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# Salinized water as a strategy for increase stocking density in *Heros severus* larviculture, an Amazonian ornamental fish

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#### ABSTRACT

The aim of this study was to evaluate the effect of different salinized water (0, 3 and 6 g L<sup>-1</sup>) and stocking densities (10, 15 and 20 larvae L<sup>-1</sup>) in *Heros severus* larviculture. The experimental design was completely randomized, in a 3x3 factorial design, with three replicates. For this, a total of 405 larvae of *H. severus* were randomly distributed in 27 aquariums (1L) according to the treatments and after 15 days all the larvae were measured, weighed and counted. Higher values for final length, length gain and specific growth rate were observed in *H. severus* larvae raised at any stocking density tested at 3 g L<sup>-1</sup> of salinized water (p < 0.05). The final weight and weight gain followed the same trend of the length, being higher in fish submitted to 3 g L<sup>-1</sup> of salinized water of 6 g L<sup>-1</sup> was detrimental to larvae weight uniformity and survival rate (p < 0.05). The fish stocked at densities of 15 or 20 larvae L<sup>-1</sup> presented the best results of final weight and weight gain (p < 0.05). The stocking density of 20 larvae L<sup>-1</sup> in salinized water at 3 g L<sup>-1</sup> is recommended for *H. severus* larviculture.

Keywords: aquaculture; cichlid; culture management; first life stages; salt addition.

#### Água salinizada como estratégia para aumentar a densidade de estocagem na larvicultura de *Heros severus*, um peixe ornamental da Amazônia

#### RESUMO

O objetivo deste trabalho foi avaliar o efeito de diferentes águas salinizadas (0, 3 e 6 g L<sup>-1</sup>) e densidades de estocagem (10, 15 e 20 larvas L<sup>-1</sup>) na larvicultura de *Heros severus*. O delineamento experimental foi inteiramente casualizado, em esquema fatorial 3x3, com três repetições. Para isso, 405 larvas de *H. severus* foram distribuídas aleatoriamente em 27 aquários (1 L), de acordo com os tratamentos e, 15 dias depois, todas foram medidas, pesadas e contadas. Maiores valores de comprimento final, ganho de comprimento e taxa de crescimento específico foram observados em larvas criadas em qualquer densidade testada a 3 g L<sup>-1</sup> de água salinizada (p < 0,05). O peso final e o ganho de peso seguiram a tendência do comprimento, sendo maiores nos peixes submetidos a 3 g L<sup>-1</sup> de água salinizada (p < 0,05). O por outro lado, a água salinizada a 6 g L<sup>-1</sup> prejudicou a uniformidade do peso e a taxa de sobrevivência das larvas (p < 0,05). Os peixes estocados nas densidades de 15 ou 20 larvas L<sup>-1</sup> apresentaram os melhores resultados de peso final e ganho de peso (p < 0,05). A densidade de estocagem de 20 larvas L<sup>-1</sup> em água salinizada a 3 g L<sup>-1</sup> é recomendada para a larvicultura de *H. severus*.

Palavras-chave: aquicultura; ciclídeo; manejo de criação; primeiras fases da vida; adição de sal.

# **INTRODUCTION**

Fish species from Cichlidae family, as the *Heros severus*, shows potential for ornamental fish farming (Abe et al., 2016; Campelo et al., 2019b). The *H. severus* is a freshwater fish endemic from the Amazon basin (Mora et al., 2007), highly appreciated in aquarium trade industry due to their peaceful behavior and its varieties of bright colors, from yellow to greenish tones in the body and intense red in the fins (Favero et al., 2010; Abe et al., 2016). Furthermore, the species has good adaptation to cultivation conditions, easily accepts the diets supplied and it is relatively easy to reproduce (Campelo et al., 2019b; Oliveira et al., 2020). However, despite all the attractive characteristics of this species, studies are still needed to optimize culture management, especially on their first life stages, aiming to define new production strategies (Abe et al., 2016; Campelo et al., 2019b).

Larviculture is considered the most important and critical stage of aquaculture production chain, once it is the phase of higher animal fragility (Evangelista et al., 2020; Reis et al., 2021; Santos et al., 2021). The larvae are sensitive to several stressful factors such as pathogenic infections, productive managements and changes in water parameters, which can lead to high mortality rates and reduce productivity (Zuanon et al., 2011; Dias et al., 2016; Abe et al., 2019). Thus, the success of larviculture is determined by the optimal culture conditions, aiming to guarantee the adequate fish development (Dias et al., 2016; Campelo et al., 2019a; Abe et al., 2021). In this scenario, the effects of salinized water and stocking density of fish are some of the factors that should be studied, since they can directly affect the larviculture management (Luz et al., 2012; Dias et al., 2016; Santos et al., 2021).

The use of an adequate stocking density is commercially beneficial, once it improves the use of tanks and water resources, in addition to maximizing economic results (Santos et al., 2021). In general, low stocking densities is related to improvement in growth performance and survival rates of fish. However it's underutilization of the space can reduce the efficiency of production (Gonçalves Junior et al., 2014; Dias et al., 2016). Furthermore, the use of inappropriate stocking densities may lead to increase competition for space, food and oxygen (Faria et al., 2011), reduce water quality and resulted on a chronic stress response which can, consequently, cause fish growth impairment (Gonçalves Junior et al., 2013; Abe et al., 2016). Thus, determining the adequate stocking density for each species and life stage is fundamental to optimize productivity, without compromising animal welfare and growth (Abe et al., 2019).

The used of salinized water in larviculture of freshwater fish is considered a good strategy to improve larval growth and survival (Jomori et al., 2013; Araújo et al., 2021). The used of salinized water benefits the Artemia nauplii, once it makes possible the microcrustacean to remain alive longer than in freshwater and thus, improving its availability and attractiveness to the larvae (Silva et al., 2019). In addition, the increase in water salt concentration decrease the ionic difference between water and fish plasma, which reduce the energy expenditure by freshwater fish to maintain its osmotic equilibrium (Salaro et al., 2012; Fabregat et al., 2015). The fish larval can redirected this energy to other physiological processes, such as fish growth, immunity and stress responses (Fisher et al., 2021; Freire and Sampaio, 2021). Salinized water also reduces the toxicity of nitrogenous compounds (Sampaio et al., 2002) and prevents the occurrence of some fish diseases and parasites (Garcia et al., 2007; Santos et al., 2020). However, inadequate levels of salinized water lead to a reduction in fish development and survival (Luz et al., 2013; Abe et al., 2015).

Ornamental fish farming success is related to the production of a high number of fish in the smallest space or volume possible; however, information on the optimal stocking density and strategies that make possible to optimize fish productivity are limited, especially during the larviculture, which may impair the others culture stages. The stocking density is directly related to the reduction of stress caused by salinized water and a study testing these two practices is very relevant. Although many authors studied the effect of interaction between salinized water and stocking density in larviculture of many freshwater fish (Abe et al., 2015; Dias et al., 2016; Santos et al., 2021), this information is missing for *H. severus* larviculture. In this context, it is essential to develop research aimed at improving production management, making the fish farming of *H. severus* more competitive and economically attractive. Thus, the present study aimed to evaluate the growth performance and survival rate of *H. severus* larvae submitted to different salinized water and stocking densities.

#### MATERIAL AND METHODS

#### Fish and experimental design

The experimental trial was carried out in Laboratório de Peixes Ornamentais, Instituto de Estudos Costeiros, Universidade Federal do Pará, Bragança, Pará, Brasil. All animal procedure and protocol described herein were approved by the Ethics Committee for the Use of Animals of the Universidade Federal do Pará, CEUA/UFPA (Approval number: 2010080719).

The *H. severus* larvae were obtained through natural reproduction of couples in laboratory of ornamental fish under controlled environmental conditions. A total of 405 larvae of *H. severus* with 7 days post-hatching and presenting an initial mean weight and length of  $5.26 \pm 1.65$  mg and  $5.57 \pm 0.68$  mm (mean  $\pm$  standard deviation), respectively, were used. All larvae did not have a yolk sac and had an ideal mouth opening for accepting exogenous food. The *H. severus* larvae were randomly distributed in 27 aquariums with a useful volume of 1 L of water. All aquariums had an individual aeration system and the laboratory was maintained under natural photoperiod of approximately 12/12 hours light/dark condition, as recommended by Veras et al. (2016).

The experiment was carried out in a completely randomized design, in a 3x3 factorial design, with three replicates for each treatment. Three concentrations of salinized water (0, 3 and 6 g  $L^{-1}$ ) and three fish stocking densities (10, 15 and 20 larvae  $L^{-1}$ ), were evaluated, for a period of 15 days. The *H. severus* larvae were fed with *Artemia* nauplii in concentration of 250 *Artemia* nauplii larvae<sup>-1</sup> day<sup>-1</sup> at a feeding frequency of four times a day, with three-hour intervals between meals, at times of 8, 11, 14 and 17 hours, as recommended by Abe et al. (2016).

All aquariums were siphoned once daily in the afternoon after 1 hour the last feeding, with partial changes of 30% of the useful volume of each aquarium were made through siphoning of the bottom of the aquaria, for withdrawal of feces and possible food residues. The *H. severus* larvae were counted during the cleaning management to adequacy the prey concentrations in case of mortality of the animals.

#### Artemia hatching

Artemia nauplii were obtained daily after incubation of 5 g  $L^{-1}$  cysts in salinized water at a concentration of 35 g  $L^{-1}$ . The containers for the hatching of the cysts (2 L) were kept under artificial lighting for 24 hours and constant aeration. After hatching, aeration was withdrawn and the living nauplii were collected from the suspension of unhatched cysts by siphoning. Artemia nauplii were then filtered through a 120 µm mesh and washed twice with running freshwater to remove impurities, ensure food quality and remove the salinized water so as not to influence the treatments. Then, the hatched nauplii were transferred to a 200 mL of water and the density was estimated by collecting a volume of 0.5 mL, to determine the mean number of nauplii per mL. The counting of the nauplii was performed out in triplicate with the aid of petri dish under stereomicroscope (QUIMIS Q714Z-2; SP, Brazil) with increase of 40×. After estimating nauplii density, the volume of Artemia nauplii to be supplied in each treatment was calculated.

#### Parameters of water quality

During the experimental period, the parameters of water quality, temperature (°C), pH, dissolved oxygen (mg L<sup>-1</sup>) and electrical conductivity (mS cm<sup>-1</sup>) were measured every 3 days at the morning time using a portable multiparameter probe (HORIBA U-50; CA, USA). The salinized water was measured daily at the afternoon time using the same portable multiparameter probe throughout the 15 days of rearing, aiming to monitor the concentration of salinized water and correct when necessary. In addition, total ammonia levels (mg L<sup>-1</sup>) were measured every 3 days at the morning time using a colorimetric method, Kit Labcon Test (Industry and Commerce of Alcon dehydrated foods; SC, Brazil).

In the 15 days of the experiment, the temperature remained at 26.06  $\pm$  0.41°C, pH at 6.95  $\pm$  0.17, dissolved oxygen at 6.39  $\pm$  0.05 mg L<sup>-1</sup> and total ammonia at 0.08  $\pm$  0.14 mg L<sup>-1</sup>. However, the electrical conductivity in the treatments without addition of salt was 0.34  $\pm$  0.11 mS cm<sup>-1</sup>, whereas in those that had the salinized water the values were of 4.98  $\pm$  0.28 mS cm<sup>-1</sup> and 9.81  $\pm$  0.17 mS cm<sup>-1</sup> for 3 and 6 g L<sup>-1</sup> of salt, respectively. Nevertheless, the parameters of water quality remained within the appropriate condition for *H. severus* larviculture (Eiras et al., 2019; Paixão et al., 2019).

#### Growth performance and survival rate

At the beginning of the experiment, for the initial biometrics, due to the small size and fragility of the fish larvae, a sample of 81 individuals (20% of the batch) were weighted with the aid of an analytical balance (GEHAKA AG200; 0.0001 g accuracy; SP, Brazil) and measured with the aid of an electronic caliper (PANTEC-150; 0.01 mm; SP, Brazil) to estimate initial mean of weight and length. At the end of the experiment, all surviving larvae were weighted and measured to determine the growth performance parameters: Final length (FL), length gain (LG) LG = final length - initial length, final weight (FW), weight gain (WG) WG = final weight - initial weight, specific growth rate (SGR) SGR = ((ln final weight - ln initial weight)/number of experiment days) \*100 (Lugert et al., 2016), uniformity of the batch for weight (WU) and length (LU) being WU = (number of fish with weight varying  $\pm 20\%$  from the average in each experimental unit/total number of fish per experimental unit) \*100 and LU = (number of fish with length varying  $\pm 20\%$  from the average in each experimental unit) \*100 (Furuya et al., 1998), and survival rate (SR) SR = (final larvae number/initial larvae number) \*100.

#### **Statistical analysis**

For confirmation of normality and homogeneity of variances, data were submitted to Lilliefors and Bartlett's tests, respectively. After assumptions were satisfied, a *two-way* analysis of variance (ANOVA) at 5% significance was performed. If no interaction between salinized water and stocking density was detected, *one-way* ANOVA (p < 0.05) of single factors was performed. Then, in the values that presented significant difference (p < 0.05), the Tukey test at 5% significance was performed. All statistical analyses were performed using SPSS (SPSS Inc., Chicago, IL, USA, version 23.0).

#### RESULTS

The values of FW and WG were higher in *H. severus* larvae submitted to salinized water of 3 g L<sup>-1</sup> (p < 0.05) in comparison to 0 and 6 g L<sup>-1</sup>, while the WU and SR were lower in *H. severus* larvae submitted to salinized water of 6 g L<sup>-1</sup> (p < 0.05) in comparison to 0 and 3 g L<sup>-1</sup>. The LU of *H. severus* larvae did not show significant difference, independent of the salinized water and stocking density used (p > 0.05). The *H. severus* larvae stocked at densities of 15 or 20 larvae L<sup>-1</sup> presented the best results of FW and WG (p < 0.05) in comparison to 10 larvae L<sup>-1</sup>, while the WU and SR of *H. severus* larvae did not show significant differences at any stocking density (p >0.05) (Table 1).

Statistical interaction between salinized water and stocking density of *H. severus* larvae was identified for the parameters of FL, LG and SGR (p < 0.05). The *H. severus* larvae stocked at densities of 10, 15 and 20 larvae L<sup>-1</sup> and submitted to 3 g L<sup>-1</sup> of salinized water presented the higher values for these parameters (p < 0.05) in comparison to the other salinized water used. On the other hand, lower values for the same parameters were observed in *H. severus* larvae stocked at densities of 10 larvae L<sup>-1</sup> when the salinized water was at 6 g L<sup>-1</sup> (p < 0.05) in comparison to the other salinized water and stocking density used (Table 2).

#### DISCUSSION

The *H. severus* larvae submitted to 3 g L<sup>-1</sup> of salinized water presented the better FW and WG. The salinized water between 2 and 4 g L<sup>-1</sup> had been previously used for the same species and provided greater values of weight gain (Eiras et al., 2019). The use of salinized water also provided better weight gain results in larviculture of other freshwater fish species that was fed with *Artemia* nauplii as live food. Such as *Astronotus ocellatus* (Jomori et al., 2013), *Betta splendens* (Dias et al., 2016), *Lophiosilurus alexandri* (Nascimento et al., 2020) and *Hypsolebias radiseriatus* (Araújo et al., 2021) kept in salinized water between 0 and 2 g L<sup>-1</sup>; and *Piaractus mesopotamicus* (Jomori et al., 2012), *Colossoma macropomum*, *Leporinus macrocephalus* (Jomori et al., 2013), *B. splendens* (Fabregat et al., 2017) and *Arapaima gigas* (Silva et al., 2019) kept in salinized water between 2 and 4 g L<sup>-1</sup>.

The used of *Artemia* nauplii as live food in freshwater fish larviculture may be the reason for the increased larvae weight gain when reared in salinized water. *Artemia* is a marine microcrustacean naturally found in saline environments and when submitted to freshwater does not survive for long time (Jomori et al., 2012; Silva et al., 2019). This leads to reduce its availability during the feeding of freshwater fish larvae,

impairing feed efficiency, larval development and water quality (Santos et al., 2015; Araújo et al., 2021). In this context, the larviculture in salinized water may have helped to increase the *Artemia* nauplii survival, extending the life time of the nauplii and making the live food available and attractive for longer. Thus, favoring higher rates of encounter and ingestion of nauplii by larvae and, consequently, better use of live food by the fish (Jomori et al., 2013; Silva et al., 2019). The salinization of water can be considered a simple low cost practice and a great alternative for increase food efficiency in freshwater fish larviculture (Silva et al., 2019; Santos et al., 2021).

The used of salinized water in freshwater fish larviculture can have positive or negative implications, with tolerance being recognized as species-dependent, varying according the fish development stage, water quality, feeding management, fish farming systems and acclimatization time (Salaro et al., 2012; Jomori et al., 2013; Takata and Luz, 2015). The *H. severus* larvae presented a WU and SR reduction when the salinized water increased from 3 to 6 g L<sup>-1</sup>. Similarly, were registered for the larviculture of *Pseudoplatystoma corruscans* (Santos and Luz, 2009), *C. macropomum, Brycon amazonicus, A. ocellatus* (Jomori et al., 2013), *Oreochromis niloticus* (Luz et al., 2013), *Pyrrhulina brevis* (Abe *et al.*, 2015), *B. splendens* (Dias et al., 2016) and *H. radiseriatus* (Araújo et al., 2021), when

**Table 1.** Growth performance and survival rate (mean values  $\pm$  salinized water and stocking densities.

Table 1. Growth performance and survival rate (mean values ± standard deviation) of *Heros severus* larvae submitted to different

Treatment	Performance indices <sup>°</sup>							
	FL (mm)	LG (mm)	FW (mg)	WG (mg)	SGR (% day-1)	WU (%)	LU (%)	SR (%)
Salinized water								
0 g L <sup>-1</sup>	12.70 ± 0.07 <sup>b</sup>	7.13 ± 0.07 <sup>b</sup>	29.85 ± 0.87 <sup>b</sup>	24.59 ± 0.87 <sup>b</sup>	11.57 ± 0.19 <sup>b</sup>	97.04 ± 3.29ª	$\begin{array}{c} 100.00 \pm \\ 0.00 \end{array}$	$100.00 \pm 0.00^{a}$
3 g L <sup>-1</sup>	13.27 ± 0.12ª	7.70 ± 0.12ª	33.58 ± 1.00ª	$28.32 \pm 1.00^{a}$	$12.35 \pm 0.20^{a}$	91.11 ± 8.40ª	$\begin{array}{c} 100.00 \pm \\ 0.00 \end{array}$	$100.00 \pm 0.00^{a}$
6 g L <sup>-1</sup>	12.26 ± 0.35°	6.69 ± 0.35°	27.17 ± 2.19°	21.91 ± 2.19°	10.92 ± 0.54°	70.44 ± 14.10 <sup>b</sup>	98.89± 1.98	96.67 ± 3.70 <sup>b</sup>
Stocking density								
10 larvae L <sup>-1</sup>	12.51 ± 0.51 <sup>b</sup>	6.94 ± 0.51 <sup>b</sup>	28.80 ± 3.04 <sup>b</sup>	23.54 ± 3.04 <sup>b</sup>	11.28 ± 0.74 <sup>b</sup>	78.89 ± 19.26	98.89 ± 1.98	$\begin{array}{c} 100.00 \pm \\ 0.00 \end{array}$
15 larvae L <sup>-1</sup>	$\begin{array}{c} 12.88 \pm \\ 0.35^{a} \end{array}$	7.31 ± 0.35ª	30.67 ± 2.55ª	25.41 ± 2.55ª	11.72 ± 0.55ª	90.61 ± 7.47	$\begin{array}{c} 100.00 \pm \\ 0.00 \end{array}$	97.78 ± 3.46
20 larvae L <sup>-1</sup>	$12.85 \pm 0.29^{a}$	7.28 ± 0.29ª	31.13 ± 1.72ª	25.87± 1.72ª	$11.84 \pm 0.37^{a}$	89.09 ± 10.10	$\begin{array}{c} 100.00 \pm \\ 0.00 \end{array}$	98.89 ± 1.73
p-value <sup>#</sup>								
Salinized water	***	***	***	***	***	***	NS	**
Stocking density	***	***	**	**	**	NS	NS	NS
Interaction	*	*	NS	NS	*	NS	NS	NS

"FL: final length; LG: length gain; FW: Final weight; WG: weight gain; SGR: Specific growth rate; WU: Weight uniformity; LU: Length uniformity; SR: survival rate; Mean values in the same column, with different letters, are significantly different by Tukey test at 5% probability (n=9); #ANOVA: NS: non-significant (p > 0.05); \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.01.

	Final length (mm)							
Salinized	Stocking density (larvae L-1)							
water (g L <sup>-1</sup> )	10	15	20					
0	$12.64\pm0.10^{\rm aB}$	$12.75\pm0.10^{\mathrm{aB}}$	$12.71\pm0.08^{\mathrm{aB}}$					
3	$13.13\pm0.12^{\mathtt{aA}}$	$13.40\pm0.15^{\rm aA}$	$13.28\pm0.08^{\mathtt{aA}}$					
6	$11.74\pm0.26^{\rm bC}$	$12.49\pm0.09^{\rm aB}$	$12.56\pm0.23^{\mathrm{aB}}$					
	Length gain (mm)							
Salinized	Stocking density (larvae L <sup>-1</sup> )							
water (g L <sup>-1</sup> )	10	15	20					
0	$7.07\pm0.10^{\rm aB}$	$7.18\pm0.10^{\rm aB}$	$7.14\pm0.08^{\rm aB}$					
3	$7.56\pm0.12^{\rm aA}$	$7.83\pm0.15^{\rm aA}$	$7.71\pm0.08^{\rm aA}$					
6	$6.17\pm0.26^{\rm bC}$	$6.92\pm0.09^{\rm aB}$	$6.99\pm0.23^{\rm aB}$					
	Specific growth rate (% day-1)							
Salinized	Stocking density (larvae L <sup>-1</sup> )							
water (g L <sup>-1</sup> )	10	15	20					
0	$11.46\pm0.13^{\rm aB}$	$11.53\pm0.20^{\mathrm{aB}}$	$11.72\pm0.20^{\mathrm{aB}}$					
3	$12.21\pm0.08^{\mathtt{aA}}$	$12.52\pm0.21^{\rm aA}$	$12.34\pm0.23^{\rm aA}$					
6	$10.17\pm0.35^{\rm bC}$	$11.11\pm0.35^{\rm aB}$	$11.46\pm0.39^{\mathrm{aB}}$					

**Table 2.** Mean values (± standard deviation) of the interactions between salinized water and stocking densities of *Heros severus* larvae\*.

\*Mean values in the same line, with different minuscule letters, are significantly different by Tukey test at 5% probability (n = 3); Mean values in the same column, with different capital letters, are significantly different by Tukey test at 5% probability (n = 3).

the salinized water increased from 2 to 4 g L<sup>-1</sup>. In addition, for *P. mesopotamicus* (Jomori et al., 2012), *L. macrocephalus* (Jomori et al., 2013), *Brycon vonoi* (Coraspe-Amaral et al., 2017) and *Pterophyllum scalare* (Eiras et al., 2019), reduction in fish weight uniformity and survival rate were observed when the salinized water increased from 4 to 6 g L<sup>-1</sup>.

Reduction in survival rate with increased the salinized water is a characteristic response in freshwater fish (Luz et al., 2013; Abe et al., 2015; Eiras et al., 2019). Once the use of high concentrations of salt in water for freshwater fish can lead to osmoregulatory imbalance, caused when the concentration of salts in water exceeds the limits of the fish homeostasis control (Becker and Baldisserotto, 2020; Mattioli et al., 2020). Situation that may compromise animal welfare, generate physiological and behavioral changes and, consequently, impair fish survival (Barton, 2002; Luz and Santos, 2008; Dias et al., 2016). Eiras et al. (2019) previously evaluated the salinized water between 0 and 8 g L<sup>-1</sup> for *H. severus* larviculture and found that 2 g L<sup>-1</sup>, or higher salinized water, led to decrease larvae survival rate. However, the present study demonstrated that the H. severus larviculture could be carried out in salinized water up to 3 g L<sup>-1</sup> without compromising the larvae survival rate.

The different stocking densities evaluated in the present study did not affect WU and SR of *H. severus* larvae. However, the FW and WG were higher in *H. severus* larvae stocked at densities of 15 and 20 larvae L<sup>-1</sup>. In fish larviculture, the use of inadequate stocking densities impairs the larvae growth, uniformity and survival, while low-stocking densities may underestimate the use of space (Dias et al., 2016; Abe et al., 2019; Santos et al., 2021). Abe et al. (2016) previously evaluated stocking densities between 1 and 20 larvae L<sup>-1</sup> for *H. severus* larviculture and recommended the stocking density of 5 larvae L<sup>-1</sup>, once higher stocking densities occasioned fish growth reduction and decrease water quality. However, the authors did not observe differences in fish survival rates in different stocking densities evaluated, indicating that high stocking densities can be used, as long as optimal culture conditions for *H. severus* larviculture are met.

The present study demonstrated that the stocking density of 20 larvae L<sup>-1</sup> is considered the best option for *H. severus* larviculture, since it increased productivity without significantly decrease of growth performance. This suggesting that the managements adopted in the present study were adequate to maintain the parameters of water quality at appropriate condition for larviculture of this fish species. Similar results, were registered during the larviculture of the Rhinelepis aspera at densities between 20 and 60 larvae L<sup>-1</sup> (Santos et al., 2012), L. alexandri at densities between 60 and 300 larvae L<sup>-1</sup> (Cordeiro et al., 2016), Monocirrhus polyacanthus at densities between 10 and 20 larvae L-1 (Ramos et al., 2016), B. amazonicus at densities between 20 and 60 larvae L<sup>-1</sup> (De Barros et al., 2019), Trichogaster lalius at densities between 5 and 40 larvae L-1 and Pethia conchonius at densities between 20 and 80 larvae L<sup>-1</sup> (Ramee et al., 2020). These results showing the importance to defining the optimal stocking density according to the fish species, farming system and management adopted.

The different salinized water and stocking densities evaluated in the present study did not affect LU of H. severus larvae. Similar results were registered for the larviculture of P. scalare at salinized water between 0 and 6 g L-1 (Eiras et al., 2019) and H. severus at densities between 1 and 20 larvae L<sup>-1</sup> (Abe et al., 2016). On the other hand, significantly difference for length uniformity were registered for the larviculture of B. splendens at salinized water between 0 and 2 g L<sup>-1</sup> (Dias et al., 2016) and Carassius auratus at densities between 5 and 25 larvae L-1 (Gonçalves Junior et al., 2014). Some cichlids have a social habit and live in shoals during the larvae stages (Abe et al., 2016; Pereira et al., 2016), such as H. severus, and negative interactions and territorialism may not have been intense enough to impair the larvae length uniformity. It is worth mentioning that the length uniformity have great relevance in the ornamental fish production, once the batch of fish uniform allows greater ease of classifications management and uniform fish are highly valued in aquarium trade industry (Dias et al., 2016; Veras et al., 2016).

Statistical interaction was identified between salinized water and stocking density of *H. severus* larvae for the parameters of FL, LG and SGR. The *H. severus* larvae stocked at density of 20 larvae L<sup>-1</sup> in 3 g L<sup>-1</sup> of salinized water presented the higher growth values. These results may be related to the reduction of the osmotic gradient, between farmed water and fish plasma, which leads to a reduction in energy requirement for osmoregulation (Salaro et al., 2012; Fabregat et al., 2015), redirecting that energy to other physiological processes, such as fish growth (Fisher et al., 2021; Freire and Sampaio, 2021). Furthermore, adequate levels of salinized water can reduce the toxic effect of ammonia and nitrite, contributing to improving the welfare of the fish and decrease the toxicity of the nitrogenous compounds (Sampaio et al., 2002). The salinized water also has provided increase stocking density in the larviculture of other freshwater fish species. Such as L. alexandri, witch increase the stocking density from 20 to 60 larvae L-1 (Luz and Santos, 2008), O. niloticus witch increase the stocking density from 1 to 30 larvae L-1 (Luz et al., 2012) and C. macropomum witch increase the stocking density from 10 to 50 larvae L<sup>-1</sup> (Santos et al., 2021), using 2 g L<sup>-1</sup> of salinized water.

The optimal stocking density is considered one of the most important factors for the ornamental fish farming success. Since small areas are used for fish production and the commercialization of individuals is carried out at the unit price, not per weight as in the marketing of edible fish (Zuanon et al., 2011). This makes necessary to define adequate stocking densities and strategies that make possible to optimize fish productivity, without compromising fish growth performance and survival (Santos et al., 2021). The present study showing that the salinized water can be an efficient strategy for better growth of H. severus larvae, probably for contribute to the longer survival time of the Artemia nauplii and reduce the osmotic stress of the fish. In addition, the salinized water allows optimizes the use of available space in fish aquarium, increasing the stocking density and the productivity of H. severus larviculture. However, the salinized water must be carefully adjusted and monitored, thus it does not exceed the limits of tolerance and impair the fish growth and survival.

### CONCLUSIONS

The use of different salinized water and stocking densities significantly interferes in the growth performance and survival rate of *H. severus* larvae. The stocking density of 20 larvae  $L^{-1}$  in salinized water at 3 g  $L^{-1}$  is recommended for *H. severus* larviculture. The salinized water can be a strategy to improve feed and productive efficiency in larviculture of this species. However, other studies must be carried out to maximize this fish species production in salinized water with the use of higher stocking densities, which allows the development of new technological information for *H. severus* larviculture.

#### **CONFLICT OF INTERESTS**

Nothing to declare.

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## **AUTHORS' CONTRIBUTIONS**

Oliveira, L.C.C.: Conceptualization, Investigation, Methodology, Data curation, Project administration, Writing original draft. Silveira, B.G.: Conceptualization, Investigation, Methodology. Nascimento, E.T.S.: Conceptualization, Investigation, Methodology. Eiras, B.J.C.F.: Conceptualization, Investigation, Methodology, Data curation, Project administration, Writing - original draft. De Moura, L.B.: Formal Analysis, Writing - review & editing. Salaro, A.L.: Formal Analysis, Writing - review & editing. Cordeiro, C.A.M.: Project administration, Supervision, Validation, Formal Analysis, Investigation, Writing - review & editing. Campelo, D.A.V.: Project administration, Supervision, Validation, Formal Analysis, Investigation, Writing - review & editing.

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