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The Effect of Stocking Density on Growth Performance and Water Quality of the Silver rasbora (*Rasbora argyrotaenia*) Fry Reared in Plastic-lined Pond

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ABSTRACT

Silver rasbora is one of the local fish species in Indonesia that gains a high public demand. To meet the increasing market demand, it is necessary to implement an optimal silver rasbora rearing system. The objective of the study was to determine the best stocking density for the growth and water quality of silver rasbora (*Rasbora argyrotaenia*) fry reared in plastic-lined ponds. The fries utilized had a mean initial body length of 1.85 ± 0.5 cm and body weight of 0.22 ± 0.05 g. The experiment lasted for 40 days and was carried out in a 500 L plastic-lined pond, with dimensions of 1 x 1 x 1 m and five treatments were evaluated (100, 150, 200, 250, 300 fish m^{-2}). The feed used was commercial floating feed with 39% protein content, and feeding was conducted three times a day (08.00 AM, 12.00 PM, and 16.00 PM) on ad satiation basis. According to the results, the stocking density showed a significant effect on body weight, final biomass, and feed conversion ratio (FCR) in all treatments. T1 resulted in the highest body weight with a value of 0.71±0.02 g, and the lowest FCR with a value of 0.90±0.01. While, T5 resulted in the highest final biomass with a value of 102.81±5.62 g. The pH concentration was the only parameter that showed a significantly different value, with the lowest value in T5 (7.02±0.0). The best water quality parameter in T5 showed values of 27.03±0.1°C (temperature), 7.02±0.03 (pH), 6.79±0.21 mgL^{-1}(DO), 2.81±0.89 mgL^{-1} (nitrate), 0.18±0.02 mgL^{-1} (nitrite), and 0.05±0.01 mgL^{-1} (TAN). A stocking density of 300 fish showed suitable conditions for rearing silver rasbora fries in plastic-lined pond.

INTRODUCTION

Silver rasbora (*Rasbora agyrotaenia*), a freshwater fish from the family Cyprinidae is commonly found in Southeast Asia, including Indonesia, and lives in flowing (rivers) to stagnant waters (rice fields and lakes) **(Nelson, 2006; Said & Triyanto, 2013)**. Silver rasbora has a high economic value in Indonesia because it can be used as both a food consumption and an ornamental fish **(Said *et al.*, 2011)**. Previous data states that in 2018, rasbora production in Indonesia reached 9,523.96 tons at 60,000 IDR or 4.19 USD **(KKP, 2021; Rukayah & Lestari, 2021)**. It is one of the popular freshwater fish for its delicious taste and high nutritional content (protein 47,54% and fat 2,9%) **(Said *et al.*, 2010; Sogandi *et al.*, 2019)**, but unfortunately, rasboras cannot be optimally cultivated and still rely on wild harvests **(Budi *et al.*, 2020)**. According to previous research, rasbora is an endangered species due to overfishing (**Muchlisin *et al.*, 2010**) and environmental pollution such as the use of pesticides, which affects the availability of rasbora in nature; therefore, aquaculture regulations should be carried out to prevent rasboras from extinction **(Prastika *et al.*, 2021)**.

Since 2010, there has been an increase in efforts to cultivate rasbora, and several studies on its development have been conducted, including habitat characteristics, feeding habits and conservation systems **(Sulastri *et al.*, 2010; Sulistiyarto, 2013; Rosadi *et al.*, 2014)**, reproductive aspects **(Said *et al.*, 2011)**, mtDNA assay **(Kusuma *et al.*, 2017)**, spawning induction using hormones **(Ningrum *et al.*, 2019)**, sperm cryopreservation to support their breeding efforts **(Muchlisin *et al*, 2020**; **Eriani *et al*, 2022**); and also the use of different stocking densities in larval stages **(Budi *et al.*, 2020)**. The majority of fish harvest has been currently directed to fulfill market demand. Hence, it is necessary to conduct appropriate fry-rearing techniques to fullfill the market demand. Information about the appropriate stocking density is very important to increase the productivity of silver rasbora fries and ensure a continuous supply.

Stocking density has a direct impact on biota growth, water quality, and production efficiency **(de Oliveira *et al.*, 2012; Tibile *et al.*, 2016)**. According to previous studies, excessive stocking density reduces the oxygen content in waters causing the fish to become stressed, susceptible to disease, and witness a decrease in both growth performance, survival rate and feed efficiency **(Martos-Sitcha *et al.*, 2020; Muchlisin *et al*, 2021).** Other studies stated that high stocking densities can cause high carbon dioxide levels due to fish respiration, thereby lowering pH and increasing acidity in the waters **(Hong *et al.*, 2019).** Furthermore, studies related to stocking density are important, because if the density is too high, it will cause a size disparity among fish, competition for food, and ammonia excretion in the waters, in addition to predation (**Ronald *et al*, 2014; Tibile *et al*, 2016)**. However, if it is not enough, then the profit from aquaculture will not be optimal **(Szczepkowski *et al.*, 2011)**. Therefore, this study aimed to obtain an appropriate method to produce quality fish and ensure a continuous supply of fries in silver rasbora hatchery activities using plastic-lined ponds by determining the effect of different stocking densities on growth parameters and water quality.

MATERIALS AND METHODS

This study was conducted at the School of Health and Life Sciences (SIKIA) Banyuwangi Campus Laboratory of Airlangga University, Banyuwangi, Indonesia.

Fish stocking

Silver rasbora fries were obtained from the Technical Services Unit of Freshwater Aquaculture Development in Umbulan (Pasuruan, East Java). Silver rasbora fries specimens were kept for 1 week in an adaptation pond before the experiment to minimize stress. During the adaptation period, the fries were fed the same type of commercial floating feed and at the same time (three times a day at 08.00 AM, 12.00 PM, and 16.00 PM) as will be carried out in the experimental process. However, before being placed in the experimental pond, the fries were selected and weighed individually, based on their length and size. The fries samples had a mean initial weight of 0.22 ± 0.05 g and a mean length of 1.85 ± 0.05 cm.

Experimental Design

A completely randomized experimental design was used in this study. This study was conducted for 40 days, using 5 treatments of different stocking density levels (100, 150, 200, 250, and 300 fishes m^{-2}) with 4 replicates. Silver rasbora fries were reared semi-outdoor using plastic-lined ponds measuring 1×1×1 m, with a water volume of 500 L and aerated with an air blower (140 L min^{-1}).

During the experiment, the feed was administered three times a day (at 08.00 AM, 12.00 PM, and 16.00 PM) using floating commercial feed with a protein content of 39%, lipid (5%), crude fiber (4%), moisture (10%), and ash (11%) (PF-500 Matahari Sakti Ltd Indonesia), with ad satiation basis. The feed was weighed before and after being given, and feeding was carried out gradually and stopped if the fish stops responding to the feed. The sampling of water quality and fish growth was conducted before morning feeding time and siphon process (carried out once every 10 days by changing the water to a maximum of 10% of the total volume of water in the fishpond).

Water Quality Measurement

Temperature and pH were measured using a pH meter (Lutron YK2001) and DO (Dissolved oxygen) was measured using a DO meter (YSI Pro20). Nitrite and nitrate were measured using a test kit (Merck MColortest 114658 and Hanna Instruments HI3874) and TAN (Total Ammonia Nitrogen) was analyzed using a spectrophotometer based on the APHA (1999) procedures. In addition, measurement of TAN, nitrite, and nitrate concentrations was carried out in the water quality analysis laboratory. The water samples were collected directly from four points in the water body using a 250 ml sample bottle and then sent to the laboratory for analysis. Meanwhile, the temperature and DO measurements were carried out in situ.

Observation Parameters

Temperature, pH, and DO were measured daily (every morning) while TAN, Nitrite, and Nitrate were measured every 10 days. Water samples were taken in the morning before feeding time and the production parameters observed include Final Biomass (FB), Weight Gain (WG), Survival Rate (SR), Total amount of given feed (TF), and Feed Conversion Ratio (FCR). Furthermore, it can be formulated FCR = TF/WG; WG = Mean final weight – mean initial weight; SR (%) = (Final number of fish/ Initial number of fish) x 100 (**Sarker *et al*, 2016**). Growth sampling was also performed every 10 days in the morning before feeding and after measuring water quality by taking 10% of biomass (T1: 10; T2: 15; T3: 20; T4: 25; T5: 30 fish) from each treatment in the experimental pond and measuring the lengths and weights individually.

Data analysis

The data obtained were processed using Microsoft Excel 2013 and then tested for normality and homogeneity using the Kolmogorov-Smirnov and Levene's test, followed by the analysis of the fish growth data using Analysis of Variance (ANOVA) at a 95% confidence interval ($\alpha = 0.05$). Subsequently, if the results were statistically significant ($P < 0.05$), the 95% Tukey Test was carried out. Water quality parameters were measured using repeated-measures ANOVA with two factors (water quality and time). Bonferroni test was used when differences between time and treatments were detected ($P < 0.05$). Statistical analysis was also performed using IBM-SPSS version 18.

RESULTS

1. Growth Performance Parameter

The use of different stocking densities had an effect on the growth parameters of silver rasbora seeds. The results were showed in Table 1.

Table 1. Growth performance of Silver rasbora fries for 40 days rearing period using the different stocking densitie

Variable	Treatment (Different Stocking Density)				
	T1 (100)	T2 (150)	T3 (200)	T4 (250)	T5 (300)
FB (g $tank^{-1}$)	69.99 ± 1.31^{a}	73.36 ± 3.15^{ab}	78.91 ± 1.73^{b}	90.58 ± 3.68^{c}	102.81 ± 5.62^{d}
TF (g $tank^{-1}$)	45.43 ± 0.01^{a}	47.75 ± 0.02^{a}	65.56 ± 0.01^{b}	79.15 ± 0.01^{c}	97.83 ± 0.02^{d}
FCR	0.90 ± 0.01^{a}	0.93 ± 0.03^{a}	1.20 ± 0.01^{b}	1.20 ± 0.14^{b}	1.30 ± 0.01^{b}
WG (g)	50.3 ± 1.87^{a}	51.6 ± 2.37^{ab}	55.8 ± 1.36^{b}	65.88 ± 2.19^{c}	77.09 ± 2.94^{d}
SR (%)	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00

FB: Final Biomass; WG: Weight Gain; TF: Total amount of Feed given; FCR: Feed Conversion Ratio; SR: Survival Rate. Values are mean ± SD. Superscript letters denote significant differences ($P < 0.05$) between treatments

The ANOVA test showed that stocking density had a significant effect ($P<0.05$) on production parameters (Final Biomass (FB), total amount of food given (TF), and Feed Conversion Ratio (FCR)) but had no significant effect ($P>0.05$) to Survival Rate (SR). The results of the study showed that the highest values of FB, TF, and FCR were found in T5 treatment using a stocking density of 300 fish m^{-2} silver rasbora fries. Tukey test showed that the FB and TF values in treatment T5 (300 fish m^{-2}) were significantly higher than in other treatments. The FCR value in treatment T5 showed a significantly higher than in treatment T1 and T2 but not significant in treatment T3 and T4. The SR value showed no significant results among all the treatments (Table 1).

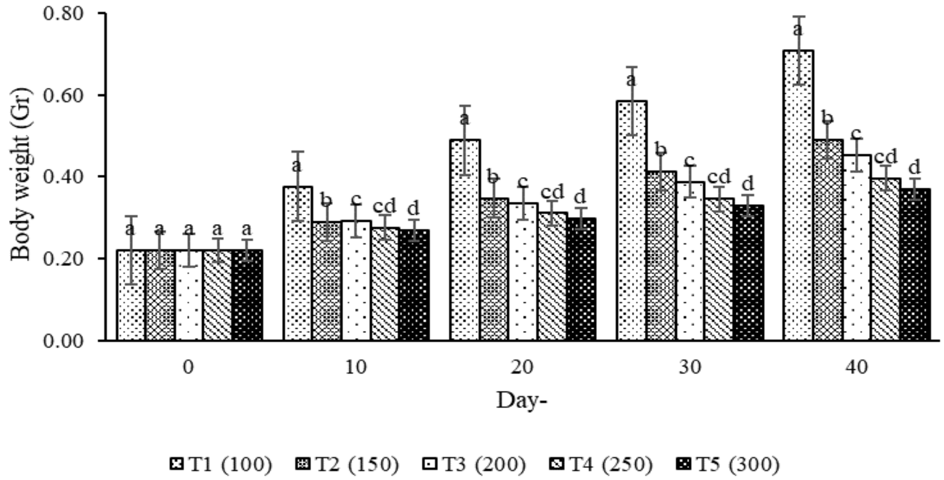

Figure 1. Body weight of Silver rasbora fries at 0, 10, 20, 30, and 40 days with different stocking density treatments. Superscript letters differ significantly (P < 0.05) between all treatments

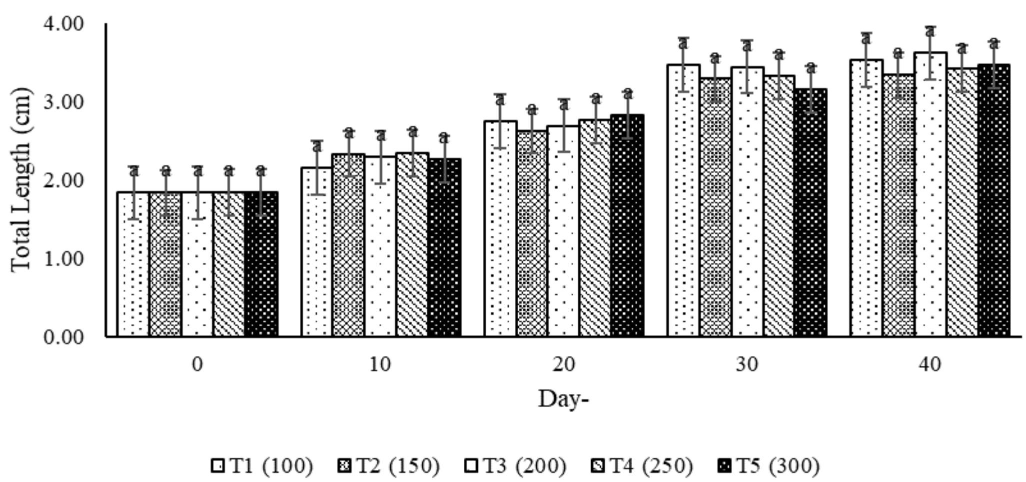

Figure 2 Total length of Silver rasbora fries at 0, 10, 20, 30, and 40 days with different stocking density treatments. Total length of silver rasbora showed did not significantly (P >0.05) among all treatments

The results showed that the use of stocking density affected the weight of the fish (Figs. 1,2). Fish weight during the rearing period showed a significant value (P<0.05) between all treatments. However, the total length did not show a significant value

(P>0.05) between all treatments. This indicates the higher the stocking density used, the lower the fish weight.

Water Quality Parameters

The results of water quality analysis using a one-way repeated measure ANOVA showed in Table 2. The results were not significantly different (P>0.05) between the use of stocking density and water quality in the form of TAN, nitrite, nitrate, DO, and temperature on rearing media of silver rasbora. However, the use of stocking density gave a significant effect ($P < 0.05$) on water quality in the form of pH value. The pH value between treatments showed a significant value ($P < 0.05$) between T5 and T1, T2, and T4 treatments. However, it was not significant (P>0.05) from the T3 treatment. In general, water quality parameters during the rearing period of silver rasbora fry can be seen in Table 2.

Table 2. Water quality of Silver rasbora fry for 40 days rearing period using the different stocking densities

Variable	T1 (100)	T2 (150)	T3 (200)	T4 (250)	T5 (300)	P value†
TAN (mg L^{-1})	0.03±0.01	0.04±0.02	0.04±0.02	0.05±0.02	0.05±0.01	0.329
Nitrite (mg L^{-1})	0.09±0.01	0.18±0.03	0.28±0.01	0.18±0.08	0.18±0.02	0.317
Nitrate (mg L^{-1})	2.79±1.41	3.10±0.71	3.22±0.65	2.69±0.75	2.81±0.89	0.967
DO (mg L^{-1})	7.06±0.14	7.02±0.16	7.01±0.14	7.07±0.07	6.79±0.21	0.340
pH	7.19±0.07^{bc}	7.24±0.07^{c}	7.07±0.03^{a}	7.16±0.11^{b}	7.02±0.03^{a}	0.000
Temperature (^{o}C)	27.5±0.4	27.4±0.4	27.5±0.1	27.6±0.1	27.3±0.1	0.114

TAN: Total ammonia nitrogen; DO: Dissolved oxygen. Values are mean ± SD. Superscript letters denote significant differences between treatments (Bonferroni test, $P < 0.05$). †Results from one-way repeated measure ANOVA (Density x Times) and Bonferroni test.

Inorganic Nitrogen

The results of the sampling every 10 days which were analyzed using a one-way repeated measure ANOVA showed that were not significantly different (P>0.05) between the use of stocking density and the concentration of inorganic nitrogen (TAN, nitrite, nitrate) on silver rasbora rearing media. The value of TAN concentration tends to increase at the beginning of the rearing period and tends to decrease at the end of the rearing period. While the value of Nitrite concentration tends to be high at the beginning of the maintenance period and tends to fluctuate until the end of the rearing period. Nitrate concentration values tend to increase until the end of the maintenance period in all treatments.

DISCUSSION

The use of different stocking densities significantly affected the growth parameters of silver rasbora fry during the experimental period. The weight of silver rasbora fry decreased with the increase in the stocking density of silver rasbora fry. The weight of the fish decreased with the increase in stocking density and this result is consistent with several studies using fish species from the family Cyprinidae (*Labeo bata*, *Puntius binotatus, Barbonymus gonionotus*) which showed a negative correlation between the stocking density and fish growth. In addition, the use of high stocking density could reduce the water quality, increase the competition for feed, and affect the aggressiveness of fish **(Rahman e*t al.*, 2015; Samad *et al.*, 2016; Hayat *et al.*, 2018)**. However, several studies showed a positive correlation between stocking density and fish growth, such as the Sciaenidae family (*Argyrosomus japonicus* and *Argyrosomus regius*) **(Pirozzi *et al.*, 2009; Millán-Cubillo *et al.*, 2016).** This result is possible with animals who prefer to live in groups to avoid **stress (de las Heras *et al.*, 2015)**. Studies on stocking density must take into consideration a variety of factors, including species selection, social interactions, water quality, and environmental conditions **(Salari *et al.*, 2012; Arifin *et al.*, 2017)**.

Furthermore, the stocking density also affected the FCR and the total amount of given feed as the higher the stocking density, the higher the FCR and TF value. However, the use of different stocking densities in the rearing of silver rasbora fry was still ideal because it showed an FCR value of less than 1.5 in all treatments. Feed conversion and efficiency are very important indicators of efficiency in the use of the feed provided because feed prices tend to rise **(Craig and Khun, 2017; Fry *et al.*, 2018)**. Moreover, the FCR value of less than 2 was still considered good in aquaculture industries **(Bag e*t al.*, 2016).** The use of optimal stocking densities in aquaculture is very necessary to reduce stress on fish and potential economic losses ***(Arifin et al.*, 2017)**.

The stocking density treatments did not affect the SR of silver rasbora fry until the end of the experimental period. The high SR in all treatments could be influenced by several factors, such as the species used and the influence of environmental factors **(Samad *et al.*, 2016)**. Stocking density is one of the most important factors in aquaculture activities because it directly affects SR, growth performance, water quality, and production efficiency in fish or shrimp farming **(Chakraborty and Mirza, 2007; Rahman and Verdegem, 2010; de Oliveira *et al.*, 2012)**. Several similar studies in the family Cyprinidae also showed results where stocking density did not affect SR **(Said and Triyanto, 2013; Rahman *et al.*, 2015; Hayat *et al.*, 2018).**

Silver rasbora is a fish of the genus Rasbora which is found on many islands in Indonesia **(Kusuma *et al.*, 2017)**. The result of water quality parameters in several Rasbora studies is shown in Table 3.

Based on observations made during the maintenance period, showed that the water quality parameters in the form of TAN, Nitrite, and Nitrate values during the maintenance period were higher than in the literature (Table 3). This could be caused by excess feed residue and the use of stocking density **(Hayat *et al.*, 2018)**. Concentrations of TAN, Nitrite, and Nitrate in the rearing medium fluctuated but increased until the end of the rearing period. The increased concentration of TAN, Nitrite, and Nitrate in rearing ponds could be due to the higher organic matter content in the rearing media from uneaten feed and fish feces **(Jiménez-Montealegre *et al.*, 2002; Torres-Beristain *et al.*, 2006; Bag *et al.*, 2016)**.

Table 3. Water quality parameters in the genus of Rasbora

Variable	During Experiment	Acceptable Range (Literature)	Notes
TAN (mg L^{-1})	0.029-0.061	0.010-0.013[c]	Larva rearing
Nitrite (mg L^{-1})	0.101-0.310	0.002-0.012[a]	Natural habitat
Nitrate (mg L^{-1})	1-3.8	0.398-3.272[a]	Natural habitat
pH	6.5-8.0	6.7-8.8[ab]	Natural habitat
DO (mg L^{-1})	6.2-7.2	6.2-7.5[ac]	Natural habitat; larvae rearing
Temperature (^{o}C)	27.1-27.7	24.5-32.0[d]	Adaptation test

[a]Sentosa and Djumanto, (2010) ; [b]Sulastri et al., (2010); [c] Budi et al., (2020); [d] Said and Triyanto, (2013)

The siphoning process that was carried out every 10 days during the rearing period in all treatments did not affect the concentration of TAN, Nitrite, and Nitrate in the rearing pond. Furthermore, the fluctuating conditions of TAN, Nitrite, and Nitrate concentrations during the rearing period indicated the occurrence of nitrification and denitrification by natural bacteria found in rearing ponds **(Durborow *et al.*, 1997; Turker *et al.*, 2003)**.

CONCLUSION

Differences in stocking density significantly affected the growth performance of BW, FB, TF, FCR, WG, and water quality (pH value) of silver rasbora (*Rasbora argyrotaenia*) fry in plastic-lined ponds. A stocking density of 100 fish 500 L^{-1} resulted in optimal growth of the fry. However, based on all production parameters and overall water quality, the use of a stocking density of 300 fish 500 L^{-1} still showed suitable conditions for cultivation activities with an FCR value of less than 1 and optimal water quality for rearing silver rasbora fries in plastic-lined ponds.

AUTHORS' CONTRIBUTIONS

All authors contributed to the conception and design of the study. Wildan Kurnia Pratama and Darmawan Setia Budi prepared the materials, collected data, and analyzed the results. Muhammad Hanif Azhar wrote the first draft of the manuscript and Ahmad Taufiq Mukti, Devrim Memiş, and Muhammad Brawijoyo Santanumurti commented on previous versions of the manuscript.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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