The effect of water pH on the incubation and larviculture of curimbatá *Prochilodus lineatus* (Valenciennes, 1837) (Characiformes: Prochilodontidae)

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Different pH levels of water in the incubation and larviculture of 'curimbatá' (*Prochilodus lineatus*) were tested to evaluate the effect of this variable on the survival and development of eggs and larvae. During incubation, pH of 5.0, 6.0, 7.0, 8.0, and 8.5 was tested. To observe the effects of pH on larvae with different hatchery histories, the following experimental design was utilised (pH incubation - pH larviculture): L 6-6; L 6-7; L 7-6; L 7-7; L 7-8; L 8-7; L 8-8; L 8.5-7, and L 8.5-8.5. At pH 5.0 and four hours after incubation, an almost total mortality of incubated eggs was seen. Eggs incubated at pH 6.0 to 8.5 had similar fertilization and survival rates at the end of incubation, although eggs and larvae incubated at pH 6.0 had a smaller diameter and total length than larvae incubated at neutral and alkaline pH. During larviculture, it was observed the highest post-larvae survival rates with incubation at pH values of 7.0 (93 ± 7%) and the largest post-larvae lengths from the L 8.5-7 treatment (9.29 ± 0.8 mm). It can be concluded that hatchery and larviculture pH directly affects the initial developmental stages of *P. lineatus*.

Diferentes níveis de pH da água foram testados na incubação e na larvicultura do curimbatá (*Prochilodus lineatus*) para avaliar seu efeito na sobrevivência e desenvolvimento de ovos e larvas. Durante a incubação, pH de 5,0; 6,0; 7,0; 8,0 e 8,5 foram testados. Para observar o efeito do pH nas larvas com diferentes históricos de incubação, foi utilizado o seguinte desenho experimental (pH incubação – pH larvicultura): L 6-6; L 6-7; L 7-6; L 7-7; L 7-8; L 8-7; L 8-8; L 8,5-7 e L 8,5-8,5. Quatro horas depois de iniciada a incubação, foi observada a mortalidade de todos os ovos incubados em pH 5,0. Ovos incubados em pH 6,0 a 8,5 tiveram taxas de fertilização e eclosão semelhantes, embora, ovos e larvas incubados em pH 6,0 apresentaram diâmetro e comprimento total inferiores às larvas incubadas em pH's neutro e alcalinos. Durante a larvicultura foi observada uma maior taxa de sobrevivência das pós-larvas que tinham sido incubadas em pH 7,0 (93 ± 7%) e pós-larvas com maior comprimento no tratamento L 8,5-7 (9,29 ± 0,8 mm). Conclui-se que o pH da incubação e da larvicultura afeta diretamente a sobrevivência e o crescimento dos estágios iniciais de *P. lineatus*.

Keywords: Fish culture, Fish larval physiology, Fish eggs physiology, Hatchery, Water quality.

Introduction

The 'curimbatá', *Prochilodus lineatus*, is the most important commercial fish species in the rivers of the La Plata basin based on biomass, and it constitutes, together with other species of the same genus, the largest fishing resource in South American Rivers (Smolders *et al*., 2000). However, in spite of the remarkable abundance of 'curimbatá' in neotropical basins in recent years, its natural stocks are starting to decline due to human population growth, which is associated with pollution, ciliary deforestation and the construction of river dams (Zaniboni-Filho & Schulz, 2003; Agostinho *et al*., 2007). Consequently, the production of 'curimbatá', as well as other migratory species, in captivity is gaining importance.

Information on the incubation of eggs and on the larviculture of South American migratory fish is scarce. Although information about the effects of water quality on

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cultivated species is lacking, most authors agree that the influence of abiotic factors in the initial growing phases on cultivated species is significant (Ferreira *et al.*, 2001; Handeland *et al.*, 2008; Zaniboni-Filho *et al.*, 2009) and crucial to the survival of eggs and larvae (Lin *et al.*, 2006). One of the critical factors that limit the rearing of aquatic organisms is pH. According to Kleerekoper (1990), the acidic, alkaline or neutral nature of the medium in which these organisms live is important because the proportion of H^+ and OH^- ions in solution extensively regulates several physiological processes that are critical to animals and plants. The same author also states that most water organisms show a remarkable preference for a particular pH in the environment. Therefore, the pH values of water have a decisive role in the composition of a biotic community.

Studies carried out in neotropical environments have shown that a preference for a pH value between 6 and 8 exists in many species (Lopes *et al.*, 2001; Townsend & Baldisserotto, 2001; Baumgartner *et al.*, 2008), although this preference can vary substantially between species and between the different stages of fish's life cycle (Ferreira *et al.*, 2001; Parra & Baldisserotto, 2007). Thus, for the rearing of a native species, it is necessary to know the appropriate parameter ranges for the culture water. The present study aims to answer two questions: (1) the ideal pH for the development of eggs and larvae; and (2) if the incubation pH influences larval development during larviculture.

To answer these questions, the present study verified the effect of pH during incubation and larviculture on the performance of 'curimbatá' eggs and larvae.

Material and Methods

The experiment was carried out in the Laboratory of Biology and Rearing of Fresh Water Species, in the Federal University of Santa Catarina's Aquaculture Department.

To evaluate how the pH of water during incubation and larviculture affects the development of 'curimbatá' (*P. lineatus*), the study was divided into two stages.

Stage I. Incubation. The first stage was measured from egg fertilization to the beginning of the first feeding of the 'curimbatá' larvae. In this stage, the following pH values were tested: 5.0 ± 0.1; 6.0 ± 0.1; 7.0 ± 0.1; 8.0 ± 0.2 and 8.6 ± 0.4. The pH treatments for the water during Stage I will hereafter be referred to as I 5; I 6; I 7; I 8, and I 8.5, respectively. These pH values were chosen based on the range of pH values generally found in the La Plata basin, natural environment of 'curimbatá'.

All eggs were obtained from the same breeders, which were induced to reproduce by the hypophysation method with previous doses (Zaniboni-Filho & Barbosa, 1996). After the extrusion and dry mixture of gametes, samples of 3.0 ± 0.1 g (approximately 3.400 eggs) were transferred to fifteen 500-mL beakers. Then water with different pH values specific to each treatment were added to allow

fertilization and hydration. The eggs were then washed and transferred to the hatching units.

Incubators of 20-L black fibreglass funnel-type were used. To make the control of the pH easier, hatching was carried out in a closed system. The oxygenation and agitation of the eggs was accomplished by artificial aeration. For each treatment, were used three replicate incubators. The pH of the water in the incubators was adjusted prior to the introduction of the eggs.

Water pH was monitored every four hours with a pHmeter YSI-60. The water pH in the different treatments was regulated with sulphuric acid, H_2SO_4 (5%), and sodium hydroxide, NaOH (10%) (Kugel & Peterson, 1989; Lopes *et al.*, 2001). Treatments were continuously aerated, but water was not changed during the experiment. Water temperature and dissolved oxygen concentration were monitored every day at 08:00 and 17:00 h with an YSI-55 oximeter. Alkalinity, hardness, and ammonia and nitrite concentrations were measured every day using colorimetric methods. The Na^+ levels in the hatching were measured with a B262 flame spectrophotometer (precision 1 mg L^{-1}) at the beginning and at the end of hatching.

To follow egg's development of the 'curimbatá' in the different treatments, samples containing 8-10 eggs were removed from each hatchery at 1, 3, 9, and 15 h after fertilization. The diameter of the hydrated egg, perivitelline space and the size of the yolk sac were measured, according to recommendations of Ahlstrom *et al.* (1976). The fertilization rate was checked according to Godinho (2007). The hatched time was recorded when the first larvae hatched.

To characterize the larvae in each treatment, biometric measurements were conducted at the moment of hatching and at the beginning of external feeding of the larvae (the end of incubation) to 20 larvae from each hatchery. The sampled larvae were fixed in 4% buffered formol for the subsequent measurement of the total length, total weight and volume of the yolk sac, according to Pavlov & Moksness (1995). The final survival rate for this stage was estimated 73 hours after fertilization by counting all the larvae.

Stage II. Larviculture. Larval growth was evaluated from the beginning of the first feeding to the 16th day of rearing. In this stage, nine treatments were tested (Fig. 1).

Once the survival rate for the incubation stage was recorded, the larvae were installed in continuously-aerated 200-L black plastic tanks in a closed system and with a total of 220 larvae $tank^{-1}$.

The larvae were fed four times each day, at 9:00; 13:00; 17:00, and 21:00 h. During the 16 days of rearing, larvae were provided with wild zooplankton, maintaining an average concentration between 4000-7000 organisms L^{-1} day^{-1}, together with *Brachionus plicatilis* supplied at a concentration of 1300 organisms L^{-1} day^{-1}. To supplement the feedings, commercial shrimp food that were sieved between 150-250 μm and that contained 50% crude protein

was supplied. To evaluate the growth of the post-larvae, seven individuals from each tank were randomly captured every four days and measured.

The water pH values, the oxygen level, and the temperature in the different treatments were regulated with the same criteria and methodology used for incubation. The Na^+ levels in the incubator water were measured with a B262 flame spectrophotometer (precision 1 mg L^{-1}) at the beginning and at the end of larviculture. The photoperiod was 16 h of light: 8 h of darkness.
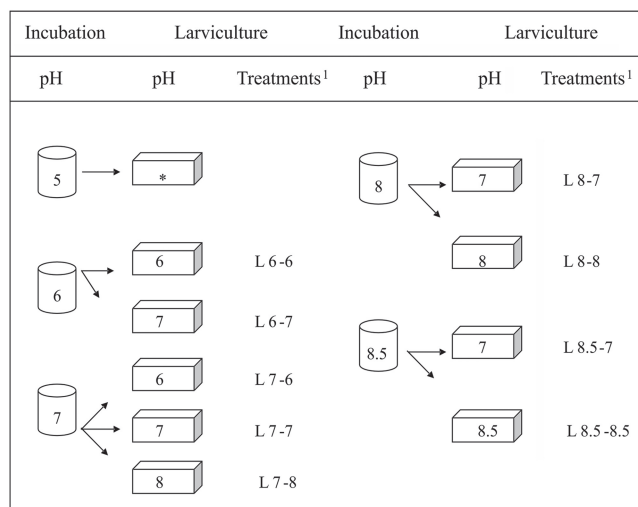

Fig. 1. Experimental design elaborated for the incubation and larviculture stages of *Prochilodus lineatus*. [1] In the treatments tested in the larviculture, the first number represents the incubation pH history and the second the pH in which the larviculture took place. The larviculture treatments will be described in this fashion throughout the text. * Treatment not accomplished due to an almost total mortality in the incubation stage.

Data analysis. To evaluate differences in eggs (total diameter and perivitelline space), larvae (total length, volume of the yolk sac and total weight) and biomass, Analysis of Variance (one-way ANOVA) was applied, with treatment as an independent factor. To evaluate the fertilization and hatching rates at the incubation stage and the survival rate in the larviculture stage, the percent data were arc-sin transformed and one-way ANOVA was used, with treatment as independent factor. The data meet the assumptions for ANOVA (normality and homocedasticity). When ANOVA results were significant, Tukey's test was applied *a posteriori* to evaluate differences between treatments. The significance level was α=0.05. All analyses were carried out in the software Statistica 7.0.

Results

Stage I. Incubation. By carrying out the experiment in a closed environment, abrupt temperature variations were avoided (24.9 ± 0.4°C). Oxygen levels were always higher than 7.5 mg L^{-1}, and total ammonia and nitrite values were never higher than 0.4 mg L^{-1} and 0.01 mg L^{-1}, respectively, over the course of the entire incubation stage. The alkalinity during this stage averaged 20 mg $CaCO_3$ L^{-1} and hardness averaged 35 mg $CaCO_3$ L^{-1}. The water pH values remained at the desired values. The adjustment of tank water to alkaline pH with NaOH increased Na^+ levels from 0.112 to 0.174 mEq L^{-1} in treatment I 8 and 0.478 mEq L^{-1} in treatment I 8.5 (F=4.128; P=0.004); in the others treatments, the Na^+ levels were similar (F=0.912; P=0.338) to the initial hatching level.

One hour after the beginning of the experiment, it was also noticed that the eggs from treatment I 5 had withered, were presenting white stains in the chorion and were clumped together. At this pH, most of the eggs floated or sank to the bottom of the incubator. Two hours after fertilization, almost all of the eggs were whitish in colour.

After 9 hours, it was noticed that in spite of a small tendency towards increased fertilization in treatment I 8.5, there were no differences among treatments I 6, I 7, I 8 and I 8.5 (F=5.612; P=0.111). Treatment I 5 had the lowest fertilization rate (Table 1). After 72 hours, by the end of the incubation stage, none of the eggs had hatched in treatment I 5, but the survival rates were similar in the other treatments (F=0.912; P=0.308) (Table 1).

Table 1. Average values ± standard deviation of the fertilization and survival rates during the incubation of 'curimbatá' *Prochilodus lineatus*. [1]Different letters in the columns indicate statistical difference in the averages by the Tukey test (P < 0.05). *Fertilization Rate was measured that according to Godinho (2007). **Survival Rate was measured by counting all larvae.

Treatment	Fertilization Rate (%)[1]*	Survival Rate (%)[1]**
I 5	1.2 ± 0.9 a	0 a
I 6	62.8 ± 24.7 b	45.7 ± 29.6 b
I 7	67.7 ± 7.7 b	51.5 ± 1.21 b
I 8	67.3 ± 6.2 b	51.2 ± 34.7 b
I 8.5	75.0 ± 2.9 b	72.1 ± 8.6 b

During incubation, it was noticed that the eggs incubated in treatment I 6 had average of diameter of 3.29 ± 0.05 mm, which was significantly smaller (F=5.891; P=0.001) than that of eggs incubated with pH values equal to or higher than 7 (3.87 ± 0.06 mm). The difference was due to the existence of significantly smaller perivitelline space for treatment I 6 (0.96 ± 0.09 mm) compared to treatments I 7 (1.49 ± 0.04 mm), I 8 (1.53 ± 0.07 mm) and I 8.5 (1.50 ± 0.08 mm) (F=6.274; P=0.001) (Table 2). These different egg diameters were verified during most of the embryonic stage, except for the first two hours, in which treatment I 8.5 presented a reduced perivitelline space (1.13 ± 0.12 mm; Table 2).

The average values for the length of *P. lineatus* larvae at hatching varied according to the water pH, presenting similar values when the water pH ranged from 7 to 8.5. However, total length was shorter for larvae incubated in treatment I 6 (F=5.498; P=0.001; Table 2).

The average volume for the yolk sac was 0.55 ± 0.07 mm³ and was not significantly different (F=1.529; P=0.214) among treatments. In the present study, hatching took place 19.5 hours after fertilization, but a delay of one hour was observed in the hatching of larvae incubated in I 6.

In the final yolk sac absorption stage, larvae cultivated in treatment I 6 were smaller (5.66 ± 0.09 mm). There was no statistical difference in the length of larvae kept at pH values between 7 and 8.5 (5.83 ± 0.03 mm; F=0.628; P=0.587). However, no difference was recorded in the volume of the yolk sac of larvae kept in all treatments at the end of the incubation (0.04 ± 0.01 mm; Table 2).

Stage II. The pH values during larviculture matched the values registered in the experimental protocol, except for the most alkaline pH, which presented notorious variations during the day (Table 3).

Similar to what was observed during the incubation stage, the temperature remained between 24°C and 25°C during the 16 days of larviculture. The dissolved oxygen values were always higher than 7.5 mg L^{-1}, and total ammonia and nitrite were not higher than 0.3 mg L^{-1} and 0.01 mg L^{-1}, respectively. The alkalinity was 20 mg CaCO$_3$ L^{-1} and the hardness was 30 mg CaCO$_3$ L^{-1}. The adjustment of tank water to alkaline pH with NaOH increased Na$^+$ levels from 0.98 to 0.315 mEq L^{-1} in treatments L 7-8 and L 8-8 and 0.821 mEq L^{-1} in treatment L 8.5-8.5 (F=6.032; P=0.001). For the others treatments, the Na$^+$ levels were similar (F=1.304; P=0.217) to the initial larviculture level.

Higher survival rates were observed in larvae incubated at pH 7 (Table 3). The final length measurements showed that larvae cultivated in treatment L 8.5-7 had the highest length values (F=3.041; P=0.029) while treatments L 6-6 and L 8-8 had the lowest values (Table 3; Fig. 2). The two treatments incubated at pH 6.0 (L6-6 and L6-7) and the L8-8 treatment had the lowest final biomass values (F=4.339; P=0.018), and the highest value was observed in treatment L 8.5-7 (F=4.538; P=0.014). The final weight of the larvae did not show any statistical differences between treatments (F=1.583; P=0.091). The results showed that, in general, the larvae of the treatments carried through larviculture at pH 7 showed the best performance (Table 3).

Table 2. Average values ± standard deviation of the Diameter of the egg, Perivitelline Space (P.S), Length of the larvae (initial and final), Volume of the Yolk Sac (initial and final) during the incubation of *Prochilodus lineatus* at the different pH values tested. [1]Different letters in the same column indicate statistical differences in the averages by the Tukey test (P < 0.05).

pH	Diameter (mm) [1]		Length (mm) [1]		Volume of the yolk sac (mm³) [1]	
	Egg	P.S	Initial	Final	Initial	Final
I 5	3.22±0.09a	0.92±0.07a	-	-	-	-
I 6	3.29±0.05a	0.96±0.09a	4.33±0.03a	5.66±0.09a	0.54±0.02	0.04±0.01
I 7	3.87±0.06b	1.49±0.04b	4.48±0.03b	5.84±0.07b	0.56±0.04	0.04±0.01
I 8	3.90±0.10b	1.53±0.07b	4.53±0.03b	5.85±0.08b	0.54±0.07	0.04±0.01
I 8.5	3.88±0.04b	1.50±0.08b	4.50±0.05b	5.81±0.10b	0.57±0.03	0.04±0.01

Table 3. Average values ± standard deviation of water pH, weight, length, biomass, and survival at the end of 16 days of larviculture for *Prochilodus lineatus* in the different treatments. [1]Different letters in the same column indicate statistical differences in the averages for the Tukey test (P < 0.05).

| Treatment	pH	Final weight (mg) [1]	Final length (mm) [1]	Survival (%) [1]	Final biomass (mg) [1]
L 6-6	6.0 ± 0.2	4.4 ± 1.9	8.19 ± 0.5a	56 ± 5a	541.2 ± 132.9a
L 6-7	7.0 ± 0.1	3.7 ± 1.4	8.56 ± 1.1ab	69 ± 4b	562.0± 124.5a
L 7-6	6.0 ± 0.2	4.5 ± 1.0	8.75 ± 0.6ab	98 ± 3c	972.0± 118.9bc
L 7-7	7.0 ± 0.1	4.2 ± 0.9	8.45 ± 0.8ab	90 ± 9c	831.6± 87.4b
L 7-8	8.0± 0.1	4.0 ± 0.7	8.25 ± 0.8ab	92 ± 12c	808.0± 171.6ab
L 8-7	7.0 ± 0.1	4.6 ± 2.1	8.50 ± 1.1ab	64 ± 9ab	648.6± 316.4ab
L 8-8	8.0 ± 0.1	3.9 ± 1.3	8.16 ± 0.6a	68 ± 10ab	585.0± 138.2a
L 8.5-7	7.0 ± 0.1	5.7 ± 0.4	9.29 ± 0.8b	82 ± 10bc	1026.0± 142.2c
L 8.5-8.5	8.6 ± 0.4	4.9 ± 1.3	8.58 ± 0.5ab	78 ± 33abc	842.8±304.3abc
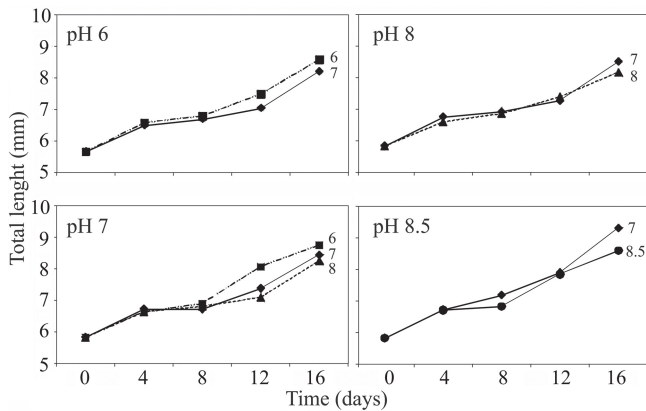
Fig. 2. Growth curves in total length of the *Prochilodus lineatus* larvae after 16 days of larviculture. The results are grouped in the different pH values used in the incubation.

Discussion

The successful incubation and larviculture of fish requires careful management of water quality parameters, since these are not only critical for the survival of eggs and larvae, but also for obtaining high quality fry.

Several studies have identified that incubations carried out in acidic waters, with pH lower than 5.5, may cause high mortality during this phase. Korwin-Kossakonski (1988) noticed in laboratory experiments that *Cyprinus carpio* larvae died after nine days in water with pH lower than 5.0. Atlantic salmon (*Salmo salar*) eggs, when exposed to water with pH between 4.0 and 5.5 in the eye pigmentation stage, had lowered hatching rates (Peterson *et al*., 1980). Swarts *et al*. (1978) have also registered lethal results for brook trout (*Salvelinus fontinalis*) eggs incubated at pH 4.75. The harmful effects of an acidic pH may be easily observed in the egg membranes through the emergence of bubbles that stick to them. This makes the eggs float on the surface of the incubator (Zaniboni-Filho, 2000). Westernhagen (1988) noticed that damage to the osmotic activity of perivitelline colloids reduced water uptake of eggs cultivated at low pH values. Together with the lower water uptake, the ability of resisting to deformations by mechanical actions decreases (Eddy & Talbot, 1983). Eddy & Talbot (1983) found an inhibition of water uptake by eggs of Atlantic salmon and rainbow trout (*Oncorhynchus mykiss*) that had recently been fertilized in water with a pH equal to or lower than 5.5.

In the present study, it was noticed that, one hour after fertilization, the different pH levels tested allowed for the fertilization of *P. lineatus* eggs. This could indicate that the pH range that was evaluated does not significantly affect the activity of spermatozoids and oocytes. Fertilization was also observed in eggs incubated at pH 5.0. These eggs, although withered and stuck to each other, presented the first embryonic stages. Four hours after fertilization, the great majority of the eggs incubated at this pH were decomposing.

In the current study, it was observed a smaller diameter of eggs incubated at pH 6.0 and observed a lower hydration of the perivitelline space compared to eggs incubated in waters with neutral to basic pH values.

The perivitelline fluid tends to allow cations such as Na^+, K^+, Ca^{2+}, Mg^{2+}, and H^+ to penetrate under normal conditions of external and internal pH, where these conditions are optimal in freshwater with neutral pH, and most detrimental in acidic media (Alderdice, 1988). Moreover, the development of embryos in acidic waters could result in a decrease of ion accumulation and, according to Westernhagen (1988), delayed overall development.

Based on the results obtained in this study, the fertilization of 'curimbatá' eggs may be possible in a pH ranging from 5 to 8.5. However, an embryonic development at pH 5.0 is harmful to the embryos and causes mortality.

Exposure to low pH prolongs the period between fertilization and hatching (Kelley, 1946; Westernhagen, 1988), and one of the effects of a pH lower than 5.5 is the inactivation of the enzyme chorianase, which promotes hatching. The pH of the incubation medium has a strong influence on the activity of hatching enzymes, which present highest activity over rainbow trout eggs at pH 8.5 (Hagenmaier, 1974), whereas this value stays between 7.5 and 8 for the Pacific salmon (*Oncorhynchus keta*) (Bell *et al*., 1969). A low pH delayed the development of Pacific herring (*Clupea pallasii*) embryos (Kelley, 1946), and a high pH (pH 10) slightly accelerated the development of zebra fish, *Danio rerio* (Johansson *et al*., 1973). In the current study, 'curimbatá' larvae incubated at pH 6.0 hatched one hour later than larvae in alkaline and neutral treatments. In addition, eggs incubated at pH 6.0 resulted in smaller larvae than those obtained from the remaining treatments.

The effect of pH on the size of recently hatched larvae has already been investigated. According to Johansson & Kihlstrom (1975), Northern pike (*Esox lucius*) larvae incubated at pH 4.2 were smaller than those cultivated at neutral pH, however they had larger yolk sacs, indicating a decreased use of this reserve. The delay in the absorption of the yolk sac at pH 5.0 was also observed in brook trout (Menendez, 1976). In rainbow trout larvae, a low pH affects metabolism by decreasing the heart rate, decreasing the time of bone formation, reducing size, lowering pigmentation and increasing the mortality rate (Nelson, 1982).

The use of a yolk sac reserve is necessary for the survival and production of healthy larvae. Larvae unable to use these reserves could die, particularly between the yolk sac absorption period and first feeding, which is a critical stage for most fish species (May, 1974). In this work, no significant difference was observed in the survival rate of 'curimbatá' eggs incubated between pH 6 to 8.5.

Neutral and slightly alkaline pH values have been recommended as appropriate for the rearing of commercial freshwater species (Boyd, 1982; Michaels, 1988). Most studies to date have been developed with fish from rivers or ponds where the pH is usually close to neutral. In Brazil,

many environments present extreme pH conditions. In the Amazon area, there are marginal ponds in the rio Negro basin that are highly acidic, with pH values below 3. In contrast, ponds subject to isolation in flooded plains in the Pantanal (wetlands) in Mato-Grosso have been found to have pH values of 11 (Esteves, 1988). In spite of the existence of extreme values, there are fish populations adapted to these conditions (Zaniboni-Filho, 2000).

Lopes *et al*. (2001) found a significantly higher survival rate, length and weight of silver catfish (*Rhamdia quelen*) larvae that were maintained at a pH between 8.0-8.5 at the end of 21 days of rearing, compared to those kept at pH values of 5.0, 6.0 and 7.0. The growth and survival of larvae is significantly reduced in flagfish *Jordanella floridae* (Craig & Baksi, 1977) and in brook trout (Kwain & Rose, 1985) at pH values between 4.5 and 5.5, but flagfish larvae presented similar growth when cultivated in pH 6.0 with pH 6.8 (Craig & Baksi, 1977). According to Zaniboni-Filho *et al*. (2009), 'curimbatá' larvae presented high mortality when raised at pH values higher than 9.7.

In this study, the highest post-larvae survival values of 'curimbatá' were recorded after incubation at pH 7 and the longest post-larvae lengths were observed for the L8.5-7 treatment. The worst values for survival and length were registered for the L6-6 treatment.

The low survival and length values obtained at the end of larviculture in the treatments with a pH incubation of 6.0 may be a reflection of the negative effects of incubation at this pH, which might have directly affected the development of embryos and larvae. The difference in the larval survival between the treatments with slightly acidic (L6-6, 56%), and neutral (L7-6, 98%) incubation pH seems to clearly indicate that the difference was due to the pH in which the incubation took place, with detrimental effects on incubation evident even at a slightly acidic pH. The high survival rate obtained in treatment L7-6 may indicate that *P. lineatus* larvae may adapt to pH 6.0 during larviculture and to neutral or alkaline pH values, which are acknowledged as ideal fish rearing media (Boyd, 1982; Michaels, 1988), as long as they have a neutral or alkaline pH incubation history.

Treatments L6-6 and L6-7 present statistical differences in survival rate. The considerable difference among the survival averages of L6-7 (69%) and L6-6 (56%) could indicate a light recovery of the post-larvae incubated in slightly acidic pH when they were transferred to a neutral pH during larviculture and may be explained by the capacity of larvae to capture ions that are important to form their body structures in the first few days of life, since the assimilation of these ions is maximized at neutral pH (Alderdice, 1988).

In the present study, significant differences were found in the length of the larvae obtained from the different treatment groups at the end of the larviculture stage and observed certain trends: (a) The larvae with incubation pH values of 8.5 were largest at the end of the larviculture stage; (b) Comparing the growth of the larvae coming from the same incubation pH, there was a slight tendency to obtain

larger post-larvae specimens when the larviculture pH was lower, where this fact was observed for most treatments, except those where the eggs were incubated at pH 6.0.

It was observed a tendency for higher fertilization and survival rates for the I 8.5 incubation group, as well as an increased growth of the larvae during the rearing stage after that incubation, where this may have been caused by the interference of a higher waterborne Na^+ concentration in this treatment, especially during the incubation stage. The amount of NaOH that was introduced in the incubators of treatment I 8.5 was necessary for the maintenance of pH values within the range established for this treatment and was significantly higher than the amount added in the remaining treatments, thus increasing the availability of Na^+ in the most alkaline treatment. The effect of this ion might have acquired significance due to the low alkalinity of the water. According to Rudy & Potts (1969), salmonid embryos capture and accumulate Na^+ in their perivitelline fluid until the eye formation stage, which is an advantage, especially when rearing takes place in waters of low alkalinity. According to Brow & Lynam (1981), the incubation of brown trout (*Salmo trutta*) eggs in water with a pH of 4.5 was most successful in water containing 10 mg L^{-1} of calcium, apart from the concentration of Na^+ (0, 1 or 10 mg L^{-1}). After incubation, they observed that the survival of fry was higher in water containing Na^+ at 1 or 10 mg L^{-1} (Brow & Lynam, 1981).

Each species has an ideal growth range within each physiochemical parameter, where this is related to its natural history. According to Leis & Trnski (1989), larvae and adults are often totally different ecologically, and may even be considered echo-species, presenting peculiarities for habitat type, feeding, and behaviour.

The results obtained in this study agree with the environmental conditions of growth and reproduction of the 'curimbatá'. According to Agostinho *et al*. (1993), South American migratory species, among which it is find the 'curimbatá', usually spawn in open waters of the main channel or tributary where the pH is usually neutral or close to neutral. The annual pH average is 7.4 ± 0.3 for the Paraná River, 7.0 ± 0.3 for the Ivinhema River (Thomaz *et al*., 1997), and 7.1 ± 0.2 for the Uruguay River (Larenze & Arrigoni, 1993). Once spawning has taken place, eggs are transported by the stream towards flooded areas and marginal ponds, where the larvae usually arrive shortly before their digestive system is totally formed (Domingues & Hayashi, 1998).

Flooded locations are seasonally submitted to harsh modifications in their physical, chemical and hydrological characteristics, which change the animal and vegetal communities. These changes are incorporated into the life cycles of several species. In flood periods, when the volume of water transported by the rivers is huge, the decomposition of plants, animal excrement, and autochthonous and allochthonous organic material causes a considerable enrichment of the water due to the great amount of nutrients

introduced in the process. This factor significantly favours the proliferation of bacteria, phytoplankton and zooplankton in the entire flooded environment, and it also influences pH reduction, through the accumulation of organic matter, as well as the wide variations of this variable due to the high concentration of phytoplankton. In adjacent ponds of the Paraná River, the natural environment of 'curimbatá', the pH presents an annual average of 6.6 ± 0.5, varying from 5.1 to 9.1. In the temporary ponds, where it can also find the initial stages of this species, the annual pH average is of 6.2 ± 0.5, with variations that oscillate between 4.9 and 6.8 (Thomaz *et al*., 1997). The data found in the present study agrees with what takes place in nature, where the incubation of eggs and larvae occur in a neutral pH and the larviculture is accomplished in environments where the pH values are lower, in spite of large variations in time.

It can be concluded that water pH directly affects the initial stages of 'curimbatá' growth and survival during incubation and larviculture. The best incubation pH for the eggs is neutral and alkaline, whereas acidic and neutral pH values are recommended for larviculture. It can be also conclude that the incubation pH strongly influences the future performance of 'curimbatá' larvae in larviculture.

Acknowledgments

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