# Survival of *Prochilodus lineatus* (Valenciennes) fingerlings exposed to acute pH changes

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**ABSTRACT.** The determination of the lethal pH levels is an essential procedure before starting the culture of a fish species, since the tolerance to pH varies according to the species. Therefore, the aim of the present study was to investigate the effect of acute changes of water pH on survival of *Prochilodus lineatus* (Valenciennes) (Characiformes, Prochilodontidae) fingerlings, a species largely used in fish culture in Brazil. Fingerlings were submitted to pH 3.5, 3.7, 3.9, 4.0, 7.0, 9.5, 9.6, 9.7, 9.8, 10.0, and 10.5 for 120 h. The 3.5 - 3.7 pH range was lethal because survival was around 3%. The survival at pH 3.9 and 4.0 were 37 and 68%, respectively. The upper pH level for survival was 9.84, because upper pH led to increased mortality. However, the best pH range for *P. lineatus* fingerling survival was 4.0 - 9.84.

**Key words:** *Prochilodus lineatus*, pH, fingerlings, *curimatã*.

RESUMO. Sobrevivência de alevinos de *Prochilodus lineatus* (Valenciennes) submetidos a valores extremos de pH. A determinação dos níveis letais de pH é informação essencial para o cultivo de determinada espécie de peixe, haja vista que a tolerância do pH varia com a espécie. Dessa forma, o objetivo do presente estudo foi investigar o efeito das mudanças do pH da água na sobrevivência de alevinos de *Prochilodus lineatus* (Valenciennes) (Characiformes, Prochilodontidae), uma espécie amplamente utilizada na piscicultura brasileira. Alevinos foram submetidos a pH de 3,5; 3,7; 3,9; 4,0; 7,0; 9,5; 9,6; 9,7; 9,8; 10,0 e 10,5 por 120 h. O intervalo de pH entre 3,5 e 3,7 foi letal, apresentado sobrevivência em torno de 3%. A sobrevivência ao pH 3,9 e 4,0 foi 37% e 68%, respectivamente. O limite máximo de pH foi 9,84, haja vista que acima deste foi observado aumento da mortalidade. Entretanto, os maiores valores de sobrevivência para alevinos de *P. lineatus* foram observados quando os valores de pH estiveram entre 4,0 e 9,84.

Palavras-chave: Prochilodus lineatus, pH, alevinos, curimatá.

#### Introduction

The carbonic gas-bicarbonate-carbonate system usually maintains water pH within the 6.0 - 8.0 range (Wilkie and Wood, 1994). Temporary upward water pH surges may be due to an increase of plants or algae photosynthetic processes (Arana, 1997). Some lakes show very alkaline pH, due to high bicarbonate and carbonate salts concentrations, e.g. lake Van, in Turkey (pH 9.8) (Danulat and Selcuk, 1992) and Magadi lake, in Kenya (pH 10) (Maetz and De Renzis, 1978). Water pH can become very acidic (lower than 4.0) when the soil has a great amount of sulfate (Simpson *et al.*, 1983), or even due to the presence of humic and fulvic acids, as in the Amazon streams (Walker, 1990).

Most species survive to acute pH changes down to pH 4.0 - 4.5 (Freda and McDonald, 1988; Zaions and Baldisserotto, 2000). The cause of death in acidic water is the inhibition of the Na<sup>+</sup>/H<sup>+</sup> exchanger in the gills due to the excess H+ (Potts, 1994), which led to a gradient too steep for further protons extrusion (Lin and Randall, 1991), reducing the Na<sup>+</sup> uptake. Moreover, high H<sup>+</sup> concentrations disrupt the tight junctions of gill epithelia, increasing ion loss by a paracellular route (Gonzalez, 1996). Survival in water pH up to 9.0 is common (Van Dijk et al., 1993; Zaions and Baldisserotto, 2000). Exposure to very alkaline water causes disturbances in internal electrolyte balance, due to reduced Na<sup>+</sup> and Cl<sup>-</sup> influx, caused by branchial transporters inhibition (Wilkie et al., 1999).

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Sometimes it is not possible, or economically not viable, to maintain the water in optimum conditions for fish culture. Therefore, the lethal pH levels determination is an essential procedure before starting the culture of a fish species, since, as already mentioned, the tolerance to pH varies according to the species. Therefore, the aim of the present study was to investigate the effect of water pH acute changes on survival of *Prochilodus lineatus* (Characiformes Prochilodontidae) fingerlings, a species largely used in Brazilian fish culture in.

#### Material and methods

Prochilodus lineatus fingerlings were bought from a local supplier near Florianópolis, State of Santa Catarina, Brazil. To determine the pH effect on the survival and general feeding behavior of P. lineatus fingerlings, groups of 10 fishes (tank biomass 89.0 ± 1.7g) were placed in continuously aerated 150-l freshwater tanks. The tank water pH values were 3.5, 3.7, 3.9, 4.0, 7.0, 9.5, 9.6, 9.7, 9.8, 10.0, and 10.5 (three replicates per treatment). Water pH was previously adjusted to the desired experimental pH by the addition of sulfuric acid or sodium hydroxide, measured three times a day (8am., 1pm., and 5pm.) with a Schott-Gertate pHmeter (precision 0.1) and adjusted when necessary. Temperature and dissolved oxygen were determined twice a day (8am. and 5pm.) with YSI 55 OD-meter. At the end of the experiment, a 500-ml sample was collected from each tank, to determine water hardness by EDTA titrimetric method (Greenberg et al., 1976).

Fish were fed *ad libitum* twice a day (9am. and 4pm.), with dry pellets produced in the laboratory (30% crude protein), and the feeding behavior was observed. Mortality was determined at 24, 48, 72, 96, and 120 h after exposure to different pH's. All feces and pellet residues were removed daily by suction, and consequently 10% of the tank water was replaced by water with the same pH.

Mean lethal acidic and alkaline pH levels for 120h were obtained using the mean survival data for each treatment, according to Holtze and Hutchinson (1989). Survival was analyzed by variance analysis and Tukey test. Data were expressed as mean  $\pm$  SD, and the minimum significance level was P < 0.05.

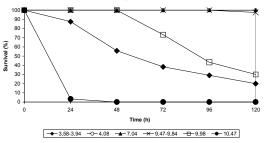
### Results

Mean values for dissolved oxygen, temperature and hardness throughout the experimental period were 9.11±0.53 mg/l, 20.6±1.9°C and 95.1±46.8 mg/l, respectively. Maximum total ammonia, non-

ionized ammonia, and nitrite values were 0.260 mg/l, 0.144 mg/l, and 0.06 mg/l, respectively.

Fingerlings submitted to extremely acidic values (3.58, 3.75, and 3.87) presented low survival rate. Mortality started 24 h after transfer to pH 3.58, while dead fish were observed in the first hours of experiment at pH 3.75, 3.87, and 3.94. However, the highest mortality in the lower exposition time was detected at pH 3.75. The survival decreased slowly with time in the other acidic pHs (Figure 1).

Fingerlings submitted to the 4.08 – 9.84 pH range showed 100% survival. It is interesting to observe that any increase water pH over 9.84 induced high mortality, since at pH 9.98 the survival was only 30%, and at pH 10.47 the mortality was almost 100% within 24 h (Table 1). After 120 h of exposure to the experimental pH, the lethal concentration values for acidic and alkaline pH were 3.96 and 9.93, respectively.



**Figure 1.** Survival of *Prochilodus lineatus* fingerlings as a function of time after transfer to the experimental pH

**Table 1.** Survival of *Prochilodus lineatus* fingerlings as a function of time after transfer to the experimental pH

pН	Survival (%) <sup>1</sup>				
(Min-Max)	24 h	48 h	72 h	96 h	120 h
3.58±0.07	100.0±0°	76.7±25.2bc	50.0±10.0bc	33.3±15.3ab	26.7±20.8a
(3.50 - 3.88)					
$3.75 \pm 0.05$	63.3±20.8 <sup>b</sup>	$3.3\pm5.8^{a}$	$3.3\pm5.8^{a}$	$3.3\pm5.8^{a}$	3.3±5.8 <sup>a</sup>
(3.67 - 3.86)					
$3.87 \pm 0.11$	90.0±10.0°	60.0±36.1 <sup>b</sup>	26.7±46.2ab	23.3±40.4 <sup>ab</sup>	13.3±23.1 <sup>a</sup>
(3.75 - 4.21)					
$3.94 \pm 0.05$	96.7±5.8°	83.3±15.3 <sup>bc</sup>	73.3±15.3 <sup>cd</sup>	56.7±15.3 <sup>bc</sup>	36.7±28.9 <sup>a</sup>
(3.81 - 4.1)					
4.08 ±0.10	100.0±0.0°	100.0±0.0°	$100.0\pm0.0^{d}$	100.0±0.0°	100.0±0.0 <sup>b</sup>
(3.89– 4.55)	4000.000	4000.000	1000.001	4000.000	400 0 . 0 ob
7.04 ±0.06	100.0±0.0°	100.0±0.0°	100.0±0.0 <sup>d</sup>	100.0±0.0°	100.0±0.0 <sup>b</sup>
(6.89 – 7.22)	1000.000	100 0 . 0 00	1000.004	1000.000	0 4 7 . F Fb
9.47 ±0.10	100.0±0.0°	100.0±0.0°	100.0±0.0 <sup>d</sup>	100.0±0.0°	96.7±5.5 <sup>b</sup>
(9.05– 9.63) 9.62 +0.19	100.010.00	100.010.00	100.0±0.0 <sup>d</sup>	100.010.00	100.0+0.0 <sup>b</sup>
9.62 ±0.19 (8.8–9.9)	100.0±0.0°	100.0±0.0°	100.0±0.0	100.0±0.0	100.0±0.0
(o.o-9.9) 9.72 ±0.17	100.0+0.0°	100 0±0 0°	100.0±0.0 <sup>d</sup>	100 0±0 0°	100.0+0.0 <sup>b</sup>
(8.98–9.99)	100.010.0	100.010.0	100.0±0.0	100.0±0.0	100.0±0.0
9.84 +0.16	100.0+0.0°	100 0±0 0°	100 0+0 0d	100.0+0.0°	93.3+11.5 <sup>b</sup>
(9.11–10.1)	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	75.5±11.5
9.98 +0.06	100 0±0 0°	100 0±0 0°	73.3+20.8 <sup>cd</sup>	43 3+25 2ab	30.0+26.5°
(9.82–10.12)	100.0±0.0	100.0±0.0	, 5.5_20.0	1010_2012	20.0_20.0
10.47 ±0.07	3.3±5.8 <sup>a</sup>	$0.0\pm0.0^{a}$	$0.0\pm0.0^{a}$	$0.0\pm0.0^{\circ}$	$0.0\pm0.0^{a}$
(10.33–10.6)					

 $<sup>^{\</sup>rm I}$  Means identified by different letters in the column were significantly different (p < 0.05) as determined by Tukey test

#### Discussion

The knowledge of the ideal range for water physicochemical parameters is a crucial factor for improving the culture of a given fish species. Water pH is a very important parameter, since ionic imbalances occur when a fish species is exposed to values outside its survival range (Zaions and Baldisserotto, 2000). In spite of being important to know the lethal pH ranges for cultured species, few studies have been made with native Brazilian teleost species.

The P. lineatus post larvae survived to exposure at pH 4.8 to 9.2 (72 h) (Zaniboni-Filho et al., 2002 - to be soon published). In this study we demonstrated that this species fingerlings showed no mortality at 4.08 to 9.84 pH range, being, therefore, more resistant than the post larvae. This result was expected, since the water pH effect on fish is related to age and developmental stage, the embryonic and larval stages being the most sensitive to pH changes (Lloyd and Jordan, 1964). Similar results were also obtained for silver catfish (Rhamdia quelen): the lethal pH for eggs of this species is 4.0 (12 h after fertilization), but recently hatched larvae (33-45 h after fertilization) are more susceptible to pH changes, since their lethal pH is 5.0 (Ferreira et al., 2000). In addition, fingerlings of the same species presented 100% survival (after 96 h) when exposed to the 4.00 to 9.0 pH range (Zaions and Baldisserotto, 2000). For P. lineatus eggs the best water pH during incubation is 7.0 and, during the larviculture, the best results were achieved with neutral or slightly acid pH (Reynalte-Tataje, 2000).

Adult *P. lineatus* are found in rivers with neutral pH, while larvae and fingerlings inhabit marginal ponds with mean annual pH of  $6.6 \pm 0.5$ , with oscillations between pH 5.1 and 9.1 (Thomaz *et al.*, 1997). Therefore, according to our results, fingerlings do not have any problems for survival in the natural water pH, as expected, but in some circumstances death may occur in the first larval stages. The pH survival range for fingerlings of this species also allows good resistance to normal pH variation in fishpond systems.

#### References

ARANA, L.V. Princípios químicos da qualidade da água em aqüicultura. Florianópolis: Editora da UFSC, 1997.

DANULAT, E.; SELCUK, B. Life history and environmental conditions of the anadromous *Chalcalburnus tarichi* (Cyprinidae) in the highly alkaline Lake Van, Eastern Anatolia, Turkey. *Arch. Hydrobiol.*, Stuttgart, v.126, p.102-125, 1992.

FERREIRA, A.A. Influência de diferentes níveis de pH no desempenho de ovos, larvas e pós-larvas de jundiá (Rhamdia quelen). 2000. Dissertação (Mestrado em Aqüicultura) - Universidade Federal de Santa Catarina, Florianópolis, 2000.

FREDA, J.; McDONALD, D.G. Physiological correlates of interspecific variation in acid tolerance in fish. *J. Exp. Biol.*, Cambridge, v.136, p.243-258, 1988.

GONZALEZ, R.J. Ion regulation in ion poor waters of low pH. In: VAL, A.L. et al. (Ed.). Physiology and biochemistry of the fishes of the Amazon. Manaus: INPA, 1996.

GREENBERG, A.E. et al. Standard methods for the examination of water and wastewater. 14. ed. Springfield: Bru-El Graphic Inc., 1976.

HOLTZE, K.E.; HUTCHINSON, N.J. Lethality of low pH and Al to early life stages of six fish species inhabiting precambrian shield waters in Ontario. *Can. J. Fish. Aquat. Sci.*, Ottawa, v.46, p. 1188-1202, 1989.

LIN, H.; RANDALL, D.J. Evidence for the presence of an electrogenic proton pump on the trout gill epithelium. *J. Exp. Biol.*, Cambridge, v.161, p.119-134, 1991.

LLOYD, R.; JORDAN, D.H.M. Some factors affecting the resistance of rainbow trout, *Salmo gairdneri* R. to acid water. *Int. J. Air Water Pollution*, Cincinati, v.8, p. 393-403, 1964

MAETZ, J.; DE RENZIS, G. Aspects of the adaptation of fish to high external alkalinity: comparison of *Tilapia grahami* and *T. mossambica*. In: SCHMIDT-NIELSEN, K. et al. (Ed.). Comparative physiology: water, ions and fluid mechanics. Cambridge: Cambridge University Press, 1978.

POTTS, W.T.W. Kinetics of sodium uptake in freshwater animals: a comparison of ion exchange and proton pump hypothesis. *Am. J. Physiol.*, Bethesda, v.226, p.R315-R320, 1994.

REYNALTE-TATAJE, D.A. Efeito do pH da água na incubação e larvicultura do curimbatá Prochilodus lineatus Valenciennes, 1847 (Characiformes, Prochilodontidae). 2000. Dissertação (Mestrado em Aqüicultura) - Universidade Federal de Santa Catarina, Florianópolis, 2000.

SIMPSON, H.J. *et al.* Brackishwater aquaculture in pyrite-bearing tropical soils. *Aquaculture*, Amsterdam, v.34, p.333-350, 1983.

THOMAZ, S.M. et al. Caracterização limnológica dos ambientes aquáticos e influência dos níveis fluviométricos. In: VAZZOLER, A.E.A. et al. (Ed.). A planície de inundação do alto rio Paraná: aspectos físicos, biológicos e socioeconômicos. Maringá: EDUEM, 1997.

VAN DIJK, P.L.M. et al. The influence of gradual acid/base status and plasma hormone levels in carp (*Cyprinus carpio*). *J. Fish Biol.*, London, v.42, p.661-671, 1993

WALKER, I. Ecologia e biologia dos igapós e igarapés. *Ciência Hoje*, Rio de Janeiro, v.11, n.64, p.44-47, 1990.

WILKIE, M.P.; WOOD, C.M. The effects of extremely alkaline water (pH 9.5) on rainbow trout gill function and morphology. *J. Fish Biol.*, London, v.45, p.87-98, 1994.

WILKIE, M.P. et al. The physiological basis for altered Na<sup>+</sup> and Cl<sup>-</sup> movements across the gills of rainbow trout

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(Oncorhynchus mykiss) in alkaline (pH = 9.5) water. Physiol. Biochem.Zool., Chicago, v. 72, n.3, p.360-368, 1999.

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

ZANIBONI-FILHO, E. et al. Influência do pH na sobrevivência de pós-larvas de curimbatá, *Prochilodus lineatus* (Characiformes, Prochilodontidae). *Boletim do Instituto de Pesca*, São Paulo, 2002. (in press).

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