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# Tolerance to temperature, pH, ammonia and nitrite in cardinal tetra, *Paracheirodon axelrodi*, an amazonian ornamental fish

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## ABSTRACT

Poor water quality condition has been pointed out as one of the major causes for the high mortality of ornamental fishes exported from the state of Amazonas, Brazil. The purpose of the current study was to define water quality standards for cardinal tetra (*Paracheirodon axelrodi*), by establishing the lower and higher for lethal temperature ( $LT_{50}$ ), lethal concentration ( $LC_{50}$ ) for total ammonia and nitrite and  $LC_{50}$  for acid and alkaline pH. According to the findings, cardinal tetra is rather tolerant to high temperature (33.3 °C), to a wide pH range (acid pH=2.9 and alkaline pH=8.8) and to high total ammonia concentration (23.7 mg/L). However, temperatures below 19.6 °C and nitrite concentrations above 1.1 mg/L  $NO_2^-$  may compromise fish survival especially during long shipment abroad.

**KEYWORDS:** 96-h  $LC_{50}$ , ornamental fish, *Paracheirodon axelrodi*, water quality.

# Tolerância a temperatura, pH, amônia e nitrito do cardinal tetra, *Paracheirodon axelrodi*, um peixe ornamental da Amazônia

## RESUMO

A má qualidade da água tem sido apontada como uma das maiores causas da alta mortalidade dos peixes ornamentais exportados pelo Estado do Amazonas, Brasil. A proposta deste estudo foi definir padrões de qualidade da água para o cardinal tetra (*Paracheirodon axelrodi*), estabelecendo a menor e a maior temperatura letal ( $LT_{50}$ ), a concentração letal ( $LC_{50}$ ) para amônia total e nitrito e  $LC_{50}$  para pH ácido e alcalino. De acordo com os resultados, o cardinal tetra é mais tolerante a temperaturas elevadas (33,3 °C), a amplos limites de pH (pH ácido = 2,9 e pH alcalino = 8,8) e a alta concentração de amônia (23,7 mg/L). Entretanto, temperaturas abaixo de 19,6 °C e concentrações de nitrito acima de 1,1 mg/L  $NO_2^-$  podem comprometer a sobrevivência dos peixes, especialmente durante longos períodos de transporte para o exterior.

**PALAVRAS-CHAVE:** 96-h  $LC_{50}$ , *Paracheirodon axelrodi*, peixe ornamental, qualidade da água.

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## INTRODUCTION

The ornamental fish trade from the Amazonas State is one of the most profitable forms of sustainable fish exploitation and comprises nearly 90% of all ornamental fish exports from Brazil (Chao et al., 2001). Most of the exported fish species are harvested from the flooded forests located in the mid Rio Negro basin and valued around US\$ 3 million per year, representing 2% of the Manaus Free Trade Zone exports (Harris & Petry, 2001). Although ornamental fish exports represent a small portion of all foreign commerce undertaken by the Amazonas State, it still comprises over 65% of the local economy in the municipality of Barcelos, involving directly and indirectly 80% of the 16,107 residents (Prang, 2001).

In the last few years, the ornamental fish exporters have recorded a reduction of 40 to 50% on their trade. Several factors have contributed for this market loss, such as, the increasing supply of higher quality species from aquaculture, the high mortality rate of wild caught fish, and the low quality of the fish exported. From 30 to 70% of the fish captured in the Amazon parish before their delivery to the final consumer (Waichman et al., 2001). According to those authors, maintaining a stable water quality condition is very important and its poor quality may become one of the main causes for the high fish mortality rates.

The cardinal tetra (*Paracheirodon axelrodi*) is the most abundant species (21%) in the mid Rio Negro basin (Chao et al., 2001). This species is also the most requested Amazonian ornamental fish in the world market, dominating the fish exports from Brazil, and representing 80% of all fish exported annually from the Amazonas State. According to Chapman et al. (1997), describing a case study, 40 out of 50 boxes of cardinal tetras imported from South America to the United States were lost as a result of mortality.

The purpose of this paper is defining water quality standards for cardinal tetra, *Paracheirodon axelrodi*, establishing the lethal levels of high and low temperatures, acid and alkaline pH and high ammonia and nitrite concentrations in the water.

## MATERIAL AND METHODS

Cardinal tetra of  $0.07 \pm 0.002$  g (mean  $\pm$  SD) were collected from forest streams (igarapés) of the mid Rio Negro basin located in the municipality of Barcelos, Amazonas State. Fish were transported to the laboratory in Manaus, Amazonas, where they were kept in 500 L holding tanks supplied with stabilized temperature ( $25 \pm 1^\circ\text{C}$ ) and aerated water, regularly fed with a commercial fish diet for at least 4 weeks prior to the experiments. Bioassays to establish tolerance limits to pH, temperature, ammonia and nitrite were performed in four 40-L test chambers equipped with an air compressor and a thermostat bath. Twenty-four hours prior to the experiments,

four groups of ten fish (10 per replicate) were transferred to test chambers where the water quality was preserved. For each test parameter, the experiments were conducted for over 96 hours, in which fish mortality was observed and water physical and chemical parameters were monitored.

In order to test fish resistance to pH, water pH was adjusted with HCl diluted solution for acid pH levels and Tris and NaOH for alkaline pH levels, which were introduced into the thermostated bath to be mixed and distributed into the test chambers. An alkaline or acid solution was added to the bath at 1-h intervals until the desired pH was achieved. The pH levels tested were: control (6.0), acid (2.6, 3.1, 3.6, 4.3, 4.7, 5.2 and 5.6) and alkaline (6.5, 7.2, 7.4, 7.8, 8.4, 8.8 and 9.3). During all the experiment, water pH was monitored every 3 hours by using a digital WTW pH-meter model pH330i.

For temperature tests, a series of progressively higher and lower water temperatures was achieved by using a programmable thermostat bath (Mod. BTD 770 - São Carlos, SP, Brazil), which controlled the gradual rising or lowering temperature to its desired point. The tested temperatures were: control ( $25^\circ\text{C}$ ), high ( $27, 29, 31, 33, 35 \pm 1^\circ\text{C}$ ) and low ( $21, 19, 17$  and  $15 \pm 1^\circ\text{C}$ ).

Regardless the tolerance test performed, the water quality variables were measured in all test chambers. In the total ammonia test, water pH was previously set to  $7.6 \pm 0.12$  by adding 1 to 2 g/L NaOH and/or Tris solutions to the thermostated bath where it was mixed and distributed evenly to all test chambers. The tests were carried out by adding a pre-established quantity of  $\text{NH}_4\text{Cl}$  solution to the bath, which were evenly distributed into the chambers. The cardinal tetras were exposed to six concentrations: 0.9, 1.4, 8.5, 13.1, 18.6, 23.7 and 35.6 mg/L of total ammonia, yielding 0, 0.022, 0.032, 0.19, 0.23, 0.31, 0.44 and 0.85 mg/L  $\text{NH}_3$  (unionized ammonia). Test chamber ammonia concentration was determined daily and the water pH was monitored constantly. Nitrite tolerance tests were performed by adding the  $\text{NaNO}_2$  solution to obtain four nitrite concentrations (0.5; 1.0; 1.5 and 2.0 mg/L  $\text{NO}_2^-$ ).

Dissolved  $\text{O}_2$ , pH, temperature and electrical conductivity were measured twice daily and water samples were collected for total ammonia and nitrite determination. Dissolved oxygen and water temperature were measured with a YSI (Yellow Springs Instruments), model 55/12 digital DOmeter; pH was measured with a WTW model D-812, electrode (WTW) type E 50 pH 0...14 - 5... +80°C digital pH-meter; and electrical conductivity was determined by using a WTW model LF-92 digital meter. Water samples were collected and the colorimetric method applied for the analysis of total ammonia ( $\text{NH}_3 + \text{NH}_4^+$ ) and nitrite ( $\text{NO}_2^-$ ) concentrations, according to Boyd & Tucker (1992).

Water quality data are reported as mean  $\pm$  SD. The mean values of different test chambers were compared by using the ANOVA analysis. The differences were considered to be significant at  $p < 0.05$  using the Tukey test. The 96-h pH (acid and alkaline),  $LC_{50}$  ammonia and nitrite concentrations (lethal concentration to 50% of a population) and  $LT_{50}$  temperature (high and low) (lethal temperature to 50% of a population) were estimated according to the trimmed Spearman-Kärber method (Hamilton et al., 1977).

## RESULTS AND DISCUSSION

Water quality remained uniform among replicates for all tested variables (pH, temperature, ammonia and nitrite) with no significant difference between physical and chemical parameter (Table 1).

The mortality rates of cardinal tetra submitted to low and high temperatures and pH are presented in Table 2.A and mortality rates to ammonia and nitrite concentration are presented in Table 2.B. Also, the 96-h  $LT_{50}$  to low and high temperature and the 96-h  $LC_{50}$  to acid and alkaline pH, total ammonia and nitrite concentrations to cardinal tetra are presented in Table 3. The toxicity action and physiological effects of low and high pH on fish have been widely studied and reviewed by many authors (Wood, 1991; Affonso et al., 2002; Lim et al., 2003; Moiseenko & Sharova, 2006; Aride et al., 2007). During our study, the pH tests with cardinal tetra showed 100% survival for pH values between 4.0 and 8.5. The 96-h  $LC_{50}$  to acid and alkaline pH were calculated as 2.9 and 8.8 respectively, indicating that cardinal tetra is highly tolerant to acid and alkaline pH. The toxicity at a given pH is affected by factors like fish species, water temperature and the amount of humic acid present in the water (Peuranen et al., 2003). The high tolerance of cardinal tetra to low pH is to be expected once water pH is very acid (around 3.5) in the environment where this species naturally occurs (black water streams) (Walker, 2001). Waichman et al. (2001), in a study to assess fish transport water conditions, found that water pH tended to increase from 4.62 to 6.15 from capturing of *P. axelrodi* until its storage at the exporter's facilities, so that the observed pH variation had little effect on the physiology of these fish.

Temperature can influence fish in multiple ways, affecting biochemical and physiological activities and can act as a lethal factor when its effect destroys the integrity of the organism (Currie et al., 1998). In the present study, the  $LT_{50}$  of cardinal tetra to low and high temperatures were 19.6 and 33.7 °C, respectively. These findings showed that fish mortality increased at temperatures below 19 °C and reached total mortality at 15 °C (Table 2). The tests with high temperatures (25 to 35 °C), showed 100% fish survival at 29 and 31 °C, resulting in total fish mortality above 35 °C. These results corroborate with Waichman et al. (2001) findings, while evaluating the water quality used for transportation of cardinal tetra captured in waters with temperatures from 29 to 31 °C. According to these authors, their findings suggest that the maintenance of cardinal tetra should be restricted to high temperatures, considering its inability in tolerating low temperatures. The tilapias, *Oreochromis*, *Sarotherodon* and *Pelvicachromis* sp., which represent popular and important warmwater aquaculture fish species for food and ornament, are also very sensitive to cold water, presenting thermal death point values between 10 and 38 °C, limiting their culture to the tropical zones (Harpaz et al., 1999). Nevertheless, there are some warmwater species called eurythermal, which can tolerate a broader range of water temperature (Wedemeyer, 1996). Eurythermal fish, such as the goldfish, *Carassius auratus*, can survive temperatures between 0 and 41 °C and short term exposures to 44 °C (Fort and Beiting, 2005), as well as the channel catfish, *Ictalurus punctatus*, which presents thermal death points between 4 and 35 °C (Wedemeyer, 1996).

Ammonia and urea are the two main nitrogenous products excreted by teleosts, with ammonia usually representing 75-90% of the nitrogenous excretion (Handy & Poxton, 1993). Ammonia toxicity to fish depends on the concentration of unionized ammonia ( $NH_3$ ). Fish branchial membranes are relatively permeable to  $NH_3$ , but not to  $NH_4^+$ , due to its molecular size. When dissolved in water, ionized and unionized forms of ammonia are in equilibrium, which is affected by water pH, temperature and salinity. Alterations in these parameters can result in the variation of the different forms of ammonia, whose concentrations can become toxic to

**Table 1** - Water quality parameters during experiments to establish cardinal tetra (*Paracheirodon axelrodi*) tolerance to pH, temperature, total ammonia and nitrite concentrations. Means  $\pm$  SD.

Parameter	Tolerance					
	Acid pH	Alkaline pH	Low Temp.	High Temp.	Ammonia	Nitrite
O <sub>2</sub> (mg/L)	7.8 $\pm$ 0.3	8.0 $\pm$ 0.4	7.9 $\pm$ 0.7	6.3 $\pm$ 0.5	6.2 $\pm$ 0.5	8.2 $\pm$ 0.6
Temp (°C)	25.0 $\pm$ 0.7	25.0 $\pm$ 0.7	-	-	25.2 $\pm$ 0.6	25.0 $\pm$ 0.8
pH	-	-	6.6 $\pm$ 0.3	6.6 $\pm$ 0.5	7.6 $\pm$ 0.12	6.7 $\pm$ 0.3
Conductivity ( $\mu$ S/cm)	31.7 $\pm$ 16	279 $\pm$ 74	8.1 $\pm$ 0.3	8.2 $\pm$ 0.2	100.0 $\pm$ 9.7	8.0 $\pm$ 0.3
Ammonia (mg/L)	0.9 $\pm$ 0.0	0.05 $\pm$ 0.0	0.02 $\pm$ 0.0	0.02 $\pm$ 0.0	-	0.02 $\pm$ 0.0
Nitrite (mg/L)	0.08 $\pm$ 0.0	0.04 $\pm$ 0.0	0.038 $\pm$ 0.0	0.04 $\pm$ 0.0	0.008 $\pm$ 0.0	-

**Table 2** - Mortality rates of cardinal tetra (*Paracheirodon axelrodi*) submitted to low and high temperatures and pH (A) and to high ammonia (total and unionized) and nitrite concentrations (B).

(A)

Low temp. (°C)	Mort. (%)	High Temp. (°C)	Mort. (%)	Acid pH	Mort. (%)	Alkaline pH	Mort. (%)
25	0	25	0	6.0	0	6.0	0
21	17	27	0	5.6	0	6.5	2.5
19	30	29	2.5	5.2	0	7.2	0
17	92	31	25	4.7	5	7.4	2.5
15	100	33	63	4.3	15	7.8	0
		35	100	3.6	45	8.4	0
				3.1	72	8.8	42.5
				2.6	100	9.3	100

(B)

Total Ammonia (NH <sub>3</sub> + NH <sub>4</sub> <sup>+</sup> ) mg/L	Mort. (%)	Unionized ammonia (NH <sub>3</sub> ) mg/L	Mort. (%)	Nitrite (mg/L)	Mort. (%)
0.0	0	0	0	0.0	0
0.9	0	0.022	0	0.5	7
1.4	2	0.032	2	1.0	40
8.5	12	0.19	12	1.5	65
13.1	15	0.23	15	2.0	100
18.6	38	0.31	38		
23.7	70	0.44	70		
35.6	75	0.85	75		

**Table 3** - Lethal lower and higher temperatures (LT<sub>50</sub>), and lethal concentrations (LC<sub>50</sub>) to acid and alkaline pH, total ammonia and nitrite to cardinal tetra (*Paracheirodon axelrodi*).

LT <sub>50</sub>		Tolerance			
Low temp.	High Temp.	LC <sub>50</sub>			
		Acid pH	Alkaline pH	Ammonia	Nitrite
19.6 (°C)	33.7 (°C)	2.9	8.8	23.7 mg/L	1.1 mg/L

fish (Arana, 1997). The exposure of freshwater or seawater fish to sublethal levels of ammonia can increase their subsequent resistance to lethal concentrations (EIFAC, 1973).

The acute and chronic toxicities of ammonia have been extensively reviewed for freshwater fishes (Wang & Walsh, 2000; Biswas et al., 2006; Reddy-Lopata et al., 2006). High levels of ammonia cause stress and produce harmful physiological response such as osmoregulatory disturb, kidneys and branchial epithelium damages (Meade, 1989; Soderberg, 1994), retarded growth, inefficient immune response (Cheng et al., 2004; Pinto et al., 2007) and reduced survival (Jobling, 1994). The findings in the current experiments indicated 100% survival of the fish in 96-h exposure to the control and 0.9-mg/L of total ammonia, while 98, 88, 85, 62, 30 and 25% of the fish survived to 1.4, 8.5, 13.1 18.6, 23.7 and 35.6 mg/L of total ammonia (or 0, 0.022 0.032, 0.19, 0.23, 0.31, 0.44 and 0.85 mg/L NH<sub>3</sub>) respectively. Lethal ammonia concentration (LC<sub>50</sub>) for cardinal tetra was

calculated to be 23.7 mg/L NH<sub>3</sub> + NH<sub>4</sub><sup>+</sup> or 0.36 mg/L NH<sub>3</sub>. The results obtained in this study are within the toxicity range suggested by Abdalla & MacNabb (1998), in which the lethal concentration of unionized ammonia for fish varies between 0.32 e 3.1 mg/L. Several authors have described the lethal levels (LC<sub>50</sub>) of total and unionized ammonia for different fish species (Lemarié et al., 2004), such as *Ictalurus punctatus*, 45 mg/L NH<sub>3</sub> + NH<sub>4</sub><sup>+</sup> and 1.6 mg/L NH<sub>3</sub> (Colt & Tchobanoglous, 1976), *Oncorhynchus mykiss*, 22 mg/L NH<sub>3</sub> + NH<sub>4</sub><sup>+</sup> and 0.3-0.6 mg/L NH<sub>3</sub> (Haywood, 1983), *Odontesthes argentinensis*, 0.76-0.96 mg/L NH<sub>3</sub> (Ostrensky & Brugger, 1992; Sampaio & Minillo, 2000), and *Cichlasoma facetum*, 2.95 mg/L NH<sub>3</sub> (Piedras et al., 2006). The data obtained indicate that the cardinal tetra may be considered as to be tolerant to ammonia, which certainly facilitates its survival, especially during transport from Barcelos to Manaus, when the total ammonia can reach high concentrations (< 12 mg/L) (Waichman et al., 2001).

Besides a wide variety of factors, size can influence fish tolerance to ammonia, as smaller fish are exposed to a higher dosage per body weight unit than larger fish, being the small fish more susceptible to unionized ammonia (Piedras et al., 2006). This fact explains the wide range of results obtained in several studies. Cavero et al. (2004) have exposed young *Arapaima gigas* to a concentration of 25 mg/L NH<sub>3</sub> + NH<sub>4</sub><sup>+</sup> or 2 mg/L NH<sub>3</sub> for 24 hours and no effect was observed on fish survival or performance.



The toxicity of nitrite to fish has received much attention in recent years, but little information is available on the susceptibility of tropical fish to this compound (Moraes et al., 1998; Martinez & Souza, 2002; Costa et al., 2004). Nitrite is the intermediate compound in the nitrification process, in which total ammonia nitrogen is converted to nitrite ( $\text{NO}_2^-$ ). Under normal conditions, nitrite is rapidly converted to non-toxic nitrate ( $\text{NO}_3^-$ ) by naturally occurring bacteria (Durborow et al., 1997). At elevated concentration, nitrite reduces blood oxygen carrying capacity by oxidizing hemoglobin (Hb) to methemoglobin (metHb), which loses the ability to bind the oxygen, and under acute concentration the oxygen carrying capacity of blood markedly decreases (Jensen, 1995). Methemoglobin gives blood a brownish color, so a visible symptom of high blood methemoglobin levels is the brown color of blood and gills (Kroupova et al., 2005).

Nitrite tolerance determination for the cardinal tetra would be a useful tool to define the environmental quality and handling standards during shipment. In our tests, all fish survived to 96-h exposure to the control, while 93, 60 and 35% of fish survived to 0.5, 1.0 and 1.5 mg/L  $\text{NO}_2^-$ , respectively. Total fish mortality was observed at 2 mg/L  $\text{NO}_2^-$ . The 96-h  $\text{LC}_{50}$  was calculated as 1.1 mg/L  $\text{NO}_2^-$ , indicating the high sensitivity of this species to nitrite. Factors affecting nitrite toxicity includes the length to nitrite exposure, fish size and weight, and fish species (Kroupova et al., 2005). Piedras et al. (2006) observed the mortality of *Cichlasoma facetum* to increasing water concentrations of nitrite, where there were 45.63% fish mortality in the higher dosages of 6.68 mg/L  $\text{NO}_2^-$ . However, Paula-Silva (1999) studied disturbances on blood tissue of *Colossoma macropomum* from the exposure to concentrations that varied from 0 to 3.6 mg/L  $\text{NO}_2^-$  and, although there was no mortality, it was concluded that sub-lethal  $\text{NO}_2^-$  concentration could damage the basic fish physiological functions, growth and reproduction. Among a variety of tests on the acute toxicity of nitrite to fish, the salmonids showed to be the most sensitive of the taxa studied up to date. Channel catfish is as sensitive to nitrite as salmonids, and tilapias are slightly less sensitive (Kroupova et al., 2005). The largemouth bass (*Micropterus salmoides*) presents high critical concentration of nitrite, as this species does not concentrate this compound in the blood plasma and thus appears to discriminate nitrite from chloride (Palachek & Tomasso, 1984).

Our study suggests that cardinal tetra can be considered tolerant to acid and alkaline pH and also to ammonia. Low temperatures (< 19 °C) and nitrite concentrations above 1.1 mg/L may compromise its survival, especially during the long exposure involved in overseas shipping and maintenance at the wholesaler's facilities.

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