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Preference behavior of silver catfish, *Rhamdia quelen*, juveniles in waters with pH gradients: laboratory experiments

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The aim of this study was to determine the preferred pH in silver catfish *Rhamdia quelen* juveniles acclimated to different water hardness and the effect of shelters and infection by *Ichthyophthirius multifiliis*. Fish were acclimated for two weeks at different water hardness levels (4, 24, 50, or 100 mg $CaCO_3$ L^{-1}) and then transferred to a polyethylene tube with a pH gradient ranging from 3.5 to 11.7 and maintaining the same hardness. The position of the fish in the pH gradient was observed at 1, 2, 4, 6, 8, 10, and 12 h after transfer. Acclimation to different water hardness did not change pH preference of uninfected silver catfish (pH 7.30-7.83), and the presence of a shelter at the preferred pH or outside this preferred pH did not change the chosen pH range, either. Consequently silver catfish favored the acid-base regulation over shelter seeking tendency. Juveniles infected with *I. multifiliis* acclimated to water hardness of 24 mg $CaCO_3$ L^{-1} preferred alkaline pH (9.08-9.79). This choice is not explained by the higher Na^+ levels at alkaline pH compared to neutral pH because infected and uninfected fish choose the same waterborne Na^+ levels in a Na^+ gradient with the same pH.

O objetivo deste estudo foi determinar o pH preferencial de juvenis de jundiá *Rhamdia quelen* aclimatados a diferentes durezas da água e o efeito de abrigos e infecção por *Ichthyophthirius multifiliis*. Os peixes foram aclimatados durante duas semanas em diferentes níveis de dureza da água (4, 24, 50 ou 100 mg $CaCO_3$ L^{-1}) e então transferidos para um tubo de polietileno com um gradiente de pH de 3,5-11,7, mantendo a mesma dureza. A posição do peixe no gradiente de pH foi observada 1, 2, 4, 6, 8, 10 e 12 h após a transferência. A aclimatação a diferentes durezas da água não afetou o pH preferencial de jundiás não infectados (pH 7,30-7,83), e a presença de um abrigo no pH preferido ou fora deste pH também não alterou a faixa de pH preferida. Portanto, jundiás favorecem a regulação ácido-base em detrimento a uma tendência de procurar abrigo. Em juvenis infectados com *I. multifiliis* aclimatados à dureza da água de 24 mg L^{-1} de $CaCO_3$ o pH preferencial é alcalino (9,08-9,79). Esta escolha não é explicada pelos maiores níveis de Na^+ em pH alcalino que em pH neutro porque peixes infectados e não infectados escolheram os mesmos níveis de Na^+ na água em um gradiente de Na^+ com o mesmo pH.

Key words: Behavior, Calcium, Hardness, *Ichthyophthirius multifiliis*, Shelter.

Introduction

Water pH plays an important role in fish homeostasis, development, and survival. Alterations of pH may cause disturbances in acid-base balance, ion regulation and ammonia excretion (Baldisserotto, 2011). The usual pH range for fish growth is 6.0 to 9.0; lower pH can occur due to the presence of acidic cations, humic and fulvic acids, and more alkaline pH can be due to high levels of carbonate and other ions (Parra & Baldisserotto, 2007). Water quality may elicit a preference or avoidance response in fish (Kroon & Housefield, 2003), and several studies have demonstrated that fish preferred a specific pH (Jones *et al.*, 1985; Nakamura, 1986; Peterson *et al*. 1989; Åtland & Barlaup 1996; Åtland, 1998; Ikuta *et al.*, 2003; Kroon & Housefield, 2003, Kroon, 2005, Scott *et al.*, 2005, Riffel *et al.*, 2012).

Silver catfish is the most often raised native species in fish cultures of South Brazil (Baldisserotto, 2009). Several studies determined the best pH (Zaions & Baldisserotto, 2000; Lopes *et al.*, 2001; Copatti *et al.*, 2005, 2011a, b) and water hardness (Townsend & Baldisserotto, 2001; Silva *et al.*, 2003, 2005; Townsend *et al.*, 2003) for survival and growth of this species. The increase of water hardness improves fish survival at very acidic and alkaline environments (Townsend &

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Baldisserotto, 2001) because Ca^{2+} stabilizes gill tight junctions and reduces their permeability, reducing diffusive ionic loss to the water (Parra & Baldisserotto, 2007; Baldisserotto, 2011). However, the effect of pH on silver catfish growth is altered by water hardness (Copatti *et al.*, 2005, 2011a, 2011b).

Water pH and hardness also affects infection of silver catfish by the ciliate protozoan *Ichthyophthirius multifiliis* (Garcia *et al.*, 2011), which causes ichthyophthiriosis, also known as "white spot disease" or "ich", damages gill epithelium and skin, and can cause the death of the host (Miron *et al.*, 2003; Carneiro *et al.*, 2005; Garcia *et al.*, 2007, 2011). Therefore, if available in the fish culture pond, it is possible that fish might choose a specific pH and/or hardness to reduce the intensity of ich infection. However, there are no studies regarding the preferred pH in fish acclimated to different water hardness levels or infected by *I. multifiliis*. Consequently, the objective of this study was to determine the preferred pH in silver catfish acclimated to different water hardness or infected by *I. multifiliis*. In addition, as silver catfish is less stressed when in a shelter (Barcellos *et al.*, 2009), an analysis was also made of whether or not the presence of shelters can change the preferred pH.

Material and Methods

Silver catfish juveniles (12.40 ± 1.33g and 11.00 ± 0.29cm – total length) were obtained from a fish culture farm near the city of Santa Maria, southern Brazil, and transferred to the Fish Physiology Laboratory at the Universidade Federal de Santa Maria. These juveniles were maintained in continuously aerated (two air pumps of 12 W each) 250 L tanks for 15 days for acclimation (temperature: 23 ± 0.1ºC, pH: 7.1-7.7, dissolved oxygen levels: 6.9 ± 0.1 mg L^{-1}). Juveniles were then divided in the following treatments (in mg $CaCO_3$ L^{-1}): 4, 24, 50, and 100, and kept for 20 days in continuously aerated 250 L tanks with the same temperature, pH and dissolved oxygen conditions than acclimation. A water hardness of 4 mg $CaCO_3$ L^{-1} was obtained using distilled water, and waterborne Na^+, Cl^-, and K^+ levels were adjusted to identical levels of the water with 24 mg $CaCO_3$ L^{-1}. A water hardness of 50 or 100 mg $CaCO_3$ L^{-1} was reached by adding $CaCl_2.2H_2O$. The photoperiod was 12h light - 12h darkness, and the luminosity of the laboratory was 0.6 lux (measured with a LI-COR photometer model LI-185B). Juveniles were fed once a day at 8:00 am with a commercial diet (Supra 42% crude protein, Alisul Alimentos S.A., Carazinho, Brazil) at 5.0% of their body mass. Residues and feces were siphoned 30 min after finishing the food, and consequently at least 20% of the water was replaced with water previously adjusted to the appropriate water hardness. Fish were fasted for 24 h prior to any experiment.

After acclimation to experimental water hardness each group was transferred to a 6m long polyethylene tube containing 50 L of water, which had been added at one end 0.5 N sulfuric acid to generate pH around 3.5 and at the other end 1N sodium hydroxide (NaOH) to obtain pH around 11.7. The solutions added at the extremities diffused through the water along the tube, creating the pH gradient, which was maintained by adding the same solutions at the extremities every two hours.

Each group (six replicates per treatment, N = 5 each) was placed in the polyethylene tube closest to its acclimation pH. Fish location at the pH gradient was visually observed at 1, 2, 4, 6, 8, 10, and 12 h after the transfer, in order to identify their preferred pH. The pH was always measured at the location at the moment of the observation. After 12 h observation the water of the tube was replaced and a new replicate was placed in the tube. Aerators were placed at the tube, and dissolved oxygen levels were maintained at 6.0 - 6.5 mg L^{-1}. Dissolved oxygen was monitored with an oxygen meter YSI (Y5512, YSI Inc., Yellow Springs, Ohio, USA) every 4 h. The pH was verified with a DMPH-2 pH meter (Digimed, São Paulo, Brazil) and water hardness by the EDTA titrimetric method (Eaton *et al.*, 2005).

The same experiment was repeated, but with the tube containing a shelter (25 mm diameter tube) placed at the preferred pH determined in the first experiment. In another series, two shelters were placed at pH other than preferred one (pH 6.5 and 8.5)

In the third experiment, silver catfish infected with *I. multifiliis* were separated into different groups according to their level of infection: 1 - 20, 21 - 50, 51 - 100, and 101 or more trophonts/fish. The white spots (trophonts) were counted with the aid of a stereomicroscope (Garcia *et al.*, 2007) in fish anesthetized with eugenol (20 µL L^{-1}) (Cunha *et al.*, 2010). The infected fish were transferred to the polyethylene tube containing the pH gradient and water hardness of 24 mg $CaCO_3$ L^{-1}. The pH preference was observed for 12h.

Silver catfish infected by *I. multifiliis* preferred an alkaline pH (see results), and as the alkaline pH was obtained by adding NaOH, waterborne Na^+ levels were 50-70% higher at alkaline pH. Therefore an additional experiment with infected and uninfected fish was carried out in polyethylene tubes with a sodium chloride gradient (adding NaCl 0.5N at one extremity). Waterborne Na^+ levels were checked every 2 h (up to 8 h) at the site preferred by the fish. The Na^+ range in the tube was 1.14±0.0 to 7.78±0.0 mmol L^{-1}, water hardness of 24 mg $CaCO_3$ L^{-1} (pH: 7.76±0.02). Waterborne Na^+levels were determined with a B262 flame spectrophotometer (Micronal, São Paulo, Brazil).

Statistical Analysis. The homogeneity of variances between groups was tested with the Levene test. The comparisons between different treatments were performed by one-way analysis of variance (ANOVA) followed by the Tukey's test using the Software Statistica version 7.0. Data were expressed as mean ± S.E.M. The minimum significance level was set at $P < 0.05$.

Results

After the silver catfish (infected with *I. multifiliis* or not) were placed in the polyethylene tubes, they swam along the entire length of the tube (along the pH gradient), but after about 10 minutes they remained as a shoal at their preferred pH range up to 12 h of exposure.

Acclimation to different water hardness levels did not significantly affect the preferred pH, which was within the 7.30-7.83 range. When the shelter was within the preferred pH range, fish remained all 12h in the shelters. The presence of shelter at the preferred pH or outside this preferred pH did not change the chosen pH range (Table 1), *i.e.*, if the shelter was outside the preferred pH, silver catfish did not use the shelters.

Mortality of silver catfish infected with *I. multifiliis* was 10%, irrespective of the number of trophonts. Infected fish choose a more alkaline pH than uninfected fish and those with 21-50 trophonts/fish preferred the highest pH between the infected fish (Table 2). In the experiment with the Na^+ gradient in the tube, silver catfish preferred the 1.14 - 5.94 mmol L^{-1} range, and there was no significant difference between uninfected and infected fish.

Discussion

Fish in the wild may respond to several environmental factors (Gunn & Noakes, 1986), and laboratory experiments may separate and clarify these responses (Peterson *et al.*, 1989). Previous study demonstrated that uninfected silver catfish juveniles at water hardness of 24 mg $CaCO_3$ L^{-1} preferred the 7.0-7.6 pH range (Riffel *et al.*, 2012). These results are within the same range observed in the present study for this water hardness, with is in agreement with the fact that juveniles of this species presented better growth at pH 7.0-7.5 than at pH 5.5 and 9.0 (Baldisserotto, 2011). Survival of silver catfish juveniles in acidic and alkaline water is improved by the addition of Ca^{2+} to the water (Townsend & Baldisserotto, 2001), and high water hardness reduced the deleterious effects of acidity (pH 5.5) on growth in soft waters (Copatti *et al.*, 2011a). In addition, growth of juveniles maintained at water hardness close to zero was higher at pH 6.0 than 7.0 and 8.0 (Copatti *et al.*, 2011b). Therefore, as silver catfish growth at different pH is altered by water hardness, it was expected that the pH preference could also be altered by adaptation to different water hardness. However, water hardness did not change pH preference (pH 7.30-7.83) in this species.

Several others species also showed preference to a specific pH (Peterson *et al.*, 1989). Japanese fat minnows (*Phoxinus lagowski*) presented avoidance behavior and their swimming region shifted from pH 6.0 to 7.0 immediately after decreasing pH and during the exposure to acidic water their swimming activity clearly decreased (Nakamura, 1986). Sockeye salmon (*Oncorhynchus nerka*), brown trout (*Salmo trutta*) and

Table 1. Preferred pH of silver catfish acclimated to different water hardness. Values are reported as mean ± SEM. Water hardness or the presence of shelter in the tube did not significantly change the preferred pH.

Time after transfer (h)	Polyethylene tube without shelter	Tube with shelter	
		Within preferred pH	Outside preferred pH
		4 mg $CaCO_3$ L^{-1}	
1	7.73 ± 0.22	7.70 ± 0.09	7.71 ± 0.07
2	7.50 ± 0.10	7.73 ± 0.61	7.70 ± 0.10
4	7.63 ± 0.25	7.75 ± 0.15	7.58 ± 0.22
6	7.54 ± 0.53	7.65 ± 0.95	7.50 ± 0.06
8	7.59 ± 0.78	7.43 ± 0.48	7.48 ± 0.28
10	7.77 ± 0.80	7.80 ± 0.20	7.77 ± 0.59
12	7.72 ± 0.88	7.85 ± 0.40	7.70 ± 0.39
		24 mg $CaCO_3$ L^{-1}	
1	7.80 ± 0.42	7.40 ± 0.24	7.65 ± 0.46
2	7.83 ± 0.51	7.82 ± 0.24	7.83 ± 0.28
4	7.57 ± 0.59	7.58 ± 0.31	7.48 ± 0.50
6	7.65 ± 0.49	7.67 ± 0.48	7.27 ± 0.24
8	7.73 ± 0.39	7.90 ± 0.50	7.54 ± 0.58
10	7.73 ± 0.55	7.84 ± 0.63	7.89 ± 0.30
12	7.57 ± 0.24	7.82 ± 0.78	7.60 ± 0.36
		50 mg $CaCO_3$ L^{-1}	
1	7.47 ± 0.19	7.46 ± 0.05	7.62 ± 0.17
2	7.45 ± 0.55	7.45 ± 0.54	7.73 ± 0.14
4	7.58 ± 0.56	7.63 ± 0.32	7.50 ± 0.30
6	7.66 ± 0.66	7.70 ± 1.13	7.43 ± 0.12
8	7.62 ± 0.61	7.55 ± 1.25	7.50 ± 0.09
10	7.80 ± 0.51	7.50 ± 1.40	7.43 ± 0.29
12	7.83 ± 0.41	7.60 ± 0.40	7.40 ± 0.09
		100 mg $CaCO_3$ L^{-1}	
1	7.55 ± 0.23	7.68 ± 0.17	7.58 ± 0.24
2	7.43 ± 0.28	7.70 ± 0.55	7.53 ± 0.50
4	7.53 ± 0.41	7.63 ± 0.71	7.57 ± 0.57
6	7.60 ± 0.21	7.80 ± 0.47	7.45 ± 0.40
8	7.30 ± 0.25	7.60 ± 0.58	7.73 ± 0.37
10	7.60 ± 0.21	7.63 ± 0.23	7.70 ± 0.06
12	7.60 ± 0.26	7.77 ± 0.28	7.63 ± 0.55

Table 2. Preferred pH of silver catfish infected with *Ichthyophthirius multifiliis* acclimated to water hardness of 24 mg $CaCO_3$ L^{-1}. Values are reported as mean ± SEM. Means identified by different letters in the rows were significantly different ($P < 0.05$) as determined by ANOVA and Tukey's comparison of mean values.

Time after transfer (h)	Uninfected	Infected fish (trophont/fish)			
		1-20	21 - 50	51 - 100	101 or more
1	7.80 ± 0.42[b]	9.08 ± 0.27[a]	9.20 ± 0.24[a]	8.33 ± 0.09[a]	8.97 ± 0.54[a]
2	7.83 ± 0.51[b]	9.05 ± 0.46[a]	9.05 ± 0.25[a]	9.27 ± 0.18[a]	9.30 ± 0.90[a]
4	7.57 ± 0.59[b]	9.08 ± 0.21[a]	8.97 ± 0.07[a]	8.75 ± 0.15[ab]	9.53 ± 0.68[a]
6	7.65 ± 0.49[b]	9.28 ± 0.32[a]	9.43 ± 0.42[a]	9.05 ± 0.15[a]	8.95 ± 0.05[a]
8	7.73 ± 0.39[c]	9.10 ± 0.25[b]	10.5 ± 0.50[a]	8.75 ± 0.50[b]	8.40 ± 0.30[bc]
10	7.73 ± 0.55[c]	9.03 ± 0.29[b]	10.8 ± 0.00[a]	9.80 ± 0.50[b]	9.35 ± 0.25[b]
12	7.57 ± 0.24[c]	9.10 ± 0.23[b]	10.6 ± 0.00[a]	9.60 ± 0.20[b]	9.45 ±0.45[b]
Overall mean	**7.70 ± 0.46[c]**	**9.10 ± 0.29[b]**	**9.79 ± 0.18[a]**	**9.08 ± 0.19[b]**	**9.14 ± 0.45[b]**

Japanese trout (*Salvelinus leucomaenis*) showed inhibition of digging and swimming behavior in slightly acidic (5.8-6.4) compared to neutral water (6.8-7.1) (Ikuta *et al*., 2003). Juvenile brook trout (*Salvelinus fontinalis*) avoided pH 4.0, 5.0 and 5.5 and these acidic pH values affected social interactions (Pedder & Maly, 1986). Common carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*) avoided pH values within the 5.5-7.0 range with preference to pH 8.4 and 7.2, respectively (Ishio, 1965).

Silver catfish remained in the shelters when they were within the preferred pH. This result is in accordance with the fact that the presence of a shelter in the tank reduced whole body plasma cortisol peak values and their duration in previously stressed silver catfish (Barcellos *et al.*, 2009). Shelter seeking tendencies were also observed in channel catfish *Ictalurus punctatus* (Brown *et al.*, 1970). The presence of the shelter provided a darkened refuge that, most likely, created a more comfortable environment and allowed a fast recovery from stress (Britz & Piennar, 1992) and better growth rates in African catfish, *Clarias gariepinus* (Hossain *et al.*, 1998). However, when the shelters were outside the preferred pH range, silver catfish did not use them. Therefore, silver catfish prefer a certain pH over shelter seeking tendency.

Silver catfish infected by *I. multifiliis* showed preference for a more alkaline pH (9.08 - 9.79) than uninfected fish (pH 7.70). This result is unexpected, since infected silver catfish presented higher mortality at this water hardness when maintained at pH 9.0 than at pH 5.0 (Garcia *et al.*, 2011). As there was a gradual reduction of "white spots" in silver catfish infected by *I. multifiliis* using NaCl (786 mmol L^{-1} Na^+) (Miron *et al.,*2003), it was hypothesized that infected fish preferred the alkaline pH due to the higher Na^+ levels at this pH, compared with neutral pH. Fish would choose the site where the infection would be reduced due to the higher salt concentration. However, there was no difference in the range of Na^+ levels chosen by infected and uninfected fish. Therefore, the reason for infected silver catfish to choose alkaline pH remained to be studied.

In conclusion, the results indicated that acclimation to different water hardness did not change pH preference of uninfected silver catfish (pH 7.30-7.83), and the presence of shelter at the preferred pH or outside this preferred pH did not change the chosen pH range. Consequently silver catfish prefer a certain pH over shelter seeking tendency. Juveniles infected with *I. multifiliis* acclimated to water hardness of 24 mg $CaCO_3$ L^{-1} preferred alkaline pH (9.03-9.79). This choice is not explained by the higher Na^+ levels at alkaline pH compared to neutral pH because infected and uninfected fish chose the same waterborne Na^+ levels in a Na^+ gradient with the same pH.

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