rate did not cause stress to fish, as represented by a cortisol level of < 2.33 (ng/mL) for all treatments. This agreed with **Sari *et al*. (2023**), finding that *H. nemurus* with < 543ng/ mL cortisol was not stressed. The present results conformed to a previous work of **Pankhurst (2010)**, reporting that the cortisol level of teleost fish at < 10ng/ mL was not stressful. Considering all results of this work, the rearing of *H. nemurus* can be best conducted at a flow rate of 20cm/ s.

CONCLUSION

The rearing of *H. nemurus* seeds at a water flow rate of 20cm/ s gave the most desirable results concerning specific growth rate, survival rate, and feed efficiency, reaching up to 5.28, 46.50, and 85.69%, respectively. This concluded that the optimum condition for the culture of *H. nemurus* was a water flow rate of 20cm/ s.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTION

Adang Saputra, Lusi Herawati Suryaningrum, Rini Widya Sari, Sinung Rahardjo, Siti Murniasih, Edy Farid Wadjdy, are the primary investigators who are in charge of developing the research proposal, approving the final draft of the manuscript, conducting laboratory work, data collection, chemical analysis-interpretation, carrying out data analysis, report drafting, and final draft checking and proofreading.

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