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PARÂMETROS DA ÁGUA ALTERAM A EXCREÇÃO DE RESÍDUOS NITROGENADOS E DE FÓSFORO E O COMPORTAMENTO DE JUVENIS DE JUNDIÁ *(Rhamdia quelen)*

TESE DE DOUTORADO

Jaqueline Ineu Golombieski

Santa Maria, RS, Brasil

2013

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Orientador: Prof. Dr. Bernardo Baldisserotto

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EFEITO DA QUALIDADE DA ÁGUA NA EXCREÇÃO DE RESÍDUOS NITROGENADOS E DE FÓSFORO E NO COMPORTAMENTO DE JUVENIS DE JUNDIÁ *(Rhamdia quelen)*

elaborada por

Jaqueline Ineu Golombieski

Como requisito parcial para a obtenção do grau de

Doutor em Biodiversidade Animal

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Santa Maria, 25 de janeiro de 2013.

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“Todo obstáculo em seu caminho será pequeno se sua vontade de vencer for grande!”

Hudson Pessini

RESUMO

Tese de Doutorado

Programa de Pós-Graduação em Biodiversidade Animal

Universidade Federal de Santa Maria

PARÂMETROS DA ÁGUA ALTERAM A EXCREÇÃO DE RESÍDUOS NITROGENADOS E DE FÓSFORO E O COMPORTAMENTO DE JUVENIS DE JUNDIÁ *(Rhamdia quelen)*

AUTOR: Jaqueline Ineu Golombieski

ORIENTADOR: Bernardo Baldisserotto

Data e Local da Defesa: Santa Maria/RS, 25 de janeiro de 2013.

No primeiro estudo analisou-se a excreção de amônia, ureia, creatinina, proteína, nitrito, nitrato e fósforo (P) em diferentes níveis de dureza da água, ácido húmico ou pH em juvenis de jundiás (*Rhamdia quelen*). Os peixes foram submetidos a diferentes níveis de dureza da água (4, 24, 50 ou 100 mg $CaCO_3$ L^{-1}), ácido húmico (0, 2,5 ou 5,0 mg L^{-1}) ou pH (5,0, 6,0, 7,0, 8,0 ou 9,0) durante 10 dias. A excreção nitrogenada global medida foi de 88,1% (244 – 423 µmol kg^{-1} h^{-1}) para amônia, 10,9% (30 – 52 µmol kg^{-1} h^{-1}) para creatinina, 0,02% (0,05 – 0,08 µmol kg^{-1} h^{-1}) para proteína, 0,001% (0,002 – 0,004 µmol kg^{-1} h^{-1}) para ureia, 0,5% (0,64 – 3,6 µmol kg^{-1} h^{-1}) para nitrito e 0,5% (0,0 – 6,9 µmol kg^{-1} h^{-1}) para nitrato, e estas proporções não foram afetadas pelos níveis de dureza da água ou ácido húmico. A excreção global de P em *R. quelen* foi 0,14 – 2,97 µmol kg^{-1} h^{-1}. A excreção de amônia em *R. quelen*, em geral, foi significativamente maior nas primeiras 12 horas após a alimentação e nenhum efeito claro dos níveis de dureza da água, ácido húmico e do pH pode ser observado sobre este padrão diário de excreção de amônia. A dureza da água afetou apenas a excreção de amônia e de P de juvenis de *R. quelen* no primeiro e quinto dias após a transferência, respectivamente. A exposição desta espécie ao ácido húmico diminuiu a excreção de amônia após 10 dias de exposição, mas não afetou a excreção de P. Um aumento no pH diminuiu a excreção de

amônia e aumentou a excreção de creatinina, mas não alterou a excreção de P em *R. quelen*. Portanto, quando houver qualquer alteração nos níveis de ácido húmico ou pH na cultura desta espécie os compostos nitrogenados devem ser monitorados, pois suas taxas de excreção são variáveis. Por outro lado, as taxas de excreção de P determinados no presente estudo são aplicáveis a uma ampla gama de condições na cultura de peixes. O objetivo do segundo estudo foi determinar o pH preferido em 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 de $CaCO_3$ L^{-1}) e então transferidos para um tubo de polietileno com um gradiente de pH de 3,5 a 11,7. 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 preferido 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 de $CaCO_3$ L^{-1} o pH preferido 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.

Palavras-chave: piscicultura, gradiente de pH, ácido húmico, dureza da água

Abstract

PhD Dissertation

Post-Graduate Program in Animal Biodiversity

Universidade Federal de Santa Maria

WATER QUALITY CHANGES ON NITROGENOUS COMPOUNDS AND PHOSPHORUS EXCRETION AND BEHAVIOR OF SILVER CATFISH (*Rhamdia quelen*) **JUVENILES**

AUTHOR: Jaqueline Ineu Golombieski

ADVISER: Bernardo Baldisserotto

Date and Place of Defense: January 25th , 2013, Santa Maria/RS.

In the first study examined ammonia, urea, creatinine, protein, nitrite, nitrate, and phosphorus (P) excretion at different water hardness, humic acid or pH levels in silver catfish *(Rhamdia quelen)* juveniles. The fish were exposed to different levels of water hardness (4, 24, 50, or 100 mg $CaCO_3$ L^{-1}), humic acid (0, 2.5 or 5.0 mg L^{-1}) or pH (5.0, 6.0, 7.0, 8.0, or 9.0) for 10 days. The overall measured nitrogen excretions were 88.1% (244 – 423 µmol kg^{-1} h^{-1}) for ammonia, 10.9% (30 – 52 µmol kg^{-1} h^{-1}) for creatinine, 0.02% (0.05 – 0.08 µmol kg^{-1} h^{-1}) for protein, 0.001% (0.002 – 0.004 µmol kg^{-1} h^{-1}) for urea, 0.5% (0.64 – 3.6 µmol kg^{-1} h^{-1}) for nitrite, and 0.5% (0.0 – 6.9 µmol kg^{-1} h^{-1}) for nitrate, and these proportions were not affected by water hardness or humic acid levels. The overall P excretion in *R. quelen* was 0.14 – 2.97 µmol kg^{-1} h^{-1}. Ammonia excretion in *R. quelen* usually was significantly higher in the first 12 h after feeding, and no clear effect of water hardness, humic acid levels and pH on this daily pattern of ammonia excretion could be observed. Water hardness only affected the ammonia and P excretion of *R. quelen* juveniles in the initial and fifth days after transfer, respectively. The exposure of this species to humic acid decreased ammonia excretion after 10 days of exposure but did not affect P excretion. An increase in pH decreased ammonia and increased creatinine excretion but did not change P excretion in *R. quelen*. Therefore, when there is any change on humic acid levels or pH in the culture of this species nitrogenous compounds must be monitored because their excretion rates are variable. On the other hand, P excretion rates

determined in the present study are applicable to a wide range of fish culture conditions. The aim of the second study was to determine the preferred pH in silver catfish *Rhamdia quelen* 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. The position of the fish in the pH gradient was observed at 1, 2, 4, 6, 8, 10, and 12 h after transference. 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.

Keywords: fish culture, pH gradient, humic acid, water hardness

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1 INTRODUÇÃO

O Brasil destaca-se no contexto mundial pela grande extensão de costa marítima e reservas de água doce que detém, além de seu clima extremamente favorável para o crescimento de organismos cultivados, com terras disponíveis e ainda relativamente baratas na maior parte do país, mão de obra abundante e crescente demanda por pescado no mercado interno. No entanto, a exploração racional e sustentável destas características para o desenvolvimento da aquicultura é ainda muito deficitária, especialmente pela lentidão de difusão de tecnologias geradas no meio científico para o meio de produção primária (SEAP, 2009).

Como existe o hábito de consumir espécies nativas, as quais são obtidas por meio da pesca, há a possibilidade de que a produção destas espécies melhore em função do aumento da demanda. No caso da região sul do Brasil, o cultivo de jundiá, *Rhamdia quelen*, apresenta-se como uma boa alternativa, pois das espécies nativas esta é a mais procurada em função de sua resistência às baixas temperaturas e seu crescimento rápido, apesar da sua produção permanecer muito abaixo das possibilidades da região (BALDISSEROTTO, 2009a).

A qualidade da água utilizada na piscicultura é muito importante para o crescimento e o desenvolvimento do jundiá, pois quanto melhor seu controle, melhor será o rendimento da criação visto que para todas as fases de vida dessa espécie são necessários níveis ideais de determinados parâmetros de água **(**BALDISSEROTTO; RADÜNZ NETO, 2004).

1.1 A espécie – *Rhamdia quelen* – jundiá

O jundiá, *Rhamdia quelen*, é uma espécie economicamente importante no Brasil e que ocorre do Sul do México à Argentina Central, apresentando 49 sinonímias, com coloração variando de marrom-avermelhado claro a cinza ardósia (SILFVERGRIP, 1996). É uma espécie que habita lagos e rios, sendo muito apreciada para consumo nos países deste

continente (PARRA *et al.*, 2008). Com hábito bento-pelágico, observado em ambientes lênticos e com fundo arenoso ou lodoso, costuma sair à noite em busca de alimentos como insetos ou peixes menores, porém também é hábil em digerir alimentos de origem vegetal, o que o caracteriza como um peixe onívoro preferencialmente piscívoro (COLDEBELLA; RADÜNZ NETO, 2002).

A produção desta espécie pode ser uma alternativa para regiões com clima temperado e subtropical (GOMES *et al.*, 2000; BARCELLOS *et al.*, 2001). Este peixe apresenta maturidade sexual no primeiro ano de vida o que mostra dois picos reprodutivos por ano (primavera e verão) com múltiplas desovas. O desenvolvimento embrionário é rápido e o desenvolvimento larval ocorre em 3-5 dias (GOMES *et al.*, 2000; BARCELLOS *et al.*, 2004). Existem alguns estudos sobre incubação (SILVA *et al.*, 2003) e larvicultura em diferentes pHs e durezas de água (LOPES *et al.*, 2001, TOWNSEND *et al.*, 2003, SILVA *et al.*, 2003, 2005).

É um peixe de fácil reprodução e com boa resistência ao manejo, apresentando excelente aceitação pelo mercado consumidor, tanto para pesca esportiva quanto para a alimentação, com características propícias ao processamento (GOMES *et al.*, 2000; BARCELLOS *et al.*, 2004; CARNEIRO; MIKOS, 2005).

1.2 Parâmetros físico-químicos da água

1.2.1 pH

O pH da água é um parâmetro muito importante a ser considerado na aquicultura já que possui um profundo efeito sobre o metabolismo e processos fisiológicos de peixes e de

todos os organismos aquáticos (ARANA, 1997; PARRA; BALDISSEROTTO, 2007). É regulado pelo sistema gás carbônico – bicarbonato – carbonato, oscilando em níveis que variam entre 6,0 a 8,0 (ZAIONS; BALDISSEROTTO, 2000). Mudanças extremas no pH da água causam alterações fisiológicas nos peixes, resultando, por exemplo, em limitação de crescimento e reprodução (ZWEIG *et al*., 1999), e por fim, levando à morte (ARIDE *et al*., 2004).

A mudança no pH da água também é responsável por significativas alterações nos mecanismos iônicos dos peixes (WOOD *et al.*, 1998; MATSUO; VAL, 2002). Desequilíbrios iônicos podem causar colapso no sistema de circulação dos peixes e eventualmente levar à morte (VAN DIJK *et al*., 1993). De acordo com Baldisserotto (2009b), a redução de pH na água desencadeia um aumento da excreção urinária de íons hidrogênio (H^+) e amônia (NH_4^+) para compensar esse problema, levando a uma redução do pH da urina. Nestas condições, os íons H^+ afrouxam as junções entre as células das membranas brânquias de modo que aumenta a perda de íons para o meio externo por difusão.

pHs extremamente ácidos causam degeneração nas brânquias e aumento na produção de muco, podendo matar o peixe por asfixia (BOYD, 1990). Em águas extremamente alcalinas, entretanto, a imediata inibição da excreção de amônia pode ocorrer, causando um aumento potencialmente letal dos níveis desta no plasma dos peixes (WILKIE; WOOD, 1994).

Com relação a alterações comportamentais observadas em peixes expostos a ambientes com pHs extremos, trabalhos na literatura mostram que ocorrem mudanças na interação social da espécie (PEDDER; MALY, 1986), inibição de cavar e de nadar contra o fluxo de água (IKUTA *et al.*, 2003) e comportamento de fuga daquele local específico, procurando um local de maior conforto (KROON, 2005).

Juvenis de jundiá podem sobreviver em uma faixa de pH de 4,0 a 9,0 em águas moles (20-30 mg $CaCO_3$ L^{-1}) sendo que fora desta faixa de pH o jundiá apresenta uma redução dos níveis corporais de Na^+ e K^+, o que pode levar a uma diminuição no crescimento e morte (ZAIONS; BALDISSEROTTO, 2000). Trabalhos com crescimento de juvenis de jundiá (COPATTI *et al.*, 2005; COPATTI *et al.*, 2011) mostraram que a exposição a água com pH 5,5 ou 9,0 reduz o crescimento quando comparado a pH 7,5. Já para larvas de jundiá o melhor crescimento é obtido com pH entre 8,0 e 8,5 (LOPES *et al.*, 2001).

1.2.2 Resíduos nitrogenados

A amônia é o poluente aquático mais comum e tóxico para peixes, composto este resultante do catabolismo das proteínas (ARANA, 1997) com sua entrada no ambiente natural através de resíduos industriais e da agricultura e também produtos naturais da quebra de nitrogênio da matéria orgânica (THURSTON; RUSSO, 1983; PIEDRAS *et al*., 2006). Estes compostos excretados pelos peixes podem chegar a concentrações tóxicas em culturas com alta densidade de peixes, reduzindo o crescimento e a produtividade (TOMASSO *et al.*, 1980; FRANCES *et al.*, 2000; MIRON *et al.*, 2008; 2011).

A toxicidade da amônia em organismos aquáticos pode ser atribuída principalmente à forma não-ionizada (NH_3) livremente difusível através das membranas biológicas (FELIPO; BUTTERWORT, 2002; WICKS; RANDALL, 2002; IP *et al.*, 2004; CHEW *et al.*, 2005), enquanto a forma ionizada (NH_4^+) é considerada menos tóxica (WILKIE, 1997). A exposição a altos níveis de amônia em peixes provoca danos branquiais (edema e fusão das lamelas) (MIRON *et al.*, 2008) e nos processos osmorregulatórios.

Neste sentido, o pH desempenha um papel importante na homeostase dos peixes, pois diminuindo-se ou aumentando-se o pH ocorrem distúrbios no balanço ácido-base, regulação

iônica e excreção da amônia (FOSS *et al.*, 2003; BALDISSEROTTO, 2009b). Níveis de amônia não-ionizada de 0,4-2,0 mg L^{-1} (depende do pH) provocam morte de jundiás em poucos dias, enquanto que o crescimento é prejudicado em níveis de NH_3 acima de 0,1 mg L^{-1} (BALDISSEROTTO; RADÜNZ NETO, 2004). A amônia é excretada principalmente através das brânquias (WILKIE, 2002; BUCKING; WOOD, 2008), e teleósteos geralmente aumentam a excreção de amônia após a alimentação (ALTINOK; GRIZZLE 2004).

Ureia, proteína e creatinina são outros resíduos nitrogenados da excreção de peixes. A ureia é excretada em quantidades significativas em algumas espécies de peixes, porém não é tóxica (WILKIE, 2002; LAM *et al.* 2008). Já a creatinina e a proteína são excretados em menores quantidades dependendo da espécie de peixe em estudo, assim como nitrito e nitrato que em algumas espécies não foram excretadas em quantidades detectáveis (KAJIMURA *et al*., 2002).

1.2.3 Dureza da água

A dureza da água é um parâmetro ambiental que pode influenciar no cultivo de peixes. Vários estudos têm demonstrado que o ajuste da dureza e pH da água melhora a sobrevivência de algumas espécies de peixes (McDONALD *et al.*, 1980, LOPES *et al.*, 2001; TOWNSEND; BALDISSEROTTO, 2001) principalmente durante os primeiros estágios de desenvolvimento (MOLOKWU; OKPOKWASILI, 2002; TOWNSEND *et al.*, 2003, SILVA *et al.*, 2003, 2005). Os íons cálcio (Ca^{2+}) e magnésio (Mg^{2+}) são os principais constituintes da dureza da água, sendo que o Ca^{2+} possui papel fundamental na regulação iônica, reduzindo a permeabilidade das brânquias e, consequentemente, o fluxo difusivo de íons para o meio aquático (GONZAL *et al.*, 1987, WURTS; DURBOROW, 1992). Nos peixes, o Ca^{2+} e o Mg^{2+} são essenciais para a formação dos ossos, coagulação do sangue e outras reações metabólicas

(BALDISSEROTTO, 2009b). Trabalho realizado com juvenis de jundiá demonstrou que a dureza da água de 30 – 80 mg $CaCO_3$ L não afeta o crescimento em pH neutro (COPATTI *et al.*, 2011).

1.2.4 Substância húmica

A maior parte da matéria orgânica dissolvida (DOM) na água doce é composta pelas substâncias húmicas, e em ecossistemas de água doce oligotróficos a concentração de carbono orgânico dissolvido (DOC) situa-se entre 1 e 100 mg L^{-1} (BANO *et al.*, 1997; McDONALD *et al.*, 2004; STEINBERG *et al.*, 2006). Segundo Canellas *et al.* (2001) e McDonald *et al.* (2004), de modo geral, é aceita a distribuição das frações humificadas em três categorias: as *huminas*, que representam a matéria orgânica intimamente ligada à fração mineral do solo, e por isso insolúvel; os *ácidos fúlvicos*, que apresentam grande quantidade de grupamentos funcionais oxigenados e são solúveis tanto em meio ácido como básico, e os *ácidos húmicos*, os quais representam a fração reativa mais estável da matéria orgânica humificada.

A quantidade e a origem da matéria orgânica podem ser consideradas os mais importantes fatores para determinar o funcionamento biótico do ecossistema de um rio, com a fração de DOM tendo um papel chave no fluxo de energia. O DOC em ambientes aquáticos representa uma das grandes atividades do carbono orgânico em reservatórios na biosfera, com um total de DOC isolado nos oceanos sendo comparável ao gás carbônico na atmosfera. Ele é um recurso vital, afetando diretamente cada rede alimentar, ou indiretamente através de mecanismos como turbidez, pH, quelação de metais e transporte de contaminantes (McDONALD *et al.*, 2004).

A relevância ecoquímica das substâncias húmicas é grandemente discutida no que diz respeito a sua capacidade para se ligar ou integrar poluentes semelhantes a xenobióticos

orgânicos e metais pesados, decrescendo sua biodisponibilidade e toxicologia (HAITZER *et al.*, 1998). Entretanto, alguns grupos funcionais também podem interagir diretamente com as membranas biológicas (TRANVIK, 1990; VIGNEAULT *et al.*, 2000).

A água doce contém uma soma variável de substâncias dissolvidas, tais como sais e compostos orgânicos, dependendo do solo no qual ocorre. Assim, a presença de ácidos húmicos e fúlvicos, formados no solo através da decomposição da matéria orgânica, pode reduzir o pH da água abaixo de 3,5, como observado nas águas negras dos rios amazônicos (PARRA; BALDISSEROTTO, 2007). Na bacia Amazônica, o termo "água negra" é aplicado à coloração escura, transparente da água, que tipicamente têm um baixo teor de íons, com elevada acidez e alta concentração de material húmico dissolvido (MATSUO; VAL, 2007).

Muitas espécies de peixes sobrevivem a mudanças agudas de pH para águas com pH abaixo de 4,0 – 5,0 ou acima de 9,0 -10,0, mas a exposição a águas mais ácidas ou alcalinas é letal em algumas horas (PARRA; BALDISSEROTTO, 2007). Numerosos rios da Bacia amazônica apresentam grande diversidade de peixes, como o Rio Negro (mais de 1000 espécies), indicando que esses peixes são altamente adaptados para condições extremas e em particular, baixos pH e concentrações de Ca^{2+} (STEINBERG *et al.,* 2006). Águas com baixo teor de íons e de baixo pH são fisiologicamente desafiadoras para peixes, principalmente devido ao seu efeito conhecido sobre a regulação iônica branquial (MATSUO; VAL, 2007). Entretanto, em águas pretas com DOC, a inibição do fluxo é atenuada, pois Wood *et al.* (2003) verificaram que o DOC natural pode atuar diretamente nas brânquias, em águas com baixo pH, para exercer um efeito protetor, o qual supõe-se que seria semelhante à ação protetora do Ca^{2+}. Por outro lado, em experimentos realizados com ácido húmico comercial em pH ácido, os referidos autores verificaram que houve uma exacerbação no efluxo iônico ao invés de efeito protetor, já que o produto comercial pode apresentar uma elevada afinidade aos íons Ca^{2+}, mobilizando este íon branquial e assim promovendo o desequilíbrio

osmorregulatório observados nos peixes expostos a pH ácido. Matsuo e Val (2007) demonstraram que cardinal tetra *Paracheirodon axelrodi* (Schultz) exposto cronicamente à substância húmica comercial, especialmente em condições de baixo pH, diminui a perda de Na^+ corporal, sugerindo que há uma proteção ao efluxo deste íon.

Trabalho realizado com jundiás expostos ao ácido húmico comercial (10, 25 e 50 mg L^{-1}) em pHs ácidos já foram realizados por Costa (2012). Este autor conclui que a presença de ácido húmico sintético na água é prejudicial para o desenvolvimento de juvenis de jundiá, pois quanto maior a concentração de ácido húmico na água, maior é a barreira água-sangue nas lamelas e a proliferação de células de cloreto nas brânquias. O ácido húmico parece proteger juvenis de jundiá contra os efeitos ionorregulatórios negativos da exposição ao pH ácido, mas isso não se reflete numa melhor sobrevivência.

1.2.5 Fósforo na água

O fósforo é um mineral essencial para peixes porque atua em vários processos metabólicos, pois além de participar da formação do ATP, faz parte do metabolismo dos fluídos corporais e aminoácidos e, em casos extremos, sua carência pode afetar a formação do esqueleto e ocasionar a morte dos animais (DATO-CAJEGAS; YAKUPITIYAGE, 1996; RODEGUTSCORD *et al.*, 2000; DIEMER *et al.*, 2011).

A concentração de fósforo na dieta dos peixes deve atender às exigências necessárias para o bom desempenho, sem comprometer a qualidade da água de cultivo (BALLESTRAZZI *et al.*, 1994). Esse mineral, juntamente com o nitrogênio, é um dos principais nutrientes que provocam a eutrofização dos corpos d'água, sendo considerado por vários órgãos fiscalizadores como elemento de alta capacidade poluidora (LAZZARI; BALDISSEROTTO, 2008).

Peixes tem a capacidade de absorver o P da água, mas a concentração deste elemento em água doce normalmente é baixa. Portanto os peixes não obtém fósforo suficiente da água e, devido ao fato do fósforo contido nos alimentos convencionais ser parcialmente indisponível, as rações para peixes são usualmente suplementadas com fontes de fósforo (LI *et al.*, 1996). Grande parte do P orgânico não é digerível e é excretado nas fezes (ROY; LALL, 2004).

1.3 Luminosidade e abrigos para jundiá

O habitat do jundiá é o fundo dos rios com baixo fluxo de água, onde o peixe passa o dia em lugares escondidos e apresentando sua maior atividade durante a noite quando sai a procura de alimentos (GOMES *et al.*, 2000). Experimentos realizados com jundiás em aquários relataram que estes apresentaram uma aversão à luz, buscando locais escuros como refúgios (PIAIA *et al.*, 1999). Trabalhos de radiotelemetria mostraram que os jundiás se concentram em áreas caracterizadas por apresentar barrancos muito inclinados com abrigos em forma de seixos grandes ou troncos submersos (SCHULZ; LEUCHTENBERGER, 2006).

A adaptação a diferentes cores e intensidades de luz (branca com 580nm, azul com 436 nm e verde com 480 nm) afeta a resposta ao estresse no jundiá (BARCELLOS *et al.*, 2006). Por outro lado, a adaptação por um período de 10 dias em tanques brancos e azuis não obteve nenhum efeito na resposta de cortisol a um estressor agudo. No entanto, um tanque de cor azul combinado com a presença de um abrigo apropriado pode reduzir a magnitude e a duração da resposta ao estresse. Em conjunto, os resultados sugerem que a melhor alternativa para manter alevinos de jundiá são tanques com paredes azuis e com abrigo (BARCELLOS *et al.*, 2009).

1.4 Jundiá e ictioftiríase

Um dos agentes patogênicos mais importantes em peixes de água doce é o protozoário ciliado *Ichthyophthirius multifiliis*, causador da ictioftiríase, também conhecida como "doença dos pontos brancos", "ictio" ou "ich" (SELOSSE; ROWLAND, 1990; TRAXLER *et al*., 1998; BUCHMANN; NIELSEN, 1999; GLEENSON *et al.*, 2000; TUMBOL *et al.*, 2001). Este parasita causa consideráveis perdas em cultura de peixes, devido à mortalidade e/ou redução no crescimento, como resultado de danos ao epitélio branquial do hospedeiro durante seu ciclo de vida, comprometendo a osmorregulação e as trocas de gases pelas brânquias (EWING *et al.*, 1994; TUMBOL *et al.*, 2001).

A aplicação de 4 g de sal (NaCl) L^{-1} é um tratamento eficaz para o ictio do jundiá, pois provoca uma gradual redução dos pontos brancos e sobrevivência de 100% (MIRON *et al.*, 2003). Três banhos terapêuticos com 10 g de sal L^{-1}, durante uma hora em intervalos de 48h também são uma boa alternativa para o controle de ictio (CARNEIRO *et al.*, 2005). Contudo, o uso de até 6,0% de sal na ração foi ineficaz no tratamento dessa infestação (GARCIA *et al.*, 2007). A infestação pelo ictio também é afetada pelo pH e dureza da água, pois em dureza de 20 mg $CaCO_3$ L^{-1} e pH 5, a infestação é menor que em pH mais altos, enquanto que em dureza 120 mg $CaCO_3$ L^{-1} em pH 7 a sobrevivência é melhor que em pH 5 (GARCIA *et al.*, 2011).

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3 OBJETIVO GERAL

Avaliar o efeito de diferentes níveis de pH, dureza da água e ácido húmico na excreção de resíduos nitrogenados em juvenis de jundiás, *Rhamdia quelen*, além de verificar o pH preferencial em exemplares aclimatados em diferentes durezas da água, e se a presença de abrigos e a infecção por *Ichthyophthirius multifiliis* modifica essa preferência.

4 OBJETIVOS ESPECÍFICOS

- Verificar o efeito de diferentes pHs na excreção de resíduos nitrogenados e fósforo em juvenis de jundiá;

- Avaliar as consequências de diferentes níveis de dureza da água na excreção de resíduos nitrogenados e fósforo em juvenis de jundiá.

- Verificar o efeito de diferentes níveis de ácido húmico na excreção de resíduos nitrogenados e fósforo em juvenis de jundiá;

- Verificar o pH preferido de juvenis de jundiás não infectados e infectados com *Ichthyophthirius multifiliis*, aclimatados em diferentes durezas da água.

- Verificar se a presença de abrigos pode modificar o pH preferido de juvenis de jundiá

5 DESENVOLVIMENTO

Neste item serão apresentados os trabalhos resultantes desta tese de doutorado:

ARTIGO I. Nitrogenous and phosphorus excretions in juvenile silver catfish (*Rhamdia quelen*) **exposed to different water hardness, humic acid and pH levels.** Artigo no prelo (2012) em *Fish Physiology and Biochemistry* . Doi: 10.1007/s10695-012-9744-8

MANUSCRITO I. Preferred pH of silver catfish *Rhamdia quelen***: effect of water hardness presence of shelter, and infection by** *Ichthyophthirius multifiliis***.** Submetido à revista Neotropical Ichthyology

5.1 Artigo I - Nitrogenous and phosphorus excretions in juvenile silver catfish (*Rhamdia quelen*) exposed to different water hardness, humic acid and pH levels

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Abstract: This study examined ammonia, urea, creatinine, protein, nitrite, nitrate, and phosphorus (P) excretion at different water hardness, humic acid or pH levels in silver catfish *(Rhamdia quelen)* juveniles. The fish were exposed to different levels of water hardness (4, 24, 50, or 100 mg L^{-1} $CaCO_3$), humic acid (0, 2.5 or 5.0 mg L^{-1}) or pH (5.0, 6.0, 7.0, 8.0, or 9.0) for 10 days. The overall measured nitrogen excretions were 88.1% (244 – 423 µmol kg^{-1} h^{-1}) for ammonia, 10.9% (30 – 52 µmol kg^{-1} h^{-1}) for creatinine, 0.02% (0.05 – 0.08 µmol kg^{-1} h^{-1}) for protein, 0.001% (0.002 – 0.004 µmol kg^{-1} h^{-1}) for urea, 0.5% (0.64 – 3.6 µmol kg^{-1} h^{-1}) for nitrite, and 0.5% (0.0 – 6.9 µmol kg^{-1} h^{-1}) for nitrate, and these proportions were not affected by water hardness or humic acid levels. The overall P excretion in *R. quelen* was 0.14 - 2.97 µmol kg^{-1} h^{-1}. Ammonia excretion in *R. quelen* usually was significantly higher in the first 12 h after feeding, and no clear effect of water hardness, humic acid levels and pH on this daily pattern of ammonia excretion could be observed. Water hardness only affected the ammonia and P excretion of *R. quelen* juveniles in the initial and fifth days after transfer, respectively. The exposure of this species to humic acid decreased ammonia excretion after 10 days of exposure but did not affect P excretion. An increase in pH decreased ammonia and increased creatinine excretion but did not change P excretion in *R. quelen*. Therefore, when there is any change on humic acid levels or pH in the culture of this species nitrogenous compounds must be monitored because their excretion rates are variable. On the other hand, P excretion rates determined in the present study are applicable to a wide range of fish culture conditions.

Keywords: ammonia, acidic water, alkaline water, waterborne calcium, humic substance

Introduction

The main nitrogenous compound excreted by fish is ammonia, which may reach toxic concentrations in high-density fish cultures and reduce growth and productivity (Tomasso et al. 1980; Frances et al. 2000; Miron et al. 2008; 2011). However, a significant proportion of nitrogenous waste may also be excreted as urea (Wood 1993), creatinine, protein (Evans et al. 2005), nitrite, and nitrate in some species (Kajimura et al. 2004). Ammonia is toxic at low concentrations, particularly in the un-ionized ammonia form (NH_3) (Wicks and Randall 2002; Ip et al. 2004; Chew et al. 2005; Miron et al. 2008). The main internal source of ammonia in fish is the catabolism of proteins, and most of this waste product is produced in the liver during the transamination of amino acids followed by the deamination of glutamate (Wicks and Randall 2002). Ammonia is mainly excreted through the gills (Wilkie 2002; Bucking and Wood 2008), and teleosts usually increase ammonia excretion after feeding (Altinok and Grizzle 2004).

Phosphorus (P) is also an important end product in fish and can affect not only the rearing water but also the whole environment. The soluble forms of phosphorus directly affect water quality, whereas the particulate form settles to the bottom of the tank or accumulates in the sediment (Ballestrazzi et al. 1998). Phosphorus excretion (69-86% of dietary P) is by the feces and urine (Lazzari and Baldisserotto 2008).

Humic substances (HS) account for 50–80% of the dissolved organic matter in non-eutrophicated freshwater ecosystems. In the ion-poor Amazonian “blackwaters”, HS protects fish against ionoregulatory effects induced by a low pH because they stimulate the uptake of essential ions, such as Na^+ and Ca^+, at extremely low pH values (3.5–4.0) (Steinberg et al. 2006; Matsuo and Val 2007). In the only study regarding ammonia excretion, exposure of stingray (*Potamotrygon* sp) to humic acid or dissolved organic carbon (DOC: 8.4 mg L^{-1})

from the Rio Negro (Amazon) blackwater did not significantly change ammonia excretion compared to water with negligible levels of DOC (0.6 mg L^{-1}) (Wood et al. 2003).

The pH plays an important role in the homeostasis of aquatic animals. An increase or decrease in pH is reported to cause disturbances in acid–base balance, ion regulation and ammonia excretion (Danulat 1995; Wood 2001). Fish exposed to alkaline waters have increased plasma ammonia levels because a significant proportion of the excreted ammonia remains as NH_3 in the water. In alkaline waters, the amount of H^+ available to react with NH_3 to produce NH_4^+ is low. Under these conditions, the NH_3 plasma–water gradient is reduced, thus decreasing NH_3 excretion and increasing accumulation in the plasma and tissues (Wilkie et al. 1996).

Brazilian fish farmers are interested in culturing silver catfish, *Rhamdia quelen* (Quoy & Gaimard 1824) (Heptapteridae), because of its good growth rate, omnivorous feeding habits, high fertilization and hatching rates, and acceptance by consumers (Gomes et al. 2000). The survival of juveniles in acidic and alkaline water is improved by the addition of Ca^{2+} to the water (Townsend and Baldisserotto 2001), and high water hardness reduced the deleterious effects of acidity (pH 5.5) on growth in soft water (Copatti et al. 2011). Ammonia excretion of *R. quelen* was determined at different life stages and the influence of fasting time on this parameter was also analyzed (Garcia et al. 2012).

There are no studies regarding the effect of water hardness on ammonia and phosphorus excretion in fish, and only a few studies regarding the effect of humic acid levels on ammonia excretion have been reported. Consequently, the objective of this study was to examine ammonia, urea, creatinine, protein, nitrite, nitrate, and phosphorus excretion at different levels of water hardness, humic acid and pH in *R. quelen* juveniles.

Materials and Methods

Fish

Silver catfish (*R. quelen*) juveniles were obtained from a fish culture near Santa Maria, southern Brazil. These juveniles were transferred to the Fish Physiology Laboratory at the Universidade Federal de Santa Maria and maintained in three continuously aerated (two air pumps of 12 W each) 250 L tanks for 15 days at room temperature of 23 ± 0.1 °C. Water has a pH of 7.8 ± 0.09, a dissolved oxygen level of 7.1± 0.1 mg L^{-1}, a water hardness level of 24 ± 0.1 mg L^{-1} $CaCO_3$, an un-ionized ammonia (NH_3) level of 0.007 ± 0.0004 mg L^{-1}, and a nitrite level of 0.04 ± 0.02 mg L^{-1}. The values of these physicochemical parameters of the water are suitable for silver catfish growth (Baldisserotto et al. 2010). The fish were maintained in 24 h darkness (except during feeding and cleaning of the tanks) because this condition is favorable for this nocturnal catfish (Piaia et al. 1999). The fish were fed a total of 3% total tank biomass once a day with commercial feed for juveniles (Supra 42% crude protein, Alisul Alimentos S.A., Carazinho, Brazil) that contained 1.98% Ca^{2+} and 1.65% P. The use of fish in the methodology of this experiment was approved by the Ethical and Animal Welfare Committee of the Universidade Federal de Santa Maria (registration number 24/2007).

Nitrogenous compounds and phosphorus excretion

The juveniles (7.28 ± 0.57 g and 10.29 ± 0.28 cm) were randomly redistributed in continuously aerated 40 L^{-1} polypropylene boxes (10 fish per box) and kept for seven days. For the water hardness experiment, the fish were exposed to 4, 24, 50, or 100 mg L^{-1} $CaCO_3$. A water hardness of 4 mg L^{-1} $CaCO_3$ was obtained using distilled water, and waterborne Na^+, Cl^- and K^+ were adjusted to identical levels of the water with hardness of 24 mg L^{-1} $CaCO_3$ by

adding NaCl and KCl. A water hardness of 50 or 100 mg L^{-1} $CaCO_3$ was reached by adding $CaCl_2.2H_2O$. In the humic acid experiment, humic acid (CAT Aldrich®, St Louis, USA) corresponding to 44% dissolved organic carbon was mixed with deionized water and stirred for 12 h before being added to the tanks at 0, 2.5 or 5.0 mg L^{-1} humic acid (nominal values). The water supply used in the experiments contained 0.2 mg L^{-1} of dissolved organic carbon (DOC), which was determined according to the methodology of Tedesco et al. (1995). In the pH experiment, the fish were exposed to either pH 5.0 (4.6 to 5.3), 6.0 (5.9 to 6.3), 7.0 (6.8 to 7.4), 8.0 (7.8 to 8.1), or 9.0 (8.7 to 9.1). The experimental pH was adjusted to an acidic or alkaline pH with 10% sulfuric acid or 10% sodium hydroxide, respectively. All experimental tests were performed with four replicates.

Fifteen minutes after feeding, all feces and residues were removed and the water was replaced and maintained at a total volume of 15 L in the boxes. Water samples were collected at 0, 2, 6, 12, and 24 h after feeding. These samples were collected on days 1, 3, 5, and 10 after transfer to the respective treatment. On the other days, approximately 50% of the water in the boxes was replaced by water with previously adjusted hardness, humic acid and pH levels. The dissolved oxygen levels and temperature through the experiments were 7.1 ± 0.1 mg L^{-1} and 23.0 ± 0.2°C, respectively.

Aquaria (four replicates) without fish were spiked with ammonium chloride (0.4 mg L^{-1}) and monopotassium phosphate (0.1 mg L^{-1}) and then sampled to determine the amount of ammonia and P that was lost. There was no significant change throughout the 10 days of the study. Fish then were placed in a 1 L aquaria (3 fish/aquaria, average total weight: 39.7 ± 0.1 g) with five replicates, fed, and left for 24 h to evaluate the nitrogenous compounds excreted by fish that had been previously adapted to different water hardness, humic acid or pH levels. After this period, water samples were collected and the ammonia (Verdouw et al. 1978), urea (Rahmatullah and Boyde 1980), creatinine (Kit Analisa®, CAT 335, Gold Analisa Diagnóstica

Ltda, Belo Horizonte, MG, Brazil), protein (Bradford 1976), nitrite and nitrate (Eaton et al. 2005) levels were analyzed.

Water analysis

Dissolved oxygen and temperature were monitored with an oxygen meter (model Y5512, YSI Inc., Yellow Springs, Ohio, USA). The pH was verified with a DMPH-2 pH meter (Digimed, São Paulo, Brazil), water hardness was analyzed by the EDTA titrimetric method (Eaton et al. 2005), and alkalinity was analyzed according to Boyd and Tucker (1992). Phosphorus levels were determined following the methodology of APHA (1999), and NH_3 was calculated as described by Colt (2002). Na^+ and K^+ levels were measured with a B262 flame photometer (Micronal, São Paulo, Brazil), Cl^- levels were measured according to Zall et al. (1956), and Ca^{2+} levels were measured as described by Antes et al. (2010). Nitrogenous compounds and phosphorus excretion were calculated according to Gonzalez et al. (1998). Standard solutions were created with analytical grade reagents (Vetec – Vetec Química Fina Ltda, Duque de Caxias, RJ, Brazil or Merck – Jacarepaguá, RJ, Brazil) dissolved in deionized water, and each standard curve was constructed with five different concentrations.

Statistical analysis

All data are expressed as the means ± standard error of mean (SEM). The homogeneity of variances between groups was tested with the Levene test. Nitrogenous compounds or phosphorus excretion of the treatment groups were compared with a two-way ANOVA (the time after feeding X the levels of water hardness, humic acid or pH) followed

by the Tukey test or the Scheirer-Ray-Hare extension of the Kruskal-Wallis test followed by the Nemenyi test. Physicochemical parameters of the water (pH, alkalinity, total and un-ionized ammonia, nitrite, Na^+, Cl^-, K^+ and Ca^{2+} ions) presented homogenous variances, and the data were compared with a one-way ANOVA followed by the Tukey test. All statistical tests used the software Statistica (version 7.0, StatSoft, Tulsa, OK). The relationships between nitrogenous compounds or phosphorus excretion and water hardness, humic acid or pH levels were calculated by linear regression with the software SigmaPlot (version 11.0, Systat Software, San Jose, CA). The minimum significance level was set at $P < 0.05$.

Results

Most water parameters did not differ between the treatments in the three experiments, but the alkalinity levels were significantly lowest at a water hardness of 4 mg L^{-1} $CaCO_3$, and the Ca^{2+} levels increased with the increase of water hardness. There was a significant positive relationship between water alkalinity and pH levels (Table 1).

There was a significant positive relationship between ammonia excretion (µmol kg^{-1} h^{-1})(y) and water hardness (x) at 2-6 h after feeding ($y = 465.3 + 27.4x – 0.2x^2$, $r^2 = 0.987$) on day 1 after transfer. However, at the other periods after feeding in this same day and on days 3, 5 and 10 after transfer, ammonia excretion did not show any significant relationship with water hardness, neither was affected by this parameter. In this experiment usually ammonia excretion was significantly lower 12-24 h after feeding. Therefore, water hardness only affected ammonia excretion in the first day after transfer and did not change the daily pattern of ammonia excretion (Figure 1).

There was a significant positive relationship between ammonia excretion (y) and humic acid levels (x) at 2-6 ($y = 640 + 78.6x + 35.92x^2$, $r^2 = 1$) and 6-12 h ($y = 436 – 20.6x +$

$25.68x^2$, $r^2 = 1$) after feeding on day 1 after transfer and 6-12 h after feeding on days 3 (y = 370 + 224x, $r^2 = 0.996$) and 5 (y = 452 + 173.4x, $r^2 = 0.972$) after transfer. On day 10 there were not significant relationships between ammonia excretion and humic acid levels, but in most intervals after feeding ammonia excretion was generally lower in fish that were exposed to humic acid than in unexposed fish. Consequently, exposure to humic acid initially increased ammonia excretion, but after 10 days it decreased this excretion. In this experiment *R. quelen* not exposed to humic acid presented higher ammonia excretion in the first 2 or 6 h after feeding, but those exposed to humic acid showed higher ammonia excretion 2-24 h after feeding, except on day 10, when higher ammonia excretion was observed at 0-6 h (Figure 2).

There was a significant negative relationship between ammonia excretion (y) and pH (x) at 6-12 h (y = 2588.9 – 287.9x, $r^2 = 0.974$) after feeding on day 3 after transfer; at 6-12 h (y = 1842.9 – 156.7x, $r^2 = 0.922$) after feeding on day 5; and at 2-6 h (y = 2770.6 – 250 x, r^2 = 0.953) and 12-24 h (y = 2340 – 222.2x, $r^2 = 0.976$) after feeding 10 days after transfer. Therefore, there is a tendency of reducing ammonia excretion with pH increase. Irrespective of pH, in this experiment ammonia excretion usually was higher in the first 6 or 12 h after feeding (Figure 3).

Overall, the measured nitrogen excretions were 88.1% (244 – 423 µmol kg^{-1} h^{-1}) for ammonia, 10.9% (30 – 52 µmol kg^{-1} h^{-1}) for creatinine, 0.02% (0.05 – 0.08 µmol kg^{-1} h^{-1}) for protein, 0.001% (0.002 – 0.004 µmol kg^{-1} h^{-1}) for urea, 0.5% (0.64 – 3.6 µmol kg^{-1} h^{-1}) for nitrite, and 0.5% (0.0 –6.9 µmol kg^{-1} h^{-1}) for nitrate, and these proportions were not affected by water hardness or humic acid levels. There was a significant positive relationship between urea, creatinine and protein excretion and pH, and a significant negative relationship was found between ammonia excretion and pH (Table 2).

Water hardness did not significantly change the P excretion on days 1, 3 and 10 after transfer. On day 5 after transfer, the highest P excretion was observed in juveniles exposed to 100 mg L^{-1} $CaCO_3$. Phosphorus excretion was significantly lower on day 1 after transfer compared with day 3, except in *R. quelen* maintained at 24 mg L^{-1} $CaCO_3$ (Figure 4A). Humic acid levels did not significantly affect P excretion on days 1, 3, 5 and 10 after transfer. Nevertheless, P excretion was significantly highest on day 10 after transfer, regardless of the humic acid levels (Figure 4B). Phosphorus excretion was not altered significantly by pH on days 1, 3 and 5 after transfer. On day 10 after transfer, the lowest P excretion was observed in juveniles exposed to pH 7.0. However, P excretion was significantly highest on days 5 and 10 after transfer compared to other days, and this was independent of pH (Figure 4C).

Discussion

Most physicochemical parameters of the water were kept within the range recommended for this species (Baldisserotto et al. 2010). Water alkalinity in the pH experiment increased with an increasing water pH because of the addition of NaOH to the water.

Ammonia excretion values in *R. quelen* were higher in the present study than the values observed in this species in Garcia et al. (2012) (450 – 850 µmol kg^{-1} h^{-1}). This result was possibly because the authors used larger fish that were fed food with lower crude protein levels (32% CP). The total nitrogen excretion rate is dependent on the species, dietary protein intake, temperature (Altinok and Grizzle 2004; Kajimura et al. 2004; Lam et al. 2008) and fish size (Leung et al. 1999; Oliva-Paterna et al. 2007). Ammonia excretion values in *R. quelen* determined in the present study were within the range observed in marble goby (*Oxyeleotris marmorata*) (Tng et al. 2008: 167 – 883 µmol kg^{-1} h^{-1}; Lam et al. 2008: 588 –

1588 µmol kg^{-1} h^{-1}) and mangrove snapper (*Lutjanus argentimaculatus*) (Leung et al. 1999: 278 – 1500 µmol kg^{-1} h^{-1}). However, these excretion values were higher than the excretion values of several other freshwater fish, as tambaqui (*Colossoma macropomum*) (331 µmol kg^{-1} h^{-1}) (Ismiño-Orbe et al. 2003), channel catfish (*Ictalurus punctatus*) (122 µmol kg^{-1} h^{-1}), goldfish (*Carassius auratus*) (205 µmol kg^{-1} h^{-1}), rainbow trout (*Oncorhynchus mykiss*) (110 µmol kg^{-1} h^{-1}) (Altinok and Grizzle 2004), tui chub (*Gila bicolor*) (100 µmol kg^{-1} h^{-1}), Tahoe sucker (*Catostomus tahoensis*) (90 µmol kg^{-1} h^{-1}), Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*) (140 µmol kg^{-1} h^{-1}), cui-ui (*Chasmistes cujus*) (45 µmol kg^{-1} h^{-1}) (McGeer et al. 1994). The urinary ammonia excretion in an 8.0 g *R. quelen* would be approximately 1.84 µmol kg^{-1} h^{-1} according to the equation presented by Bolner and Baldisserotto (2007). Based on the results of daily excretions under similar conditions, the urinary ammonia excretion in the present study would be 0.14 - 0.30% of the total ammonia excretion in *R. quelen*. The oscar (*Astronotus ocellatus*) has also presented low ammonia excretion rates through urine (less than 2% of the excretion rate across the gills) (Wood et al. 2009).

In the present study ammonia excretion in *R. quelen* usually was significantly higher in the first 12 h after feeding, in agreement with a previous study of Garcia et al. (2012) with the same species. No clear effect of water hardness, humic acid levels and pH on this daily pattern of ammonia excretion could be observed. Higher ammonia excretion occurred between 2 and 11 h after feeding in *O. mykiss*) (van Weerd et al. 1995; Dosdat et al. 1996; Bucking and Wood 2008), aerolate grouper (*Epinephelus areolatus*), mangrove snapper *L. argentimaculatus* (Leung et al. 1999), and tambaqui (*C. macropomum*) (Ismiño-Orbe et al. 2003).

Ammonia and creatinine were the most important forms of nitrogenous waste in *R. quelen*. Ammonia is responsible for 50-84% of the total nitrogenous waste excreted in most fish (Jobling 1981; Altinok and Grizzle 2004; Kajimura et al. 2004; Lam et al. 2008), but of

the few species in which creatinine+creatine excretion were measured (Kajimura et al. 2004), only anchoveta (*Engraulis ringens*) (McCarthy and Whitledge 1972) and carp (*Cyprinus carpio*) (Smith 1929) presented excretion percentages (15.9-30.1 and 4.4-6.5 %, respectively) as high as *R. quelen*. However, McCarthy and Whitledge (1972) stated that the high creatinine excretion in *E. ringens* is most likely related to the high level of activity in the chambers and/or the poor health of the specimens. The *R. quelen* specimens used in the present experiment were apparently healthy and were not active in the chambers. In addition, approximately 50% of the creatinine output was sourced from the gut in *O. mykiss* (Kajimura et al. 2004). Therefore, the high creatinine excretion by *R. quelen* was most likely not related to health problems. In comparison to *R. quelen*, much higher protein (3-11%) and urea excretion (6-10%) values were observed for *O. mykiss* (Kajimura et al. 2004) and *L. argentimaculatus* (13-21%) (Lam et al. 2008), as well as a higher urea excretion (6.3%) in fasted *C. macropomum* (Ismiño-Orbe et al. 2003), channel catfish (*I. punctatus*) (17.5%), goldfish (*C. auratus*) (17.8%) and *O. mykiss* (15.9%) (Altinok and Grizzle 2004).

Waterborne Ca^{2+} reduces gill membrane permeability and prevents diffusive ion loss to water (Parra and Baldisserotto 2007). Therefore, *R. quelen* would spend less energy for osmoregulation at a higher level of water hardness, and consequently, less protein would be used, and less ammonia (and possibly P) would be excreted. However, the absence of a water hardness effect on ammonia and P excretion on most days is consistent with the fact that water hardness from 30 to 180 mg L^{-1} $CaCO_3$ does not affect *R. quelen* juvenile growth in neutral water (Copatti et al. 2011).

The effect of humic acid levels on ammonia excretion in *R. quelen* was dependent on the time of exposure. Initially exposure to humic acid increased ammonia excretion, but after 10 days it decreased this excretion. Unpublished observations of our laboratory verified that *R. quelen* exposed to high concentrations of humic acid (10 – 50 mg L^{-1}) presented lower feed

intake and weight gain that unexposed fish. Probably the lower ammonia excretion in *R. quelen* observed in the present study after 10 days of exposure to humic acid is related to lower feed intake in these specimens. A similar study verified that a rapid exposure (2 h, pH 6.3) of the stingray (*Potamotrygon* sp) to the blackwater of the Rio Negro, Amazon (8.4 mg L^{-1} dissolved organic carbon - DOC) or a similar humic acid (Aldrich) level did not significantly change ammonia excretion compared to exposure to water with negligible levels of DOC (0.6 mg L^{-1}) (Wood et al. 2003).

Ammonia excretion in *R. quelen* decreased with the increase of water pH, and this relationship became more evident the longer the exposure time. The increase of water pH also decreased the ammonia urinary excretion rate and increased the plasma ammonia in this species (Bolner and Baldisserotto 2007), which is consistent with the findings of the present study. Alkaline water also causes inhibition of ammonia excretion in several freshwater teleosts (Scott and Wilson 2007) and the subsequent increase in plasma ammonia in *O. mykiss* (Wright and Wood 1985; Wilkie and Wood 1995; Wilkie et al. 1996; Wilson et al. 1998; Laurent et al. 2000). Ammonia excretion in the gills of freshwater fish is via the Rhesus (Rh) glycoproteins situated in the basolateral and apical membranes (Wright and Wood 2009; Weihrauch et al. 2009) and its rate of excretion is thought to be determined by the magnitude of the NH_3 partial pressure gradient across the gills (Salama et al. 1999). An inhibition of ammonia excretion is attributed to a disruption of this gradient because of a reduction in the H^+ ions available to trap NH_3 as NH_4^+ in the gill boundary layer (water layer next to external surface of the gill) (Wright et al. 1989).

The increase in water pH increased creatinine, urea and protein excretion in *R quelen*. However, the importance of urea and protein on nitrogenous excretion in this species is minimal; therefore, the increase in creatinine excretion appears to compensate for the reduction of ammonia excretion at an alkaline pH. This is the first study to demonstrate an

increase in creatinine excretion related to exposure to an alkaline pH. In *O. mykiss*, exposure to pH 10 resulted in decreased ammonia excretion because of a reduction in the ammonia production rate rather than a detoxification to glutamine or urea (Wilson et al. 1998). Perch (*Perca fluviatilis*) exposed to pH 9.5 also decreased ammonia excretion and did not increase urea excretion (Scott et al. 2005). Nitrite and nitrate excretion in *R. quelen* in all experiments were consistent with the low values observed by Kajimura et al. (2004) for *O. mykiss*.

Phosphorus is an essential dietary mineral to fish, but in aquacultural effluents, it is considered a source of pollution by many regulatory agencies. Uneaten food and unavailable dietary P in feces are the two primary contributors in fish farm effluents (Riche and Brown 1996). The overall P excretion in *R. quelen* was 0.14 - 2.97 μmol kg^{-1} h^{-1} (fed 1.65% P). Similar values of phosphorus excretion were observed in European seabass (*Dicentrarchus labrax*) (78.5 g) (0.18 and 0.14 μmol kg^{-1} h^{-1}, fed a pelleted and extruded diet containing 2.0% P) (Ballestrazzi et al. 1998) and for seabass (*Lates calcarifer)* juveniles (0.99 g) (2.48 μmol kg^{-1} h^{-1}, fed a diet with 2.64% P) (Tantikitti et al. 2005).

In conclusion, the water hardness levels in this study only affected the ammonia and P excretions of *R. quelen* juveniles on the initial and fifth days, respectively, after transference. The exposure of this species to humic acid (up to 5 mg L^{-1}) decreased ammonia excretion after 10 days but did not affect P excretion in *R. quelen* juveniles. An increase in pH decreased ammonia and increased creatinine excretion but did not change the P excretion in *R. quelen*. Therefore, when there is any change on humic acid levels or pH in the culture of this species nitrogenous compounds must be monitored because their excretion rates are variable. On the other hand, P excretion rates determined in the present study are applicable to a wide range of fish culture conditions.

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Table 1. Physicochemical parameters of the water in the experiments.

	Water hardness (mg L^{-1} $CaCO_3$)			
Parameters	4	24	50	100
pH	7.8 ± 0.08	7.7 ± 0.04	7.9 ± 0.20	7.9 ± 0.02
Alkalinity*	10 ± 0.01[b]	66 ± 0.01[a]	68 ± 0.01[a]	62 ± 0.01[a]
Total ammonia (mg L^{-1})	0.05 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.06 ± 0.00
NH_3 (mg L^{-1})	0.001 ± 0.00	0.002 ± 0.00	0.002 ± 0.00	0.001 ± 0.00
Nitrite (mg L^{-1})	0.011 ± 0.01	0.018 ± 0.01	0.020 ± 0.01	0.019 ± 0.01
Na^+ (mmol L^{-1})	0.90 ± 0.14	1.20 ± 0.13	1.54 ± 0.17	1.21 ± 0.08
K^+ (mmol L^{-1})	0.07 ± 0.0[a]	0.09 ± 0.00[a]	0.10 ± 0.01[a]	0.10 ± 0.01[a]
Cl^+ (mmol L^{-1})	1.49 ± 0.17[a]	1.80 ± 0.03[a]	1.94 ± 0.31[a]	1.60 ± 0.00[a]
Ca^{2+} (mmol L^{-1})	0.02 ± 0.001[c]	0.41 ± 0.005[b]	0.43 ± 0.01[b]	0.73 ± 0.01[a]

	Humic acid (mg L^{-1})		
Parameters	0	2.5	5.0
pH	7.6 ± 0.10	7.5 ± 0.07	7.6 ± 0.04
Alkalinity*	35 ± 5.90[a]	21 ± 3.90[b]	14 ± 1.80[b]
Hardness*	4.7 ± 0.60[a]	8.7 ± 1.52[a]	16.3 ± 0.29[b]
Total ammonia (mg L^{-1})	0.05 ± 0.00	0.07 ± 0.00	0.07 ± 0.00
NH_3 (mg L^{-1})	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00
Nitrite (mg L^{-1})	0.018 ± 0.01	0.020 ± 0.01	0.019 ± 0.01
Na^+ (mmol L^{-1})	0.06 ± 0.008[a]	0.01 ± 0.002[b]	0.04 ± 0.01[a]
K^+ (mmol L^{-1})	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.01
Cl^+ (mmol L^{-1})	0.21 ± 0.01[b]	0.37 ± 0.01[a]	0.31 ± 0.01[a]

	pH (units)				
Parameters	5.0	6.0	7.0	8.0	9.0
Alkalinity*	7 ± 0.93	16 ± 5.30	23 ± 3.50	103 ± 7.55	183 ± 28.35
Hardness*	30 ± 2.00	29 ± 1.76	27 ± 1.76	26 ± 1.15	24 ± 0.04
Total ammonia (mg	0.05 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.00

L^{-1})					
NH_3 (mg L^{-1})	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
Nitrite (mg L^{-1})	0.02 ± 0.0	0.02 ± 0.0	0.02 ± 0.0	0.02 ± 0.0	0.02 ± 0.0
Na^+ (mmol L^{-1})	0.04 ± 0.0^b	0.04 ± 0.0^b	0.05 ± 0.0^b	0.06 ± 0.0^b	0.11 ± 0.0^a
K^+ (mmol L^{-1})	0.08 ± 0.00	0.12 ± 0.02	0.11 ± 0.02	0.13 ± 0.02	0.09 ± 0.01
Cl^+ (mmol L^{-1})	0.13 ± 0.01	0.16 ± 0.03	0.18 ± 0.03	0.16 ± 0.04	0.17 ± 0.05

Means ± SEM. The 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. * Alkalinity and hardness: mg $L^{-1}CaCO_3$.

Table 2. Nitrogenous compound excretions (μmol kg^{-1} h^{-1} and in parentheses % of total) of *Rhamdia quelen* exposed to different levels of pH, water hardness and humic acid.

	pH (units)				
	5.0	6.0	7.0	8.0	9.0
Ammonia	423.2 ± 8.3	377.0 ± 6.2	358.1 ± 6.3	301.5 ± 8.4	244.6 ± 4.2
	(90.1)	(88.7)	(88.1)	(84.0)	(80.7)
Creatinine	44.1 ± 0.9	46.8 ± 0.9	46.5 ± 1.5	50.9 ± 1.4	52.7 ± 1.1
	(9.4)	(11.0)	(11.4)	(14.2)	(17.2)
Urea	0.002 ± 0.0	0.002 ± 0.0	0.002 ± 0.0	0.002 ± 0.0	0.002 ± 0.0
	(0.0004)	(0.0005)	(0.0005)	(0.0006)	(0.0007)
Protein	0.05 ± 0.0	0.06 ± 0.0	0.05 ± 0.0	0.08 ± 0.0	0.08 ± 0.0
	(0.01)	(0.01)	(0.01)	(0.02)	(0.03)
Nitrite	0.64 ± 0.4	1.20 ± 0.9	0.78 ± 0.2	2.30 ± 1.1	1.60 ± 0.1
	(0.1)	(0.3)	(0.2)	(0.6)	(0.5)
Nitrate	1.67 ± 0.4	0.0 ± 0.0	1.1 ± 0.6	4.04 ± 1.0	4.20 ± 0.2
	(0.4)	(0.0)	(0.3)	(1.1)	(1.4)
		Water hardness (mg $L^{-1}CaCO_3$)			
		4	24	50	100
Ammonia		351.8 ± 9.1	348.7 ± 6.4	319.1 ± 9.5	360.5 ± 6.1
		(89.7)	(89.9)	(88.1)	(89.5)
Creatinine		37.6 ± 0.6	36.5 ± 0.8	34.0 ± 0.6	37.8 ± 0.8
		(9.6)	(9.4)	(9.4)	(9.4)
Urea		0.003 ± 0.0	0.004 ± 0.0	0.003 ± 0.0	0.003 ± 0.0
		(0.001)	(0.001)	(0.001)	(0.001)
Protein		0.06 ± 0.0	0.07 ± 0.0	0.06 ± 0.0	0.05 ± 0.0
		(0.02)	(0.02)	(0.02)	(0.01)
Nitrite		2.70 ± 0.8	0.99 ± 0.6	1.94 ± 0.8	1.15 ± 0.2
		(0.7)	(0.3)	(0.5)	(0.3)
Nitrate		0.0 ± 0.0	1.4 ± 0.5	6.9 ± 1.0	3.2 ± 0.9

	(0.0)	(0.4)	(1.9)	(0.8)
		Humic acid (mg L^{-1})		
		0	2.5	5.0
Ammonia		351.9 ± 9.1	317.9 ± 5.9	275.5 ± 8.7
		(89.7)	(89.1)	(89.6)
Creatinine		37.6 ± 0.6	35.0 ± 1.2	30.1 ± 1.0
		(9.6)	(9.8)	(9.8)
Urea		0.003 ± 0.0	0.003 ± 0.0	0.002 ± 0.0
		(0.0008)	(0.0008)	(0.0007)
Protein		0.06 ± 0.0	0.05 ± 0.0	0.08 ± 0.0
		(0.02)	(0.01)	(0.03)
Nitrite		2.70 ± 0.8	3.61 ± 1.2	0.79 ± 0.4
		(0.7)	(1.0)	(0.3)
Nitrate		0.0 ± 0.0	0.06 ± 0.0	1.03 ± 0.0
		(0.0)	(0.0)	(0.3)

The values are reported as the means ± SEM. The relationships in the pH experiment are described by the following equations: ammonia: $y = 102.77 – 2.35x$, $r^2 = 0.919$; creatinine: $y = –0.52 + 1.88x$, $r^2 = 0.931$; urea: $y = 0.00005 + 0.00007x$, $r^2 = 0.942$; and protein: $y = –0.019 + 0.005x$, $r^2 = 0.781$, where y = % of total excreted and x = pH

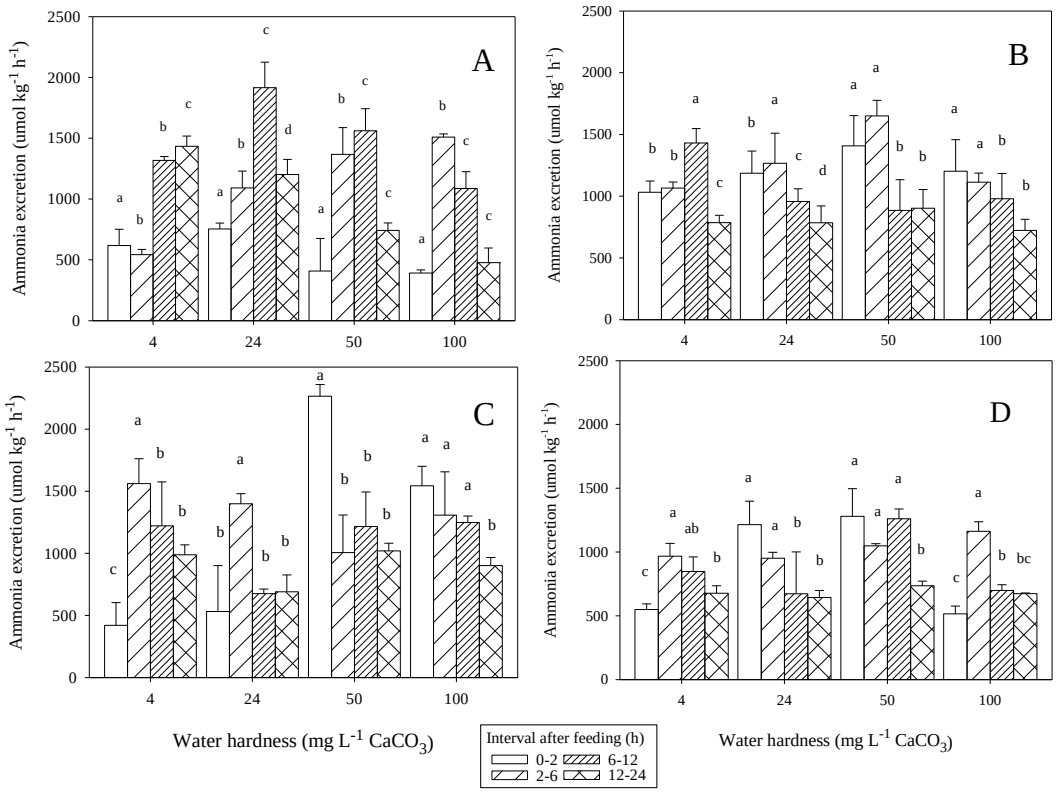

Figure 1. Ammonia excretion in silver catfish as a function of water hardness at days 1 (A), 3 (B), 5 (C) and 10 (D) after transfer. Different letters indicate significant difference between intervals after feeding in the same treatment ($P < 0.05$) as determined by the Scheirer-Ray-Hare extension of the Kruskal-Wallis test and the Nemenyi test. There was no significant effect of water hardness. The values are reported as the means ± SEM.

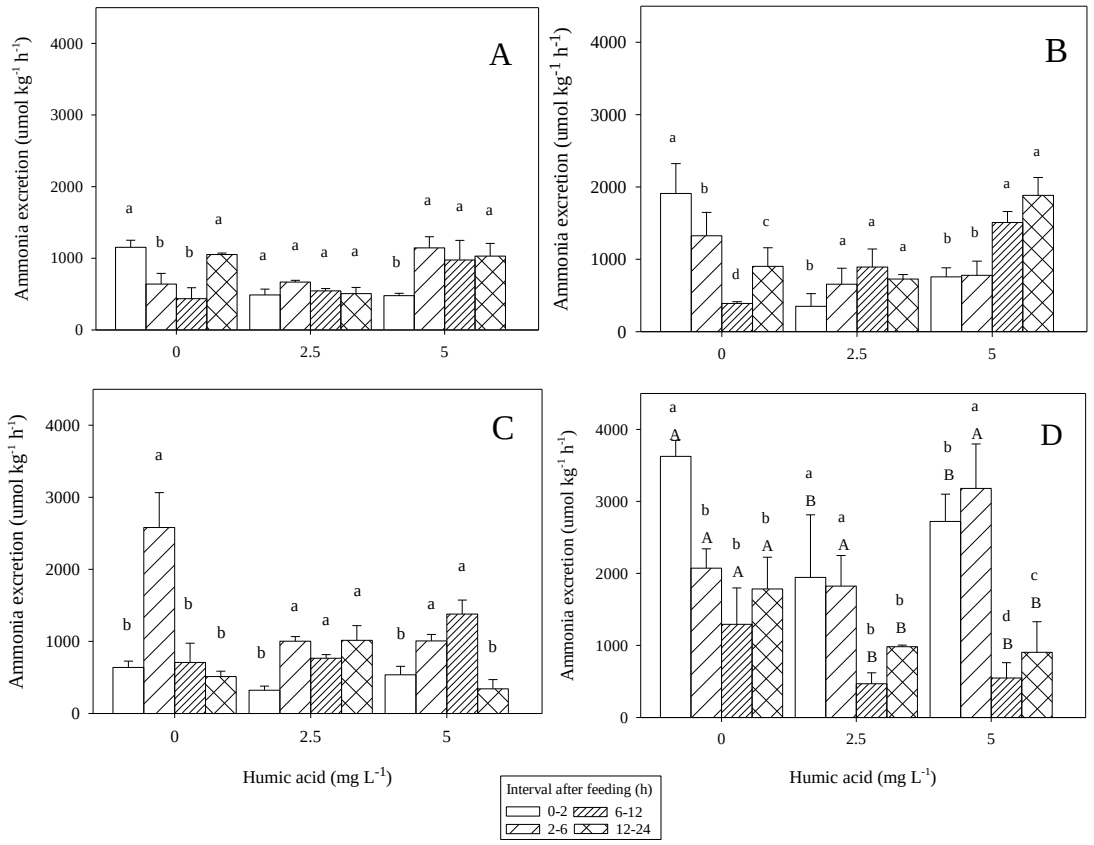

Figure 2. Ammonia excretion in silver catfish as a function of humic acid at days 1 (A), 3 (B), 5 (C) and 10 (D) after transfer. Means identified by different lowercase letters between interval after feeding and different capital letters between treatments were significantly different ($P < 0.05$) as determined by analysis the Scheirer-Ray-Hare extension of the KrusKal-Wallis test followed by the Nemenyi test. The values are reported as the means ± SEM.

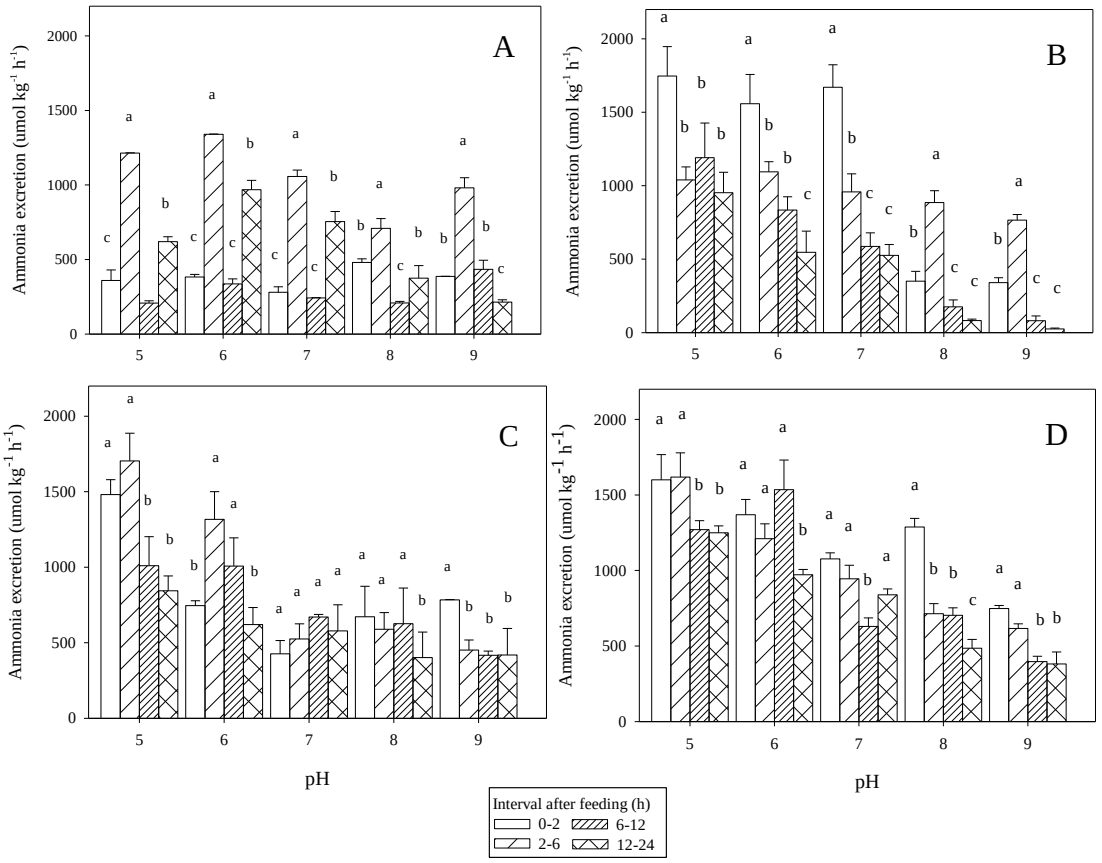

Figure 3. Ammonia excretion in silver catfish as a function of pH at days 1 (A), 3 (B), 5 (C) and 10 (D) after transfer. Means identified by different lowercase letters between interval after feeding and different capital letters between treatments were significantly different ($P < 0.05$) as determined by analysis the Scheirer-Ray-Hare extension of the KrusKal-Wallis test followed by the Nemenyi test. The values are reported as the means ± SEM.

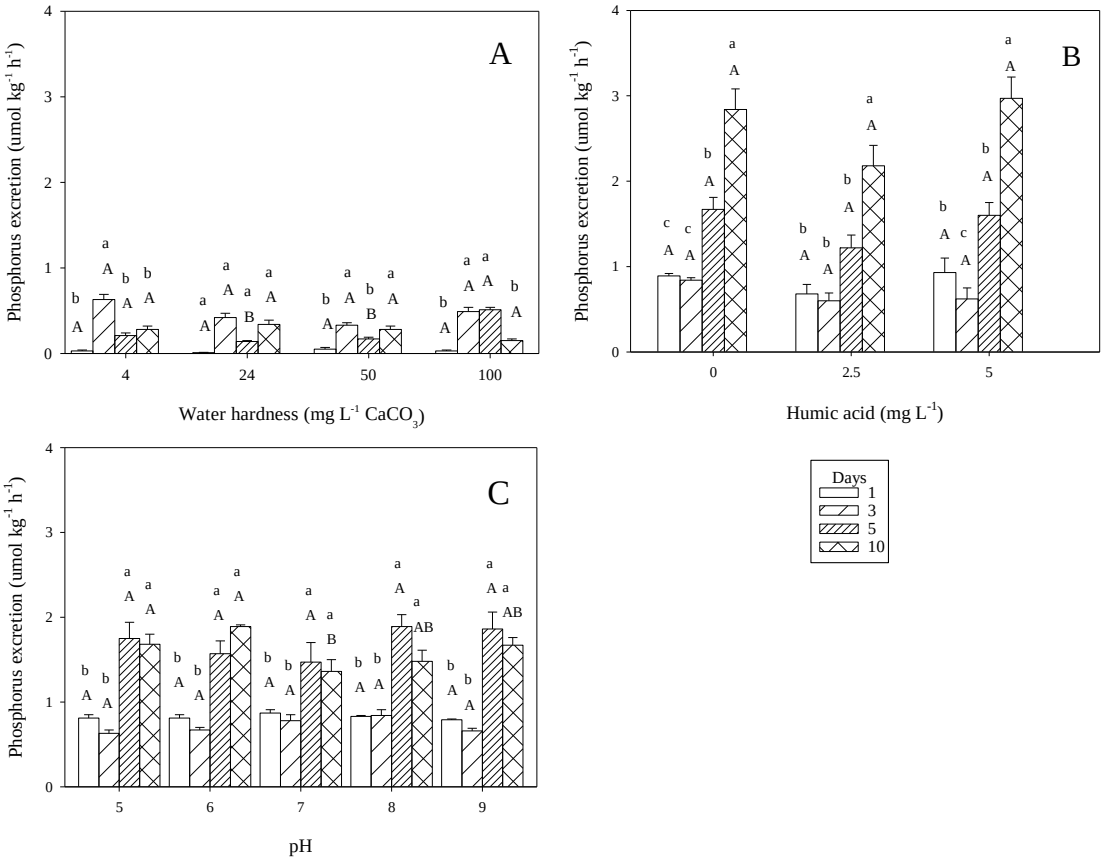

Figure 4. Phosphorus excretion in silver catfish as a function of water hardness (A), humic acid (B) and pH (C) at days 1, 3, 5 and 10 after transfer. The means identified by different lowercase letters between days (within each treatment) or capital letters between treatments were significantly different ($P < 0.05$) as determined by an ANOVA and Tukey's comparison of the mean values. The values are reported as the means ± SEM.

5.2 MANUSCRITO I - Preferred pH of silver catfish *Rhamdia quelen*: effect of water hardness presence of shelter, and infection by *Ichthyophthirius multifiliis*

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Running Head: Hardness, shelter, ictio and preferred pH

Abstract: The aim of this study was to determine the preferred pH in silver catfish *Rhamdia quelen* 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. The position of the fish in the pH gradient was observed at 1, 2, 4, 6, 8, 10, and 12 h after transference. 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.

Resumo: O objetivo deste estudo foi determinar o pH preferido de jundiás *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. 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 $CaCO_3$ L^{-1} 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: water quality, behavior, calcium, parasite, pH gradient

Introduction

Water quality may elicit a preference or avoidance response in fish (Kroon & Housefield, 2003) and 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). Several studies demonstrated that fish preferred a neutral pH against acidic or alkaline waters (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 south Brazil (Baldisserotto, 2009). Several studies determined the best pH (Zaions & Baldisserotto, 2000; Lopes *et al.*, 2001; Copatti *et al.*, 2005, 2011) 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 & Baldisserotto, 2001; Parra & Baldisserotto, 2007). Silver catfish is affected by the ciliate protozoan *Ichthyophthirius multifiliis*, 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). 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) 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. 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 (temperature: 23 ± 0.1ºC, pH: 7.1-7.7). 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 $CaCl_2.2H_2O$. Juveniles were fed once a day at 8:00 a.m. 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 furnishing the food, and consequently at least 20% of the water was replaced with water previously adjusted to the appropriate water hardness.

After acclimation to experimental water hardness each group (fasted 24h) was transferred to a 6m long polyethylene tube (200 mm diameter) 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 transference, 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 (one at each end), 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. multifilis* 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).

This study was approved by the Ethics Committee on Animal Experimentation of UFSM under registration number 24/2007.

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. multifilis* 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). Usually, the initial response of fish to an environmental perturbation is to change their

behavior (Nakamura, 1986). Uninfected silver catfish juveniles preferred neutral pH (pH 7.30-7.83) at all water hardness levels tested. These results are within the same pH range (pH 7.0-7.6) observed previously for this species at water hardness of 24 mg $CaCO_3$ L^{-1} (Riffel *et al.*, 2012). In agreement with these results at this water hardness, juveniles presented better growth at pH 7.0-7.5 than at pH 5.5 and 9.0 (Baldisserotto, 2011). Several others species also showed preference to a neutral 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 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).

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.*, 2011). However, water hardness did not change pH preference in this species.

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 favored the acid-base regulation 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. multifilis* 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 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 (9.03-9.79). 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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Table 1. Preferred pH of silver catfish acclimated to different water hardness.

Time after transference (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

Values are reported as mean ± SEM. Water hardness or the presence of shelter in the tube did not significantly change the preferred pH.

Table 2. Preferred pH of silver catfish infected with *Ichthyophthirius multifiliis* acclimated to water hardness of 24 mg $CaCO_3$ L^{-1}, after different times of the transference.

		Infected fish (trophont/fish)			
Time (h)*	Uninfected	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}**

Values are reported as means ± 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 transference

6 CONCLUSÃO GERAL

- O principal resíduo nitrogenado excretado por juvenis de jundiá é a amônia, seguida da creatinina.

- A dureza da água somente afetou a excreção de amônia e de fósforo de juvenis de jundiá no primeiro e quinto dias após a transferência, respectivamente.

- A exposição desta espécie ao ácido húmico diminuiu a excreção de amônia após 10 dias de exposição, mas não afetou a excreção de fósforo.

- O aumento do pH da água diminuiu a excreção de amônia e aumentou a de creatinina, mas não alterou excreção de fósforo em *R. quelen*.

- A aclimatação de jundiás 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.

- Em juvenis infectados com *Ichthyophthirius multifiliis* aclimatados à dureza da água de 24 mg $CaCO_3$ L^{-1}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.