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Short Communication

Larviculture of *Deuterodon iguape* (Eigenmann, 1907) in low salinity: Impacts on larval performance

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ABSTRACT

The larviculture of freshwater fish in closed systems depends largely on the use of live feed, particularly *Artemia* sp. nauplii. Hatching and maintaining these nauplii in saline water can improve their survival, which in turn benefits the growth and health of freshwater fish larvae. This study aimed to evaluate the viability of larviculture of the freshwater species *Deuterodon iguape* under different salinity conditions. Larvae of *D. iguape* obtained through captive breeding were distributed across five treatments with different salinities: T1 (freshwater), T2 (2 ppt), T3 (5 ppt), T4 (10 ppt) and T5 (15 ppt). A total of 20 50-L aquariums were used, and fish growth and water quality parameters were monitored throughout the 42-day experiment. Water quality parameters, including dissolved oxygen, oxygen saturation, electrical conductivity, and alkalinity, were significantly influenced by salinity. Survival rates were notably lower in brackish water treatments, despite higher specific growth rates in surviving larvae. Larviculture of *D. iguape* in brackish water did not yield positive commercial outcomes compared to freshwater. The species exhibited low tolerance to saline conditions, and the increase in salinity adversely affected multiple water quality parameters without improving larval survival, despite better growth performance in survivors.

Keywords: Aquaculture; *Deuterodon iguape*; Survival rate; Brackish water; Zootechnical performance.

Larvicultura de *Deuterodon iguape* (Eigenmann, 1907) em baixa salinidade: Impactos no desempenho larval

RESUMO

A larvicultura de peixes de água doce em sistemas fechados depende amplamente do uso de alimento vivo, especialmente náuplios de *Artemia* sp. A eclosão e manutenção desses organismos em água salinizada podem favorecer sua sobrevivência e, consequentemente, o crescimento e a saúde das larvas de peixes. Este estudo avaliou a viabilidade da larvicultura de *Deuterodon iguape*, espécie dulcícola endêmica, sob diferentes condições de salinidade. Larvas obtidas por reprodução em cativeiro foram distribuídas em cinco tratamentos: T1 (água doce), T2 (2 ppt), T3 (5 ppt), T4 (10 ppt) e T5 (15 ppt). Foram utilizados 20 aquários de 50 litros cada um, e foram monitorados o crescimento dos peixes e a qualidade da água ao longo de 42 dias. Parâmetros como oxigênio dissolvido, saturação, condutividade elétrica e alcalinidade foram significativamente influenciados pela salinidade. As taxas de sobrevivência foram menores nos tratamentos com água salobra, apesar da maior taxa de ganho específico nas larvas sobreviventes. A larvicultura de *D. iguape* em água salinizada não gerou resultados comerciais positivos em comparação à água doce. A espécie mostrou baixa tolerância às condições salinas, e o aumento da salinidade impactou negativamente vários parâmetros de qualidade da água sem promover melhorias na sobrevivência, apesar do melhor desempenho observado nas larvas sobreviventes.

Palavras chave: Aquicultura; *Deuterodon iguape*; Sobrevivência; Água salobra; Desempenho.

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INTRODUCTION

Deuterodon iguape (Eigenmann, 1907), commonly referred to as the Atlantic Forest lambari, is endemic to the Ribeira de Iguape River on Brazil's southeast coast. Like other closely related small characins, collectively known as lambari (*Astyanax* spp.), it is primarily marketed for human consumption as a snack and as live bait for sport fishing (Sabbag et al., 2011; Silva et al., 2011). The species' rapid growth, short life cycle, plankton-feeding habits, ease of handling, and continuous reproductive capacity make it a promising candidate for aquaculture(Castilho-Almeida, 2007; Henriques et al., 2021).

Larviculture of freshwater fish in closed systems relies on live feed, particularly *Artemia* sp. nauplii (Lipscomb et al., 2023). As a crustacean from hypersaline environments, *Artemia* exhibits low survival in freshwater (Beux & Zaniboni Filho, 2006), and its mortality in culture tanks negatively affects larviculture by reducing larval feeding, impairing development, and contributing to water quality deterioration (Dairiki et al., 2022).

One strategy to improve nauplii survival is to increase the salinity of the culture water. The use of brackish water has been successfully applied to several neotropical stenohaline species, often producing results comparable to or better than those observed in freshwater (Jomori et al., 2013; Luz & Santos, 2010; Santos & Luz, 2009). In the case of *D. iguape*, this approach may be particularly feasible due to the species' natural adaptation to estuarine oligohaline waters (Henriques et al., 2021).

In addition, the presence of marinas and companies focused on nautical tourism, particularly recreational fishing, generates a consistent demand for live baits such as *D. iguape* (Henriques et al., 2018). The decline of traditional live bait stocks in estuarine and marine environments, combined with the species' natural ability to survive in estuarine waters, has driven the search for strategies to improve *D. iguape*'s adaptation to brackish conditions, highlighting both ecological and market-driven incentives for the development of sustainable larviculture practices. Thus, the aims of this study were to evaluate whether larviculture of *D. iguape* in low-salinity water can improve larval survival and to assess the associated changes in water quality parameters under different salinity conditions.

MATERIAL AND METHODS

Adult *D. iguape* were obtained under license (SISBIO number 71205-1) and maintained as breeders. Reproduction was induced hormonally using pituitary extract, following the methodology described by Lopes et al. (2013). All procedures complied with the ethical principles for animal experimentation (Ethics Committee on Animal Experimentation, Fishery Institute, No. 02/2018). A total of 2,000 newly hatched larvae were used in this study. At seven days after hatching (DAH), which marked the beginning of the experiment, larvae presented an average weight of 4.5 ± 0.2 mg and an average total length of 4.2 ± 0.3 mm. At this stage, larvae were counted individually and transferred at a density of two larvae per liter into 50-L glass aquariums, each operating as an independent static system and equipped with aeration, and an internal foam filter, following larviculture protocols established for other freshwater species (Jomori et al., 2013; Luz & Santos, 2010). Temperature was maintained by controlling the experimental room using air conditioning, which was kept at 26°C throughout the experiment. The larvae were assigned to five treatments (T) with four replicates each. The treatments corresponded to different salinity levels:

- T1: freshwater (control);
- T2: 2 ppt;
- T3: 5 ppt;
- T4: 10 ppt;
- T5: 15 ppt.

In this study, direct transfer of larvae from freshwater to salinized water was adopted, as preliminary trials indicated no advantage of gradual acclimation, with mortality remaining below 10% after 24 hours.

The experimental aquariums were maintained under a 12:12 h light:dark photoperiod, controlled by an analog timer. Weekly, 50% of the water (25 L) in each aquarium was replaced to remove waste and other materials not captured by the filtration system. Dead larvae were collected and counted daily during tank cleaning to monitor survival and ensure that feeding levels remained appropriate throughout the experimental period. All larvae were fed exclusively with *Artemia* nauplii. For hatching, 2-L incubators were prepared with water at 5 ppt salinity and maintained at 28°C under strong aeration and 15 W LED lighting for 24 hours. *Artemia* cysts (2 g per incubator) were added, and after hatching, the nauplii were separated from the cysts, rinsed with running water, and three 1-mL samples were collected to estimate nauplii density. Nauplii counting was performed in a glass Petri dish under a 40× magnification stereomicroscope. After determining the density, the volume of *Artemia* concentrate to be provided in each aquarium was calculated. Each larva was fed 200 nauplii per day, divided into four daily meals. Feeding was subsequently adjusted weekly, with an increment of 100 nauplii per larva, according to larval development and survival.

Water quality was monitored daily. The parameters measured

included temperature, dissolved oxygen, oxygen saturation, electrical conductivity, pH, alkalinity, ammonia, nitrite, nitrate, and phosphorus. Measurements were performed using a multiparameter probe (Hanna, model 9828) and a multiprocess digital colorimeter (Alfakit, ATP 100). Salinity was maintained constant in each treatment throughout the experimental period, as it was the main factor under investigation. It was monitored daily and adjusted when necessary to ensure stability. During feeding, nauplii were filtered through a fine mesh to minimize the introduction of external water and prevent unintended changes in salinity.

At the end of the 42-day experiment, the larvae were fasted for 12 hours before final biometry. Measurements were performed using a stereoscopic microscope and an analytical balance with 0.0001 mg accuracy. Based on total length (mm), weight (mg), and mortality records, the following performance parameters were calculated:

Survival (S%) (Eq. 1):

$$S = 100 \times (Fxf/Fxi) \quad (1)$$

Where: Fxf: the number of larvae at the end of the experiment;

Fxi: the initial number.

Specific growth rate (SGR %) (Eq. 2):

$$SGR = 100 \times (\ln Wf - \ln Wi) / t \quad (2)$$

Where: Wf: the final average weight; Wi: the initial average weight; t: the duration of the trial in days.

Size uniformity (U%) (Eq. 3):

$$U = (N1/N) \times 100 \quad (3)$$

Where: N1: the number of larvae within ± 10% of the mean weight in each experimental unit; N: the total number of larvae per tank (Nagata et al., 2010).

The experiment was conducted as a completely randomized design. Mean values of the four replicates per treatment were used for statistical analysis. Survival data, expressed as percentages, were transformed according to Eq. 4:

$$\text{Transformed variable} = \text{arcsen}\sqrt{x} \text{ prior to analysis} \quad (4)$$

All data were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test, after confirming normality (Kolmogorov-Smirnov's test) and homoscedasticity (Levene's test). For treatments in which complete mortality occurred, water quality measurements obtained after the death of the last surviving fish were excluded from statistical analyses because they no longer represented active larviculture conditions. Statistical analyses were performed using the R software package.

RESULTS

Salinity significantly influenced the growth performance of *D. iguape* larvae (Table 1). An increase in length and weight was observed for increasing water salinity, which was possibly a direct response to the lower stocking density caused by lower survival rates, as well as the potentially higher survival of nauplii at higher salinities. Larvae reared in saline water showed lower size uniformity and, owing to reduced final densities, exhibited higher specific growth rates. In addition, survival was significantly greater in the freshwater control (Fig. 1).

Throughout the experiment, water temperature remained within the recommended range for *D. iguape* (25.1–27.2°C; Henriques et al., 2021). As expected, salinity significantly affected key water quality parameters. Dissolved oxygen and oxygen saturation decreased with increasing salinity and over time, reaching the lowest values in the highest salinity treatments

Table 1. Productive performance parameters (mean ± standard deviation) of *Deuterodon iguape* larviculture with saline water during the 42 day experimental period (n = 10)*.

Parameters	Salinity				
	Zero	2	5	10	15
Initial length (mm)	4.20 ± 0.30	4.20 ± 0.30	4.20 ± 0.30	4.20 ± 0.30	4.20 ± 0.30
Initial weight (mg)	4.50 ± 0.20	4.50 ± 0.20	4.50 ± 0.20	4.50 ± 0.20	4.50 ± 0.20
Final length (mm)	5.10 ± 0.60	5.10 ± 0.60	5.30 ± 0.40	5.80 ± 1.10	-
Final weight (mg)	48.60 ± 8.20^{b}	50.2 ± 7.40^{b}	52.30 ± 6.30^{b}	72.81 ± 9.90^{a}	-
SGR weight (% Body Weight)	3.91 ± 0.150^{c}	4.15 ± 0.21^{c}	4.86 ± 0.33^{b}	5.46 ± 0.29^{a}	-
Uniformity (%)	71.20 ± 5.30^{a}	60.40 ± 5.10^{b}	56.40 ± 7.50^{b}	36.50 ± 9.60^{c}	-

*lines with different letters indicate significant differences between treatments (Tukey test, $p < 0.05$).

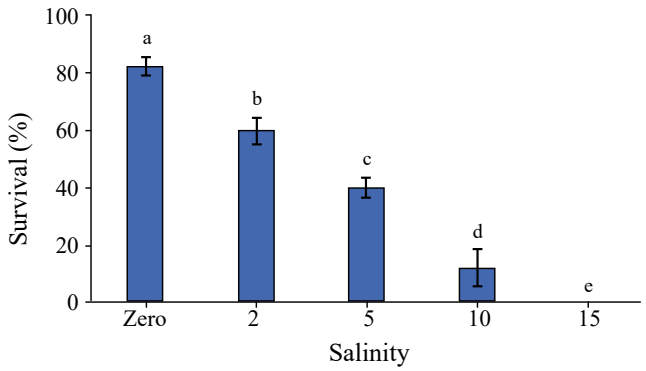

Figure 1. Survival (mean ± standard deviation) in larvae of *Deuterodon iguape* in brackish water during the 42 day experimental period. Bars with different letters indicate significant differences between treatments (Tukey's test, $p < 0.05$).

by week 5 (oxygen: 4.8–5.1 mg·L^{-1}; saturation: 76.5–78.6%; Table 2). Electrical conductivity increased progressively with salinity, from 26 µS·cm^{-1} in the control to over 55 µS·cm^{-1} in the highest salinity treatment, reflecting the sodium chloride content of each treatment. Alkalinity also increased with salinity, ranging from ~14–16 mg $CaCO_3$·L^{-1} in the control to ~25–28 mg $CaCO_3$·L^{-1} in the highest salinity, underscoring clear physiological differences from freshwater that can directly impact larval osmotic balance.

Ammonia and nitrite concentrations also differed significantly among treatments (Table 3). Complete mortality in the 15 ppt treatment occurred during week 5. Therefore, water quality measurements obtained thereafter are presented as unavailable (–) and were excluded from statistical analyses. Ammonia increased over time, particularly in intermediate salinities, reaching values up to 0.80 mg·L^{-1}. Nitrite followed a similar pattern, with higher concentrations observed in saline treatments. These differences may reflect the mortality observed during the initial phases of larviculture, as well as the decomposition of uneaten food and dead *Artemia* nauplii. Nitrate and phosphorus concentrations exhibited an increasing trend throughout the experimental period, which is consistent with the accumulation of metabolic wastes associated with larval growth and feeding activity.

Table 2. Water quality parameters (mean ± standard deviation) in larviculture of *Deuterodon iguape* during the 42 day experimental period (n = 5)*.

Parameter	Salinity					
	Week	0	2	5	10	15
Temperature (°C)	1	25.10 ± 0.60	25.10 ± 0.60	25.10 ± 0.20	25.30 ± 0.50	25.30 ± 0.70
	2	26.30 ± 0.30	26.30 ± 0.30	26.20 ± 0.60	26.30 ± 0.40	26.10 ± 0.50
	3	26.10 ± 0.40	26.10 ± 0.40	26.20 ± 0.30	26.10 ± 0.40	26.30 ± 0.20
	4	25.60 ± 0.50	25.60 ± 0.50	25.70 ± 0.50	25.40 ± 0.80	25.50 ± 0.60
	5	26.40 ± 0.80	26.40 ± 0.80	26.50 ± 0.50	26.30 ± 0.40	26.40 ± 0.50
	6	27.20 ± 0.40	27.20 ± 0.40	27.10 ± 0.20	27.10 ± 0.30	27.00 ± 0.40
Oxygen (mg·L^{-1})	1	6.30 ± 0.40	6.40 ± 0.30	6.20 ± 0.50	6.00 ± 0.40	5.80 ± 0.50
	2	5.80 ± 0.20^a	5.70 ± 0.20^a	5.70 ± 0.20^a	5.40 ± 0.10^b	5.00 ± 0.30^b
	3	5.50 ± 0.30^a	5.40 ± 0.30^a	5.50 ± 0.20^a	5.20 ± 0.10^b	4.80 ± 0.30^b
	4	5.90 ± 0.40^a	5.60 ± 0.30^a	5.60 ± 0.40^a	5.00 ± 0.20^b	5.00 ± 0.20^b
	5	6.00 ± 0.20^a	5.70 ± 0.30^a	5.60 ± 0.30^a	5.00 ± 0.20^b	4.90 ± 0.40^b
	6	6.30 ± 0.30^a	6.10 ± 0.40^a	6.00 ± 0.50^a	5.10 ± 0.30^b	5.10 ± 0.30^b
Oxygen saturation (%)	1	94.20 ± 2.20^a	92.20 ± 2.30^a	90.80 ± 3.90^a	82.10 ± 4.10^b	80.90 ± 3.70^b
	2	90.20 ± 3.10^a	88.60 ± 3.50^a	89.10 ± 4.70^a	80.20 ± 4.60^b	83.20 ± 5.60^b
	3	88.60 ± 4.30^a	87.40 ± 4.30^a	87.30 ± 3.80^a	80.60 ± 3.70^b	80.20 ± 3.30^b
	4	89.50 ± 3.20^a	87.10 ± 3.60^a	85.90 ± 3.60^a	80.20 ± 3.70^b	80.10 ± 3.60^b
	5	86.70 ± 4.70^a	83.10 ± 3.70^a	84.30 ± 4.80^a	79.10 ± 2.90^b	78.60 ± 3.40^b
	6	90.10 ± 4.80^a	88.10 ± 5.80^a	89.70 ± 4.90^a	76.50 ± 5.40^b	76.60 ± 4.90^b
Conductivity (µS·cm^{-3})	1	25.80 ± 5.10^b	26.50 ± 4.10^b	28.60 ± 6.20^b	35.30 ± 5.40^b	44.80 ± 6.70^b
	2	29.80 ± 3.10^b	30.10 ± 2.10^b	29.50 ± 3.80^b	35.20 ± 3.10^b	47.20 ± 3.10^a
	3	30.20 ± 3.60^c	31.20 ± 2.60^c	30.50 ± 2.10^c	39.30 ± 4.10^b	48.90 ± 3.20^a
	4	34.80 ± 2.60^c	35.90 ± 1.60^c	33.80 ± 3.60^c	40.40 ± 3.70^b	50.00 ± 4.20^a
	5	34.90 ± 3.60^c	35.30 ± 2.30^c	34.80 ± 3.80^c	45.60 ± 2.10^b	52.30 ± 5.70^a
	6	38.90 ± 3.40^c	40.10 ± 4.30^c	39.70 ± 5.30^c	50.10 ± 4.30^b	55.10 ± 3.90^a

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Continuation.

Parameter	Salinity					
	Week	0	2	5	10	15
pH	1	6.80 ± 0.20	6.90 ± 0.20	7.00 ± 0.40	7.20 ± 0.30	7.40 ± 0.30
	2	6.80 ± 0.30	6.90 ± 0.30	7.00 ± 0.20	7.20 ± 0.30	7.50 ± 0.20
	3	6.90 ± 0.40	7.00 ± 0.40	7.00 ± 0.20	7.20 ± 0.20	7.50 ± 0.40
	4	7.00 ± 0.30	7.20 ± 0.30	7.00 ± 0.50	7.20 ± 0.30	7.60 ± 0.30
	5	7.00 ± 0.40	7.20 ± 0.40	6.90 ± 0.40	7.10 ± 0.50	7.60 ± 0.40
	6	6.90 ± 0.30	7.10 ± 0.30	6.90 ± 0.20	7.00 ± 0.40	7.60 ± 0.30
Alkalinity (mg $CaCO_3 \cdot L^{-1}$)	1	16.20 ± 1.60^b	16.20 ± 1.50^b	18.60 ± 4.10^b	22.10 ± 2.20^b	28.40 ± 3.10^a
	2	15.90 ± 3.60^c	15.90 ± 3.40^c	17.30 ± 2.60^c	23.30 ± 1.60^b	28.60 ± 3.60^a
	3	15.50 ± 2.40^b	15.50 ± 2.10^b	17.10 ± 5.10^b	22.40 ± 2.40^b	26.30 ± 1.60^a
	4	15.10 ± 3.60^c	15.10 ± 3.20^c	16.00 ± 2.10^c	21.00 ± 2.10^b	25.10 ± 2.30^a
	5	14.40 ± 3.10^c	14.80 ± 3.30^c	15.50 ± 2.30^c	19.30 ± 1.10^b	25.40 ± 1.80^a
	6	13.80 ± 3.20^c	14.90 ± 3.40^c	15.10 ± 2.50^c	19.00 ± 1.10^b	25.50 ± 2.50^a

*Averages on lines with different letters indicate significant differences between treatments (Tukey's test, $p < 0.05$).

Table 3. Nitrogen series and phosphorus (mean ± standard deviation) in larviculture of *Deuterodon iguape* during the 42 day experimental period (n = 5)*.

Parameter	Salinity					
	Week	0	2	5	10	15
Ammonia ($mg \cdot L^{-1}$)	1	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
	2	0.15 ± 0.02^b	0.20 ± 0.10^b	0.20 ± 0.10^b	0.25 ± 0.15^b	0.55 ± 0.15^a
	3	0.25 ± 0.10^b	0.20 ± 0.10^b	0.25 ± 0.10^b	0.35 ± 0.13^b	0.80 ± 0.20^a
	4	0.30 ± 0.10^a	0.30 ± 0.10^a	0.30 ± 0.10^a	0.32 ± 0.11^a	0.00 ± 0.00^b
	5	0.30 ± 0.10^a	0.30 ± 0.10^a	0.30 ± 0.10^a	0.40 ± 0.20^a	0.00 ± 0.00^b
	6	0.50 ± 0.15^a	0.40 ± 0.15^a	0.50 ± 0.15^a	0.40 ± 0.12^a	-
Nitrite ($mg \cdot L^{-1}$)	1	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 002	0.06 ± 0.02	0.06 ± 002
	2	0.06 ± 0.02^b	0.08 ± 0.02^b	0.09 ± 0.03^b	0.09 ± 0.03^b	0.15 ± 0.02^a
	3	0.02 ± 0.01^c	0.03 ± 0.01^c	0.07 ± 0.04^b	0.15 ± 0.09^b	0.25 ± 0.05^a
	4	0.05 ± 0.01^b	0.05 ± 0.01^b	0.07 ± 0.02^b	0.20 ± 0.07^a	0.30 ± 0.08^a
	5	0.04 ± 0.02^b	0.05 ± 0.02^b	0.06 ± 0.02^b	0.15 ± 0.05^a	0.15 ± 0.06^a
	6	0.06 ± 0.02	0.07 ± 0.02	0.06 ± 0.02	0.08 ± 0.02	-
Nitrate ($mg \cdot L^{-1}$)	1	0.68 ± 0.12	0.71 ± 0.15	0.72 ± 0.15	0.77 ± 0.18	0.66 ± 0.11
	2	0.98 ± 0.32	0.93 ± 0.21	0.97 ± 0.22	0.96 ± 0.30	0.95 ± 0.21
	3	1.01 ± 0.35	1.04 ± 0.42	1.08 ± 0.41	1.12 ± 0.31	1.03 ± 0.35
	4	1.20 ± 0.14	1.22 ± 0.18	1.25 ± 0.18	1.28 ± 0.32	1.34 ± 0.45
	5	1.56 ± 0.20	1.61 ± 0.22	1.65 ± 0.43	1.70 ± 0.34	1.70 ± 0.31
	6	2.65 ± 0.70	2.69 ± 0.65	2.76 ± 0.65	3.01 ± 0.98	-
Phosphorous ($mg \cdot L^{-1}$)	1	0.31 ± 0.13	0.33 ± 0.17	0.33 ± 0.15	0.38 ± 0.11	0.35 ± 0.12
	2	0.45 ± 0.22	0.55 ± 0.21	0.49 ± 0.21	0.68 ± 0.19	0.87 ± 0.24
	3	0.55 ± 0.25	0.52 ± 0.18	0.65 ± 0.26	0.88 ± 0.27	1.00 ± 0.20
	4	0.62 ± 0.38	0.59 ± 0.28	0.70 ± 0.41	0.97 ± 0.31	1.51 ± 0.22
	5	0.81 ± 0.32	0.95 ± 0.36	0.97 ± 0.53	1.56 ± 0.43	1.86 ± 0.41
	6	1.21 ± 0.61	1.30 ± 0.51	1.41 ± 0.69	2.21 ± 0.71	-

*Means within a row followed by different letters differ significantly among treatments (Tukey's test, $p < 0.05$). Measurements obtained after total mortality were excluded from statistical analyses; –: unavailable data due to complete mortality of larvae in the 15 ppt treatment during week 5.

Overall, the combination of osmotic stress, reduced dissolved oxygen, and altered nitrogenous compounds in saline treatments likely contributed to the lower survival and reduced size uniformity of *D. iguape* larvae, despite the higher SGR observed in the remaining individuals.

DISCUSSION

Freshwater fish exposed to changes in environmental salinity undergo metabolic and physiological adaptations to enhance survival, including adjustments in ion transport and altered permeability of the gills, kidneys, and intestines (Baldisserotto, 2009). The larval rearing stage, however, is highly sensitive to osmotic stress, which likely led to increased mortality at higher salinities in the present study. This sensitivity can be attributed to the underdevelopment of key osmoregulatory organs during early larval development. During the larval phase, the osmoregulatory function shifts gradually from the skin to the gills, which become the main osmoregulatory site, but this transition is incomplete in early larvae (Varsamos et al., 2005). Larval osmoregulatory capacity is limited by the progressive development of ionocytes in the gills, the kidney, and the urinary bladder, and the ability to produce hypotonic urine at low salinity increases accordingly with ontogeny (Takvam et al., 2021; Charmantier et al., 2022). As a result, early larvae are particularly vulnerable to osmotic stress and altered ionic composition compared to later developmental stages (Agarwal et al., 2024), which might explain the low tolerance of *D. iguape* larvae to salinity (Luz et al., 2008).

Additionally, the reduction in survival at higher salinities likely decreased stocking density and consequently favored larval growth in the remaining individuals, as observed in other freshwater species where lower density increases individual growth even when total survival is low (Oliveira et al., 2022). This pattern may also have been influenced by higher nauplii survival at higher salinities, increasing food availability per surviving larva. In addition to the reduction in stocking density, the enhanced growth observed in larvae reared at 10 ppt may also be partially associated with improved feeding opportunities resulting from the prolonged survival of *Artemia* nauplii under saline conditions. *Artemia* is naturally adapted to hypersaline environments and remains viable for longer periods in brackish water than in freshwater, increasing prey availability and allowing larvae to feed over extended periods. Similar responses have been reported in several freshwater and neotropical species, where low to moderate salinities improved feeding efficiency and growth despite variable effects on survival (Conceição et al., 2010; Jomori et al., 2013). Furthermore, moderate salinity may temporarily reduce the osmotic gradient between body fluids and the external environment, decreasing part of the energetic costs associated with ion regulation and allowing a greater proportion of assimilated energy to be allocated to somatic growth. This mechanism has been documented in several teleost species and may help explain the significantly greater final weight and specific growth rate observed in the surviving *D. iguape* larvae at 10 ppt. However, the potential energetic benefits associated with improved food availability and osmoregulatory adjustments were insufficient to compensate for the negative effects of salinity on survival, indicating that growth enhancement occurred only among the relatively small fraction of larvae capable of tolerating the imposed osmotic conditions. However, in the present study, increased salinity also altered water quality parameters, as conductivity and alkalinity increased, while dissolved oxygen and oxygen saturation decreased, and ammonia and nitrite concentrations differed among treatments. The rapid mortality of *Artemia* nauplii in freshwater may also have contributed to elevated ammonia concentrations and reduced dissolved oxygen levels in those treatments.

Although ammonia and nitrite concentrations remained within ranges generally considered acceptable for freshwater fish (< 1 $mg \cdot L^{-1}$ ammonia; < 0.5 $mg \cdot L^{-1}$ nitrite; < 40 $mg \cdot L^{-1}$ nitrate), larvae are more sensitive to nitrogenous compounds than adults, and values above 0.25 ppm ammonia may cause stress and histological damage (Araújo et al., 2021). In this study, ammonia reached up to 0.80 $mg \cdot L^{-1}$ and nitrite up to 0.30 $mg \cdot L^{-1}$ in some treatments, which, while not acutely lethal, may have contributed to larval stress. Besides, the larvae were exposed to altered ionic composition due to NaCl addition, which may have been particularly stressful for a species adapted to oligotrophic Atlantic Forest streams. *D. iguape* is endemic to small coastal rivers and streams of São Paulo state in the Atlantic Forest, including the Ribeira de Iguape River basin, where water is typically oligotrophic (Leonardo et al., 2008). Species adapted to oligotrophic environments are often sensitive to changes in ionic composition, as their osmoregulatory physiology is optimized for low-ion waters. Larvae are particularly sensitive to such changes because their osmoregulatory organs are still developing, making them less capable of compensating for ionic imbalances compared to adults (Varsamos et al., 2005). The relationship between water chemistry and ion regulation is critical for teleost survival and growth, as changes in water hardness and ionic composition can disrupt osmoregulatory function (Parra & Baldisserotto, 2019). The combination of osmotic stress, reduced dissolved oxygen, and altered water

chemistry likely contributed to the high mortality observed in saline treatments.

In this study, salinities of 10 and 15 ppt resulted in extremely low survival rates, indicating that these conditions are unsuitable for *D. iguape* larviculture. Recent work on related freshwater species confirms that while moderate salinization may improve growth under certain stocking densities, higher salinities are detrimental to survival and size uniformity (Oliveira et al., 2022; Manchin-Bertolini et al., 2025). The rapid mortality of *D. iguape* larvae at 6 $g \cdot L^{-1}$ NaCl aligns with findings by Manchin-Bertolini et al. (2025), who reported zero survival of *Astyanax altiparanae* larvae after 10 days at 0.6% NaCl (6 $g \cdot L^{-1}$), and significantly reduced survival at 0.4% NaCl ($p = 0.0010$). Both Characiformes species from Atlantic Forest streams show similar salinity intolerance during larval development. Therefore, freshwater remains the most suitable condition for *D. iguape* larviculture.

Larviculture of freshwater fish fed with *Artemia* spp. benefits from increased salinity, which extends nauplii survival, thereby enhancing larval growth and survival (Jomori et al., 2013). However, the effects of saline water on SGR and overall performance remain inconsistent across freshwater species. In this sense, it was already determined that larvae of several freshwater species, including *Hoplias lacerdae*, *Colossoma macropomum*, *Brycon amazonicus*, *Astronotus ocellatus*, and *Leporinus macrocephalus*, can tolerate salinities of 2–4 $g \cdot L^{-1}$ without adverse effects on growth or survival (Jomori et al., 2013; Luz & Portella, 2002).

Recent work with the yellowtail tetra *Astyanax altiparanae*, a related characid from the same basin, demonstrated that 0.2% NaCl (2 $g \cdot L^{-1}$) improved survival and growth compared to freshwater, while ≥ 0.4% (≥ 4 $g \cdot L^{-1}$) significantly reduced survival (Manchin-Bertolini et al., 2025). However, studies have shown that a salinity of 8 $g \cdot L^{-1}$ significantly reduces the survival of *Rhamdia quelen* larvae (Fabregat et al., 2015). Similarly, *Pyrrhulina brevis* larvae exposed to salinities of 0, 2, 4, and 6 $g \cdot L^{-1}$ exhibited mortality greater than 65% at salinities ≥ 4 $g \cdot L^{-1}$, whereas salinities ≤ 2 $g \cdot L^{-1}$ resulted in better survival, indicating a clear relationship between mortality and salinity at levels below 4 $g \cdot L^{-1}$ (Abe et al., 2015). In the present study, even low salinities (2–6 $g \cdot L^{-1}$) resulted in markedly lower survival compared to freshwater, suggesting that *D. iguape* larvae are more sensitive than *A. altiparanae* and other related characids. Phylogenetic and ecological differences likely explain this variation in salinity tolerance among closely related species.

In the present study with *D. iguape*, salinity produced contrasting biological responses. While the surviving larvae reared at 10 ppt exhibited significantly greater final weight and SGR than those maintained in freshwater, these improvements occurred simultaneously with substantial reductions in survival and size uniformity. Such results indicate that salinity acted as a selective environmental stressor, favoring the growth of a limited number of tolerant individuals while reducing overall production efficiency. Although higher salinity extended the lifespan of *Artemia* nauplii, and resulted in higher SGR in surviving larvae, this did not translate into improved larval survival or total biomass production. The higher SGR observed in saline treatments likely resulted from reduced stocking density due to mortality, rather than a direct benefit of salinity. The young larvae of *D. iguape* likely experienced higher energy expenditure for osmoregulation and increased sensitivity to saline conditions, despite the species' tolerance for low salinity as adults (Barbieri et al., 2019).

In other studies, conducted with lambari fish, acute exposure of *Astyanax bimaculatus* and *D. iguape* to varied salinities induced physiological and behavioral alterations, including increased specific ammonia excretion, changes in specific oxygen consumption, and reduced swimming acceleration over short periods of time (Barbieri et al., 2019; Carneiro, 2019), which may help explain the lower survival observed under long-term exposure in the present study. Nonetheless, evidence suggests that these effects may be transient, with individuals potentially stabilizing through physiological adjustments to the new salinity conditions. In related species, such as *Astyanax altiparanae*, larvae from 21 days of age tolerated salinities of up to 6% without adverse effects on performance or survival (Rufino, 2021), indicating that the negative responses observed here could be influenced by additional factors—such as age, exposure duration, nutrition, or water quality—rather than salinity alone.

Salinity tolerance increases markedly at the transition from larva to juvenile, as osmoregulatory functions are hormonally controlled and linked to developmental changes (Varsamos et al., 2005), and blood osmolality changes progressively during postembryonic development, reflecting maturation of osmoregulatory capacity (Charmantier et al., 2022). Thus, a possible direction for future studies on *D. iguape*'s adaptation to brackish waters is to focus on juveniles, since larvae are generally more sensitive to salinity changes than older life stages (Zhao et al., 2015). In previous trials, *D. iguape* juveniles exposed abruptly to different salinities (2.5, 5, 7.5, 10, and 12.5 $g \cdot L^{-1}$) showed changes in hematological parameters (serum glucose, hemoglobin, and total protein) and mild to moderate histological alterations in gills and kidneys, indicating limited tolerance to sudden saline exposure (Barbieri et al., 2019).

Gradual acclimation, through stepwise increases in salinity up to previously established tolerable limits, combined with close management of water parameters (*e.g.*, more frequent partial water exchanges and careful adjustment of pH and alkalinity), may improve survival and performance under brackish conditions and should be considered in future studies.

CONCLUSION

Considering the findings of this study, although the use of brackish water initially appeared potentially beneficial, larviculture of *D. iguape* under saline conditions did not yield positive results compared to freshwater. *D. iguape* larvae seems to be highly sensitive to osmotic imbalance, and even low salinities significantly altered key water quality parameters, including electrical conductivity, alkalinity, dissolved oxygen, and oxygen saturation. The combination of osmotic stress, reduced dissolved oxygen, and elevated ammonia and nitrite concentrations likely contributed to the high mortality observed in saline treatments. This reduction in mortality led to lower stocking densities, which in turn appears to have increased the SGR of the surviving larvae, despite their reduced size uniformity. Therefore, freshwater remains the most suitable condition for *D. iguape* larviculture.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

Data will be available upon request.

AUTHORS' CONTRIBUTIONS

Formal Analysis: Olier, B.S.; **Investigation:** Olier, B.S., Sousa, O.M.; **Data curation:** Olier, B.S., Sousa, O.M., Maganhe, B.L.; **Writing – original draft:** Olier, B.S., Sousa, O.M., Maganhe, B.L.; **Conceptualization:** Henriques, M.B., Sanches, E.G.; **Resources:** Henriques, M.B., Sanches, E.G.; **Supervision:** Henriques, M.B., Sanches, E.G.; **Validation:** Henriques, M.B., Sanches, E.G.; **Final approval:** Maganhe, B.L.

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DECLARATION ON THE USE OF ARTIFICIAL INTELLIGENCE TOOLS

During the preparation of this manuscript, artificial intelligence (AI) tools were used solely for grammar and language verification. No AI tool was used to generate scientific content, analyze data, interpret results, or draft the intellectual content of the manuscript. The authors reviewed and approved all final text and take full responsibility for the work.

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