EGG INCUBATION AND LARVAL REARING OF PIAVA, *Leporinus obtusidens*: EFFECT OF pH

Mário Augusto GOSMANN[1] and Alex Pires de Oliveira NUÑER[1]

ABSTRACT

The aim of this study was to evaluate the effect of the pH of the water during *Leporinus obtusidens* egg incubation and larval rearing. During incubation of the eggs pH 5.0, 7.0 and 9.0 were tested. pH 7.0 produced the best results for fertilization rate, hatching, and weight of hatched larvae (29.62 ± 6.01%, 23.57 ± 2.81% and 0.63 ± 0.01 mg, respectively). As pH 5.0 caused total mortality of eggs, the pH range was expanded in larval rearing that used pH 5.0, 6.0, 7.0, 8.0 and 9.0. pH 5.0 and 6.0 were lethal to the larvae at 24 and 72 h, respectively. At pH 9.0, higher survival (87.22 ± 3.47%) and final weight (3.82 ± 0.19 mg) were registered, whereas the final length of the larvae was the same (P>0.05) for pH 7.0, 8.0, and 9.0. Incubation at extreme pH values was harmful to the development of the *L. obtusidens* eggs, while at neutral pH (7.0), the best hatching rate and weight of hatched larvae were obtained. During larval rearing, the acidic pH was lethal to the larvae within just a few hours of exposure, whereas higher larvae survival and final weight were registered at pH 9.0.

Keywords: Anostomidae; acidic water; alkaline water

INCUBAÇÃO DE OVOS E LARVICULTURA DA PIAVA, *Leporinus obtusidens*: EFEITO DO pH

RESUMO

O objetivo deste estudo foi avaliar o efeito do pH da água durante a incubação dos ovos e a larvicultura de *Leporinus obtusidens*. Durante a incubação dos ovos foram testados os pH 5,0, 7,0 e 9,0. Em pH 7,0 foram obtidas as maiores taxas de fertilização e de eclosão e o maior peso das larvas eclodidas (29,62 ± 6,01%, 23,57 ± 2,81% e 0,63 ± 0,01 mg, respectivamente). Como o pH 5,0 causou mortalidade total de ovos, a faixa de pH testada na larvicultura foi ampliada, tendo sido testados os pH 5,0, 6,0, 7,0, 8,0 e 9,0. Os pH 5,0 e 6,0 foram letais para as larvas em 24 e 72 h, respectivamente. Em pH 9,0 foram registrados os maiores valores de sobrevivência (87,22 ± 3,47%) e peso final (3,82 ± 0,19 mg), enquanto o comprimento final das larvas foi o mesmo (P>0,05) em pH 7,0, 8,0 e 9,0. A incubação em valores extremos de pH foi prejudicial ao desenvolvimento dos ovos de *L. obtusidens*, enquanto em pH neutro (7,0) foram obtidas as maiores taxas de eclosão e peso das larvas eclodidas. Durante a larvicultura, o pH ácido foi letal para as larvas após poucas horas de exposição, enquanto a maior sobrevivência e peso final foram registrados em pH 9,0.

Palavras chave: Anostomidae; água ácida; água alcalina

Artigo Científico: Recebido em 02/06/2014 – Aprovado em 14/05/2015

[1] *Laboratory of Freshwater Fish Biology and Cultivation, Aquaculture Department, Federal University of Santa Catarina (UFSC). Rodovia Francisco Thomaz dos Santos, 3532 – CEP: 88066-260 – Florianópolis – SC – Brazil. e-mail: mario.gosmann@mpa.gov.br; alex.nuner@ufsc.br (corresponding author)*

INTRODUCTION

The pH can greatly influence aquatic environments by acting on many chemical and biological processes (WETZEL, 2001). As pH can produce histological changes that may affect the growth and the reproduction (FERREIRA *et al*., 2001), it has an important role in the metabolism and physiology of fish (PARRA and BALDISSEROTTO, 2007). Extremely high or low pH values affect ionic regulation and may lead to fish death (SCOTT *et al*., 2005), especially during the early stages of development. Very high acidity conditions lead to a decrease of the plasma and body ions concentration (BALDISSEROTTO, 2011), which increases mucus production and may degenerate gills, causing asphyxiation (BOYD and TUCKER, 1998). This rapid ion loss increases the hematocrit and hemoglobin in blood plasma, causing a disturbance in the internal volume of fluids, leading to circulatory failure (BALDISSEROTTO, 2011). Strongly alkaline pH values inhibit the excretion of ammonia, which raises the intracellular pH, causing negative effects on muscle function (SCOTT *et al*., 2005) and promoting a state of internal alkalosis, which diminishes the H^+ concentration in the blood plasma, thus reducing the exchange of Na^+ in the external environment (BALDISSEROTTO, 2011).

The piava, *Leporinus obtusidens* (Valenciennes 1837), is distributed in the basins of the São Francisco River, Paraná River, and Uruguay River (ZANIBONI FILHO and SCHULZ, 2003). This species performs reproductive migrations and total spawning (REYNALTE-TATAJE and ZANIBONI FILHO, 2010). The piava belongs to the order Characiformes (Anostomidae), and is well known in commercial and sport fishing. In addition, it has an excellent quality of meat and great acceptance in the market, achieving high sale prices in some regions of Brazil (REYNALTE-TATAJE and ZANIBONI FILHO, 2010). The species is omnivorous, feeding on insects, fish, and vegetable scraps, which are important characteristics for the cultivation of this species (REYNALTE-TATAJE and ZANIBONI FILHO, 2010).

Since pH plays a very important role in the development of fish, the goal of the present study was to evaluate its effect on the incubation of eggs and larval rearing of *L. obtusidens*.

MATERIAL AND METHODS

The experiments were conducted at the Laboratory of Freshwater Fish Biology and Fish Culture of the Federal University of Santa Catarina in January/2012.

Experiment I: Incubation

First, we evaluated the effect of the pH on the development of *L. obtusidens* eggs. The eggs were obtained from breeders induced to spawn with carp pituitary extract according the method described by ZANIBONI-FILHO and BARBOSA (1996). After fertilization, the eggs were transferred to cylindrical-conical incubators with 10 L of water at 25 °C in pH to be tested in the proportion of 0.3 g of eggs L^{-1} (1 g = 2,020 eggs), with constant light and aeration.

The pH values tested were 5.0, 7.0 and 9.0, using a completely random design with three replicates. The pH control was carried out every hour with a pH meter (Digimed DM-2P), and was adjusted using either a solution of 0.1 N sulfuric acid to acidify the water or a solution of sodium hydroxide (10%) to alkalify the water.

The diameter of the eggs and the perivitelline space were measured at 1, 3, and 9 hours after fertilization (HAF); the fertilization rate (FR) was estimated 7 HAF, and the hatching rate at 24 HAF. To calculate the FR (FR = number of fertilized eggs / total number of eggs in the sample x 100), three samples of eggs (n = 220) were removed from each incubator, and the total number of eggs and fertilized eggs were recorded. The hatching rate (HR = number of viable larvae hatched / [number of dead eggs + number of viable larvae] x 100) was obtained from the analysis of three samples of eggs and larvae (n = 220) from each incubator. The egg diameter, perivitelline space, and length of newly hatched larvae were measured with a micrometric eyepiece (10x) in a stereoscopic microscope. The weight of newly hatched larvae was measured using an analytical balance (0.001 g).

The temperature and the concentration of dissolved oxygen were measured every two hours

with an YSI 550A oxymeter. Alkalinity and hardness were measured as described by GOLTERMAN *et al*. (1978), and the concentration of total ammonia was quantified at the beginning and at the end of the experiment using the method described by KOROLEFF (1976).

The perivitelline space of the eggs was measured and later analyzed by linear regression and covariance analysis using GraphPad Prism® 5. The hatching rate, total length, and weight of the larvae hatched at pH 7.0 and 9.0 were compared by a t test at a significance level of 0.05. ANOVA was applied to alkalinity, hardness and total ammonia concentration data, followed by Tukey test when necessary.

Experiment II: Larval rearing

Larval rearing was performed to evaluate the effect of pH on *L. obtusidens* larval rearing. Hormonal induction and reproduction procedures were the same as described in Experiment I, with the exception that incubation occurred exclusively at pH 7.0. One day after hatching, the larvae were transferred to 8 L rectangular tanks with a water temperature of 25 ^{o}C and constant aeration at a density of 10 larvae L^{-1}. The initial weight and the total length of the larvae were 0.62 ± 0.05 mg and 4.77 ± 0.04 mm, respectively. Exogenous feeding of the larvae using *Artemia* sp. as live food was started 24 hours after starting the experiment.

The experimental design and pH adjustment procedures were the same as described in Experiment I, but as pH 5.0 caused total mortality of eggs, its range was expanded in larval rearing that used pH 5.0, 6.0, 7.0, 8.0 and 9.0. At the end of the larviculture, the final weight (W), final total length (TL), and survival (S = number of living larvae at the end of the trial / initial number of larvae x 100) of the larvae were evaluated. The weight was measured using an analytical balance (0.001 g) and the total length was measured with micrometric eyepiece (10x) in a stereoscopic microscope.

The temperature and electric conductivity were measured with an YSI 63 multiparameter twice a day, and the dissolved oxygen concentration was measured with a YSI 550A. Alkalinity and hardness were measured according to GOLTERMAN *et al*. (1978), and the total ammonia concentration was quantified using the method described by KOROLEFF (1976) at the beginning and at the end of the experiment. For pH 7.0, 8.0, and 9.0, the concentrations of sodium, calcium, and magnesium in the water were measured at the beginning and at the end of experiment by the atomic absorption spectrometry method. For pH 5.0 and 6.0, ion concentrations were measured only at the beginning of the experiment. The final larval weight, length, and the survival were analyzed by regression and covariance analysis using the GraphPad Prism® 5 software.

The tanks were siphoned daily for cleaning and the volume of water that was removed was replaced. Dead larvae removed in siphoning were counted to estimate mortality.

RESULTS

Experiment I: Incubation

During egg incubation, the temperature (25.5 ± 0.10 ^{o}C), dissolved oxygen (7.58 ± 0.06 mg L^{-1}), alkalinity (50.8 ± 0.83 mg L^{-1} $CaCO_3$), total ammonia (0.10 ± 0.03 mg L^{-1}) and hardness (62.7 ± 7.35 mg L^{-1} $CaCO_3$) were the same for the different pH values (P>0.05). The pH influenced the diameter of eggs and the perivitelline space at 1 HAF, but not at 3 or 9 HAF (Table 1). At pH 5.0, chorion disruption occurred and all of the eggs died before hatching. Disruption also occurred at pH 9.0, but larvae hatched at a rate much lower than at pH 7.0. This disruption interfered with the calculation of the fertilization rate and, therefore, this rate was calculated only for the eggs incubated at pH 7.0 (Table 2). While the larvae that hatched in pH 7.0 and pH 9.0 (Table 2) had the same total length (P>0.05), the larvae weight was higher at pH 7.0 (P<0.05).

Experiment II: Larval rearing

The variation in water quality variables did not influence the larvae performance (Table 3). The concentration of Na^{+} showed variations for the pH values between the beginning and end of the experiment, which was in contrast to $Ca2^{+}$ and Mg^{2+}, which remained unchanged (Table 3).

Table 1. Diameter (mm) and perivitelline space of the eggs (mm) (mean ± standard deviation) of *Leporinus obtusidens* at 1, 3, and 9 hours after fertilization (HAF) at different pH values and regression models relating egg variable with pH.

Egg variable	HAF	pH 5.0	pH 7.0	pH 9.0	Model
Diameter (mm)	1	1.61 ± 0.03	2.32 ± 0.03	2.17 ± 0.03	Y = 0.14X + 1.05; R^2 = 0.55; P<0.05
	3	1.63 ± 0.03	2.44 ± 0.04	1.93 ± 0.03	P>0.05
	9	*	2.42 ± 0.06	*	
Perivitelline space (mm)	1	0.37 ± 0.03	0.58 ± 0.04	0.55 ± 0.02	Y = 0.04X + 0.18; R^2 = 0.63; P<0.05
	3	0.37 ± 0.03	0.59 ± 0.04	0.45 ± 0.07	P>0.05
	9	*	0.59 ± 0.03	*	

* = *chorion disruption*

Table 2. Fertilization rate (%), hatching rate (%), total length (mm) and weight (mg) (mean ± standard deviation) of the hatched *Leporinus obtusidens* larvae at pH 7 and 9.

Variable	pH	
	7	9
Fertilization rate (%)	29.6 ± 6.01	*
Hatching rate (%)	23.6 ± 2.81[a]	16.3 ± 2.19[b]
Total length (mm)	3.6 ± 0.11[a]	3.4 ± 0.05[a]
Weight (mg)	0.6 ± 0.01[a]	0.5 ± 0.01[b]

*Different letters denote statistical differences. * = chorion disruption.*

Table 3. Water quality (mean ± standard deviation) during larval rearing of *Leporinus obtusidens* at different pH values.

Variable		pH				
		5.0	6.0	7.0	8.0	9.0
Temperature (°C)		25.5 ± 0.1	25.6 ± 0.1	25.5 ± 0.4	25.5 ± 0.4	25.6 ± 0.4
Dissolved oxygen (mg L^{-1})		7.77 ± 0.05	7.52 ± 0.21	7.48 ± 0.19	7.60 ± 0.18	7.74 ± 0.23
Alkalinity (mg L^{-1} $CaCO_3$)		39.5 ± 1.9	41.5 ± 2.3	45.3 ± 3.4	50.8 ± 1.5	53.3 ± 2.6
Total ammonia (mg L^{-1})		*	0.03 ± 0.05	0.12 ± 0.11	0.24 ± 0.19	0.12 ± 0.11
Hardness (mg L^{-1} $CaCO_3$)		68.69 ± 3.20	68.17 ± 4.40	71.84 ± 1.35	60.05 ± 2.28	49.74 ± 1.52
Conductivity (µS cm^{-1})		179.6 ± 15.1	172.4 ± 17.4	271.4 ± 30.9	352.4 ± 25.0	611.0 ± 13.3
Na^+ (mg L^{-1})	B	2.01	2.61	10.31	23.00	28.00
	E	*	*	36.00	54.00	145.00
Ca^{2+} (mg L^{-1})	B	4.35	4.38	4.85	3.41	3.41
	E	*	*	4.16	3.27	3.27
Mg^{2+} (mg L^{-1})	B	0.75	0.77	0.77	0.76	0.67
	E	*	*	0.82	0.73	0.60

* = *total larvae mortality; B = ion concentration at beginning of the experiment; E = ion concentration at the end of the experiment.*

The lower pH values markedly influenced the survival of *L. obtusidens* larvae. At pH 5.0, the larvae survival was 16.67 ± 7.26% after 12 hours and was zero within 24 hours. At pH 6.0, survival was 43.44 ± 0.16% at 24 h, 14.11 ± 0.05% at 36 hours, and zero after 72 hours. The final survival in pH 7.0, 8.0 and 9.0 was 78.89 ± 11.09, 47.78 ± 5.85 and 87.22 ± 3.47%, respectively.

In pH 9.0, better survival condition was observed (Figure 1), with survival being minimally reduced until 72 hours. Larvae in pH 7.0 presented a very good condition, while the survival was strongly reduced in pH 8.0. After 72 hours, survival remained stable and practically unaltered for all of the tested pH values (Table 4).

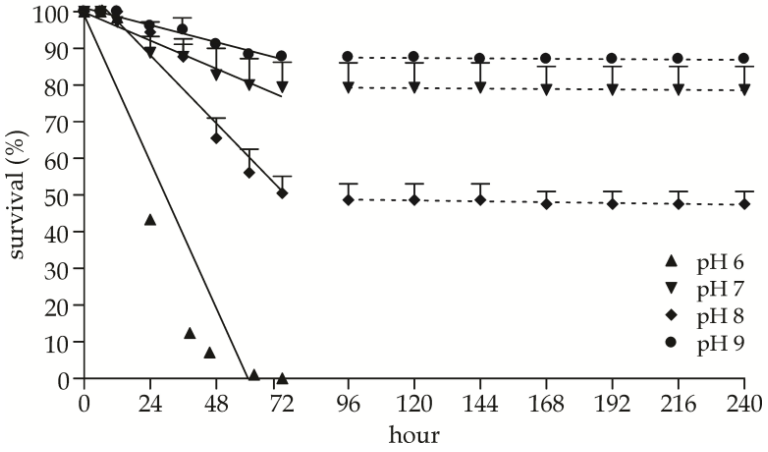

Figure 1. Survival (%) of *Leporinus obtusidens* larvae at different pH levels over time.

Table 4. Regression models relating survival of *Leporinus obtusidens* larvae and pH levels for distinct periods: 0–72 hours and 96–240 hours.

pH	0-72 h Models	R^2	96-240 h Models	R^2
6.0	Y = 99.0 - 1.664X	0.87	*	
7.0	Y = 99.8 - 0.320X	0.95	Y = 80.0 - 0.005X	0.65
8.0	Y = 106.7 - 0.773X	0.93	Y = 49.9 - 0.010X	0.65
9.0	Y = 101.1 - 0.194X	0.97	Y = 88.1 - 0.004X	0.65

* = *total mortality.*

The final length of the larvae for pH 7.0, 8.0, and 9.0 was 7.56 ± 0.17 mm, 6.34 ± 0.05 mm, and 8.36 ± 0.05 mm, respectively, but the difference was not significant (P>0.05), however the final weight of the larvae was influenced by the increase in pH (Figure 2).

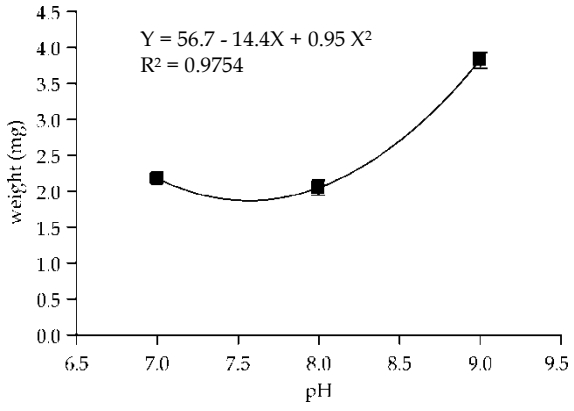

Figure 2. Final weight of the *Leporinus obtusidens* larvae after 10 days of rearing at different pH values.

DISCUSSION

Experiment I: Incubation

Most teleost fish species are adversely affected by acidic pH values below 6.0 and at alkaline pH values above 9.0 (PARRA and BALDISSEROTTO, 2007). However, this effect is also related to age and to the stage of development, with embryonic and larval being the most sensitive stages (MILLER *et al*., 1988).

In the present study, pH 5.0 was lethal for the eggs, which was also reported for *Rhamdia quelen* by FERREIRA *et al*. (2001), who observed total mortality of eggs at pH 4.0. Mortality at an acidic pH may be related to the high concentration of H^+ ions in the external medium, which inhibits the uptake of Na^+ (BALDISSEROTTO, 2011), which is essential for the osmoregulation of embryos and larvae (BENTLEY, 1990; VARSAMOS *et al*., 2005). Hard water can have a protective effect of the eggs and larvae against ionic losses (BALDISSEROTTO, 2011), but since the water

hardness for pH 5.0 was considered soft, it favored the loss of ions and internal fluids by osmotic forces, causing the wilting of the eggs and the chorion disruption.

At pH 7.0, the diameter of the eggs and the perivitelline space were similar to other species of *Leporinus*. For *Leporinus macrocephalus*, was observed a mean diameter of the eggs and the perivitelline space of 2.2 ± 0.1 mm and 0.6 ± 0.1 mm, respectively (REYNALTE-TATAJE *et al*., 2001).

Despite the chorion disruption of eggs at pH 9.0, a condition that may be related to the low water hardness that did not inhibit the excessive absorption of water by the eggs, causing their break up (SPADE and BRISTOW, 1999), the larval development was not fully interrupted, and some viable larvae were observed at the end of the experiment.

The fertilization rate obtained in this study was lower than those reported by REYNALTE TATAJE and ZANIBONI FILHO (2010) for *L. obtusidens* (48.5 ± 43.5%), SATO *et al*. (2000) for *L. friderici* (48.1%), and REYNALTE-TATAJE *et al*. (2001) for *L. macrocephalus* (94.5 ± 3.3%). The total length of newly hatched larvae at pH 7.0 and 9.0 was similar (P>0.05) and slightly higher than that reported by SATO *et al*. (2000) for *L. elongatus* (2.9 ± 0.1 mm) and for *L. macrocephalus*, which presented a total length of 2.39 ± 0.12 mm (REYNALTE-TATAJE *et al*., 2001). Better conditions for development were obtained at pH 7.0, since the weight of the newly hatched larvae was higher in that condition (P<0.05).

Experiment II: Larval rearing

A pH of 6.0 was lethal for *L. obtusidens* after 72 h of exposure and, similar to the eggs, mortality could be related to a combination of the low water hardness and reduced uptake of Na^+ from the external medium (BALDISSEROTTO *et al*., 2009; BALDISSEROTTO, 2011). *Prochilodus lineatus* larvae developed at pH range of 4.8–5.6, but no survival was observed for pH values less than 4.6 (ZANIBONI FILHO *et al*., 2009). While *R. quelen* larvae survived exposure to pH 6.0 (FERREIRA *et al*., 2001), survival, weight, and length were negatively affected (LOPES *et al*., 2001).

At pH 9.0, the survival rate was higher than that observed for the other levels tested. For *P. lineatus*, similar results were found, with survival rates exceeding 90% in the pH range between 8.7 and 9.2 (ZANIBONI FILHO *et al*., 2009). *Rhamdia quelen* larvae exposed to pH 8.6 showed a survival rate of 79% (LOPES *et al*., 2001). At pH 7.0, the survival was also high and similar to the rate of other species that inhabit the same watershed, such as *P. lineatus* larvae, for which a survival rate of 76.7% was reported at the same pH (ZANIBONI FILHO *et al*., 2009). The pH 8.0 did not provide the same benefits to larvae as pH 9.0, since only 50% of the larvae survived at the end of the study.

The higher concentration of Na^+ at pH 9.0 could directly influence the survival and final weight of the larvae, since Na^+ has a very important role in osmoregulation in fish (VARSAMOS *et al*., 2005) and is essential for larval development (BENTLEY, 1990). In alkaline waters, the low concentration of H^+ ions in the external environment may lead to decreased levels of Na^+ in the blood plasma (BOLNER and BALDISSEROTTO, 2007). However, with a large availability of Na^+ in the external medium, this decrease can be reversed by osmoregulation (SCOTT *et al*., 2005). Despite the concentration of Na^+ at pH 8.0 being higher than at pH 7.0, the weight and survival of larvae was lower (P<0.05). Therefore, the availability of Na^+ in the external medium was apparently not enough to reduce the adverse effect of the alkaline pH.

The concentrations of $Ca2^+$ and Mg^{2+} were similar between conditions at the beginning and at the end of the larval rearing of *L. obtusidens*. These ions are of great importance in ionic regulation, affecting membrane permeability and the loss of ions to water (SILVA *et al*., 2005). $Ca2^+$ is also involved in skeletal development and is the primary element in the calcification of collagen. Therefore, its deficiency may impair the development of larvae and cause stress (BLANKSMA *et al*., 2009). Mg^{2+} plays an important role in energy metabolism and protein synthesis, and its deficiency causes loss of appetite, reduced growth, and renal alterations (DABROWSKI, 1986).

In the present study, mortalities occurred primarily during the first 72 hours of the experiment, and after that time, survival remained constant. According to MILLER *et al*. (1988), fish

larvae are more sensitive to changes in the external environment when compared to most other developed stages, such as juveniles of *L. obtusidens*, which were exposed to pH 5.5 and 9.0 without showing mortality (COPATTI and AMARAL, 2009). Similarly, *R. quelen* juveniles exposed to pH values between 4.0 and 9.0 showed a survival rate close to 100% (ZAIONS and BALDISSEROTTO, 2000).

CONCLUSIONS

Incubation at extreme pH values was harmful to the development of the *L. obtusidens* eggs, while at neutral pH the best hatching rate and weight of hatched larvae were obtained. During larval rearing, an acidic pH was lethal for larvae within just a few hours of exposure, whereas at pH 9.0, higher larvae survival and final weight were observed.

ACKNOWLEDGMENTS

We would like to thank the Federal Agency of Support and Evaluation of Postgraduate Education (CAPES) for the scholarship awarded to the first author.

REFERENCES

BALDISSEROTTO, B. 2011 Water pH and hardness affect growth of freshwater teleost. *Revista Brasileira de Zootecnia*, *40*(suppl. especial): 138-144.

BALDISSEROTTO, B.; COPATTI, C.E.; GOMES, L.C.; CHAGAS, E.C.; BRINN, R.P.; ROUBACH, R. 2009 Calcium fluxes in *Hoplosternum littorale* (tamoatá) exposed to different types of Amazonian waters. *Neotropical Ichthyology*, *7*(3): 465-470.

BENTLEY, P.J. 1990 Unidirectional fluxes of Na^+, Cl^- and water in fingerling channel catfish, *Ictalurus punctatus*. *Comparative Biochemistry and Physiology*, *Part A: Physiology*, *97*(2): 195-199.

BLANKSMA, C.; EGUIA, B.; LOTT, K.; LAZORCHAK, J.M.; SMITH, M.E.; WRATSCHKO, M.; DAWSON, T.D.; ELONEN, C.; KAHL, M.; SCHOENFUSS, H.L. 2009 Effects of water hardness on skeletal development and growth in juvenile fathead minnows. *Aquaculture*, *286*(3-4): 226-232.

BOLNER, K.C.S. and BALDISSEROTTO, B. 2007 Water pH and urinary excretion in silver catfish *Rhamdia quelen*. *Journal of Fish Biology*, *70*(1): 50-64.

BOYD, C.E. and TUCKER, C.S. 1998 *Pond aquaculture water quality management*. Boston: Kluwer Academic Publishers, 700p.

COPATTI, C.E. and AMARAL, R. 2009 Osmorregulação em juvenis de piava, *Leporinus obtusidens* (Characiformes: Anostomidae), durante trocas do pH da água. *Biodiversidade Pampeana*, *7*(1): 1-6.

DABROWSKI, K.R. 1986 Ontogenetical aspects of nutritional requirements in fish. *Comparative Biochemistry and Physiology, Part A: Physiology*, *85*(4): 639-655.

FERREIRA, A.A.; NUÑER, A.P.O.; ESQUIVEL, J.R. 2001 Influência do pH sobre ovos e larvas de jundiá, *Rhamdia quelen* (Osteichthyes, Siluriformes). *Acta Scientiarum. Biological Sciences*, *23*(2): 477-481.

GOLTERMAN, H.; CLYMO, R.S.; OHNSTAD, M.A.M. 1978 *Methods for physical and chemical analysis of fresh water*. Oxford: Blackwell Scientific Publications Ltd. 213p.

KOROLEFF, F. 1976 Determination of ammonia. In: GRASSHOF, K. *Methods of seawater analysis*. Weinheim: Verlag Chemie. p.117-181.

LOPES, J.M.; SILVA, L.V.F.; BALDISSEROTTO, B. 2001 Survival and growth of silver catfish larvae exposed to different water pH. *Aquaculture International*, *9*(1): 73-80.

MILLER, T.J.; CROWDER, L.B.; RICE, J.A.; MARSCHALL, E. 1988 Larval size and recruitment mechanisms in fishes: Toward a conceptual framework. *Canadian Journal of Fisheries and Aquatic Sciences*, *45*(9): 1657-1670.

PARRA, J.E.G. and BALDISSEROTTO, B. 2007 Effect of water pH and hardness on survival and growth of freshwater teleosts. In: BALDISSEROTTO, B.; MANCERA, J.M.; KAPOOR, B.G. *Fish osmoregulation*. New Hampshire, USA: Science Publishers. p.135-150.

REYNALTE-TATAJE, D.A. and ZANIBONI FILHO, E. 2010 Cultivo de piapara, piava, piau - gênero *Leporinus*. In: BALDISSEROTTO, B. and CARVALHO-GOMES, L. *Espécies nativas para a*

piscicultura no Brasil. Santa Maria, Brazil: UFSM. p.73-92.

REYNALTE-TATAJE, D.A.; ZANIBONI-FILHO, E.; MUELBERT, B. 2001 Stages of embryonic development of the piavuçu *Leporinus macrocephalus* (Garavello & Britski, 1988). *Acta Scientiarum*, *23*(4): 823-827.

SATO, Y.; FENERICH-VERANI, N.; VERANI, J.R.; VIEIRA, L.J.S.; GODINHO, H.P. 2000 Induced reproductive responses of the Neotropical Anostomid fish *Leporinus elongatus* Val. under captive breeding. *Aquaculture Research*, *31*(2): 189-193.

SCOTT, D.M.L.M.C. and WILSON, R.W. 2005. The effect of high pH on ion balance, nitrogen excretion and behaviour in freshwater fish from an eutrophic lake: a laboratory and field study. *Aquatic Toxicology*, *73*(1): 31-43.

SILVA, L.V.F.; GOLOMBIESKI, J.I.; BALDISSEROTTO, B. 2005 Growth and survival of silver catfish larvae, *Rhamdia quelen* (Heptapteridae), at different calcium and magnesium concentrations. *Neotropical Ichthyology*, *3*(2): 299-304.

SPADE, S. and BRISTOW, B. 1999 Effects of increasing water hardness on egg diameter and hatch rates of striped bass eggs. *North American Journal of Aquaculture*, *61*(3): 263-265.

VARSAMOS, S.; NEBEL, C.; CHARMANTIER, G. 2005 Ontogeny of osmoregulation in postembryonic fish: A review. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *141*(4): 401-429.

WETZEL, R.G. 2001 *Limnology: lake and river ecosystems*. Third ed. San Diego, USA: Academic Press. 1006p.

ZAIONS, M.I. and BALDISSEROTTO, B. 2000 Na^+ and K^+ body levels and survival of fingerlings of *Rhamdia quelen* (Siluriformes, Pimelodidae) exposed to acute changes of water pH. *Ciência Rural*, *30*(6): 1041-1045.

ZANIBONI-FILHO, E. and BARBOSA N.D.C. 1996 Priming hormone administration to induce spawning of some Brazilian migratory fish. *Revista Brasileira de Biologia*, *56*(4): 655-659.

ZANIBONI-FILHO, E.; NUÑER, A.P.O.; REYNALTE-TATAJE, D.A.; SERAFINI, R.L. 2009 Water pH and *Prochilodus lineatus* larvae survival. *Fish Physiology and Biochemistry*, *35*(1): 151-155.

ZANIBONI FILHO, E. and SCHULZ; U.H. 2003 Migratory fishes of the Uruguay River. In: CAROLSFELD, J.; HARVEY, B.; ROSS, C.; BAER, A. *Migratory fishes of South America: biology, fisheries and conservation status*. Victoria, Canada: World Fisheries Trust/World Bank/IDRC. p.159-194.