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Dissolved oxygen and ammonia levels in water that affect plasma ionic content and gallbladder bile in silver catfish

Níveis de oxigênio dissolvido e amônia na água afetam o conteúdo iônico do plasma e da bile vesicular em jundiá

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ABSTRACT

*Ionic contents (Na^+, K^+ and Cl^-) of plasma and gallbladder bile (GB) of juveniles silver catfish, **Rhamdia quelen** (156.1±0.2g, 28.2±0.3cm), were determined in three different times (0, 6 and 24h) after exposure to: a) control or high dissolved oxygen (DO = 6.5mg L^{-1}) + low NH_3 (0.03mg L^{-1}); b) low DO (3.5mg L^{-1}) + low NH_3; c) high DO + high NH_3 (0.1mg L^{-1}); and d) low DO + high NH_3. High waterborne NH_3 or low DO levels increased plasma and GB ion levels. These parameters might have followed different mechanisms to affect osmoregulation since a synergic effect of these variables was detected.*

Key words: *nitrogen compound, hypoxia, ion levels, jundiá, osmoregulation.*

RESUMO

*O conteúdo iônico (Na^+, K^+ e Cl^-) do plasma e da bile vesicular (BV) de juvenis de jundiá, **Rhamdia quelen** (156,1±0,2g, 28,2±0,3cm), foi determinado em três diferentes tempos (0, 6 e 24h) após exposição a: a) controle ou alto oxigênio dissolvido (OD = 6,5mg L^{-1}) + baixa NH_3 (0,03mg L^{-1}); b) baixo OD (3,5mg L^{-1}) + baixa NH_3; c) alto OD + alta NH_3 (0,1mg L^{-1}); e baixo OD + alta NH_3. Alta concentração de amônia ou baixo oxigênio dissolvido na água aumentaram os níveis iônicos no plasma e na BV. Aparentemente, os efeitos osmorregulatórios desses parâmetros podem estar relacionados a mecanismos distintos, pois foi detectado efeito sinérgico sobre essa alteração osmorregulatória.*

Palavras-chave: *amônia, hipóxia, níveis iônicos, jundiá, osmorregulação.*

INTRODUCTION

Waterborne ammonia is composed of NH_3 (unionized ammonia) and NH_4^+ (ionized ammonia), NH_3 being the most toxic form to fish as it can readily diffuse through cell membranes and is highly soluble in lipids (BOYD & TUCKER, 1998). The main process of ammonia excretion in freshwater fish is by the passive transcellular diffusion of NH_3 down a concentration gradient (WRIGHT & WOOD, 1985; RANDALL & WRIGHT, 1987; CLAIBORNE & EVANS, 1988). Exposure to high waterborne NH_3 levels reduced plasma Na^+ in channel catfish (***Ictalurus punctatus***) (TOMASSO et al., 1980) and in rainbow trout (***Oncorhynchus mykiss***), and also increased Na^+ efflux in rainbow trout (TWITCHEN & EDDY, 1994). However, it did not change plasma ion levels in other experiments (WILSON & TAYLOR, 1992; VEDEL et al., 1998).

Low dissolved oxygen (DO) levels have a marked effect on many physiological processes in fish, for example reducing feeding (WILHELM FILHO et al., 2005). Hypoxic conditions reduced plasma osmolarity in traíra (***Hoplias malabaricus***) (SAKURAGUI et al., 2003), changed net ion fluxes in silver catfish, ***Rhamdia quelen*** (ROSSO et al., 2006), but had no effect on plasma Na^+ levels in European sea bass (***Dicentrarchus labrax***) exposed to four different DO levels (CECCHINI & CAPUTO, 2003).

The silver catfish (Quoy and Gaimard, 1824, Heptapteridae) occurs from southern Mexico to Central

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Argentina, and is the native freshwater species most raised in southern Brazil (BALDISSEROTTO, in press). It is a suitable species for fish culture because it has a good growth rate and is able to use numerous kinds of nutrient sources (LAZZARI et al., 2006; MELO et al., 2006). The lethal concentration (96h) of unionized ammonia for this species is 1.45 at pH 7.5 (MIRON et al., 2008), and it can survive at dissolved oxygen levels down to 1.68mg L^{-1}, but chronic exposure to levels below 5.2mg L^{-1} reduces growth (BRAUN et al., 2006).

Fish under hypoxia are likely to experience high ammonia levels (WALSH et al., 2007). Decreased DO levels increases ammonia toxicity in freshwater fish (THURTSON et al., 1981; WAJSBROT et al., 1991; SERAFINI et al., in press), but to our knowledge, no study have analyzed the effect of these parameters on fish osmoregulation. Therefore, this study investigated the ionic content of plasma and gallbladder bile of silver catfish exposed to different dissolved oxygen and ammonia levels.

MATERIAL AND METHODS

Silver catfish juveniles of similar body size were obtained from a local fish farm and maintained in continuously aerated 250L tanks for at least one week prior to experiments. They were maintained in 24h darkness (except during feeding and cleaning of the tanks), because this is a favorable condition to this nocturnal catfish (PIAIA et al., 1999), and fed once a day with commercial feed for juveniles (Supra, 42% CP, Alisul Alimentos S.A., Carazinho, Rio Grande do Sul, Brazil) until apparent satiety, up to 24h before the experiment.

After acclimation fish were separated in twelve 250-L tanks, yielding four treatments (three replicates each treatment) (seven fish per tank): a) control or high dissolved oxygen (DO = 6.5±0.12mg L^{-1}) + low NH_3 (0.03±0.01mg L^{-1}); b) low DO (3.5±0.18mg L^{-1}) + low NH_3; c) high DO + high NH_3 (0.1±0.019mg L^{-1}), and d) low DO + high NH_3. Both high NH_3 and low dissolved oxygen were chosen because are sublethal values that affect silver catfish growth (MIRON, 2004; BRAUN et al., 2006).

Fish were collected at 0, 6 and 24h after exposure to the treatments and dipped in ice-water slurry (2.4kg ice: 3.6L water) for 5min for anaesthetizing. Blood was collected from the caudal vein with heparinized 1m-L syringes and centrifuged at 2,000rpm for 5min to separate the plasma. After the abdomen was opened, the gallbladder bile was removed and the liquid collected and placed in Eppendorf tubes. Plasma and gallbladder bile were stored at -20ºC for later analyses. The methodology of this experiment was approved by the Ethical and Animal Welfare Committee of the Santa Maria Federal University.

Chloride concentration was measured according to ZALL et al. (1956). Sodium and potassium concentrations were measured with a B262 flame spectrophotometer (Micronal, São Paulo, Brazil). Standard solutions were made with analytical grade reagents (Vetec or Merk) dissolved in deionized water, and each standard curve was made with five different concentrations. Water dissolved oxygen and temperature were monitored with an oxygen meter (model Y5512, YSI Inc., Yellow Springs, USA). The pH levels were verified with pH meter DMPH-2 (Digimed, São Paulo, Brazil), total ammonia was determined according to BOYD & TUCKER (1992) and NH_3 according to PIPER et al. (1982). These parameters were measured every hour throughout the 24-h cycle. Dissolved oxygen levels were maintained through aeration with air and/or nitrogen, while NH_3 levels were reached by adding concentrated NH_4Cl solution. Water hardness was analyzed by the EDTA titrimetric method, alkalinity and nitrite according to BOYD & TUCKER (1992). These parameters were measured at the beginning and end of the experiment and showed no significant difference among treatments (Table 1).

All data are expressed as mean ± SEM. As Levene test showed homogeneity of variances, comparisons among treatments and times were performed by two-way analysis of variance and Tukey test. The minimum significance level was set at $P<0.05$.

RESULTS AND DISCUSSION

In this study, silver catfish showed plasma ion levels at time 0h similar to values determined in other studies of this species (BORGES et al., 2004; BECKER et al., 2006). Moreover, plasma Na^+, K^+ and Cl^- levels of fish transferred to high DO + low NH_3 treatment remained unchanged from 0 to 24h (Table 2), indicating that fish were not stressed. In fact, stressed freshwater fish increase their gill permeability due to intensification of gill ventilation rate, which contributes to ion losses (McDONALD et al., 1991; McDONALD & MILLIGAN 1997) and decrease of plasma Na^+ and Cl^- levels (PIERSON et al., 2004).

Low DO levels (3.5mg L^{-1}) for 6-24h increased plasma Na^+, K^+ and Cl^- levels in the silver catfish irrespective to NH_3 levels in the water (Table 2). In disagreement with the present results, ROSSO et al. (2006) demonstrated that exposure to 2.5-3.5mg L^{-1} DO levels for 1h increased Na^+ and Cl^- loss in silver catfish. However, these ion losses in hypoxia observed by ROSSO et al. (2006) were similar to control values 24h later, but intermediate measurements were not performed. Consequently, a recovery of ion losses in

Table 1 – Biometric data of silver catfish juveniles and physicochemical values of the water for the different treatments.

Parameters	High DO + low NH_3	Low DO + low NH_3	High DO + high NH_3	Low DO + high NH_3
Weight (g)	155.1±0.3	157.3±0.2	156.6±0.1	155.8±0.1
Length (cm)	27.4±0.2	28.9±0.4	28.1±0.2	27.8±0.1
Temperature (°C)	24.4±0.1	24.5±0.1	24.4±0.1	24.3±0.1
pH	7.32±0.09	7.40±0.12	7.42±0.13	7.35±0.09
Water hardness (mg $CaCO_3$ L^{-1})	19.3±1.3	19.2±1.2	19.4±1.5	18.8±1.4
Alkalinity (mg $CaCO_3$ L^{-1})	24.0±0.7	23.0±0.9	25.0±0.5	23.0±0.4
Nitrite - maximum (mg L^{-1})	0.07	0.07	0.07	0.07
Na^+ (mmol L^{-1})	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01
K^+ (mmol L^{-1})	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01
Cl^- (mmol L^{-1})	0.08±0.01	0.07±0.01	0.06±0.01	0.09±0.01

Means ± SEM. No significant difference occurred among treatments (one-way Anova, P > 0.05).

this species could appear between 1h and 24h, thus justifying the results of the present study. Traíra maintained at 1.16mg L^{-1} for 30 min reduced plasma osmolarity (SAKURAGUI et al., 2003), and goldfish (***Carassius auratus***) exposed to 0.75mg L^{-1} for 24h presented lower plasma Cl^- levels (SOLLID et al., 2003). Therefore, very low DO levels caused ion loss in freshwater teleosts, but mildly hypoxic levels (as 3.5mg L^{-1} or higher) might not have this osmoregulatory effect. Supporting this possibility, four different DO levels (5.12, 7.76, 12, and 20mg L^{-1}) did not affect plasma Na^+ levels in the European sea bass (CECCHINI & CAPUTO, 2003).

In the present study, silver catfish exposed to high NH_3 levels (0.1mg L^{-1}) and maintained at pH 7.38 for 6-24h presented higher plasma Na^+, K^+ and Cl^- levels compared to controls (Table 2). CARNEIRO et al. (in press) demonstrated that 0.4mg L^{-1} NH_3 (pH 8.0) significantly decreases plasma cortisol levels in this species after exposure from 5h to 96h. Increased plasma cortisol levels can increase gill and gut permeability to water and ions (WENDELAAR BONGA, 1997), which may decrease plasma Na^+ and Cl^- in freshwater fish (PIERSON et al., 2004). Consequently, reduction of plasma cortisol levels due to the exposure to high NH_3 levels (0.1mg L^{-1}) would lead to opposite results and explain the findings of the present study. Effects of water NH_3 on plasma ion levels seems to be species dependent and vary according to water pH and NH_3 levels (Table 3).

Bile is a hepatic secretion with ion levels similar to plasma (GROSELL et al., 2000). Between meals hepatic bile is stored in the gallbladder, which reabsorbs water, Na^+ and Cl^-, changing the ionic composition of

Table 2 - Time-dependent effects of dissolved oxygen and NH_3 levels on Na^+, K^+ and Cl^- in the plasma and gallbladder bile of silver catfish. Means±SEM. Different capital letters in the same column indicate difference between treatments at the same time (6 and 24h) (P<0.05). Different lower case letters in the same column indicate difference over times in the same treatment (P<0.05).

Time (hours)	Plasma (mmol L^{-1})			Gallbladder bile (mmol L^{-1})		
Treatments	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-
0						
High oxygen + Low NH_3	160.1 ± 2.1a	3.5 ± 0.5a	110.1 ± 2.1a	180.1 ± 2.1a	7.0 ± 0.5a	20.0 ± 1.1a
6						
High oxygen + Low NH_3	162.1 ± 1.8Ca	3.7 ± 0.4Ca	112.1 ± 1.8Ca	182.0 ± 1.8Ca	7.4 ± 0.4Ca	22.0 ± 1.2Ca
Low oxygen + Low NH_3	200.2 ± 2.2Ba	10.5 ± 0.4Ba	128.0 ± 2.2Ba	232.1 ± 2.2Ba	16.1 ± 0.4Ba	36.0 ± 1.1Ba
High oxygen + High NH_3	202.1 ± 2.1Ba	11.1 ± 0.4Ba	127.0 ± 2.1Ba	230.1 ± 2.1Ba	15.9 ± 0.4Ba	35.1 ± 1.3Ba
Low oxygen + High NH_3	220.0 ± 2.3Aa	14.7 ± 0.4Aa	140.1 ± 2.3Aa	250.1 ± 2.3Aa	19.1 ± 0.4Aa	42.1 ± 1.3Aa
24						
High oxygen + Low NH_3	163.2 ± 1.9Ca	3.6 ± 0.5Ca	114.0 ± 1.9Ca	184.1 ± 1.9Ca	7.2 ± 0.5Ca	23.1 ± 1.2Ca
Low oxygen + Low NH_3	182.1 ± 2.0Bb	7.5 ± 0.5Bb	120.1 ± 2.0Bb	220.0 ± 2.0Bb	11.2 ± 0.5Bb	30.0 ± 1.0Bb
High oxygen + High NH_3	185.0 ± 2.0Bb	7.7 ± 0.5Bb	121.1 ± 2.0Bb	221.1 ± 2.0Bb	10.8 ± 0.5Bb	31.0 ± 1.4Bb
Low oxygen + High NH_3	223.0 ± 2.2Aa	14.6 ± 0.4Aa	142.1 ± 2.2Aa	248.0 ± 2.2Aa	18.9 ± 0.5Aa	40.0 ± 1.2Aa

Table 3 – Effects of waterborne NH_3 on plasma ion levels of some teleost species.

Species	NH_3 (mg L^{-1})	pH	Time of exposure (h)	Effects
Ictalurus punctatus[1]	0.11	7.0	24	no change in plasma Na^+ and Cl^-
	1.03	8.0	24	lower plasma Na^+, no change in plasma Cl^-
Oncorhynchus mykiss[2]	0.11	-	8	slight increase on Na^+ loss
	0.48	7.0	24	higher Na^+ loss and 17% mortality
Oncorhynchus mykiss[3]	0.23	7.9	-	no change in plasma Na^+ and Cl^-
	0.07-0.3	8.25	-	no change in plasma Na^+ and Cl^-
Rhamdia quelen[4]	0.1	7.38	24	higher plasma Na^+, Cl^- and K^+

[1]TOMASSO et al. (1980); [2]TWITCHEN & EDDY (1994); [3]VEDEL et al. (1998); [4]present study.

this secretion and producing the gallbladder bile (GB) (REUSS, 1989). Several authors have reported that fasted teleosts have higher Na^+ and K^+ and lower Cl^- levels in the GB than plasma (HUNN, 1969, 1972; BALDISSEROTTO et al., 1990; BALDISSEROTTO & MIMURA, 1997). In the present study, the same was observed in silver catfish GB irrespective to treatment and times of exposure. Changes in GB ion levels of silver catfish exposed to low DO or high NH_3 follow the same pattern of plasma ion levels (Table 2). The increase in plasma ion levels probably increased ion levels in the hepatic bile, because it maintains ion levels similar to plasma. Thus, the higher the ion levels in the hepatic bile, the higher the GB ion levels.

The osmotic cost is around 10% of the total fish energy budget (BOEUF & PAYAN, 2001) and therefore the lower growth in silver catfish due to high waterborne NH_3 (MIRON, 2004) or low DO levels (BRAUN et al., 2006) might be a consequence of the osmoregulatory cost supposed in the present study. Despite there are no studies of combined effects of low oxygen and high NH_3 on osmoregulation, reduction of DO levels might increase NH_3 toxicity. For example, exposure to hypoxia decreased ammonia excretion in silver catfish (ROSSO et al., 2006), and a synergistic effect of high NH_3 and low DO levels is extremely lethal to dourado, ***Salminus brasiliensis*** (SERAFINI et al., in press). In conclusion, exposure of silver catfish to high waterborne NH_3 or low DO levels increased plasma and GB ion levels. In addition, the osmoregulatory effects of high waterborne NH_3 and low DO might not be due to the same mechanism, because the combination of these factors has a synergistic effect on this osmoregulatory disturbance.

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