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Salinity and prey concentration on larviculture of killifish *Hypsolebias radiseriatus* (Cyprinodontiformes: Rivulidae)

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ABSTRACT. The aim of this study was to investigate the tolerance of *Hypsolebias radiseriatus* larvae to different salinities, and the effects of different prey concentrations and water salinities on the larviculture of this species. Salinity tolerance was tested by subjecting newly-hatched larvae to 96 hours of osmotic shock testing (experiment I) and gradual acclimatization (experiment II) of the following salinities: freshwater (control), 2, 4, 6 and 8 g of salt L^{-1}. A third experiment (experiment III) evaluated three water salinities (S_0 - freshwater, S_2 - 2 g of salt L^{-1} and S_4 – 4 g of salt L^{-1}) and three initial daily prey concentrations (100, 300 and 500 artemia nauplii $larva^{-1}$). In experiments I and II, survival was only influenced by the salinity of 8 g of salt L^{-1} ($p < 0.01$). After 35 days, weight was only influenced by prey concentration ($p < 0.05$), with the highest value being with 500 artemia nauplii $larva^{-1}$. The lowest survival was for 4 g of salt L^{-1} and for 100 artemia nauplii $larva^{-1}$. *H. radiseriatus* larviculture can be carried out in salinity of up to 2 g of salt L^{-1} and initial daily prey concentrations with 500 artemia nauplii $larva^{-1}$.

Keywords: artemia nauplii; annual fish; freshwater; larvae; salt.

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Introduction

The term 'killifish' is a generic classification for all species of oviparous fish of the order Cyprinodontiformes and family Rivulidae. Most Rivulidae fishes are popularly known as annual fishes, and they live in ephemeral environments such as pools, that obligatorily dry out seasonally, which causes the death of adult individuals (Gonçalves, Souza, & Volcan, 2011). The annual killifish occur only in African and South American (Costa, 2008). Annual killifish are known to be the only vertebrates whose embryos can, at some point depending on environmental conditions, opt for distinct developemental pathways of either direct development or diapause (Podrabsky, Garrett, & Kohl, 2010).

Among these species, are those of the genus *Hypsolebias*, which are distributed in the basins of the São Francisco, Tocantins, Medium Jequitinhonha and Lower Jaguaribe rivers, and in isolated basins in Northeast Brazil (Costa, Amorim, & Braganca, 2014; Ponzetto, Britzke, Nielsen, Parise-Maltempi, & Alves, 2016). The species of killifish *Hypsolebias radiseriatus* is phylogenetically related to a complex of species linked to the killifish *Hypsolebias flavicaudatus* group (Ponzetto et al., 2016). There have been few studies on killifish larviculture, among which are those that have focused on the effect of temperature on the initial development of killifish *Austrolebias nigrofasciatus* (Volcan, Fonseca, Figueiredo, Sampaio, & Robaldo, 2012) and *Austrolebias wolterstorffi* (Fonseca, Volcan, Sampaio, Romano, & Robaldo, 2013). Another study investsigated the larviculture of Gulf killifish *Fundulus grandis* and evaluated the replacement of artemia nauplii in the initial feed with a prepared diet (Patterson et al., 2016). However, until the execution of this study, there was no published data on the larviculture of *H. radiseriatus*.

In 2013, the Chico Mendes Institute for Biodiversity Conservation (ICMBio), through the Brazilian Ministry of Environment, approved the implementation of the National Plan for the Conservation of Endangered Rivulidae Fishes (PAN Rivulidae), with 53 species of particular interest. *Ex situ* studies of endangered species are among the actions proposed in this plan, which suport projects to maintain genetically viable populations in captivity and the development of appropriate reproductive management protocols for different groups of killifish (Instituto Chico Mendes de Conservação da Biodiversidade

[ICMBio], 2013). Furthermore, killifish have been marketed as ornamental fish due to the variety of their colors and shapes, thus adding value to the production of these species.

The Artemia nauplii has been the main live feed provided during the intensive larviculture of neotropical freshwater fish species, because it is size compatible with larva mouth opening and meets nutritional needs of fish at this stage (Jomori, Carneiro, Malheiros, & Portella, 2003; Lombardi & Gomes, 2008; Rocha, Carvalho, & Sampaio, 2008; Phelps, 2010). In addition, at the beginning of exogenous feeding, the concentration of live food daily offered is of great relevance, because when used in adequate quantity, it can positively affect growth (Santos & Luz, 2009; Santos, Pedreira, & Luz, 2012; Luz & Portella, 2015; Santos, Correia, & Luz, 2015) and increase survival (Takata, Silva, Costa, Melillo Filho, & Luz, 2014).

Among the managments used in freshwater fish larviculture, the use of salinity is of particular interest because it can increase the survival of artemia nauplii, which are used as live food (Jomori, Luz, & Portella, 2012). Tests with 96-hour trials have been conducted to assess tolerance of freshwater fish larvae to different salinities (Fashina-Bombata & Busari 2003; Luz & Santos 2008a; Wang, Guo, Zhao, Liu, & Lu, 2015). Larviculture has been performed using low salinities (2 to 4 g of salt L^{-1}), which have proven to be a beneficial management practice for several freshwater species, such as neotropical trahira *Hoplias lacerdae* (Luz & Portella, 2002) dwarf marbled catfish *Lophiosilurus alexandri* (Luz & Santos, 2008b); flannel-mouth characiform *Prochilodus costatus*; long-whiskered catfish *Pseudoplatystoma corruscans* (Santos & Luz, 2009) and armored catfish *Rhinelepis aspera* (Luz & Santos, 2010); and pacu *Piaractus mesopotamicus* (Jomori et al., 2012); oscar *Astronotus ocellatus*, *Brycon amazonicus*, cachama *Colossoma macropomum* and *Leporinus macrocephalus* (Jomori, Luz, Takata, Fabregat, & Portela, 2013); and Nile tilapia *Oreochomis niloticus* (Luz, Santos, Melillo Filho, Turra, & Teixeira, 2013). The positive effect of slightly salinized water for these species can be attributed to the reduction of energy expenditure for ionic and osmotic regulation in salinized water, in relation to freshwater (Morgan, Sakamoto, Grau, & Iwama, 1997). Other aspects related to the use of salt for freshwater fish are the reduction of toxicity of nitrogen compounds (Yanbo, Wenju, Weifen, & Zirong, 2006; Sampaio, Wasielesky, & Miranda-Filho, 2002) and the preventive effect on the proliferation of diseases (Souza-Bastos & Freire, 2009; Németh, Horváth, Felföldi, Beliczky, & Demeter, 2013).

The aim of the present study was to evaluate the tolerance of *H. radiseriatus* larvae to different salinities and the effectiveness of its larviculture under different prey concentrations and water salinities.

Material and methods

Three experiments were carried out according to the protocol of the Ethical Commission on Animal Use (Protocol CEUA: 344/2016). Fertilized fish eggs were supplied by the Zoobotany Foundation of Belo Horizonte, Brazil, which were derived from the breeding stock of fish caught in a natural environment with the authorization of the State Institute of Minas Gerais Forests (License Scientific for Fishing - Category D - No. 011-09) in the city of Jaíba, Minas Gerais, where a new population of this species was found (15°21'29.00"S 43°41'19.50"W). The group of broodstock, composed of 2 males and 28 females, were kept in a 160 L aquarium.

The three experiments were performed using larvae hatched from embryonated eggs, kept in 1L-beakers containing freshwater at 28°C. Prior to their first feeding, the newly hatched larvae were transferred to experimental units. For initial biometry, a sample of 20 newly hatched larvae were weighed on an analytical balance (Mars AY220-0.001g, Santa Rita do Sapucaí, Brazil) and measured for total length with a digital caliper (Starret Ltda., Itu, Brazil, series 799A).

Experiment I. Salinity tolerance - osmotic shock

In order to assess osmotic shock, we prepared water of different salinities, and the salinity, temperature, conductivity and pH were daily measured with the aid of an appropriate instrument (Hanna Instruments Combo pH and EC Tester, Woonsocket, USA) (Table 1). Non-iodized coarse salt (MARISAL LTDA - ingredients: sodium chloride and anti-foaming INS 535 sodium ferrocyanide) was used for water salinization.

After hatching, the larvae (4.98 ± 0.7 mm and 1.02 mg) of the same spawn were transferred directly to beakers containing 1 L of water at a density of 10 larvae L^{-1}, supplementary aeration, and at the following salinities: freshwater (control), 2, 4, 6 and 8 g of salt L^{-1}. A completely randomized design of the five different water salinities was used, with three replicates each. The larvae were observed every hour for the first four hours, and thereafter every 24 hours until the total testing period of 96 hours. The experimental units were maintained in a static system without water renewal, at a constant temperature of 28ºC and monitoring of salinity (Table 1). Each

unit was aerated by using a porous stone, in order to maintain dissolved oxygen levels above 5.0 mg L^{-1}. During this period, the larvae were not fed, and dead larvae were quantified and removed. At the end of the experimental period, survival and biometry (weight and length) of the larvae were evaluated.

Table 1. Mean values (± standard deviation) for physico-chemical variables of the different water salinity treatments used for testing osmotic shock and gradual acclimatization tolerance by *Hypsolebias radiseriatus* larvae.

	Salinity (g of salt L^{-1})				
	Freshwater	2	4	6	8
	Osmotic shock (Experiment I)				
pH	8.29 ± 0.12	8.13 ± 0.09	8.13 ± 0.08	8.10 ± 0.07	8.06 ± 0.09
Temperature (ºC)	28.00 ± 0.28	28.00 ± 0.32	28.15 ± 0.30	28.20 ± 0.35	28.00 ± 0.45
Conductivity (mS cm^{-1})	0.30 ± 0.07	4.17 ± 0.05	8.29 ± 0.09	12.35 ± 0.10	16.35 ± 0.10
	Gradual acclimatization (Experiment II)				
pH	8.22 ± 0.38	8.06 ± 0.43	8.03 ± 0.47	7.98 ± 0.47	7.95 ± 0.42
Temperature(ºC)	28.05 ± 0.24	28.05 ± 0.24	28.00 ± 0.27	28.05 ± 0.26	28.05 ± 0.28
Conductivity (mS cm^{-1})	0.24 ± 0.08	4.23 ± 0.10	8.25 ± 0.29	12.23 ± 0.49	16.27 ± 0.79

Experiment II. Salinity tolerance - gradual acclimatization

Newly hatched larvae (4.98 ± 0.7 mm and 1.02 mg) of the same spawn were transferred to beakers containing 1 L of fresh water at a density of 10 larvae L^{-1}. For acclimatization to the different salinity concentrations, 2 g of salt L^{-1} was added every two hours until the final concentrations of 2, 4, 6 and 8 g of salt L^{-1} were reached (Table 1). A completely randomized design with 5 different water salinities and three replicates each was used. Mortality was assessed every 24 hours for a total period of 96h. The experimental units were maintained in a static system without water renewal, at a constant temperature of 28ºC, and each unit was aerated by using a porous stone, in order to maintain dissolved oxygen levels above 5.0 mg L^{-1}. During this period, the animals were not fed, and dead larvae were quantified and removed. At the end of the experimental period, survival and biometry (weight and length) of the larvae were evaluated.

Experiment III. Larviculture in different water salinities and prey concentrations

Newly hatched larvae (4.9 ± 0.5 mm and 0.904 mg) were transferred to 27 experimental units containing 2 L of useful volume of water at a density of 4 larvae L^{-1}. The experimental units were maintained in a thermostat-controlled bath system with water temperature of 28°C. Each unit was aerated by using a porous stone, in order to maintain dissolved oxygen levels above 5.0 mg L^{-1}.

According to the data collected from the experiments 1 and 2, three water salinities were tested (S_0 - freshwater, S_2 – 2 g of salt L^{-1} and S4 – 4 g of salt L^{-1}), along with three daily prey concentrations (Table 2). Non-iodized coarse salt (MARISAL LTDA - ingredients: sodium chloride and anti-foaming INS 535 sodium ferrocyanide) was used for water salinization. The prey concentrations used were based on previous studies with larvae of other species of freshwater fish (Santos & Luz, 2009; Luz & Portella, 2015). The experiment was conducted in a 3×3 factorial scheme with three replicates each. Larvae were fed newly hatched artemia nauplii twice a day, at 9 a.m. and 4 p.m.

Table 2. Daily concentrations of *Artemia nauplii* (P) (nauplii $larva^{-1}$) offered to the larvae of *Hypsolebias radiseriatus* during 35 days of feeding, for the different salinities tested.

Treatments	Feeding period						
	1-5 days	6-10 days	11-15 days	16-20 days	21-25 days	26-30 days	31-35 days
	Daily prey concentrations (nauplii $larva^{-1}$)						
P_{100}	100	150	225	338	506	760	1,139
P_{300}	300	450	675	1,013	1,520	2,280	3,420
P_{500}	500	750	1,125	1,688	2,532	3,798	5,697

After 15 and 35 days, survival and biometry of all animals were evaluated. For biometric measurements, larvae were anesthetized in eugenol solution (80 mg L^{-1}), weighed and measured for total length, as previously described, and then subsequently returned to the culture (Cordeiro et al., 2016).

Daily, prior to the supply of the 4 p.m. feeding, approximately 50% of the water volume of the experimental units was renewed. For renewal, water of the different salinities was prepared separately

according to the treatments and at the same temperature of the culture. Water pH, temperature, salinity and total ammonia were checked daily prior to the morning feeding. Ammonia values were measured using a commercial colorimetric kit (Alcon Labcon, Camboriú, Brazil), while pH, temperature and salinity values were measured as previously described.

Statistical analysis

The data were analyzed by the software Assistat 7.7. Data from experiments 1 and 2 were submitted to ANOVA and the means were compared using Tukey's test at 5% probability. The survival from experiment 1 and 2 underwent arcosine transformation before being analyzed. Data from experiment 3 were submitted to parametric factorial ANOVA and means were compared using Tukey's test at 5% probability. Survival from experiment 3 underwent arcsine transformation before being analyzed.

Results and discussion

The Table 3 shows the results of survival, weight and total length (L_T) after 96h for the osmotic shock test (experiment I) and gradual acclimation (experiment II). In both experiments, survival was influenced only at the salinity of 8 g of salt L^{-1} ($p < 0.05$). In the salinity tests, the negative effect of osmotic shock on larval weight, starting at 6 g of salt L^{-1}, was not evident during gradual acclimatization, and there was no differences among treatments. This finding also indicates that *H. radiseriatus* larvae can tolerate the different salinities when acclimatized. Total length was also affected ($p < 0.05$) by salinity, but only in the salinity of 8 g of salt L^{-1} in the treatment with osmotic shock; there were no differences among treatments with acclimation ($p > 0.05$). The survival of *H. radiseriatus* larvae was negatively affected by the salinity of 8 g of salt L^{-1} for both the osmotic shock and gradual acclimatization experiments. In a study of *Rhamdia quelen* larvae, at the beginning of exogenous feeding, the survival rate for a 96h salinity tolerance test, without acclimatization, began to show an effect at 6 g of salt L^{-1} (Fabregat et al., 2015). In a study of newly hatched *L. alexandri* larvae, the survival was of 100% up to 4 g of salt L^{-1}, followed by total mortality at 6 g of salt L^{-1} (Luz & Santos, 2008a). Taken together, these results indicate that salinity tolerance is species-specific. However, in the present study, salinity acclimation to 8 g of salt L^{-1} provided greater survival than via osmotic shock. This finding indicates that gradual acclimatization favors the succesfull of larvae during the process of osmoregulation.

Table 3. Mean values (± standard deviation) of survival (%), weight (mg) and total length (TL; mm) of *Hypsolebias radiseriatus* larvae 96 hours after the initiation of the osmotic shock tolerance test (Experiment I) and gradual acclimatization (Experiment II) in different salinities.

Variables	Water salinity (g of salt L^{-1})				
	Freshwater	2	4	6	8
	Osmotic shock (Experiment I)				
Survival	100.0^{a}	100.0^{a}	93.3±0.58^{a}	86.7±1.15^{a}	43.3±1.52^{b}
Weight	0.99±0.06^{a}	0.78±0.07^{ab}	0.88±0.04^{ab}	0.69±0.07^{b}	0.64±0.13^{b}
TL	4.94±0.06^{a}	4.83±0.06^{ab}	4.78±0.18^{ab}	4.79±0.01^{ab}	4.60±0.05^{b}
	Gradual acclimatization (Experiment II)				
Survival	100.0^{a}	100.0^{a}	100.0^{a}	93.3±1.15^{a}	76.6±0.58^{b}
Weight	0.92±0.07^{a}	0.76±0.10^{a}	0.71±0.04^{a}	0.66±0.20^{a}	0.71±0.13^{a}
TL	4.96±0.08^{a}	4.85±0.06^{a}	4.83±0.15^{a}	4.82±0.067^{a}	4.74±0.08^{a}

Means in the same line followed by the same letter do not differ statistically according to Tukey's test ($p > 0.05$).

During larviculture, pH was not affected by salinity, prey concentration or the interaction between these factors (SxP; $p > 0.05$) (Table 4). For total ammonia, there was no effect of salinity or the interaction (SxP; $p > 0.05$), however, prey concentration did have an effect ($p < 0.01$), with higher values for P500. During larviculture (experiment III), the water quality was maintained with regard to pH, indicating that both the salinities and prey concentrations tested did not affect this parameter. However, the lowest pH value for experiment III in relation to I and II can be attributed to the increase of the organic matter input. For total ammonia, an increase in prey concentration led to a deterioration of water quality, regardless of the salinity tested. This result is due to the greater amount of food offered, as well as the greater excretion of feces by larvae and static system used in the experiment. Similar results were recorded for the larvae of *P. corruscans*, *P. costatus*, *L. alexandri* (Santos & Luz, 2009) and *H. larcedae* (Luz & Portella, 2015) when using high prey concentrations. Therefore, when using higher concentrations of prey, the use of water recirculation system,

mechanical and biological filters, ammonia concentrations could probably be minimized (Luz et al., 2012). Nevertheless, additional studies are needed for determining the tolerance of *H. radiseriatus* larvae to total ammonia under different culture conditions.

Table 4. Mean values (± standard deviation) for physico-chemical variables for the water salinities (S) and prey concentrations (P) tested on *Hypsolebias radiseriatus* larviculture.

Statistics	*F* values	
	pH	Total ammonia
Salinity (S)	2.84^{ns}	0.60^{ns}
Prey concentration (P)	1.37^{ns}	17.06^{**}
Interaction SxP	2.83^{ns}	2.61^{ns}
Salinity	Means	
S_0	6.90 ± 0.11	0.44 ± 0.27
S_2	6.85 ± 0.10	0.56 ± 0.37
S_4	6.95 ± 0.08	0.58 ± 0.65
Prey concentration	Means	
P_{100}	6.86 ± 0,12	0.17 ± 0.18^{b}
P_{300}	6.91 ± 0,09	0.47 ± 0.23^{b}
P_{500}	6.93 ± 0,10	0.94 ± 0.46^{a}

Means in the same columm followed by the same letter do not differ statistically according to Tukey's test ($p > 0.05$). $^{*}(p < 0.05)$; $^{**}(p < 0.01)$; ^{ns}(not significative).

It is worth mentioning that during the first 15 days of larviculture, the weight of larvae in 2 g of salt L^{-1} was greater than that in freshwater and in 4 g of salt L^{-1} at the highest prey concentration tested. It is known that the addition of salt to water reduces the level of ammonia in the blood in fish, because in fresh water there is less availability of the Na^{+} ion for the realization of the exchange through the gills of NH_4^{+} present in the blood (Yanbo et al., 2006; Sampaio et al., 2002). According to the same authors, the gills are preferred for absorption by chloride ions, which results in a competitive exclusion of nitrite absorption in salinized water in relation to fresh water. This fact may have contributed to the better performance of *H. radiseriatus* larvae at higher levels of total ammonia in 2 g of salt L^{-1}. Furthermore, at 4 g of salt L^{-1}, the negative effect may have been potentiated by salinity. The salinity of 4 g of salt L^{-1} also impaired the performance of *L. alexandri* larvae at higher densities (60 larvae L^{-1}), when compared to salinity of 2 g of salt L^{-1} at the same density (Luz & Santos, 2008b). According to these authors, the lower performance may have been a consequence of the negative effects associated with the higher salinity and stocking density tested.

Table 5 presents the performance and survival from *H. radiseriatus* larviculture. Survival after 15 days was only affected by salinity ($p < 0.01$), with lower values for S_4 (Table 5). After 35 days, survival was influenced by salinity ($p < 0.01$) and prey concentration ($p < 0.05$), but not by their interaction ($p > 0.05$), with the the lowest survival for S_4 and for P_{100}. During the first 15 days of feeding, the different prey concentrations had no effect on the survival of *H. radiseriatus*. Similar results were observed for p. *corruscans* over the first five days, for *P. costatus* and *L. alexandri* over the first 10 days (Santos & Luz, 2009), for *R. aspera* over the first 7 days (Santos et al., 2012) and for *H. larcedae* over the first 15 days of feeding (Luz & Portella, 2015). These studies indicate that, in the studied species, survival is not affected by prey concentration during the initial phase of development. However, from 16 to 35 days of feeding, the initial prey concentration of P_{500} positively influenced the survival of *H. radiseriatus*. This finding may be due to a lack of sufficient food for larvae developing at the lower prey concentrations, a hypothesis also supported by the lower weight of these animals. Thus, further tests with higher prey concentrations for larvae of this age are required. In addition, culture protocols for freshwater species use live feed for usually up to two weeks, when the use of formulated diets begins, which makes it difficult to establish the ideal amount of food to be offered over longer periods of growth for many species.

Regarding water salinity, survival was only impaired at the salinity of 4 g of salt L^{-1} at 15 and 35 days. These data are in agreement with the initial tolerance to osmotic shock observed in the experiment I. Still, in spite of the greater tolerance acquired during gradual acclimatization (experiment II), long periods of maintenance in a salinity of 4 g of salt L^{-1} can be harmful to animals. The decrease in survival rate observed for the higher salinities tested is related to the increased energy expenditure required for the processes of osmoregulation (Kilambi 1980). As in the present study, the salinity of 2 g of salt L^{-1} can be used for larviclture of other freshwater species, such as *H. lacerdae* (Luz

& Portella, 2002), *L. alexandri* (Luz & Santos, 2008b), *P. corruscans* (Santos & Luz, 2009), *P. mesopotamicus* (Jomori et al., 2012) and *R. quelen* (Fabregat et al., 2015).

Table 5. Mean values (± standard deviation) of total length (L_T; mm), weight (*W*; mg), daily specific growth rate (SGR; % day^{-1}) and survival (S %) during larviculture of *Hypsolebias radiseriatus* in different water salinities (S) and prey concentrations (P).

Statistics	Valores de F							
	L_T 15 days	L_T 35 days	*W* 15 days	*W* 35 days	SGR 1-15 days	SGR 16-35 days	S 15 days	S 35 days
Salinity (S)	53.30^{**}	1.70^{ns}	20.00^{**}	0.27^{ns}	13.39^{**}	1.06^{ns}	25.55^{**}	33.48^{**}
Prey conc. (P)	44.68^{**}	2.12^{ns}	29.47^{**}	4.05^{*}	$20{,}43^{**}$	0.02^{ns}	1.65^{ns}	4.6^{*}
Inter. S x P	7.19^{**}	0.07^{ns}	3.82^{*}	0.19^{ns}	0.97^{ns}	0.69^{ns}	0.2^{ns}	1.05^{ns}
Averages by salt concentration								
S_0	12.18 ± 1.40	15.57 ± 1.86	19.49 ± 7.88	40.65 ± 13.37	19.91 ± 2.99^{a}	3.82 ± 1.24	93.06 ± 9.08^{a}	79.17 ± 8.84^{a}
S_2	12.85 ± 12.43	15.84 ± 0.02	25.87 ± 12.43	46.83 ± 20.35	21.56 ± 3.62^{a}	3.11 ± 1.23	87.50 ± 12.50^{a}	80.56 ± 12.67^{a}
S_4	9.51 ± 2.11	12.06 ± 2.25	11.96 ± 5.91	39.33 ± 34.42	16.28 ± 4.07^{b}	4.71 ± 3.36	52.78 ± 15.02^{b}	43.06 ± 15.45^{b}
Means for prey concentration (P)								
P_{100}	9.65 ± 1.19	11.94 ± 4.71	9.86 ± 3.39	24.60 ± 15.40^{b}	15.42 ± 3.09^{b}	3.86 ± 2.54	72.22 ± 21.45	61.11 ± 17.05^{b}
P_{300}	12.43 ± 1.19	14.96 ± 5.88	21.03 ± 5.55	49.07 ± 24.85^{ab}	20.76 ± 1.86^{a}	3.97 ± 2.48	77.78 ± 23.20	65.28 ± 24.83^{ab}
P_{500}	12.47 ± 17.89	16.57 ± 0.02	26.43 ± 12.55	53.15 ± 20.83^{a}	21.59 ± 4.11^{a}	3.97 ± 2.48	83.33 ± 21.65	76.39 ± 21.14^{a}
CV%	6.31	33.46	24.4	54.44	11.51	60.20	16.66	16.31

Means in the same columm followed by the same letter do not differ statistically according to Tukey's test ($p > 0.05$). *($p < 0.05$); **($p < 0.01$); ^{ns}(not significative).

After 15 days, total length showed effect for salinity, prey concentration and the interaction between these factors ($p < 0.01$). For each prey concentration, the lowest values were for the highest water salinity tested (Table 6). For the salinities of S0 and S2, the shortest length was obtained for P100, while for S4 the greatest length was with P300. After 35 days, salinity, prey concentration and their interaction did not show effects on total length ($p > 0.05$). After 15 days, weight showed an effect of salinity ($p < 0.01$), prey concentration ($p < 0.01$) and their interaction ($p < 0.05$) (Table 5). In S0 and S2 the greatest weight was for the highest concentraton of P500 ($p < 0.05$), while for S4, the weight were similar among prey concentrations ($p > 0.05$) (Table 6). Within the P100 treatment, there were no differences among animals in the different salinities ($p > 0.05$), however, for P300 and P500, greater weight was obtained in salinity S2 ($p < 0.05$). At 35 days, weight was only influenced by prey concentration ($p < 0.05$), with the greatest values being for P500 (Table 5). The specific growth rate (SGR) during the first 15 days experienced an effect of salinity ($p < 0.01$) and prey concentration ($p < 0.01$), but not of their interaction ($p > 0.05$) (Table 5). The lowest SGR was for S4 and P100. From 16 to 35 days, the SGR did not show an effect of salinity, prey concentration or their interaction ($p > 0.05$). Similar results were found for *H. lacerdae* (Luz & Portella, 2015); and *P. corruscans*, *P. costatus* and *L. alexandri* (Santos & Luz, 2009). However, due to the growth variability among the individuals of the same experimental unit, the statistical test was not able to detect SGR differences between 16-35 days of feeding. In general, prey concentrations of P300 and P500 in freshwater or 2 g of salt L^{-1} produced greater total lengths, while the greatest weight was produced in P500 and S2 after 15 days of *H. radiseriatus* larviculture. As for survival, salinity at 4 g of salt L^{-1} should not be recommended at this early stage as it also impairs performance. As shown, the ideal salinity and the daily prey concentration depend on the species being cultured, which emphasizes the importance of this study for the adoption of the best management practices for *H. radiseriatus* larviculture.

Table 6. Interaction of daily prey concentration × salinity mean values (± standard deviation) for total length (L_T; mm) and weight (*W*; mg) after 15 days of feeding for *Hypsolebias radiseriatus* larvae.

Salinity	Daily prey concentration		
	P_{100}	P_{300}	P_{500}
		L_T – 15 days	
S_0	10.48 ± 0.13^{Ba}	12.43 ± 0.18^{Aab}	13.64 ± 0.45^{Aa}
S_2	10.21 ± 0.37^{Ba}	13.65 ± 0.56^{Aa}	14.70 ± 0.87^{Aa}
S_4	8.25 ± 1.03^{Bb}	11.21 ± 0.93^{Ab}	9.07 ± 1.17^{Bb}
		W – 15 dias	
S_0	10.39 ± 0.88^{Ba}	20.32 ± 3.48^{Aab}	27.75 ± 2.83^{Ab}
S_2	12.30 ± 3.15^{Ca}	26.81 ± 2.65^{Ba}	38.51 ± 9.13^{Aa}
S_4	6.89 ± 3.56^{Aa}	15.97 ± 3.88^{Ab}	13.03 ± 6.88^{Ac}

Means followed by the same letters (A, B for lines and a, b, c for columns) do not differ by the Tukey's test ($p > 0.05$).

Conclusion

The present study represents the first investigation about the larviculture of *H. radiseriatus* under varying conditions of salinity and prey concentration. It demonstrated that larviculture of this species can be successfully carried out in a salinity of 2 g of salt L^{-1} and initial daily prey concentrations with 500 artemia nauplii $larva^{-1}$, with positive effects on growth and survival. The survival of *H. radiseriatus* larvae was negatively affected by the salinity of 8 g of salt L^{-1} for both the osmotic shock and gradual acclimatization.

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