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THE EFFECT OF STOCKING DENSITY ON THE SURVIVAL AND GROWTH OF SILVER RASBORA (*RASBORA ARGYROTAENIA*) LARVAE

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Abstract

The purpose of this study was to assess the effect of different stocking densities on the survival and growth of silver rasbora (*Rasbora argyrotaenia*) larvae as new aquaculture species. A total 3,000 larvae aged 2 days after hatching (mean body weight = 1.08 ± 0.06 mg; mean total length = 3.14 ± 0.17 mm) were assigned into five stocking density treatments (*A* – 10, *B* – 20, *C* – 30, *D* – 40, *E* – 50 individuals L^{-1}; four replicates per treatment) and reared for 21 days. The stocking density affected both survival and growth of silver rasbora larvae ($P < 0.05$). The highest survival rate and individual growth occurred at 10 fish L^{-1} treatment. Based on the highest biomass gain, the optimum stocking density treatment was 20 fish L^{-1}. We suggest using 20 fish L^{-1} stocking density in silver rasbora larviculture because estimated more efficient and profitable economically.

Introduction

Silver rasbora (*Rasbora argyrotaenia*) is a tropical freshwater bentopelagic fish that can be found in the Asian region including Mekong, Chao Phraya and Mae Khlong basins, Malay Peninsula to Borneo, Java and Sumatra in Indonesia; occurs mainly in rivers and enters flooded fields (CAPULI and BAILLY 2019). Silver rasbora is a newly cultivated species that have economic values as consumption and ornamental fish in Indonesia and surrounding countries (ADAWIYAH et al. 2019, HERAWATI et al. 2017). The captive production of this fish is limited, so the fulfillments of

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the demands are mainly depending on wild capture (ROSADI et al. 2014). On the other hand, the silver rasbora farming is still developing, remains largely incomplete, and requires a lot of basic information about its cultivation especially for the young life-stages to increase the production to overcome over exploitation on wild.

The fry availability is a limiting factor for fish farmers and affects the reliability of silver rasbora farming and other fish also. Thus, during the larval phase, increasing survival and growth must be one of the research priorities for many species (SLATER et al. 2018). Nevertheless, to the best of our knowledge, there is no literature on the rearing of silver rasbora larvae under the intensive conditions. At present, larvae production is usually under suboptimal conditions. For this reason, commonly silver rasbora juveniles are produced in low quantity and quality.

Rearing practices, feeding strategies, and environmental conditions affect the success of larval production (COWAN al. 2000, KESTEMONT et al. 2003). Stocking density of larvae is known to influence larval rearing performance. Stocking density effects in young stages have been studied in many species of freshwater aquaculture (GOMES et al. 2000, SAHOO et al. 2004, SZKUDLAREK and ZAKĘŚ 2007). However, the stocking density effects on survival and growth may vary (MERINO et al. 2007, NIAZIE et al. 2013), depending on conditions of rearing, fish age, and species (SAOUD et al. 2008). In that sense, some researches have shown that survival rate and growth are negatively influenced by stocking density increase (EL-SAYED 2002, KEER et al. 2018, SAHOO et al. 2004); other studies have found no effects of stocking density on survival or growth rate (KAISER et al. 1995, NIAZIE et al. 2013) while some of them found that low stocking density negatively affects growth (MILLAN-CUBILLO et al. 2016). These statements show that more detailed characterization of the effects of stocking density on survival and growth in silver rasbora larvae is crucial. The aim of this study was to assess the effect of different stocking densities on the survival and growth of silver rasbora larvae.

Materials and Methods

This research was conducted from January to March 2019 in the aquaculture facilities and laboratory at the Airlangga University, Banyuwangi campus (East Java, Indonesia). The study was conducted in compliance with the Law of the Republic of Indonesia Number 18 of 2002 concerning the National System of Research, Development and Application of Science and Technology under the oversight and approved by the

Faculty of Marine Science Universitas Airlangga (based on the letter of assignment from the Dean of Faculty of Marine Science Universitas Airlangga, 1751/UN3.1.16/PPd/2018).

Origin of larvae

Silver rasbora brood fish (approximately 1 year old) were obtained from Technical Implementation of Development Unit of Freshwater Aquaculture of Umbulan (Pasuruan, East Java, Indonesia). A total of two females (5.20 ± 0.14 g body weight and 8.60 ± 0.56 cm total length; average ± SD) and 6 males (4.62 ± 0.07 g of body weight and 7.50 ± 0.40 cm of total length; average ± SD) were mated and spawned naturally using palm tree fibers as spawning substrate in a glass aquarium (40 x 50 x 50 cm^3). The fecundity, fertilization rate, and hatching rate observed were 439.20 ± 22.89 egg per g fish body weight, 94.40 ± 1.80%, and 93.30 ± 0.41%, respectively. After spawning, the brood fish were removed from the aquarium and the eggs attached to the substrate left to hatch for around 2 days. The water quality parameters that were measured during the incubation (2 times per day at 07.00 AM and 04.00 PM) can be seen in Table 1.

Table 1

The water quality parameters measured during the incubation of silver rasbora egg

Parameters	Mean ± SD	Range
Dissolved Oxygen [ppm]	6.82 ± 0.38	6.2–7.5
Temperature [ºC]	24.94 ± 0.60	24–27
pH	7.24 ± 0.32	6.9–7.5
Total ammonia nitrogen [ppm]	0.012 ± 0.001	0.010–0.013

Experimental design and rearing

The experiment was conducted semi outdoor (in a room that is not fully enclosed, some parts of the room roofless), no photoperiods manipulation in fish rearing was set. A total of 3,000 larvae aged 4 days post fertilization (DPF) were taken and measured (n = 100, 1.08 ± 0.06 mg body weight, 3.14 ± 0.17 mm total length; average ± SD) and then assigned to 20 cylinder plastic tank (10 L capacity; 30 cm diameter) with 5 L volume of water with gentle aeration to maintain dissolved oxygen level. A total of 50% water was exchanged every 3 days to maintain the water quality at 09.30 AM. Water for rearing was obtained from the well and was pumped to the reservoir prior to supply to the rearing containers. The water quality in each tank was measured in the morning at 09.00 AM before water exchange and is presented in Table 2.

Table 2

Water quality parameters of silver rasbora larvae rearing at different stocking densities (*A*–*E*; see Table 3)

Paramaters	*A*	*B*	*C*	*D*	*E*
Dissolved oxygen [ppm]	6.49 ± 0.36	6.52 ± 0.38	6.56 ± 0.39	6.56 ± 0.36	6.44 ± 0.40
pH	7.46 ± 0.30	7.37 ± 0.34	7.41 ± 0.36	7.44 ± 0.34	7.33 ± 0.33
Total ammonia nitrogen [ppm]	0.013 ± 0.005	0.013 ± 0.006	0.019 ± 0.004	0.018 ± 0.005	0.013 ± 0.004

The 21-day experiment was started at 4 days post fertilization (DPF further on), a few days after mouth opening and when ability of larvae to feed on artemia nauplii was confirmed. To observe effects of stocking density on the survival and growth, larvae were reared at five different stocking densities as summarized in Table 3. A completely randomized design with four replicates was conducted in the experiment. The stocking density treatments were based on Indonesian National Standards (SNI 7733:2011) on stocking densities of goldfish larvae (*Carassius auratus*). A total 20 artemia nauplius per day per fish larvae was given as feed with the frequency of feeding 4 times a day which is 06.00 AM, 12.00 AM, 05.00 PM, and 09.00 pm.

Table 3

Stocking densities of silver rasbora larvae in five experimental treatments

Experimental treatment	Stocking density [lavae L^{-1}]	Total number of larvae per tank
A	10	50
B	20	100
C	30	150
D	40	200
E	50	250

Observation and measurements of larvae

Larvae sampling in experimental treatments was done at 4 DPF, 11 DPF, 18 DPF, and 25 DPF. At least 10% of the total number of larvae at each stocking density (n = 5–25) was selected as the size of the sample and frequency of sampling while considering the time required and technical limitations of sampling procedure as well as larvae handling caused stress. Sampled larvae were anesthetized with rapid cooling anesthetic method using ice slush exposure at 0°C (CHEN et al. 2013) and total body length [TL, mm] of larvae was measured with a micrometer under a stereo-microscope (based on fish size and magnification, precision varying from 0.05 to 0.1 mm). A digital scale with a precision of 0.1 mg used for body weight [BW, mg] measuring. Fish were returned to their respective tank after individual measurements. After sampling, no mortality was observed. Mortalities were observed in the end of treatment at 25 DPF by counting the number of initial larvae reduced by surviving larvae.

Observed parameters

The effects of stocking density on growth and survival were determined by calculating the following parameters.

Survival rates (SR), expressed as a percentage, and was calculated based on formula:

$$SR\ [\%] = [(N_f / N_i) \cdot 100]$$

where:
N_i and N_f – the initial and the final number of larvae, respectively.

Fish biomass gain per liter [BG, g L^{-1}] or was calculated following the formula:

$$BG = [(N_f\, BW_f - N_i\, BW_i) / V]$$

where:
N_i and N_f – the initial number and the final number of larvae
BW_i and BW_f – the initial average body weight [mg] and the final average body weight [mg]
V – the tank volume in L.

Length gain [LG, mm] was calculated based on formula:

$$LG = TL_f - TL_i$$

where:
TL_i and TL_f – initial and final average total length [mm].

The specific growth in body weight [SGR_{BW}, % per day] was calculated following formula:

$$SGR_{BW} = [(\ln BW_f - \ln BW_i) / D) \cdot 100]$$

where:
BW_i and BW_f – the initial and final body weights of fish
D – the experiment duration in days.

The specific growth in total length [SGR_{TL}, % per day] was calculated using the same approach, as $SGR_{TL} = [(\ln LT_f - \ln LT_i) /21] \cdot 100$

where:
LT_i and LT_f – the initial and final body lengths of fish.

Data analysis

Data were analyzed statistically by ANOVA test with 95% confidence level (the each analyzed variable data confirmed normal distribution and homogeneity of variances) and continued with Duncan Multiple Range Test (DMRT) using SPSS 17.0 software.

Results

The growth and survival of silver rasbora larvae reared at five different densities is showed in Table 4. Based on the results, the stocking density has significant effect ($P < 0.05$) on all parameters observed.

Table 4

Effects of stocking density (*A–E*; see Table 3) on survival and growth of silver rasbora larvae from 4 to 25 days post fertilization

Parameters*	*A*	*B*	*C*	*D*	*E*
SR [%]	97.08 ± 3.44^{a}	86.04 ± 7.18^{b}	87.22 ± 8.30^{ab}	75.63 ± 15.63^{b}	87.00 ± 2.48^{b}
BW_i [mg]	1.08 ± 0.06	1.08 ± 0.06	1.08 ± 0.06	1.08 ± 0.06	1.08 ± 0.06
BW_f [mg]	31.45 ± 1.21^{a}	28.69 ± 0.95^{b}	19.13 ± 0.63^{c}	13.56 ± 0.75^{d}	10.97 ± 0.32^{e}
BG [g L^{-1}]	0.29 ± 0.02^{b}	0.47 ± 0.05^{a}	0.47 ± 0.05^{a}	0.37 ± 0.09^{a}	0.43 ± 0.02^{a}
TL_i [mm]	3.14 ± 0.17	3.14 ± 0.17	3.14 ± 0.17	3.14 ± 0.17	3.14 ± 0.17
TL_f [mm]	15.44 ± 0.53^{a}	13.37 ± 0.24^{b}	12.80 ± 0.21^{c}	11.75 ± 0.36^{d}	10.48 ± 0.29^{e}
LG [mm]	11.92 ± 0.42^{a}	9.99 ± 0.37^{b}	9.40 ± 0.32^{b}	8.33 ± 0.46^{c}	7.16 ± 0.37^{d}
SGR_{BW} [% day^{-1}]	15.87 ± 0.32^{a}	15.44 ± 0.21^{a}	13.70 ± 0.14^{b}	12.21 ± 0.27^{c}	11.23 ± 0.09^{d}
SGR_{TL} [% day^{-1}]	7.04 ± 0.30^{a}	6.56 ± 0.29^{ab}	6.31 ± 0.27^{bc}	5.87 ± 0.33^{cd}	5.47 ± 0.27^{d}

*SR – survival rate (n = 4); BW_i – initial body weight (n = 100); BW_f – final body weight (n = 5–25); BG – biomass gain (n = 4); TL_i – initial total length (n = 100); TL_f – final total length (n = 5–25); LG – length gain (n = 4); SGR_{BW} – specific growth rate for body weight (n = 4); SGR_{TL} – specific growth rate for total length (n = 4). Values are means ± SD. Superscript letters denote significant differences ($P < 0.05$) between treatments

Based on the data overall, the survival and growth of silver rasbora larvae decreased with stocking density increasing, where the lowest survival and growth was observed on the highest density of the larval rearing, except in biomass gain (BG) (see Table 4 and Figure 1) Biomass gain in lowest stocking density treatment was significantly lower than in all other treatments (Table 4).

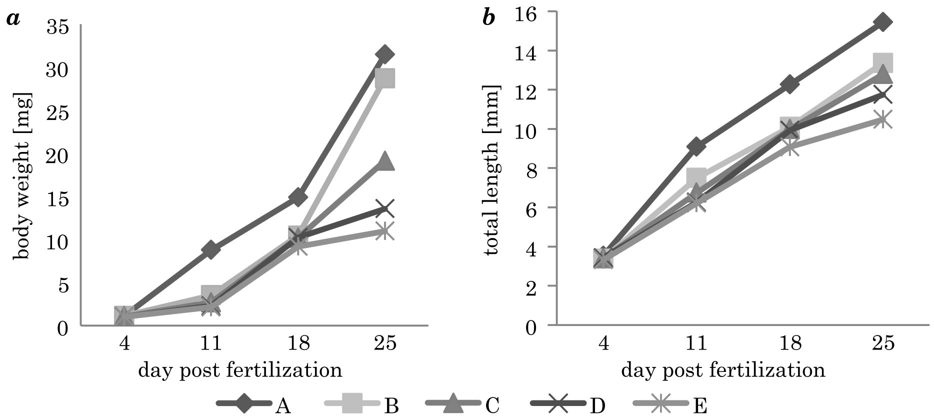

Fig. 1. Body weight (*a*) and total length (*b*) of silver rasbora larvae (n = 5–25) at 4, 11 and 25 days post fertilization in the five stocking density treatments (*A*–*E*; see Table 3)

Discussion

Overall in the present study, stocking density increase had negative effects on the individual fish growth of silver rasbora larvae at the end of study. In contrast, the yield of fish production, shown in current study as fish biomass gain (BG), tended to increase with higher stocking densities. These results are similar to previous study on pikeperch (*Sander lucioperca*) (SZKUDLAREK and ZAKĘŚ 2007) and giant gourami (*Osphronemus goramy*) (ARIFIN et al. 2019), where high stocking densities led to lower growth but positively influenced biomass gain. On the other hand, stocking density had no effects on growth of African catfish (*Clarias gariepinus*) (KAISER et al. 1995) and goldfish (*Carasius auratus*) (NIAZIE et al. 2013); and conversely in meagre (*Argyrosomus regius*) (MILLÁN-CUBILLO et al. 2016), increasing stocking density positively effects on growth. The results in other studies indicated that the effects of stocking density on the fish larvae growth are different.

Based on the present study, we found that the stocking density had an effect on the survival of larvae although no cannibalism and no aggressive behavior was observed throughout the experiment and also no difference in water quality in each treatment (Table 2). The highest survival rate was obtained on the lowest stocking density treatment. Survival rate tended to decrease in other treatments, meaning that mortality increases with increasing stocking density above 10 fish L^{-1}. Lower survival rates also observed in Reba carp (*Cirrhinus reba*) fry at high stocking densities were related to stronger food and space competition and also increased stress (KEER et al. 2018). The relationship between stocking density and stress in relation to mortality in silver rasbora needs to be investigated further in order to find clear regulations in this regard.

The highest survival rate occurred on the lowest stocking density treatment, mortality increased with increasing stocking density above 10 fish L^{-1}, and stocking density increase had negative effects on the growth of silver rasbora larvae ($P < 0.05$). Although the higher stocking density led to the lower growth and survival, the increase of biomass gain in higher stocking density is considered to determine the optimum density for production efficiency. At 20 fish L^{-1} stocking density treatments obtained the biomass gain did not differ to other treatment; however the individual growth parameters were higher compared to the higher stocking densities. It showed that using of 20 fish L^{-1} stocking density in silver rasbora larviculture estimated more efficient and profitable economically. So we recommend using 20 fish L^{-1} stocking density for application on the silver rasbora hatchery.

Conclusions

The stocking density affected survival and growth of silver rasbora larvae ($P < 0.05$). The highest survival rate and individual growth occurred at 10 fish L^{-1} treatment. However, considering the highest biomass gain, the optimum stocking density treatment was 20 fish L^{-1}.

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