


# Slightly salinized water enhances the growth and survival of *Arapaima gigas* larvae

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## Abstract

The aim of this study was to evaluate the survival and growth of *Arapaima gigas* larvae reared in slightly salinized water. Pirarucu larvae ( $183.1 \pm 41.2$  mg and  $3.4 \pm 0.3$  cm) were stocked in PVC tanks (20 L;  $n = 4$ ; 40 larvae per tank) in a static system. *A. gigas* larvae were reared in increasing levels of salinized water (0, 1, 2, 3 and 4‰) for a period of 15 days. Fish were fed six times a day with *Artemia* nauplii (2,900 nauplii per larvae per meal in the beginning). The salinized water increased by at least four times the *Artemia* nauplii lifespan, which prolonged the time of live food supply to *Arapaima* larvae. Pirarucu larvae kept in freshwater presented the lowest values in weight gain, final weight and survival. Larvae reared in water with 4‰ of salinized water showed the highest final weight. Slightly salinized water enhances the growth and survival of *A. gigas* larvae and it can be a simple practice with low cost that can be applied in the routine of pirarucu larviculture.

## KEYWORDS

*Artemia* sp nauplii, paiche, pirarucu, salinity

## 1 | INTRODUCTION

*Arapaima gigas* is a carnivorous and obligate air-breathing fish, native throughout the Amazon basin. Its common name “Pirarucu” is of Tupi origin (pira = fish and urucu = red) which corresponds to the intense dominant colouration at the extremities of its scales (Venturieri & Bernardino, 1999). Pirarucu has become an important species to aquaculture due to its fast growth and high nutritional quality of flesh (Cortegano et al., 2017). It is considered one of the largest freshwater fish, reaching between 2 and 3 metres and weighing 200 kg (Imbiriba, 2001; Nelson, 2006; Saint Paul, 1986). It tolerates high stocking density, being resistant to handling, presents rapid growth of 10–12 kg in the first year of breeding (Brandão, Gomes, & Chagas, 2006; Rebaza, Rebaza, & Deza, 2010; Tavares-Dias, Araújo, Gomes, & Andrade, 2010). In 2016, approximately 8 million tons of

this species were produced in Brazil (IBGE, 2016), which has been increasing in the last few years in this country (Lima, Tavares-Filho, & Moro, 2018). However, it is necessary to increase early *A. gigas* supply to meet the growing demand and to decrease the price of juveniles for its grow-out phase (Nuñez et al., 2011).

Larviculture requires more attention due to the large morpho-physiological transformations that occur in the fish (Portella et al., 2014; Zouiten, Khemis, Besbes, & Chantal, 2008). In the beginning of its exogenous feeding, the majority of fish larvae are dependent on live food, such as *Artemia* nauplii, a brine shrimp, which are the most commonly used in aquaculture. The cysts of *Artemia* nauplii are easy to obtain and hatch, as well as being resistant to abiotic variables, manipulation and diseases. These microorganisms are attractive to feed fish larvae and widely used in the world aquaculture due to their high swimming ability, nutritional value (in many cases,

*Artemia* need to be enriched) and adequate size, varying from 400 to 600  $\mu\text{m}$  (Conceição, Yúfera, Makridis, Morais, & Dinis, 2010; Lavens & Sorgeloos, 1996).

Several studies using *Artemia* nauplii in the first exogenous feed of fish larvae demonstrate an increase in the growth and survival rates for many species, especially for carnivorous species (Conceição et al., 2010; Jomori, Portella, & Luz, 2012; Tesser & Portella, 2011). However, *Artemia* nauplii present low life span when added in freshwater (Merchie, 1996) and protocols to support the *Artemia* nauplii survival in freshwater are necessary to improve the larviculture of high economic value fish, such as pirarucu.

An alternative protocol is the use of slightly saline water in larviculture indoor tanks. The use of NaCl in freshwater can extend the *Artemia* nauplii's lifetime, increase fish feeding and reduce water quality problems related to the mortality of nauplii in hypo-osmotic solution (Beux & Zaniboni Filho, 2006). Furthermore, it is possible to reduce energy consumption by the osmotic differences between the animal and the external environment and consequently increasing fish larvae growth (Fazio, Marafioti, Arfuso, Piccione, & Faggio, 2013; Sampaio & Bianchini, 2002).

Salinized waters promoted good growth in *Carassius auratus* with maximum 12‰ salinity and 31°C temperature (Imanpoor, Najafi, & Kabir, 2012) and *Mugil liza* (Lisboa, Barcarolli, Sampaio, & Bianchini, 2015) reared at salinity 24‰. The highest survivability of *Pelecus cultratus* larvae was obtained in salinity equal to 3‰ (Kujawa et al., 2017). Reduced mortality of *Ictalurus punctatus*, *C. auratus*, *Morone saxatilis* and *Acipenser oxyrinchus desotoi* challenged by *Flavobacterium columnare* and held in 1.0‰ salinity indicated that this level of salinity could be useful for reducing losses to columnaris in culture systems where salinity can be easily adjusted (Altinok & Grizzle, 2001). However, studies with salinity management with Neotropical freshwater species are still incipient. Therefore, this study aimed to propose new protocols to increase the lifetime of *Artemia* nauplii for its capture by pirarucu larvae.

## 2 | MATERIAL AND METHODS

### 2.1 | Hatching *Artemia* nauplii

In order to obtain *Artemia* nauplii (High 5 cysts, INVE Aquaculture), 50 g of cyst was used in incubators with a capacity of 15 litres in saline water (30‰), pH 8.0, at a temperature of 28°C, with constant aeration, kept under a 100 W or 1507 lumens lamp, for a period of 22 hr. The cysts were previously hydrated for 1 hr in fresh water. After the incubation period, the *Artemia* nauplii were filtered, quantified and supplied to the pirarucu larvae.

### 2.2 | Survival *Artemia* nauplii to increasing salinity

After hatching, *Artemia* nauplii were added in increasing levels of sodium chloride (NaCl): 0, 1, 2, 3 and 4‰ at a density of 10 nauplii/ml in 20 Beakers (200 ml;  $n = 4$ ) with constant aeration during 40 hr. After

the homogenization of each experimental unit, a sample of 1 ml of the solution was collected (1 to 6, 8, 10, 12, 14, 24, 32 and 40 hr post *Artemia* nauplii hatching) (water and *Artemia* nauplii;  $n = 3$ ) and the number of alive and dead (immovable) nauplii was registered to calculate *Artemia* nauplii survival rate (lifespan).

### 2.3 | Fish performance

*Arapaima gigas* larvae were obtained from a natural spawning in a commercial fish farm (Coari/AM, Brazil). Larvae were collected when they started to swim to the water surface together with the breeding male and subsequently transported to Fish Farming Station of INPA, Manaus/AM, Brazil. The experiment was carried out in a completely randomized experimental design ( $n = 3$ ) with five treatments, containing increasing levels of sodium chloride (NaCl): 0, 1, 2, 3 and 4‰ in water from artesian well. Groups of 40 pirarucu larvae (initial body weight  $183.1 \pm 41.2$  mg and initial length  $3.4 \pm 0.3$  cm) were distributed in the experimental units (circular polyethylene tanks, with a useful volume of 20 L), in a static system. Larvae were fed every 2 hr (7, 9, 11, 13, 15 and 17 hr) with *Artemia* nauplii newly hatched (2,900 nauplii per larvae per meal in the beginning). The quantities were increased (10% per day) over the 15 days of the trial. Fish management was according to the ethics of animal use by the Brazilian Society of Laboratory Animal Science (COBEA).

Water quality parameters such as pH ( $6.87 \pm 0.42$ ), dissolved oxygen ( $7.29 \pm 0.11$  mg/L) and temperature ( $26.6 \pm 0.9^\circ\text{C}$ ) were daily monitored by digital dissolved oxygen meter - YSI 85 and pH meter - 10A EcoSense. The tanks were cleaned daily and the total exchange water was carried out once a day. At the end of the experiment, fish were assessed to determine final weight, weight gain (WG = final weight - initial weight), survival ( $S = [\text{final number of fish} - \text{initial number of fish}] \times 100$ ) and condition factor using the reference Nehemia, Maganira, and Rumisha (2012).

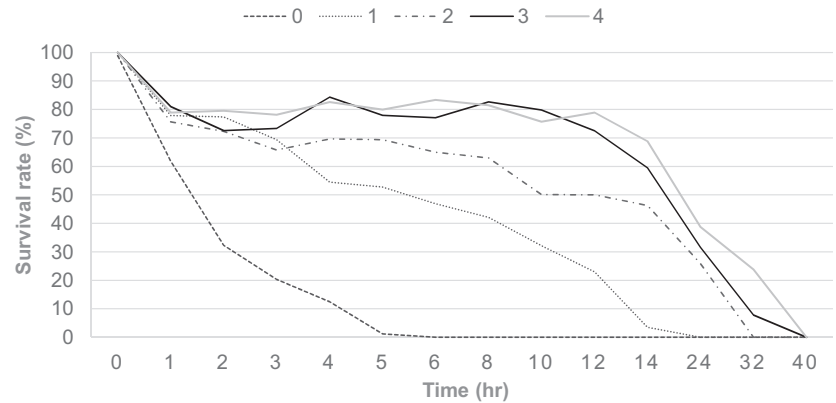
### 2.4 | Statistical analysis

Data were submitted to exploratory analysis by outlier data test, variance homogeneity and range of the response variable. Data were evaluated using an ANOVA followed by post-hoc Tukey test, using the PRISM® statistical package. Optimum salinity concentration in the water was determined by polynomial regression analysis using of the STATISTICA 10.0 software.

## 3 | RESULTS

### 3.1 | Survival *Artemia* nauplii to increasing salinity

The lifespan of *Artemia* nauplii added in freshwater was up to 6 hr post hatching (HPH). Subsequently, the survival of *Artemia* nauplii submitted to 1‰ de NaCl was increased up to 24 HPH. This low increase in NaCl result in a survival rate four times higher than *Artemia* nauplii added in freshwater water. The *Artemia* nauplii maintained in a NaCl concentration of 2‰ survived up to 32 HPH,



**FIGURE 1** Survival rate of *Artemia* nauplii in salinities between 0 to 4 ‰

followed to *Artemia* nauplii at 3‰ and 4‰ of NaCl that survived up to 40 HPH (Figure 1).

### 3.2 | *Arapaima* larvae growth performance

The increasing addition of NaCl in *Arapaima* larvae water production during 15 days promoted better results in productive performance. Only animals kept exclusively in freshwater presented the lowest values in weight gain, final weight and survival (Table 1).

After 15 days, the total length of all *Arapaima* larvae was similar. The larvae maintained at concentrations of 4‰ NaCl presented the final weight ( $850.75 \pm 56.72$  mg) with medium values differing only from the fish maintained in freshwater ( $757.49 \pm 10.23$  mg) (Figure 2). The weight gain was increasing as the concentration was increased (Table 1) and significant differences were observed just between animals reared in 4 and 0‰ of NaCl in the water.

The survival rates were similar to all pirarucu larvae submitted to slightly saline water and freshwater, except the ones submitted to 1‰ in comparison with the freshwater. Animals raised in freshwater had the lowest survival rates. The condition factor were similar in whole treatments, with the 4‰ treatment being the only exception (Table 1).

## 4 | DISCUSSION

The survival of *Artemia* nauplii was directly influenced by the saline concentration of the water, with low survival when the salinity decreases, as observed by Beux and Zaniboni Filho (2006). Our results showed a short survival period of *Artemia* nauplii when added to freshwater (no more than 6 hr). On the other hand, the lifespan of 80% of *Artemia* nauplii added in water with increasing salinity levels was prolonged up to 14 hr after hatching. The adaptation of Brine Shrimp, *Artemia* sp nauplii (High5 cysts, INVE) from freshwater through brackish into marine waters shows the exceptional

capacities of this animal group to survive with salinity changes. Osmoregulation is the main physiological mechanism that maintains the hypo-osmotic homeostasis of these animals (Thabet, Ayadi, Koken, & Leignel, 2017).

The higher survival of *Artemia* nauplii in waters with higher salt concentration could be an important factor that contributed to the pirarucu weight gain. Similar final length for all *A. gigas* larvae submitted to different saline concentrations indicates a possible spectrum to be used without damage to the animal growth. Moreover, the additional energy requirements for fish osmoregulation to maintain in hypo or hyperosmotic environments can hamper the fish growth when compared to those kept in an isosmotic environment or with an ideal saline spectrum (Pérez-Robles, Re, Giffard-Mena, & Díaz, 2012). Energy cost associated with osmoregulation can vary according the different ontogenetic developmental stages and the response to salinity may be species-dependent (Romano, Syukri, Karami, Omar, & Khalid, 2017). In some cases, the salinized water could impair the development. For *Clarias gariepinus* larvae, 10‰ of the salinity caused death within 48 hr (Britz & Hecht, 1989). Exposure to higher- and long-term salinity, significantly affects the growth and physiological response of *Clarias batrachus* (Sarma, Prabakaran, Krishnan, Grinson, & Kumar, 2013).

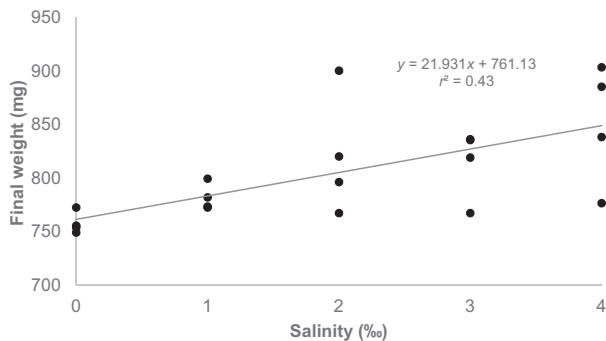
*Arapaima gigas* larvae maintained at 4‰ NaCl present a higher final weight than animals kept in freshwater. The increased salinity modifies the specific gravity of the medium reducing the energy floatation and saving food energy – around 10%–50% (Marshall & Bryson, 1998) – for body growth (Lam & Sharma, 1985). Moreover, changes in salinity result in different water intake rates, affecting the digestive enzymes activity by the salinity of the intestinal tract (Boeuf & Payan, 2001).

Many studies mention that suitable saline water reduce physiological stress (Jomori et al., 2012), the ionic and osmotic differences between external and internal fluids (Lam & Sharma, 1985; Riley, Hirano, & Grau, 2003; Wurts, 1995) and support the best results in productive performance (Amornsakun, Vo, Petchsupa, Pau, & Hassan, 2017; Britz & Hecht, 1989; Fiúza et al., 2015). Studies with freshwater fish have determined better productive results for many

**TABLE 1** Zootechnical variables of pirarucu larvae kept in slightly salinized water\*

Treatment variables	0	1	2	3	4
Final weight (mg)	757.49 ± 10.23 <sup>b</sup>	781.57 ± 12.53 <sup>ab</sup>	820.79 ± 57.19 <sup>ab</sup>	814.36 ± 32.53 <sup>ab</sup>	850.75 ± 56.72 <sup>a</sup>
Weight gain (mg)	574.49 ± 10.23 <sup>b</sup>	598.87 ± 12.53 <sup>ab</sup>	637.79 ± 57.19 <sup>ab</sup>	631.36 ± 32.53 <sup>ab</sup>	667.75 ± 56.72 <sup>a</sup>
Final height (cm)	5.03 ± 0.10 <sup>a</sup>	5.08 ± 0.08 <sup>a</sup>	5.27 ± 0.14 <sup>a</sup>	5.15 ± 0.05 <sup>a</sup>	5.17 ± 0.13 <sup>a</sup>
Survival rate (%)	95.63 ± 3.15 <sup>b</sup>	100.00 ± 0.00 <sup>a</sup>	96.00 ± 1.22 <sup>ab</sup>	97.50 ± 2.04 <sup>ab</sup>	98.13 ± 1.25 <sup>ab</sup>
Condition factor	0.0058 ± 0.0006 <sup>b</sup>	0.0058 ± 0.0006 <sup>b</sup>	0.0058 ± 0.0006 <sup>b</sup>	0.0058 ± 0.0006 <sup>b</sup>	0.0060 ± 0.0006 <sup>a</sup>

\*Means (n = 4) ± SD. Means followed by equal letters, in the lines, do not differ, by Tukey's test, at 5% level.

**FIGURE 2** Final weight of *Arapaima gigas* larvae at increasing salinities

fish species in concentrations ranging from 0.5–2‰ (Jomori, Luz, Takata, Fabregat, & Portella, 2013; Luz, Costa, Ribeiro, Silva, & Rosa, 2013; Santos & Luz, 2009).

The final survival of pirarucu larvae was similar to all group of fish reared in the slightly saline water. It indicates the positive action of NaCl to improve the resistance of animals in the most critical phase of life. Several studies indicate that the use of NaCl in neotropical fish species larviculture contributes to similar or superior survival and growth results in relation to larvae reared in freshwater (Jomori et al., 2013; Luz & Santos, 2008; Santos & Luz, 2009) and tolerance to different salinity gradients is species-dependent. In addition, the salt concentrations tested can act as growth facilitators, resulting in highest weight gain. The use of slightly salinized water can be a simple practice with low cost to be applied in the routine of pirarucu larviculture.

The inverse relationship between survival rates and increasing salinities was reported for several freshwater fish, including, *Heterobranchus longifilis* (Fashina-Bombata & Busari, 2003), *Pimelodus maculatus* (Weingartner & Zaniboni Filho, 2004) and *Lophiosilurus alexandri* (Luz & Santos, 2008). However, this statement is correct until the salinity tolerance limit is reached. Improper salinity for freshwater species like some tetras and many catfish causes problems because they are sensitive to salt (Noga, 2010). Salinity stress may occur if young freshwater salmonids (parr) are prematurely transferred to saltwater before they are ready to undergo transformation into marine adapted fish (smolts). The knowledge of the *Arapaima* larvae tolerance in salinities up to 4‰ can be a useful

tool for the use of NaCl in prophylactic procedures against pathogens (Dewi, Siallagan, & Suryanto, 2018; Magundu, Rasowo, Oyoo-Okoth, & Charo-Karisa, 2011).

## 5 | CONCLUSION

The use of NaCl concentration up to 4‰ resulted in higher weight gain and condition factor than pirarucu larvae reared in freshwater, possibly due to increasing the duration of *Artemia* survival and thus availability to the larvae and/or reducing energy requirements for osmoregulation. Future studies comparing NaCl with natural sea salt aiming at the survival of *Artemia* nauplii in addition to parasite control need to be carried out for the larviculture of this species. Besides that, the impact of discharged salinized water into receiving streams needs to be evaluated to avoid environmental problems, establishment of the nutritional values that the *Artemia* nauplii provide for pirarucu larvae and if the 4‰ concentration of NaCl does not affect the osmoregulation of the larvae.

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