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Effects of different stocking densities on growth performance of *Tor soro* fingerlings under recirculation aquaculture system

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Effects of different stocking densities on growth performance of *Tor soro* fingerlings under recirculation aquaculture system

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Abstract. Intensive aquaculture is an attempt to farm fish at a high stocking density. High stocking density in *Tor soro* can affect growth, survival, and feed efficiency. Therefore, intensive aquaculture usually adopts several technologies and one of them can be found in the form of a recirculation aquaculture system. This study aimed to examine optimal stocking density supporting *Tor soro* performance under the recirculation aquaculture system. Four different stocking densities were used as treatments: 2 fingerlings/L, 3 fingerlings/L, 4 fingerlings/L and 5 fingerlings/L. The experiment followed a completely randomized design experiment with four replications. *Tor soro* fingerlings sized 4-5 cm were reared in a 48 × 48 × 30 cm3 recirculated aquarium, with a water debit of 40 L in each tank for 60 days. Growth and survivals of fingerlings differed significantly between high and low stocking densities. The highest growth occurred to fingerlings at 4 stocking densities per liter (1.67±0.06) and the lowest growth occurred to the group with 5 fingerlings/L (0.34±0.56). Stocking density also significantly affected fingerling survivals where the first group attained 100% survivals, followed by a group of 4 fingerlings/L with (96±4.00%). Results from this study recommend stocking the fingerlings at a density of 4 /L to maintain optimal growth conditions.

Keywords: Growth performance, recirculation, stocking density, *Tor soro*

1. Introduction

Mahseer (*Tor* sp) has gained a lot of attention in aquaculture in the past three decades [1] because of their high consumer demand and fast-declining populations in the wild. Therefore, a lot of effort has been invested in domesticating fishes from this *Tor* genera. Previous studies have succeeded in developing breeding of mahseer in captivity applying various technologies such as hormonal spawning induction [2], cryopreservation [3, 4], and hybridization among [5]. Research on stocking densities for mahseer showed that in captivity, better growth is obtained at lower density see: [6, 7, 8], while aquaculture desires a species that can withstand crowded conditions.

Tor soro is an intensely studied species in Indonesia beside *Tor douronensis*, *Tor tambra* and *Tor tambroides*. Compared to the three other *Tor* sp. experiments in artificial induction of *T. soro* has yielded the best results with more than 93% hatching rate, 82.11% larval survival and only 2.47% of larval abnormality [9].

In their natural habitat, these fish inhabit a freshwater system possessing high water qualities. They are abundant in waters with low turbidity and high dissolved oxygen concentration [10, 11, 12]. Six stations assessed to investigate water qualities of *Tor* sp. by Subagja and Marson [11] provide basic information on the fish water quality requirements. They live in fast flowing water with a speed of 0.25-1.2 m/second, DO concentration from 5.7-10.56 mg/L, free CO2 is 4.4 mg/L, and pH ranges from 6.5-7.5. To obtain water qualities that can mimic the characteristic of the fish in its natural habitats, a recirculation aquaculture system (RAS) was used in this study.

Recirculation aquaculture system (RAS) is a technology that was initially developed to minimize aquaculture impact on the environment. RAS can reduce excessive use of water in fish farms and minimize nutrient load to the surrounding water bodies receiving waste water from the farm [13]. RAS uses filters (mechanical, chemical, and biological) to continuously remove ammonia, accumulate waste from the water, and then reuse the cleaned-filtered water to cultivate the fish [14]. Accordingly, RAS will be suitable to use for *Tor* sp. aquaculture.

Apart from the beneficial effect RAS provides to the environment, the system can also overcome the density issue in the rearing pond or tank. The continuous removal of excess nutrients from the water body enhances the water's carrying capacity, and more fish can be stocked in the tank [15, 16]. Therefore, our study aimed to examine the appropriate stocking density of *Tor soro* when cultured under the recirculation aquaculture system.

2. Materials and methods

2.1. Preparation of cultivation

Materials for the study consist of 12 units aquariums as rearing tanks, one unit filter tank, and one water tank. The filter tank was filled with zeolite, palm fiber, and sand as the physical filter and equipped with bioball as biofilter (figure 1). Water from each aquarium was continuously flown to the filter unit for filtration. The clean water (output from the filter unit) was collected in the water tank to be redistributed to each rearing tank.

Figure 1. Arrangement of the recirculation aquaculture system used in the study.

Fingerlings selected for the study were obtained from artificial spawning of brood stocks which were bred in breeding laboratory of Instalasi Plasma Nutfah Balai Riset Perikanan Budidaya Air Tawar dan Penyuluhan Perikanan (BRPBATPP), Cijeruk, Bogor, Jawa Barat. The size of the fish at the beginning of the experiment was 3-4 cm length weighed at 0.65 gram in average.

2.2. Methods

A completely randomized design was employed in the study with one factor (stocking densities) and four levels consisting of group A (2 individual/L, B (3 individuals/L), C (4 individuals/L), and D (5 individuals/L). The experiment was run for 60 days from October to December 2020 in the hatchery of Instalasi Plasma Nutfah Balai Riset Perikanan Budidaya Air Tawar dan Penyuluhan Perikanan (BRPBATPP), Cijeruk, Bogor, Jawa Barat. Following the Wedemeyer and Yasutake [17] protocol, glucose was quantified with a spectrophotometer.

Samplings for growth parameters were conducted three times during the experiment at day first, day 30th, and day 60th. Water quality parameters were measured every ten days, while glucose level was quantified on day 30th.

2.2.1. Observed parameters. Parameters observed for *T. soro* performance are specific growth rates were calculated based on Huisman [18] (equation 1), absolute growth length (equation 2), fish survivals was calculated according to Zonneveld *et al* (equation 3) and feed conversion ratio (equation 4).

$$\mathrm{SGR} = \frac{(\mathrm{Ln\ Wt} - \mathrm{Ln\ W0})}{\mathrm{t}}\ 100 \qquad (1)$$

Where:
SGR = specific growth rates
Wt = weight at the end of experiment (gram)
W0 = weight at the beginning of experiment (gram)
T = experiment duration (days)

$$\mathrm{L} = \mathrm{Lt} - \mathrm{L0} \qquad (2)$$

Where:
L = absolute growth (cm)
Lt = length of fish at the end of the experiment (cm)
L0 = lenght of fish at the beginning of the experiment (cm)

$$\mathrm{Sr} = \frac{\mathrm{Nt}}{\mathrm{N0}}\ 100 \qquad (3)$$

Where:
Sr = survival rate (%)
Nt = the number of fish at the end of the experiment
N0 = the number of fish at the beginning of the experiment

$$\mathrm{FCR} = \frac{\mathrm{F}}{(\mathrm{Wt+D}) - \mathrm{W0}} \qquad (4)$$

Where:
FCR = feed conversion ratio
F = weight of total feed given to the fish (g)
D = Weight of dead fish (g)

2.2.2. Data analysis. ANOVA was analyzed using Minitab 20.0. Any significant parameters to the growth of *Tor soro* was then analyzed with Duncan post-hoc test.

3. Results and discussion

3.1. Results

Data analysis that was run after 60 days of fish rearing demonstrated that stocking densities (treatments) affect the performance of *Tor soro* fingerlings (table 1) in all growth parameters, survival, and FCR. Duncan post-hoc test resulted in treatments forming three groups that differ significantly. The highest SGR results were given by group 3 (fish group with 4 fingerlings/l) whilst the lowest SGR occurred to group 4 which was stocked with 5 fingerlings/l. The best absolute growth (body mass) and absolute length were also performed by group C (4 individuals/L).

Table 1. Resume of ANOVA results for growth parameters, survival and FCR of *Tor soro.*

Treatments	SGR	Absolute Growth	Absolute Length	Survival	FCR
A (2 individual/L)	1.50 ± 0.03^{b}	0.90 ± 0.02^{b}	1.81 ± 0.08^{b}	100.00 ± 0.00^{a}	2.09 ± 0.05^{a}
B (3 individual/L)	1.57 ± 0.02^{b}	0.94 ± 0.01^{b}	1.91 ± 0.20^{b}	95.208 ± 4.73^{a}	2.06 ± 0.11^{a}
C (4 individual/L)	1.67 ± 0.06^{c}	1.00 ± 0.4^{c}	1.96 ± 0.12^{b}	96.56 ± 4.00^{a}	1.92 ± 0.16^{b}
D (5 individual/L)	0.34 ± 0.56^{a}	0.2 ± 0.3^{a}	1.69 ± 0.44^{a}	77.00 ± 9.54^{b}	2.94 ± 0.52^{c}

Note: Subscripts indicate differences between groups after Duncan Post-hoc test.

Group A performed better compared to all other groups in terms of survival, with all fingerlings surviving at the end of this study. However, post-hoc test showed that it is not significantly different from group B and C. The smallest food conversion ratio occurred to group C (1.92±0.16), which differed from the rest of the groups.

ANOVA test on glucose concentration also shows that stocking densities were significantly affected. Group D has the highest concentration with 91.7 mg/dL which is far higher and significantly different from groups A, B and C. Those other three groups were categorized into one group after Duncan post-hoc test, which means that they are not significantly different from one to another.

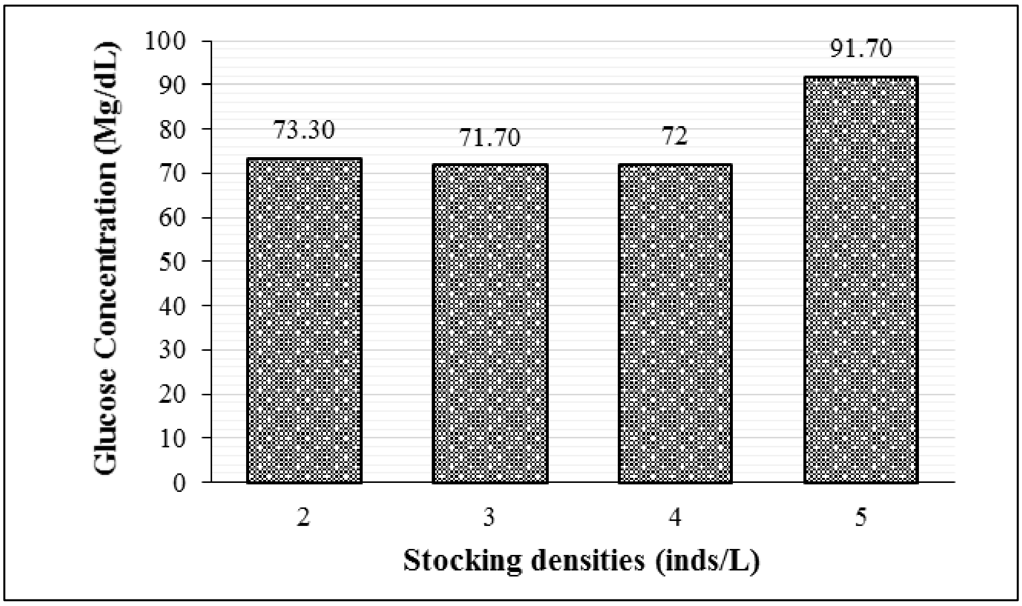

Figure 2. Blood glucose concentration average of *Tor soro* for each group treatment.

Water parameters concentration measured during the experiment resulted in all parameters were still in the range of living requirement of *Tor soro* (table 2).

Table 2. Water parameter measurement results of *Tor soro* rearing experiment with RAS.

Parameters	Stocking Densities			
	2 inds/L	3 inds/L	4 inds/L	5 inds/L
Temperature (°C)	25.5-25.7	25.6-26.2	25.6-26.3	25.7-25.7
DO (mg/L)	6.7-7.10	6.7-7.10	6.7-7.10	6.7-7.10
pH	7.10-7.39	7.10-7.27	7.10-7.39	7.10-7.31
Nitrites (mg/L)	0.16–0.17	0.16–0.17	0.15–0.17	0.15–0.16
Nitrates (mg/L)	4.97–5.04	4.97–4.98	4.40–5.28	2.20-4.78
Alkalinity (mg/L CaCO3)	74.98–75.42	75.02–76.03	75.92 – 77.67	61.03–67.32
Total Hardness (mg/L CaCO3)	110-120	110-120	120-130	110-130
TAN (mg/L)	0.04–0.06	0.04–0.05	0.04–0.05	0.04–0.05

3.2. Discussion

Data analysis of growth parameters demonstrates that group three (4 individuals/L) performs the best among all other groups. It has the highest SGR, absolute growth, absolute length, and significant differences from all other groups. Even though the survival rate was higher at group one, it is not significantly different from group three.

This finding contradicts the common aquaculture sense where fish grow better at lower stocking density. Studies have shown that both low and high stocking density can induce stress in fish see: Alanärä and Brännäs [19], Barlaya *et al* [20], Brown *et al* [21], Jorgensen *et al* [22] and therefore optimum stocking density must be evaluated on a species basis. We expect the better results occurred to the group with higher density may be affected by the nature of *Tor soro* which demonstrates schooling behavior and/or the use of a recirculation system for cultivation. Fish without schooling behavior spend 100-300% spend more energy for swimming compared to those which form schooling. While swimming, fish that do not form schooling need to change linear and angular momentum and swimming pace alterations to avoid collision with other fish. Schooling also reduces the aggressive response of the fish inside the tanks and saves some smaller amounts of energy [23]. The energy conserved can then be used to grow. Other research also suggested that fish reared under RAS have better growth and feeding performances at higher stocking density than the flow-through water system where performances are better at lower density [24].

Group three was also the most efficient food conversion ratio compared to the rest of the groups. Group D with 5 individuals/L gave the highest value for FCR. We assumed that there was a competition for food resources within this group because of crowding, where some dominating fish consumed more food. Mahseer lives in shoals [25] and schooling fish usually exhibits domination during food competition when food is limited [26, 27]. The higher variance value (0.52) in group D compared to other groups of which their FCR variance is less than 0.2 also supports that FCR is not evenly distributed among the fish and indicates some domination. According to Abdan *et al* [28], optimum stocking density will result in even growth of the fish within the group.

Measurement of blood glucose levels resulted in Group A, B and C having glucose concentration under normal conditions, i.e. 40-90 mg/dL [29]. ANOVA results also suggest that the three treatments had a similar effect when the fish were tested for stress. The higher glucose level at the group with five fingerlings/L, which exceeds the normal level, indicates this stocking density is not suitable for *Tor soro* rearing. Group D, which was stocked with 5 fingerlings/L, showed they are under pre-stress condition. This also explains the poor performance of growth and feedings in group D. Therefore, 4 individuals per liter is the optimum stocking density for the fish.

Finally, water quality parameters measurement showed that by keeping four parameters, i.e. temperature, DO, pH, and alkalinity, at the optimum range of *Tor soro* living conditions, all other parameters including nitrites, nitrates, and TAN concentration hardness were also kept under their optimum range.

4. Conclusion

A recirculation aquaculture system can rear *Tor soro* fingerlings because it can continuously ensure good water quality. The optimum stocking density proposed to farm the fish at the fingerlings stage was 4 individuals/L to be able to achieve optimum growth under the recirculation aquaculture system.

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