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EFFECTS OF DIFFERENT SALINITY LEVELS ON GROWTH AND PHYSIOLOGICAL RESPONSE OF *Tor soro* JUVENILE

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ABSTRACT

Tor soro is one of the most economically important native freshwater fish species in Indonesia. Nonetheless, the insufficient data regarding its salinity tolerance makes a thorough investigation of this issue imperative. This research was conducted to determine the effects of salinity on the growth and physiological response of *Tor soro* juveniles at optimum salinity levels. This study used *Tor soro* (total length: 5.0 ± 0.08 cm; initial weight: 2.0 ± 0.06 g) with five salinity level treatments (0, 2, 4, 6, and 8 ppt) and the stocking density of 15 fish per aquarium (three replications). Fish were maintained for 30 days and fed with commercial aquafeed. In this study, the best growth was found in 2 ppt (length gain: 0.37 ± 0.05 cm; weight gain: 0.23 ± 0.01 g; specific growth rate in length: 1.38 ± 0.16 % day^{-1}; specific growth rate in weight: 0.78 ± 0.05% day^{-1}) which is significantly higher than 4, 6, and 8 ppt ($p<0.05$), but it is not significantly different from 0 ppt ($p>0.05$). The results of the physiological response showed that there were no significant stress responses in *Tor soro* juvenile for all salinity treatments ($p>0.05$). There was no effect of 2 ppt salinity on the growth parameters compared to controls, but salinities above ppt had a significant detrimental effect. Fish Exposure to salinities did not have any stress effect as shown by physiological indicators.

KEYWORDS: *Tor soro*; salinity; fish physiology; growth; fish stress

INTRODUCTION

Tor soro is a commercially important Indonesian freshwater fish species (Gustiano *et al.*, 2013; Arifin *et al.*, 2020). As one of the native freshwater fish targeted for domestication, investigation of the ideal environmental conditions that would facilitate the growth and development of this species is necessary (Radona *et al.*, 2015). This species is in high demand among consumers because of its thick, tender, and delicious meat (Qudus *et al.*, 2012). Apart from being consumed, *Tor soro* has a high albumin concentration, so that it can be used as a source of fish serum albumin (Zakiah, 2016). In addition, water quality is a crucial element in the growth of *Tor soro*. Special consideration must be given to maintain the water quality for *Tor soro* farming. Ideally, water quality variables such as pH, ammonia, nitrite, nitrate, dissolved oxygen, carbon dioxide, temperature, and salinity should be maintained within the optimum range for this species (Sneddon *et al.*, 2016). One of the most important environmental elements that might influence fish metabolism is salinity (Bœuf & Payan, 2001). Changes in the salinity will change the dynamic balance of fish osmotic pressure (Yu *et al.*, 2021). Variations in the influence of salinity on immunological function between species or experimental situations may be attributable to the tolerance of animals to hyperosmotic environments, such as euryhaline fish that can cope with the salinity fluctuations (Yada & Tort, 2016).

The effect of salinity on growth and physiological responses has been extensively investigated in pre-

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vious studies, such as those conducted by Ath-thar & Gustiano (2010) on Nile tilapia *Oreochromis niloticus* which found optimal growth results on 15 ppt. Dahril (2017) who examined the effect of salinity on the growth of red tilapia found that salinity affects the weight gain of red tilapia when it is higher than 17 ppt. Meanwhile, a previous study (Mozanzadeh *et al.*, 2021) observed the effect of salinity on *Acanthopagrus latus* juvenile and found that raising water salinity from 6 ppt to 12 ppt greatly enhanced the growth performance of this species. Furthermore, Yunus *et al.* (2020) analyzed the rise in glucose and haemoglobin levels in giant gourami *Osphronemus gouramy* as a physiological response to salinity changes. Based on this information, research on various salinity levels is required to determine the optimal salinity levels for fish growth and physiology. However, *Tor soro* data related to salinity experiment are still lacking. Therefore, this study aims to determine the effects of salinity on growth and physiological responses of *Tor soro* juveniles.

MATERIALS AND METHODS

The study was carried out from September to December 2021 at the Research Station for Freshwater Fisheries Germplasm, Cijeruk, Bogor, West Java which belongs to Research Institute for Freshwater Aquaculture and Fisheries Extension. *Tor soro* juveniles used in this study are the second generation of stock collected from Kuningan, West Java, which had been domesticated at Research Station for Freshwater Fishery Germplasm. Juveniles tested (total length: 5.0 ± 0.08 cm; weight: 2.0 ± 0.06 g) were reared in the aquariums (50 × 50 × 50 cm) with stocking density of 15 fish/aquarium. During the experiment, the juveniles were given commercial feed (PF 500 prime feed; protein: 39%; lipid: 5%) twice every day at 08:00 and 15:00.

The completely random design experiment was carried out using five different salinity treatments, 0, 2, 4, 6, and 8 ppt with three replicates. The salinity level was obtained by mixing seawater and freshwater (groundwater) to reach the certain amount (2, 4, 6, and 8 ppt), while 0 ppt was obtained by mixing aquadest and groundwater in the aquariums until the salinity was closed to zero (~0.5 ppt). Each experiment lasted for 30 days. Length and weight measurement were carried out every 10 days for 30 days. At the initial (day 0) and final (day 30) days, blood samples were collected and analysed to determine the physiological response of *Tor soro* juveniles.

The observed growth parameters are weight gain, length gain, specific growth rate in weight, specific growth rate in length, and survival rate, while the observed physiological response is the level of blood glucose and whole-body cortisol. The levels of cortisol and glucose were acquired for each treatment in accordance with the methodology outlined by Prakoso *et al*. (2019). To collect the blood samples, fish were anesthetized using an anaesthetic solution (2-phenoxyethanol, dose 0.5 ml/L water). The blood samples were taken from the caudal part of juveniles. The blood samples were dripped on the blood glucose check strip, and then the blood glucose level was read using the glucometer (Accu-Chek Active, Germany). Whole body cortisol was taken by grinding the tested fish. The samples were then centrifuged to get the plasma. Plasma samples were analysed using an ELISA DRG Cortisol Kit and Microplate Photometer (Biosan HiPo MPP-96). For the supporting parameters, the observed water qualities are temperature, pH, dissolved oxygen, and conductivity. Temperature and dissolved oxygen measurements were measured with dissolved oxygen meter (Lutron DO-5510), pH measurement was carried out with a pH meter (ATC), while conductivity was measured using the Lamotte Con 6 Plus Conductivity Meter.

All of the collected data was analysed statistically using RStudio with “dplyr” and “ggplot2” packages. A one-way ANOVA was performed to compare the effects of the different salinity on the growth and physiological performances of *Tor soro* juveniles. A significant difference was applied as $p<0.05$.

RESULTS AND DISCUSSION

In terms of growth performance (Table 1), the length gain, weight gain, specific growth rate in length, and specific growth rate in weight for *Tor soro* juveniles were significantly higher at 2 ppt compared to 4 to 8 ppt ($p<0.05$), but not significant compared to 0 ppt (control) ($p>0.05$). The survival rate of *Tor soro* juveniles were not significantly different from 0 to 6 ppt ($p>0.05$), except for 8 ppt ($p<0.05$). In the meantime, physiological responses of *Tor soro* (Table 2) showed that the cortisol level at initial day of experiment was significantly higher than those of final day of experiment ($p<0.05$), while the cortisol levels within treatments at the end of experiment was not significantly different ($p>0.05$). In terms of glucose levels, no significant differences were found ($p>0.05$).

Table 1. Growth performance of *Tor soro* juveniles under different salinity treatments

Parameters	Salinity (ppt)				
	0	2	4	6	8
LG (cm)	0.37 ± 0.05[d]	0.42 ± 0.04[d]	0.25 ± 0.03[c]	0.12 ± 0.01[b]	0.04 ± 0.02[a]
WG (g)	0.21 ± 0.01[d]	0.23 ± 0.01[d]	0.18 ± 0.02[c]	0.10 ± 0.02[b]	0.05 ± 0.01[a]
SGRL (%)	1.24 ± 0.17[d]	1.38 ± 0.16[d]	0.82 ± 0.11[c]	0.41 ± 0.05[b]	0.14 ± 0.07[a]
SGRW (%)	0.69 ± 0.03[d]	0.78 ± 0.05[d]	0.59 ± 0.06[c]	0.36 ± 0.05[b]	0.16 ± 0.04[a]
SR (%)	100 ± 0[b]	100 ± 0[b]	100 ± 0[b]	100 ± 0[b]	93.33 ± 0.06[a]

Descriptions: Different superscript in the same row indicate significant differences (P < 0,05); LG = Length Gain; WG = Weight Gain; SGRL = Specific Growth Rate in Length; SGRW = Specific Growth Rate in Weight; SR = Survival Rate

Table 2. Profile of whole-body cortisol and blood glucose levels of *Tor soro* juveniles at different salinity exposure

Experiment	Initial (Day 0)	Final (Day 30)				
		0 ppt	2 ppt	4 ppt	6 ppt	8 ppt
Cortisol (ng/mL)	51.88 ± 11.23[b]	26.35 ± 13.43[a]	26.25 ± 5.00[a]	26.23 ± 7.21[a]	22.33 ± 10.02[a]	29.63 ± 1.98[a]
Glucose (mg/dL)	48.80 ± 15.27[a]	42.33 ± 3.79[a]	43.67 ± 4.04[a]	44.33 ± 4.16[a]	45.67 ± 3.21[a]	49.33 ± 5.51[a]

Descriptions: Different superscript in the same row indicate significant differences (P < 0.05)

The highest growth was found at salinity 2 ppt compared to 4 to 8 ppt, but not significantly different compared to 0 ppt. It means that comparing to the control, no growth improvement was found on *Tor soro* juveniles by increasing salinity levels. Similar pattern was found on previous study on striped snakehead *Channa striata* (Prakoso *et al.*, 2018) and same species at smaller size (Widyastuti *et al.*, 2021), while no detrimental impacts was found on growth of Malaysian mahseer hybrid until 9 ppt (Ain *et al.*, 2021). Fish can survive and grow on the salinity exposure because they can regulate body fluids to maintain a level of osmotic pressure that is close to normal (Wedemeyer, 1996; Takei & Balment, 2009). The higher the level of salinity compared to its natural habitat, the higher the osmotic workload to balance the pressure of the osmolarity of the media and the fish body so that the energy wasted on osmotic performance becomes greater than for growth (Rahmawati *et al.*, 2013). In relation to the present study, the findings from this study indicated the salinity tolerance of *Tor soro* juveniles to 8 ppt despite no improvement on their growth performance. In this case, *Tor soro* juveniles regulated their body fluids to maintain a normal osmotic pressure, enabling them to survive and thrive in varying salinity levels. This results in a greater energy expenditure on osmotic performance, which can hinder the growth of *Tor soro* juveniles.

This study showed that the cortisol levels from 0 to 8 ppt were not significantly different, which means no stress was found according to cortisol and glucose levels. However, this study found lower growth performance on higher salinity. In this case, changes in salinity did not have an impact on the cortisol levels of *Tor soro* juveniles because they did not surpass the threshold of tolerance. Nevertheless, as the salinity level deviates from its natural habitat, the osmotic workload increases, necessitating a balance between the osmolarity of the media and the fish body. Consequently, the energy expended on osmotic performance becomes more consequential than that expended on growth, resulting in lower growth performance at higher salinities. This pattern is similar with previous study on Nile tilapia *Oreochromis niloticus* at salinity exposure from 0 to 12 ppt (Elabarany *et al.*, 2017), common carp *Cyprinus carpio* at salinity exposure from 0 to 7.5 ppt (Saravanan *et al.*, 2018), and dwarf gourami *Trichogaster lalius* at salinity exposure from 0 to 6 ppt (Ramee *et al.*, 2020). On the other hand, some previous studies found that increasing salinity affected to the rising of cortisol levels, such as in rohu *Labeo rohita* exposed to salinity from 0 to 14 ppt (Patel *et al.*, 2022), rosy barb *Pethia conchonius* exposed to salinity from 0 to 6 ppt (Ramee *et al.*, 2020), and Nile tilapia *Oreochromis niloticus* exposed to salinity 10 and 15 ppt (Mohamed *et al.*, 2021). Meanwhile, previous study on the juvenile of *Lophiosilurus alexandri* showed the increase of cortisol level at salinity higher than 10 ppt with the salinity tolerance up to 7.5 ppt (Mattioli *et al.*, 2017). Those studies show that each freshwater fish species has different limit on tolerating salinity increase. Changes in salinity influenced the cortisol level, particularly when it is higher than tolerance limit. Fish stress levels are determined by measuring cortisol, which is the major stress response (Shreck & Tort, 2016). Cortisol is one of the hormones that has a significant role in the adaptation of fish to environmental salinity variations (Brevesa, 2014; Saravanan *et al.*, 2018; Mancera & McCormick, 2019).

Similar with the results of cortisol levels, this study showed no significant differences on glucose levels within treatments. However, different growth occurred within salinity treatment. At this point, the energy allocated towards osmotic regulation to control blood glucose level becomes more significant than that allocated towards growth of *Tor soro* juveniles, leading to reduced growth rate at elevated salinity levels. This result was similar with the study conducted by Elabarany *et al.* (2017) on Nile tilapia *Oreochromis niloticus* with salinity exposure from low to 12 ppt. Meanwhile, another previous studies on grey mullets *Mugil cephalus*, spotted snakehead *Channa punctata*, and rohu *Labeo rohita* showed significant increase of glucose after salinity changes (Prakoso *et al.*, 2015; Prakoso *et al.*, 2016; Mst Khatun *et al.*, 2020; Patel *et al.*, 2022). According to the results from this study and previous studies, *Tor soro* juveniles could keep their glucose homeostasis without increasing the blood glucose significantly, whereas most of fish species experience increased glucose levels by salinity changes due to their stress response. The high blood glucose levels indicate that the fish are experiencing stress caused by the influence of environmental salinity levels that are not in line with their natural habitat (Phuc *et al.*, 2017). However, in this study, no significant differences were found from 0 to 8 ppt. It means that 0-8 ppt are the acceptable salinity range for *Tor soro* juveniles. Angadi (2021) explained that fish can regulate glucose homeostasis at salinity exposure. This is achieved through an increase in glucose levels and a decrease in glycogen levels, especially in liver tissues, in response to an increase in the activity of Na^+-K^+-ATPase enzymes and ions in the osmoregulation organs. Those results above revealed that each species have different response in regulating glucose homeostasis under salinity stress.

In this study, dissolved oxygen levels in each treatment ranged from 4.96-5.06 mg/L, while pH was in the range of 7.53-7.79 and temperature was in the range of 27.68-28.27°C. No significant differences were found between each treatment for these parameters ($P>0.05$). On the other hand, the average results of conductivity ranged from 0.027-14.79 ms with significant differences found ($P<0.05$) within salinity treatments (Table 3).

Table 3. Observed water quality parameters on *Tor soro* juvenile's rearing media subjected to different salinity treatments.

Parameters	Salinity (ppt)				
	0	2	4	6	8
Dissolved oxygen (mg/L)					
08.00	5.05 ± 0.14[a]	5.05 ± 0.11[a]	5.00 ± 0.19[a]	5.00 ± 0.24[a]	5.06 ± 0.11[a]
15.00	5.02 ± 0.19[a]	4.96 ± 0.14[a]	4.99 ± 0.17[a]	4.96 ± 0.25[a]	5.03 ± 0.11[a]
Temperature (°C)					
08.00	27.74 ± 0.57[a]	27.78 ± 0.38[a]	27.94 ± 0.53[a]	27.68 ± 0.53[a]	27.68 ± 0.49[a]
15.00	27.85 ± 0.71[a]	27.91 ± 0.53[a]	28.27 ± 0.69[a]	27.80 ± 0.34[a]	27.90 ± 0.65[a]
pH					
08.00	7.93 ± 0.06[a]	7.62 ± 0.09[a]	7.59 ± 0.12[a]	7.54 ± 0.08[a]	7.80 ± 0.16[a]
15.00	7.99 ± 0.07[a]	7.53 ± 0.06[a]	7.66 ± 0.09[a]	7.53 ± 0.09[a]	7.78 ± 0.13[a]
Conductivity (mS)	0.027 ± 0.03[a]	5.40 ± 0.21[b]	7.86 ± 0.32[c]	12.42 ± 0.10[d]	14.79 ± 0.21[e]

The water quality data showed no significant differences on dissolved oxygen, temperature, and pH, which indicates that all treatments have similar environmental condition except the salinity treatment itself, resulting in different of conductivity. Fahrudin (2019) explained that greater conductivity value is positively correlated with salinity level in the rearing media. The pattern of conductivity increased with increase in salinity from the present study was similar with the previous studies reported by Iqbal *et al.* (2012) on Nile tilapia *Oreochromis niloticus* reared in the concrete tanks with different salinity treatments (800, 1600, 2400, 3200, and 4000 ppm) and Mubarik *et al.* (2019) on common carp *Cyprinus carpio* reared in the glass aquaria with salinity treatment from 0 to 3 ppt. The present study found that the increase in conductivity reduced the growth performance of *Tor soro* juveniles. Another previous study on tilapia *Oreochromis niloticus* reared in earthen ponds reported by Makori *et al.* (2017) also showed a negative correlation between growth and conductivity. Meanwhile, the above previous studies conducted by Iqbal *et al.* (2012) and Mubarik *et al.* (2019) showed positive correlation between conductivity and growth. Those results indicated that the effect of water conductivity varied among the different fish species. Dennis *et al.* (1995) hypothesized that the allotment of energy towards ion regulation may lead to a decline in the fish condition, thereby explaining the associations with conductivity. However, according to statement from Copp (2003), most of the existing evidence indicated that the correlations between fish body condition and water conductivity were likely coincidental. This was potentially due to underlying and more extensive geographical patterns in ecosystem function.

CONCLUSION

The growth parameters of *Tor soro* juveniles did not exhibit any significant changes in response to a salinity of 2 ppt compared to the control group. However, salinity exceeding 2 ppt resulted in a significant and detrimental effect. The physiological indicators did not demonstrate any stress effect on *Tor soro* juveniles resulting from exposure to salinities.

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